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Review

Leuconostoc dextransucrase and dextran: production, properties and applications

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Abstract: This review covers the production, properties and applications of the biopolysaccharide dextran; this biopolymer can be produced via fermentation either with *Leuconostoc mesenteroides* strains and other lactic acid bacteria or with certain *Gluconobacter oxydans* strains. The former strains convert sucrose into dextran with the dextransucrase enzyme whereas the latter convert maltodextrins into dextran with the dextran dextrinase enzyme. Emphasis is mainly focused on *Leuconostoc* strains as producer organisms of dextransucrase and dextran types. In addition to industrial fermentation processes producing the enzymes and/or the dextrans, biocatalysis principles are also being developed, whereby enzyme preparations convert sucrose or maltodextrins, respectively, into (oligo)dextrans. The chemical and physical properties of different dextrans are discussed in detail, together with the characteristics and molecular mode of action of dextransucrase. Subsequently, useful applications of dextran and some problems associated with undesirable formation of dextran are outlined.

Keywords: *Leuconostoc*; dextransucrase; *Gluconobacter*; dextran dextrinase; oligodextrans

INTRODUCTION

The name 'dextran' was first used by Scheibler[1] in 1874 when he found that the mysterious thickening of cane and beet sugar juices was caused by a carbohydrate of empirical formula ($C_6H_{10}O_6$) having a positive optical rotation.[1] Previously, in 1861, Pasteur[2] had shown that these slimes were caused by microbial action[2] and van Tieghem[3] named the causative bacterium *Leuconostoc mesenteroides*.[3] Later investigators showed that dextran can be formed by several bacterial species and that it is not a well defined substance with specific properties.

Dextrans are now defined as homopolysaccharides of glucose that feature a substantial number of consecutive $\alpha(1,6)$-linkages in their major chains, usually more than 50% of the total linkages. These α-D-glucans also possess side-chains, stemming mainly from $\alpha(1,3)$- and occasionally from $\alpha(1,4)$- or $\alpha(1,2)$-branched linkages. The exact structure of each type of dextran depends on its specific producing microbial strain[4] and hence on the specific type of dextransucrase(s) involved.

DEXTRANSUCRASE (DSase)

General

The great majority of dextrans in nature are synthesised from sucrose by dextransucrase enzymes, secreted mainly by *Leuconostoc, Streptococcus* and *Lactobacillus* species.[5–9] Dextrans can also be synthesised from maltodextrins by dextran dextrinase activity of certain *Gluconobacter* strains[10,11] and the chemical synthesis of an essentially unbranched dextran has also been reported.[5,12] *Leuconostoc mesenteroides* NRRL B-512F dextransucrase has received most attention and the resulting dextran is produced commercially.[13–16] This dextransucrase is secreted in relatively large amounts into the culture supernatant with a minimum number and quantity of related contaminating enzymes and it forms a high molecular weight, soluble dextran. This may be contrasted with other strains of *L mesenteroides* and with the dental-plaque streptococci, which form both soluble and insoluble dextrans and elaborate more than one type of dextransucrase, together with relatively large levels of related, contaminating enzymes such as invertase and levansucrase.[7,13,17] For these reasons, *L mesenteroides* NRRL B-512F has served as an important model in studying the structure of dextran and the mechanism of dextran biosynthesis by dextransucrase.[4]

Purification and characteristics of dextransucrase

Dextransucrase (EC 2.4.1.5) is an extracellular glucosyltransferase (GTF), which catalyses the transfer of D-glucopyranosyl residues from sucrose to dextran,

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while fructose is released.[4,8] Whereas *Streptococcus* species produce this enzyme constitutively, dextransucrase synthesis in wild-type strains of *L mesenteroides* is usually induced by growth on sucrose.[4] Recently, Quirasco *et al*[18] demonstrated low dextransucrase yields in *L mesenteroides* NRRL B-512F cultures growing on D-glucose or D-fructose. Also, constitutive mutants have been selected.[19–22] Numerous studies have been devoted to the purification and characterisation of dextransucrase of *L mesenteroides* NRRL B-512F.[15,23–25] The enzyme was shown to occur in multiple molecular forms as a result of enzyme aggregation[15,26,27] and is typically associated with its polysaccharide product, making purification rather difficult. Miller *et al*[24] succeeded in circumventing the problems of low enzyme yield and dextran contamination by purifying dextransucrase with a combination of dextranase treatment and ion-exchange and affinity chromatography.

The enzyme appears to have an initial molecular mass of 170 kDa, a p*I* value of 4.1 and a Michaelis–Menten constant (K_m) for sucrose of ~12–16 mM. The purified dextransucrase has a pH optimum of 5.0–5.5 and a temperature optimum of 30 °C. Low levels of calcium are necessary for optimal enzyme production and activity.[15] Some authors have reported dextransucrase activation by dextran addition,[26,27] whereas others did not[24] find this effect. According to Robyt *et al*,[28] dextransucrase contains an allosteric site to which dextran binds, thereby inducing a favourable conformation for the synthesis of dextran from sucrose.

Mode of action mechanisms of dextransucrase

Mechanistic studies have shown that B-512F dextran, which is synthesised from sucrose by dextransucrase, is formed via glucosyl intermediates. The glucosyl moieties are then transferred to the reducing end of a growing glucanosyl chain, which is covalently linked to the active site of the enzyme. The mechanism proposed by Robyt and others for the synthesis of a sequence of α(1,6)-linked glucose residues in B-512F dextran involves two nucleophiles at the active site, which attack sucrose and displace fructose to give two β-glucosyl intermediates[29,30] (Fig 1).

Pulse and chase studies with ^{14}C-labelled sucrose have indicated that dextrans from both *L mesenteroides* NRRL B-512F and *Streptococcus mutans* 6715 were synthesized by addition of glucose to the reducing end of the growing dextran chain and that the mechanism of chain elongation involves two-site insertion.[31–33] Recent data point towards a differing mode of action,[4] though the mechanism involved could be strain dependent (see Section 'Structure–function relationship of dextransucrase and related glucansucrases').

According to Robyt and Eklund,[29,30] the C-6 OH group of one of the glucosyl residues attacks C-1 of the other to form an α(1,6)-linkage and one glucosyl residue is transferred to the other. The freed nucleophile attacks another sucrose molecule forming

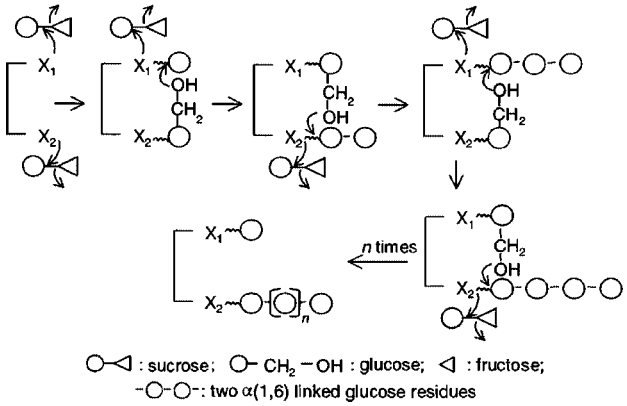

Figure 1. Mechanism proposed for the synthesis of α(1,6)-glucan by B-512F dextransucrase. X_1 and X_2 represent nucleophiles at the active site.[8]

a new enzyme glucosyl intermediate. The C-6 OH group of this new glucosyl intermediate attacks C-1 of the isomaltosyl unit (the growing dextran chain), which is actually transferred to the glucosyl residue. The glucosyl and dextranosyl units are alternately transferred between the two nucleophiles as the dextran chain is elongated at the reducing end. The elongation is terminated and the chain is released by acceptor reactions, one of which may be with an exogenous dextran chain to give a branch linkage.[8,29]

An additional requirement for the reaction to take place is the transfer of a hydrogen ion to the displaced fructosyl moiety of sucrose. Chemical modification of dextransucrase showed that two imidazolium groups of histidine were essential for dextran synthesis. It was postulated that these two imidazolium groups donate their hydrogen ions to the leaving fructose units (Fig 2) and that the resulting imidazole group, in a second step, becomes reprotonated by abstracting a proton from the attacking C-6 OH group of the glucosyl-enzyme intermediate, facilitating the nucleophilic attack and the formation of the α(1,6)-linkage.[30]

The introduction of other sugars besides sucrose (such as maltose) in the dextransucrase digests leads to the synthesis of oligosaccharides at the expense of high molecular weight dextrans. The glucosyl residue from sucrose is diverted from the synthesis of dextran and is transferred to a free hydroxyl group of those sugars that were called acceptors.[34,35] Many sugars act as acceptors and various authors have classified them according to their capacity to divert glucosyl residues from dextran to form oligosaccharides and to their effect on the rate of reaction.[35–39] Among all acceptors tested, maltose and isomaltose have been demonstrated to be the most effective.[35,36] Because of its high acceptor efficiency and its availability, maltose has been the most widely used acceptor for the synthesis of oligosaccharides by dextransucrase.[40–42] Some acceptor products can, in turn, be used as acceptors and a series of homologues acceptor products may result.[34,35,43] For example, a series of 6^2-isomaltodextrinosyl maltoses result when maltose is the acceptor.[37] With other acceptors, the first product

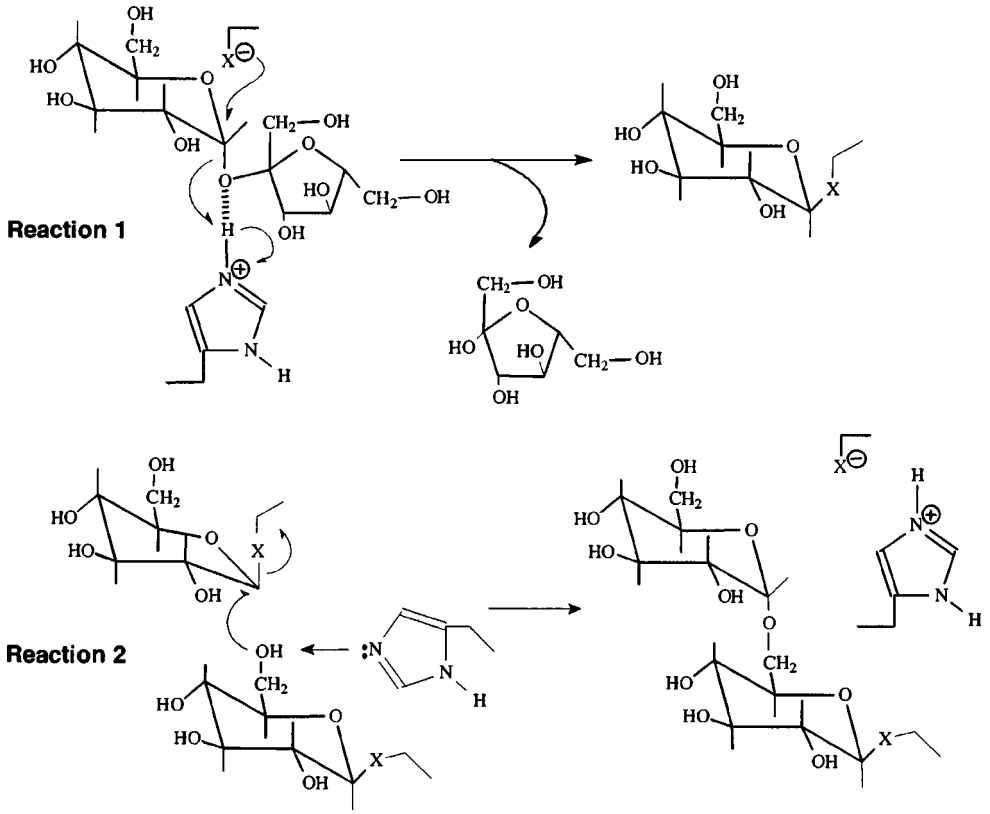

Figure 2. Mechanism for the cleavage of sucrose and the formation of an α(1,6)-glycosidic bond by dextransucrase. Reaction 1: nucleophilic displacement and protonation of the fructose moiety to form a glucosyl-enzyme intermediate. Reaction 2: formation of an α(1,6)-glycosidic bond by attack of a C-6 hydroxyl group on to the C-1 of the glucosyl-enzyme complex; the attack is facilitated by abstraction of a proton from the hydroxyl group by the imidazole group.[8]

may be a poor acceptor or a non-acceptor and no series is obtained, as in the case of fructose as an acceptor where the unusual disaccharide leucrose (α-D-glucopyranosyl-(1,5)-D-fructose) is the product.

Mechanistic studies have indicated that the acceptor interacts with the covalent enzyme-glucosyl or enzyme-dextranosyl intermediates to release the glucose or dextran chain from the enzyme-active site, with the formation of a covalent linkage between glucose or dextran and the acceptor. When the acceptor displaces the dextran from the active site, the polymerisation of the dextran chain is terminated (Fig 3).[29,35] Numerous workers have examined the complex acceptor kinetics to obtain quantitative information on oligosaccharide yield, composition and distribution.[23,36,38,39,42,44] Several authors have examined the use of such acceptor reactions to produce directly clinically sized dextran, which is momentarily produced by controlled acid hydrolysis of native dextran, followed by organic solvent fractionation.[23,39,44–46] Kim and Day[46] have developed yet another method to produce clinical dextran. The new process involves a mixed culture of *Lipomyces starkeyi*, a yeast which produces dextranase, in combination with *L mesenteroides*. Clinical dextran could be prepared with good productivity by controlling the growth of both microorganisms and the enzyme reaction conditions.

When a high molecular weight dextran chain is the acceptor, a C-3 OH of the dextran acceptor chain attacks the C-1 of the glucosyl or dextranosyl units of

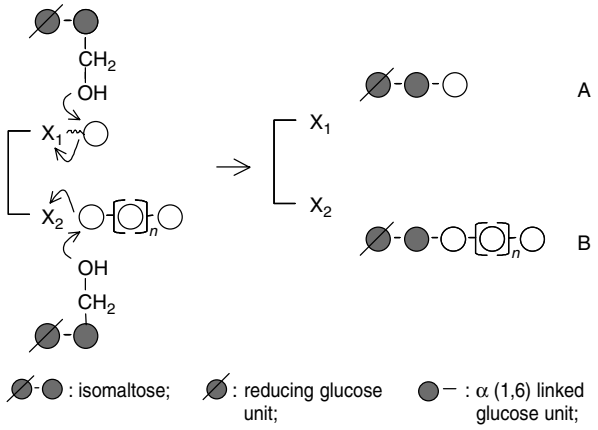

Figure 3. Mechanism of acceptor reactions for B-512F dextran sucrase. A carbohydrate such as isomaltose displaces the glucosyl unit attached to the active site of the enzyme to form a trisaccharide, isomaltotriose (A), and the glucanosyl unit to form isomaltose-reducing end-terminated glucan (B).[47]

the enzyme complex to release glucose or dextran and form a branch linkage between the acceptor dextran and the glucose or dextran chain from the enzyme (Fig 4).[8,47] This has thrown doubt upon earlier assumptions that secondary linkages in dextrans were formed through the action of a separate branching enzyme.[7] Indeed, it has recently been shown at the molecular level that the formation of α(1,2)-branched linkages are due to a second catalytic domain on one dextransucrase enzyme.[48,49]

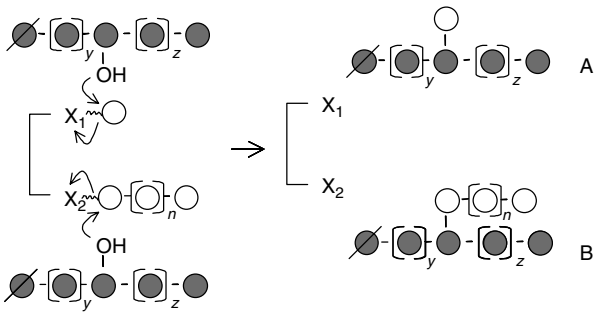

Figure 4. Mechanism for forming branch linkages in dextran. An exogenous dextran chain displaces the glucosyl unit forming an α(1,3)-glucosyl dextran (A) or displaces the dextranosyl unit forming an α(1,3)-dextranosyl dextran (B).[8]

Bozonnet *et al*[48] and Fabre *et al*[49] reported on the *L mesenteroides* NRRL B-1299 dextransucrase gene, *dsrE*, which they isolated, sequenced and cloned in *Escherichia coli*; the recombinant enzyme was shown to be an original glucansucrase which catalyses the synthesis of α(1,6)- and α(1,2)-linkages. The nucleotide sequence of the *dsrE* gene consists of an open reading frame of 8508 bp coding for a 2835 amino acid protein with a molecular mass of 313 267 Da. This is twice the average mass of the glucosyltransferases (GTFs) known so far, which is consistent with the presence of an additional catalytic domain located at the carboxy terminus of the protein and of a central glucan-binding domain (GBD), which is also significantly longer than in other glucansucrases.[50] From sequence analysis, DSR-E was classified in the glucoside-hydrolase family 70, where it is the only enzyme to have two catalytic domains, CD1 and CD2. The recombinant protein DSR-E synthesizes both α(1,6)- and α(1,2)-glucosidic linkages in transglucosylation reactions using sucrose as the donor and maltose as the acceptor. To investigate the specific roles of CD1 and CD2 in the catalytic mechanism, truncated forms of *dsrE* were cloned and expressed in *E coli*. Gene products were then small-scale purified to isolate the various corresponding enzymes. Dextran and oligosaccharide syntheses were performed. Structural characterisation by ^{13}C NMR spectroscopy and/or high-performance liquid chromatography (HPLC) showed that enzymes devoid of CD2 synthesised products containing only α(1,6)-linkages. On the other hand, enzymes devoid of CD1 modified α(1,6) linear oligosaccharides and dextran acceptors through the formation of α(1,2)-linkages. Therefore, each domain is highly regiospecific, CD1 being specific for the synthesis of α(1,6)-glucosidic bonds and CD2 only catalysing the formation of α(1,2)-linkages. This recent finding elucidates the mechanism of α(1,2) branching formation and allows one to engineer a novel transglucosidase specific for the formation of α(1,2)-linkages. This enzyme can be very useful to control the rate of α(1,2)-linkage synthesis in tailor-made dextran or oligosaccharide production.[49]

Structure–function relationship of dextransucrase and related glucansucrases

Over 30 sucrose glycosyltransferase genes (from *Leuconostoc* sp., *Streptococcus* sp., etc) have now been sequenced and their catalytic sites have been identified by comparison with the enzymes from family 13 of the glycoside-hydrolases (the α-amylase family). Both families share related mechanistic and structural characteristics.[50,51] These results allowed dextransucrase to be classified in the glycoside-hydrolase (GH) family 70.[50,52] Only one catalytic site has been identified in all dextransucrases of known sequences, except for the dextransucrase (DSR-E) from *L mesenteroides* NRRL B-1299, which is the one—in addition to α(1,6)-linkages—forming α(1,2)-linkages, rather than α(1,3)- or α(1,4)-linkages.

These recent data weaken the above-mentioned general mode of action mechanism proposed earlier for dextransucrase.[29,30]

Devulapalle and co-workers[51,53] have identified the catalytic triad involved in the formation of the glucosyl-enzyme complex as two aspartic acids and one glutamic acid residue. One of the aspartic acids is involved in the formation of the covalent intermediate.[54,55] The mechanistic role of these residues has been confirmed by site-directed mutagenesis.[51,53] The acid–base catalyst which donates its proton is a glutamic acid residue; conserved histidine in the active site is proposed to stabilize the glucosyl-enzyme complex, but not to act as a nucleophile.

Also, in the case of DSR-E, Fabre *et al*[49] recently clearly showed that truncated enzymes with only one active site are totally active in producing either only α(1,6)-linkages or only α(1,2)-linkages. In this context, several authors have suggested that another mode of synthesis involving only one catalytic site could operate[50,54,55] and that elongation could also occur from the non-reducing end. Such a mechanism has recently been demonstrated at the molecular level for amylosucrase from *Neisseria polysaccharea*; this is a GTF that uses sucrose to produce mainly a linear polymer, composed of α(1,4)-glucopyranosyl residues, similar to amylose.[56]

Substrate specificity of dextransucrase

Dextran cannot be produced by dextransucrase from glucose, mixtures of glucose and fructose or any other naturally occurring sugar; sucrose is absolutely required.[8]

The relatively high energy (16.7–20.9 kJ mol^{-1}) of the acetal–ketal linkage joining the glucose and fructose moieties of sucrose is utilised by the enzyme to synthesise the α(1,6)-linkages of the main chain (Fig 2). No ATP or cofactors are required.[4] Although sucrose is the only naturally occurring substrate, α-D-glucopyranosyl fluoride and *p*-nitrophenyl-α-D-glycopyranoside are also substrates for glucansucrases, although at rates much lower than that for sucrose.[8]

APPLICATIONS OF DEXTRANSUCRASE

Industrial production of dextran

To date, commercial dextran production is mainly accomplished by growing cultures of *L mesenteroides* in media containing sucrose, an organic source of nitrogen such as peptone, growth factors, certain trace minerals and phosphate. The bacterium is facultatively anaerobic or microaerophilic and fermentations are not aerated. Operative production factors include initial pH (typically 6.7–7.2), temperature (about 25 °C), initial sucrose concentration (usually 2%) and fermentation time (usually 24–48 h).[4] Little further process control takes place. During the first 20 h of fermentation, the culture pH falls to ~5.0 because of the formation of organic acids, favourably near the optimal pH of dextransucrase. Dextran branching appears to increase at elevated temperatures.[57] Dextran is harvested from the fermentation medium by alcohol precipitation and purified by further precipitation after redissolution in water. Cell debris is removed by centrifugation. This conventional process has the disadvantage of propagating cells, producing the enzyme and synthesising dextran under a single set of conditions that change during the course of the fermentation and, therefore, are only transitorily optimal for any one of the three stages.[16]

It has long been recognised that dextran could also be produced enzymatically using cell-free culture supernatants that contain dextransucrase. This allows dextran synthesis under controlled conditions and yields a purer polymer.[16] Conditions in the enzymic method are more constant and easier to control than in the whole-culture method; furthermore, the product is more uniform and easier to purify.[8]

The enzyme-producing fermentations were optimised by improving the different culture characteristics.[58] The optimal pH for enzyme production was 6.5–7.0, whereas the optimal pH for enzyme activity was 5.0–5.2. Fermentations needed to be carried out at 23 °C.[16] Higher yields of dextransucrase were obtained by control of the sucrose level in the fermentation broth around 0.5–1.0%. It was shown that dextran synthesis follows Michaelis–Menten kinetics up to 200 mM sucrose. At higher sucrose concentrations (>200 mM), it has been proposed that sucrose binds at a low-affinity, third sucrose binding site that allosterically changes the conformation of the active site of dextransucrase in such a way as to prevent the interaction of the two glucosyl groups to give dextran elongation.[59] *L mesenteroides* requires the growth factors nicotinic acid, thiamine, panthothenic acid and biotin, together with the amino acids valine and glutamic acid.[60] Robyt and Walseth[15] reported that the production of dextransucrase was increased twofold by the addition of 0.005% calcium chloride; treatment of *L mesenteroides* cells with the mutagen nitrosoguanidine, resulted in the selection of a mutant (designated B-512FM) which produced 300 times more enzyme than the parent strain.[37] Dextransucrase has also been immobilised, although this approach may be most useful for production of oligosaccharides.[61–63]

Since sucrose has to be used as an inducer for enzyme synthesis, simultaneous production of dextran and dextransucrase inevitably occurs. This causes a viscous culture and the formation of a complex of dextran with the enzyme has often interfered with the isolation and purification of dextransucrase.[7,34] In this context, constitutive mutants able to produce the enzyme in the absence of sucrose have been selected. Mutagenesis of cells of *L mesenteroides* B-512F resulted in constitutive mutants capable of producing dextransucrase in media containing sugars other than sucrose, such as glucose, fructose and maltose, without the simultaneous synthesis of dextran.[19–21] Enzymatic synthesis offers advantages of product molecular weight and quality control, and also the benefit of obtaining fructose as a valuable co-product. However, this approach has been largely ignored for commercial production, presumably for economic reasons.[4]

Synthesis of oligosaccharides

Oligosaccharides are now being used in foods as a source of energy, as a nutriceutical or as a sweetener. However, knowledge related to their informative biological function and their role in cell–surface interactions is increasing rapidly and is greatly stimulating the field of glycotechnology.[50] In addition to their traditional use, oligosaccharides find more and more novel applications in the food, feed, pharmaceutical or cosmetic sectors as stabilisers, bulking agents, immunostimulating agents and prebiotic compounds able to stimulate the growth of beneficial bacteria of the intestinal or skin microflora.[50,64]

The primary physiological function of dextransucrase consists of synthesising high molecular weight dextran. However, the enzymatic activity can be redirected from glucan synthesis towards oligosaccharide synthesis when an efficient acceptor is added to the reaction mixture.[29] A large variety of glucansucrases, which synthesise different types of osidic bonds, are now available for oligosaccharide synthesis.[65]

The glucooligosaccharides (GOS) produced by the action of dextransucrase from *L mesenteroides* NRRL B-512F on maltose and sucrose are composed of a maltose residue located at the reducing end and additional glucosyl residues, all $\alpha(1,6)$-linked.[35,45]

When dextransucrase from *L mesenteroides* NRRL B-1299 was used as a catalyst with maltose as acceptor, it was possible to obtain GOS which present one or more D-glucosyl units linked via $\alpha(1,2)$-osidic bonds.[41,42,64,66] Oligosaccharides containing $\alpha(1,2)$-linked residues are highly resistant to the attack of digestive enzymes; hence they are used as prebiotics in cosmetic and human nutritional applications.[50]

Remaud *et al*[67] investigated the acceptor reactions catalysed by dextransucrase from *L mesenteroides* NRRL B-742. They found that 19% of the acceptor

products, isolated from the linear oligosaccharides using a glucanase treatment, contained α(1,3)-branch linkages.

STRUCTURE OF *Leuconostoc mesenteroides* DEXTRAN

General

The particular dextrans which were used initially in synthetic blood plasma substitutes came into their role more through force of circumstances than through known superiority for the purpose. When the clinical use of dextran was initiated in Sweden in 1944, only a small number of dextran types had been reported and not all of these had been well characterised chemically.[4] It was certain however, that these dextrans were homologous polymers of glucose with predominantly α(1,6)-linkages. Numerous factors appeared to influence the properties and the amount of microbially produced dextran. Previously reported dextrans had varied in solubility, viscosity, specific rotation and content of nitrogen, phosphorus and ash.[68] This evidence of the variations among dextrans indicated the need for systematic chemical and physical studies to determine the range of diversity of the dextran polysaccharides from different microorganisms.[13]

Heterogeneity in dextran preparations

In 1954, Jeanes *et al*[13] characterised and classified dextrans from 96 bacterial strains and established that the chemical and physical properties of dextrans vary greatly according to the bacterial strain from which they were derived. These 96 strains belonged to five genera, then named *Acetobacter, Leuconostoc, Streptococcus, Betabacterium* and *Streptobacterium*. All except two of these strains produced their dextrans from sucrose. Two *Acetobacter* strains (now *Gluconobacter oxydans*) transformed amylaceous dextrin into dextran (see Section 'Dextrans synthesised by *Gluconobacter* dextran dextrinase (DDase)').

The sucrose-derived dextrans were characterised through determination of the chemical nature and proportion of glucosidic linkages present by periodate oxidation, through measurement of specific rotation, viscosity and infrared absorption and through observations on the solubility and the physical appearance of the highly hydrated gums and of their aqueous solutions.

One constant feature of all the dextrans was their structural component, which appeared to be almost exclusively the anhydroglucopyranose unit of α configuration. The other chemical and the physical characteristics of the dextrans covered wide ranges of values. The α(1,6)-glucosidic linkages constituted from 50 to 97% of the total linkages. As determined by periodate oxidation analysis, the non-α(1,6)-linkages were of two types, the α(1,4)-like and the α(1,3)-like. The intrinsic viscosity, solubility and nature of the dextran-precipitates gave continuous spectra of values. According to Wilham *et al*,[69] three types of heterogeneity appeared to occur in dextran preparations. These types were designated (a) gross heterogeneity of the culture, due to the presence of polysaccharides insoluble in the culture medium or precipitated at alcohol concentrations below or above the range for dextran (35–65%), (b) size heterogeneity, due to the broad distribution of molecular sizes, and (c) structural heterogeneity, due to the presence of different kinds and proportions of glucosidic linkages. It was pointed out by Wilham *et al*[69] that the requirements for any specific use may be more adequately provided by one particular dextran than by another. It became apparent, therefore, that further commercial utilisation of dextrans must be based on the inherent chemical and physical properties of individual dextrans.

Determination of structural features of dextran

Types of linkages

The initial procedures developed in 1954 by Jeanes *et al*[65] based on optical rotation, infrared spectra and periodate-oxidation reactions were suited for preliminary examination of dextran structures, but precluded obtaining fully reliable data.

The types and configurations of the linkages in a large number of dextrans have been determined by characterising disaccharide fragments obtained by acid hydrolysis and acetolysis of dextrans. Partial acid hydrolysates of dextrans invariably contain the disaccharide isomaltose (α-D-Glc*p*-(1,6)-D-Glc). In addition, traces of kojibiose (α-D-Glc*p*-(1,2)-D-Glc), nigerose (α-D-Glc*p*-(1,3)-D-Glc) and maltose (α-D-Glc*p*-(1,4)-D-Glc) have been found in acid hydrolysates of some highly branched dextrans.[7]

The failure to isolate secondary-linked disaccharides from the acid hydrolysates of most dextrans was attributed to the fact that secondary D-glucopyranosidic linkages are inherently more acid labile than (1,6)-D-glucosidic linkages.

The order of stability to acid hydrolysis of D-glucopyranosidic linkages is reversed under the conditions of acetolysis. As a consequence, acetolytic, degradative procedures have enabled secondary-linked disaccharide fragments to be obtained from a large number of native dextrans.[7]

Jeanes *et al*[65] also developed a method for analysing the types and proportions of the D-glucopyranosidic linkages in native dextrans from the amounts of periodate ion reduced and formic acid produced during the periodate oxidation. However, the fact that certain, differently linked, D-glucopyranosyl residues can only be determined collectively (because they react with equal amounts of periodate ion) limits the use of this method.

Methylation analysis is widely used to determine the position of linkages between component monosaccharide residues in oligo- and polysaccharides.[70] This is usually achieved by treating the carbohydrates with methylsulfinyl carbanion to form polyalkoxide ions,

followed by methylation with methyl iodide. The methylated carbohydrates are then hydrolysed and the partially methylated monosaccharides reduced and acetylated. The resulting alditol acetates of methylated sugars are separated by gas chromatography and identified by their retention times and mass spectra.[70] Using this methodology, Lindberg and Svensson[14] analysed dextran from *L mesenteroides* NRRL B-512F. They obtained mixtures of 2,3,4,6-tetra-*O*-methyl-, 2,3,4-tri-*O*-methyl and 2,4-di-*O*-methyl-D-glucose. The presence of other tri- and di-*O*-methyl-D-glucoses could not be established, demonstrating that all main chain residues were (1,6)-linked and that all branches started from 3-positions.

Methylation analysis on dextran, produced by *Gluconobacter oxydans* grown on maltodextrins, demonstrated the presence of (1,6)- and (1,4)-linkages with (1,4,6)-branching points.[71]

Sawai *et al*[72] used isomaltodextranase from *Arthrobacter globiformis* T6 for the hydrolysis of 14 native dextrans. Paper chromatography of the dextran digests revealed that this dextranase produced, in addition to isomaltose, one or two trisaccharides (isomaltose residues substituted by (1,2)-, (1,3)- or (1,4)-α-D-glucopyranosyl groups at the non-reducing D-glucopyranosyl residues). The relative amounts of these trisaccharide products appeared to indicate the approximate relative amounts of a particular linkage among the dextrans or the relative amounts of two kinds of linkages of each dextran.

Dextran structures have often been elucidated by use of degradative enzymes of known specificity, followed by oligosaccharide identification by means of thin-layer chromatography, HPLC or ^{13}C NMR spectroscopy.[18,37,63,66,73,74]

Frequency and distribution of branching

Covacevich and Richards[75] used an endodextranase from *Pseudomonas* UQM 733 to study the distribution of branch points in dextran from *L mesenteroides* NRRL B-512F. The specificity of the enzyme was such that, for effective action, it required more than five successive α(1,6)-linked glucopyranosyl units without branch points. Unfortunately, it was then not possible to reach any exact conclusion about the distribution of branches, but certain general comments concerning the regularity and concentration of branch points could be made with this method. In the meantime, chromatographic systems have been described for the analysis of carbohydrates in dextran hydrolysates and for distinction of different oligosaccharides[76].

Length of branches

Indications of the lengths of the branch chains in native dextrans stemmed initially from the results of statistical analysis of fragments of low molecular weight, obtained from acid hydrolysates of dextrans.[7] More specific information concerning the lengths of the branches in several dextrans has also been obtained by sequential degradation of fragments from dextrans that had been catalytically oxidised or *p*-toluenesulfonylated.[77]

Lindberg and Svensson[14] studied the length of branches in dextran by catalytic oxidation of the polysaccharides to carboxydextrans, followed by partial acid hydrolysis, isolation and characterisation of the aldobiouronic acids formed. The fact that the rate of acid hydrolysis of methyl α-D-glucopyranoside was drastically lowered when the hydroxymethyl group on C-5 is replaced by a carboxyl group prompted the suggestion that it might be possible to stabilise structural segments in a dextran with respect to acid hydrolysis by catalytically oxidising the hydroxymethyl groups in the dextran to carboxyl groups and then identify them by characterising the acidic oligosaccharides obtained on hydrolysing the oxidised dextran with acid.[7]

Other studies on dextran side-chains relied on the separation of enzymic degradation products by paper chromatography or, when improved resolution was required, by HPLC.[78]

Kobayashi and Matsuda[79] demonstrated that the glucoamylase from *Rhizopus niveus* can be used to remove D-glucose residues from the non-reducing ends of dextran, its action being stopped at the branch points, thus giving information on the side-chain length.

Degree of polymerisation

Manners *et al*[80] described a method which is suitable for the routine estimation of the polymerisation degree (DP_n) of polymers of D-glucose. After reduction with borohydride, the glucan is hydrolysed with acid and the amount of sorbitol produced is estimated by an enzyme-catalysed oxidation reaction. Sorbitol dehydrogenase catalyses the following reversible reaction:

$$\text{sorbitol} + \text{NAD}^+ \rightleftharpoons \text{D-fructose} + \text{NADH} + \text{H}^+$$

At pH values in the range 9.0–10.0 and in the presence of excess NAD^+, the oxidation of sorbitol is effectively quantitative; the reduction of NAD^+ may be followed by measurement of the extinction at 340 nm.

The D-glucose content of the hydrolysate is determined by a reductometric method or by use of D-glucose oxidase and the DP_n is then calculated from the relative amount of D-sorbitol:

$$DP_n = \frac{\text{mol glucose} + \text{mol sorbitol}}{\text{mol sorbitol}}$$

The acid hydrolysate of the borohydride-reduced polysaccharide could also be treated with chloromethylsilane and the trimethylsilyl (TMS) derivatives of the reducing sugars and alditols could be separated and estimated by gas–liquid chromatography.[74,80]

Molecular weight distribution

The following parameters need to be defined to characterise a heterogeneous or polydisperse dextran:

the weight-average molecular weight, M_w, represents an average in which each polymer molecule present is weighed according to its weight fraction.[81] or

$$M_w = \frac{\sum N_i M_i^2}{\sum N_i M_i} = \frac{\sum w_i M_i}{\sum w_i}$$

where w_i = weight fraction, N_i = number of molecules of mass M_i and M_i = molecular mass. The number-average molecular weight, M_n, represents the ratio of the total weight of all molecules in a sample to the total number of molecules in the sample,[81] or

$$M_n = \frac{\sum N_i M_i}{\sum N_i}$$

where N_i = number of molecules of mass M_i and M_i = molecular mass.

For a polydisperse system, M_w is always higher than M_n and the ratio M_w/M_n, known as the polydispersity, is a measure of the range of the molecular weight distribution. Alsop *et al*[82] have reviewed the methods for determining the molecular weight and molecular weight distribution of dextran.

Light scattering, ultracentrifugation and small-angle neutron scattering are methods that can be used for measuring M_w. M_n can be determined by membrane osmometry or on the basis of colligative properties. A more frequently used method for the determination of M_n is that of 'end group' or functional group analysis. In this method, the reducing groups present in dextran (it is assumed with reasonable confidence that each dextran molecule is terminated by a reducing aldehyde group) are caused to react with a reagent and the product is estimated spectrophotometrically.

Molecular weights can also be determined by viscometry. This is a simple, rapid technique requiring inexpensive equipment. It is not an absolute method, however. Careful correlation procedures are necessary so that viscosity can be related mathematically to the molecular weight of dextrans determined by other methods.[83–85] The methods described so far give only average molecular weights of the whole sample and therefore give only limited information regarding the polymer. The first method used to obtain a complete molecular weight distribution (MWD) of the polymer involved fractional precipitation of polymer fractions by solvent precipitation.[69,82] The average molecular weight of each fraction was then measured using one of the above techniques and on this basis an MWD curve was drawn. Clearly this method is very time consuming. This problem was overcome by the use of size-exclusion HPLC techniques, which provide not only rapid and sensitive methods for the determination of weight-average and number-average molecular weights, but also provide molecular weight distribution data for the polymer under investigation.[71,74,82,86]

Kim and Day[46] were the first to report the use of scanning electron microscopy to investigate the molecular weight distribution of dextran. The interaction of the polymer with a ruthenium red solution containing OsO_4 resulted in an insoluble complex, the appearance of which was indicative of the size of the polymer.

Dextran types synthesised by dextransucrase

In 1954, Jeanes *et al*[65] determined the approximate structure of several dextrans. At that time, however, the nature of the branch linkages, their distribution and the length of the branch chains had not been definitely determined. Later, methylation analysis of *L mesenteroides* B-512F dextran showed that it contained 95% α(1,6)-linkages and 5% α(1,3)-branch linkages. Since then, a great deal of research on the structure has been performed on dextrans obtained from various strains of *L mesenteroides* and *Streptococcus mutans* using methylation, periodate oxidation and ^{13}C NMR spectroscopy.[73,74]

Dextrans can be divided into three classes based on structural features: class 1 dextrans contain a main chain of consecutive α(1,6)-linked glucosyl residues, with branching at position 2, 3 or 4; class 2 dextrans contain non-consecutive α(1,3)- and α(1,6)-linkages and α(1,3)-branch linkages; and class 3 dextrans contain consecutive α(1,3)-linkages and α(1,6)-branch linkages. Class 1 comprises most of the polysaccharides, usually thought of as dextrans.[50]

L mesenteroides B-512F dextran is the classical dextran (class 1) containing a high percentage (95%) of consecutive α(1,6)-linkages and a relatively low percentage (5%) of α(1,3)-branch linkages. There is some controversy over the lengths of the branch chains. Some chemical studies have indicated that most branch chains are single α(1,3)-linked glucose residues. Physical studies, however, have indicated that the branch chains are relatively long with 50–100 α(1,6)-linked glucose residues. Consideration of the mechanism of synthesis of the branch linkages suggests that the structure is a mixture of branch chains of single glucose residues and long chains of many α(1,6)-linked glucose residues.

The L-fraction (less soluble) of *L mesenteroides* B-1355 has a structure identical with that of B-512F dextran.[65] Another structure related to that of a B-512F dextran is the L-fraction from *L mesenteroides* B-742. It contains 87% α(1,6)-linkages but has 13% α(1,4)-branch linkages instead of α(1,3)-branch linkages. Because of the α(1,4)-branch linkages, however, the behaviour of this dextran differs from that of B-512F dextran; it is much more resistant to endodextranase hydrolysis than would be expected for a molecule having only α(1,3)-branch linkages. It has been suggested that an α(1,4)-branch linkage imparts a different conformation to the dextran molecule than does an α(1,3)-branch linkage. This difference in conformation may account for the differences in endodextranase susceptibility.[50,65]

Only three examples of class 2 dextrans are known, namely the S-fractions (soluble) from *L mesenteroides* B-1355, B-1498 and B-1501, which have ~50% α(1,6)- and ~50% α(1,3)-linkages.[50] The structure is an alternating sequence α(1,6)- and α(1,3)-linked glucosyl residues. These glucans are not true dextrans as they do not have a consecutive sequence of α(1,6)-linkages. Their physical and chemical properties are distinct from those of class 1 dextrans and they are completely resistant to endodextranase hydrolysis. For these reasons, they are termed alternan instead of dextran.[87,88] Recently, a mutant derivative of strain NRRL B-1355 was reported to produce a third polysaccharide, an insoluble α-D-glucan containing linear (1,3)- and (1,6)-linkages with (1,2)- and (1,3)-branch points.[89,90]

L mesenteroides B-742 fraction S also contains material similar to alternan in that it has 50% α(1,3)-linkages. However, unlike alternan, the α(1,3)-linkages are all branch linkages. The B-742 fraction S is a class 1 dextran with main chains of consecutive α(1,6)-linked glucose residues to which single α(1,3)-linked glucosyl residues are attached. This dextran has the highest possible degree of branching and exhibits a comb-like structure completely resistant to endodextranase hydrolysis.[50] Under some conditions of biosynthesis not all glucosyl residues in the α(1,6)-linked main chain are attached to α(1,3)-linked glucosyl residues, giving an incomplete comb structure. Another dextran related in structure to the B-742 S-dextran is the soluble dextran produced by *S mutans* 6715. It contains a relatively high percentage (35%) of α(1,3)-branch linkages. Like B-742 S-dextran, the branch chains are primarily single glucose residues attached to an α(1,6)-linked main chain. This dextran exhibits a comb-like structure, but because of the lower percentage of α(1,3)-branch linkages, the gaps are wider or more numerous. If the α(1,3)-linkages are regularly spaced, however, the resulting structure is an alternating comb polymer with regular gaps between every other glucose residue on the main chain (Fig 5).

In addition to the soluble dextran, *S mutans* 6715 produces an insoluble glucan, which contains mostly (>93%) consecutive α(1,3)-linked glucose residues; two *L mesenteroides* strains, B-523 and B-1149, also produce insoluble α(1,3)-linked glucans. These are class 3 dextrans, frequently called mutans instead of dextrans because of their high percentage of consecutive α(1,3)-linkages.[50]

L mesenteroides B-1299 produces an L- and an S-dextran with α(1,6)-linked main chains with single α(1,2)-linked glucosyl residues along the chain.

The linkages found in these varied but related dextrans are given in Table 1; a diagrammatic representation of the different structures is given in Fig 5.

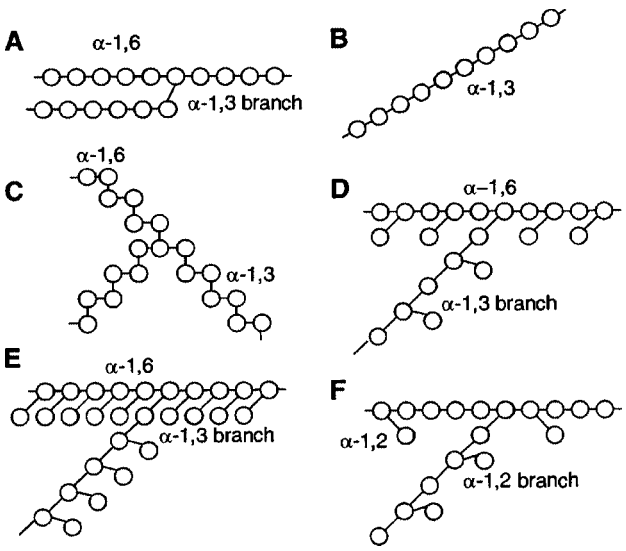

Figure 5. Diagrammatic representation of the structures of the different glucans synthesised by different glucansucrases from various *Leuconostoc mesenteroides* and *Streptococcus mutans*. (A) *L mesenteroides* B-512F; (B) *S mutans* insoluble mutan; (C) *L mesenteroides* B-1355 alternan; (D) *S mutans* soluble dextran; (E) *L mesenteroides* B-742 S-dextran; (F) *L mesenteroides* B-1299 dextran.[8]

Table 1. Linkages in different dextrans as obtained by methylation analysis[8]

		Linkages (%)				
Dextran[a]	Solubility[b]	α-(1 → 6)	α-(1 → 3)	α-(1 → 3)Br[c]	α-(1 → 2)Br	α-(1 → 4)Br
Lm B-512F	S	95	5			
Lm B-742	S	50		50		
Lm B-742	L	87				13
Lm B-1299	S	65			35	
Lm B-1299	L	66		7	27	
Lm B–1355	S	54	35	11		
Lm B–1355	L	95		5		
Sm 6715	S	64		36		
Sm 6715	I	4	94	2		

[a] *Lm* = *Leuconostoc mesenteroides*; *Sm* = *Streptococcus mutans*.
[b] S = soluble; L = less soluble; I = insoluble.
[c] Br = branch linkage.

DEXTRANS SYNTHESISED BY *Gluconobacter* DEXTRAN DEXTRINASE (DDase)

Cultures of *Acetobacter capsulatus* NCTC 4943 (now *Gluconobacter oxydans* ATCC 11 894) and *A viscosus* NCTC 7216 (now *G oxydans* ATCC 11 895), when grown on dextrin (but not when grown on other common carbohydrates) produced abundant amounts of polysaccharide material, which had serological properties similar to dextran, produced from sucrose by *L mesenteroides*.[10] Information on the constitution of the '*Acetobacter* dextran' was obtained by direct comparison with four reference polysaccharides, ie dextran isolated from sucrose broth culture of *L mesenteroides*, oyster glycogen, corn amylopectin and the dextrin used as the substrate in the medium (Table 2).

From the data summarized in Table 2, it is evident that the major features of the polyglucoside produced by *G oxydans* resembled those of *Leuconostoc* dextran and differed from those of glycogen, amylopectin and the dextrin, from which the product was formed.[91] The slight release of reducing sugar on treatment with amylase and the slight capacity of the *Gluconobacter* product to be coloured by iodine could be attributed, at least in part, to traces of amylaceous material accompanying the bacterial polysaccharide in its isolation from the dextrin broth culture. It is noteworthy, however, that treatment with saliva (although not with wheat β-amylase) caused some decrease in the opalescence of solutions of the *Gluconobacter* polysaccharide and some loss of capacity of the polysaccharide to be precipitated by 35% alcohol. These observations suggested that the *Gluconobacter* polysaccharide molecules might have contained one or more internally located α-amylase-sensitive linkages.[91]

The hydrolysate of the *Gluconobacter* polysaccharide was found to consist almost entirely of glucose. The high optical rotation and the infrared absorption spectrum both indicated α-glycosidic linkages. Absorption peaks consistent with $\alpha(1,6)$-linkages were also present, but no absorption attributable to $\alpha(1,3)$-linkages was detected. Methylation analysis revealed three components: 2,3,4,6-tetra-, 2,3,4-tri- and 2,3-di-*O*-methyl-D-glucose. The *G oxydans* polysaccharide was thus shown to consist essentially of $\alpha(1,6)$-linked chains containing $\alpha(1,4)$-branching points, of which the average $\alpha(1,6)$-linked chain length was about 13 glucose units.[11]

Jeanes *et al*[65] suggested the *Gluconobacter* dextran to be composed of 90% $\alpha(1,6)$-linkages and 10% $\alpha(1,4)$-linkages by the periodate oxidation method. However, these investigations were performed on dextran obtained from *G oxydans* cultures containing starch hydrolysate as a substrate.

In 1993, Yamamoto *et al*[92] described the synthesis of dextran from maltotetraose (G4) by the action of purified intracellular *Gluconobacter* dextran dextrinase (**DDase**). They demonstrated that the '*Acetobacter* dextran' was a highly branched glucan. Their results indicated the existence of $\alpha(1,4)$-linked branches on $\alpha(1,6)$-linked chains and that half of the $\alpha(1,4)$-linkages did not participate in branching. Native *Gluconobacter* dextran was calculated to be constructed with 6.23 branching points and 6.53 $\alpha(1,4)$-linked glucosyl residues per 100 glucosyl units. The molecular weight was guessed to range between several million and tens of millions of daltons. The size of *Gluconobacter* dextran was somewhat smaller than the commercial *Leuconostoc* dextran.[92] Sims *et al*[74] investigated the structure of dextran formed during *G oxydans* fermentations on maltooligosaccharides with varying degrees of polymerisation. They obtained a similar dextran structure to that obtained from maltotetraose with purified DDase and concluded that the linkage composition of the glucan was largely independent of the size of the initial substrate.

In a study of Mountzouris *et al*,[71] *G oxydans* cells were used to convert maltodextrins (DE 17–20) to polymeric material. The resulting dextran had a molecular weight in the range 6.6–38 kDa, and in some cases small amounts of high molecular weight dextran (>4500 kDa) were also formed. The polymer could not be separated from the high molecular weight maltodextrin part and appeared not to be highly branched.

Table 2. Properties of *G oxydans* polysaccharide compared with those of *Leuconostoc* dextran and polysaccharides of the starch class[91]

	Conversion to maltose (%)					Periodate oxidation	
Polysaccharide	α-Amylase	β-Amylase	Colour with I_2 (2 g l^{-1} polymer solution)	Rate of acid hydrolysis: $K_1 \times 10^3$ (min^{-1})	Serological activity[a]	Periodate consumed (mol)	Formic acid liberated (mol)
Gluconobacter glucan	1.0	0.2	None[b]	11	+++	1.89	0.82
Leuconostoc dextran	0.0	0.0	None[b]	10	+++	1.83	0.84
Oyster glycogen	71	33	Red–brown	41	0	1.02	0.08
Corn amylopectin	93	61	Dark purple	54	0	1.06	0.04
Dextrin	86	64	Deep maroon	52	0	1.22	0.22

[a] +++, Precipitation beyond 1:1 million dilution of the polysaccharide with type 2 and type 20 pneumococcal antisera and from 1:10 000 to 1:50 000 dilution with type 12 pneumococcal antiserum.
[b] A greenish colour was produced, however, when a 10-fold more concentrated solution of the polysaccharide was treated with iodine.

Recently, Suzuki *et al*[93] analysed dextran synthesised by extracellular *Gluconobacter* DDase from maltotriose. The ratio of $\alpha(1,4)$- to $\alpha(1,6)$-glucosidic linkages was calculated to be 1:20, whereas the average molecular mass of the polymer was estimated to be about 1270 kDa.

The carbon source and mass transfer limitations are among a range of factors known to affect the molecular mass, the pattern and number of residues, the degree of branching and the yield of microbial exopolysaccharide production.[94] The differences in the type of substrate (maltodextrins instead of maltotetraose or maltotriose) and the nature of the catalyst (*Gluconobacter* cells or extracellular DDase instead of purified intracellular enzyme) could then possibly account for the large differences between the dextran molecular weight reported by Mountzouris *et al*,[71] Suzuki *et al*[93] and Yamamoto *et al*.[92] Other factors that could have affected the dextran molecular weight relate to the presence of acceptor compounds, to the $\alpha(1,6)$ to $\alpha(1,6)$ secondary transglucosylation action mode of DDase or to the presence of a dextranase in the *G oxydans* cell suspensions.[71]

The authors have optimised the production of *G oxydans* dextran dextrinase and its use as a biocatalyst for transglucosylation reactions; also, the intracellular and extracellular form of dextran dextrinase was compared with respect to their activity and characteristics.[95–100]

USES OF DEXTRAN

Dextrans are commercially important polysaccharides and there is a considerable volume of literature devoted to the numerous uses of native dextrans, partially degraded dextrans and their derivatives.[4,50,101]

Dextran of relatively low molecular weight can be used as a therapeutic agent in restoring blood volume for mass casualties. The initial impetus for the commercial production was for this use. Natural dextran with a molecular weight of about 5×10^8 Da is unsuitable as a blood-plasma substitute. The optimum size for blood-plasma dextran, so-called clinical dextran, is 40 000–100 000 Da.[8] Material with too low a molecular weight is rapidly lost from the circulation by removal via the kidneys and is therefore therapeutically ineffective and material with too high a molecular weight can interfere with normal coagulation processes of the blood.[16,17,101] Clinical fractions of dextran with molecular weights of 70 000, 60 000 and 40 000 Da (designated dextran 70, 60 and 40, respectively) are at present available for replacing moderate blood losses.[101] The rationale behind the use of dextran is related to the retention of dextran molecules in the circulation compared with crystalloids, which rapidly penetrate vascular membranes. It has been shown that each gram of dextran will retain in the circulation ~20 ml of water, making dextran the plasma volume expander of choice. The essential feature of dextran 40, in addition to plasma volume expansion, is blood flow improvement, presumably mediated by reduction of blood viscosity and inhibition of erythrocyte aggregation.[4,101]

Dextran produced by *L mesenteroides* B-512F is the material of choice for clinical dextran because it has a low degree of antigenicity and a high percentage (95%) of $\alpha(1,6)$-glycosidic linkages.[4,65,101] The latter is important as the enzymes in the human body can only slowly hydrolyse the $\alpha(1,6)$-linkages in contrast to $\alpha(1,4)$-linkages of starch and glycogen, which are rapidly hydrolysed by human α-amylases. Furthermore, the $\alpha(1,6)$-linkage imparts high water solubility to the molecule, in contrast to material having $\alpha(1,3)$-linkages or β-linkages. Thus, B-512F dextran has the low antigenicity, high water solubility and high biological stability in the human bloodstream required for a suitable blood-plasma substitute.[8] An inevitable consequence of infusing colloids into the blood is the risk of hypersensitivity reactions. Dextran-induced anaphylactic reactions (DIAR) appeared to be related to chemical structure. Dextrans of higher molecular weights and/or with greater proportions of non-$\alpha(1,6)$-linkages caused a greater incidence of allergic reactions.[101] Hypersensitivity reactions observed in the initial development of dextran as a blood extender are now reduced owing to a modification of the dextran and by preinjecting patients with a low molecular weight dextran (MW 1000) as a monovalent hapten.[102] Approximately 20% of the population would have an anaphylactic response, which is the limiting factor in the routine use of clinical dextran as a blood-plasma substitute. Nevertheless, during a disaster, when blood plasma is scarce, clinical dextran as a blood-plasma substitute might save lives.[8]

Many different types of esters and ethers of dextran provide macromolecules with diverse properties and negative, positive or neutral charges. Their properties depend on the type of substituent, the degree of substitution and the molecular weight of the dextran.

The most widely used dextran derivative is obtained by the reaction of an alkaline solution of dextran with epichlorohydrin to give cross-linked chains. The product is a gel that is used as a molecular sieve. With its commercial introduction in 1959 by Pharmacia Fine Chemicals (Uppsala, Sweden), cross-linked dextran revolutionised the purification and separation of biochemically important macromolecules such as proteins, nucleic acids and polysaccharides. Commercial cross-linked dextran is known as Sephadex®(the name is derived from SEparation PHArmacia DEXtran).[8,103] Several types of Sephadex have been developed, eg the G-series (G-10 to G-200), with different degrees of cross-linking, giving different molecular exclusion limits. The G-numbers actually refer to the amount of water absorbed by the dry beads. Sephadex G-10, the highest cross-linked material, regains about 1 ml of water per gram of dry gel and Sephadex G-200, the lowest cross-linked material, has a water regain of about 20 ml per gram of dry gel.[8]

Dextrans are also an essential component of Sephadex derivatives, including carboxymethyl (CM) Sephadex, diethylaminoethyl (DEAE) Sephadex, diethyl(2-hydroxypropyl)aminoethyl (QAE) Sephadex, sulfopropyl (SP) Sephadex[4] and other modern separation gels such as Sephacryl and Superdex.[103]

Intravenously administered iron dextran is used to alleviate iron deficiency anaemia.[104] The shortcoming of oral iron supplementation is the gastrointestinal tract's limited capacity for iron absorption. Most orally consumed iron flows untouched through the alimentary tract.[105] An iron dextran complex named Infed®, has been licensed for general use in the USA.[106] Special iron dextran preparations have also been developed to enhance magnetic resonance imaging techniques.[101]

The sulfate ester of dextran has anticoagulant properties similar to those of heparin, a naturally occurring polysaccharide containing carbohydrate sulfate esters. It also interacts with β-lipoproteins and as such has found applications in analytical and preparative procedures.[16] Dextran sulfate has an affinity for sites that bind nucleic acids and is a potent inhibitor for ribonuclease. More recently, it has been studied as an antiviral agent, particularly in the treatment of the human immunodeficiency virus (HIV).[107] The possible use of dextran sulfate as an anti-HIV drug is based on laboratory tests, showing its inhibition of reverse transcriptase, plus the fact that oral dextran sulfate has been used against arteriosclerosis for 20 years in Japan, without harmful side-effects.[108,109]

Mercaptodextran has a higher affinity for heavy metal ions, such as silver, mercuric, cupric and auric ions, than most other thiols and chelating agents. This affinity, combined with low toxicity, suggests possible uses in acute heavy metal poisoning and in environmental cleanup of heavy metal contamination.[8]

Most major photographic companies have taken patents on the improved effects achieved by incorporating dextrans into X-ray and other photographic emulsions resulting in economies in silver usage without loss of fineness of grain.[16,103]

Dextrans are also used in several formulations for the eye and skin care market. Dextrans and derivatives offer many advantages as ingredients for cosmetics, where excellent biocompatibility, moisturising properties and proven stability are high on the list of requirements.[103] The incorporation of dextran in bakery products improves softness, crumb texture and loaf volume.[110] Dextrans have also been used in clinical nutrition, in fructose syrup and as additives in products such as candies and ice cream.[111] Dextrans are not explicitly approved as food additives in the USA or in Europe,[4,111] although *L mesenteroides* is a GRAS-status organism, present in many fermented foods. However, patents have recently been granted related to the use of dextran preparations in baked products.[110,112]

Many other potential uses for dextrans have been described, such as emulsifying and thickening agents, high-viscosity gums, explosives, deflocculants in paper products, secondary recovery of petroleum, oil drilling muds, soil conditioners and surgical sutures.[113]

The growing interest in application-specific dextrans can be partly accredited to important product features such as hydrophilicity, stability, purity and ability to form clear and stable solutions. However, another feature stands out and recently seems to have generated the most interest: the fact that dextrans are derived from renewable resources and are degraded by ecological systems.

PROBLEMS ASSOCIATED WITH DEXTRAN FORMATION

Dextrans and dental caries

Considerable evidence now exists to indict sucrose as the agent that initiates dental caries (an infective disease that breaks down tooth enamel and dentine) in persons living in technologically advanced communities. In molar teeth, caries may develop in occlusal fissures as a result of bacterial fermentation of impacted food material. However, lesions on the more numerous 'self-cleansing' smooth surfaces of teeth cannot form in a similar way. A prerequisite to the development of smooth-surface lesions appears to be the accumulation of a layer of polysaccharide-containing plaque on the tooth surface.[7]

Cariogenic microorganisms secrete extracellular glucosyltransferases that polymerise the glucose moiety of sucrose into glucan polymers, forming a pellicle on the tooth surface.[114] Many studies have reported the isolation of extracellular glucosyltransferase enzymes from culture media of oral bacteria.[114–117] Ugarte and Rodriguez[118] demonstrated the presence *in vivo* of glucosyltransferase in a crude homogenate of dental plaque from children.

The principal organism found in dental caries is *Streptococcus mutans*. The cariogenic potential of this bacterium is thought to reside in its ability to produce water-soluble (dextrans) and water-insoluble glucans (mutans) from sucrose.[17,119] These polysaccharides make up dental plaque that adheres to the enamel of the teeth, holding myriads of *S mutans* cells in close proximity to the enamel; the polysaccharides also provide an anaerobic environment for the bacteria. Dextrans and mutans thus promote dental caries by facilitating the colonisation of cariogenic bacteria on the tooth surface, but also through their presence as structural components or reserve carbohydrates in dental plaque. The gradual breakdown of reserve carbohydrates to fermentable sugars and their conversion to organic acids are a key factor in the cariogenic process, as they enable a low pH to be maintained at the tooth surface for a prolonged period of time and in the absence of exogenous carbohydrates. The acids are held in close contact with the teeth, attacking the enamel and resulting in dental lesions.[8,17,119]

Dextran problems in sugar refineries

The growth of *Lactobacilli* and *Leuconostoc* microorganisms is generally accepted to be an important factor contributing to the post-harvest deterioration of cane and beet sugar. Apart from causing significant losses of sucrose and souring the cane or beet with organic acids, these bacteria are able to convert sucrose into dextrans that can seriously lessen the efficiency with which the deteriorated cane or beet may be processed.[7,120,121] Contamination in refineries is propagated by the circulation of sweet water and slime can be produced in preferential locations.[122] Insoluble dextrans contribute to the clogging of filters, pipelines and tanks and may seriously interfere with the removal of suspended matter at the clarification stage of raw sugar manufacture. These gums also encourage the formation of scale that results in heat losses in the sucrose juice evaporators. The viscosities of raw sucrose juices and syrups are increased by the presence of soluble dextrans; these have undesirable effects on the filtration, clarification, boiling and affination processes. In addition, it has been demonstrated that soluble dextrans retard the rate of crystallisation of sucrose and also adversely affect the crystal shape.[122,123]

According to DeStefano and Irey,[124] however, dextrans are only a partial contributor to most of these problems, notably the crystallisation and filterability aspects. They state that dextran is difficult to analyse because of the relatively low parts per million levels usually present and because of its physical characteristics. It is a series of similar molecules with a wide range of molecular weights, typically with a bimodal or even trimodal molecular weight distribution.[43] To complicate matters further, *L mesenteroides* is just one of many organisms in the juice, producing extracellular products that may be carried over into the sugar in varying degrees. In addition, polymeric materials from the soil and from the plant itself may also find their way into the process.[124] The overall effect on processing is a marked decrease in sucrose recovery, reduced factory and refinery efficiency and lower quality of the final products.[125]

Dextrans and food spoilage

L mesenteroides is associated, in combination with other lactic acid bacteria, with sauerkraut production. In addition to acidification, *L mesenteroides* is responsible for flavour compounds but also, in some cases, for spoilage by dextran production. In the rum industry, *Leuconostoc*'s dextran formation causes problems from milling to fermentation. In cured meat products, slime-forming *Leuconostoc* strains have been identified among the discolouring, off-odour and off-flavour producing lactic acid bacteria. In addition, ropy strains have been isolated from vacuum-packed cooked meat products.[126]

Owing to their acid, hop and alcohol tolerance, certain acetic acid bacteria may grow in beer and cause spoilage.[127,128] The only factor limiting their growth in beer is lack of oxygen. Some species, however, can manage with only a minimal amount of oxygen.[129] In addition to beer acetification, these organisms may cause 'ropiness' or sliminess of beer through the synthesis of gelatinous dextran.[130–132] Film formation and turbidity caused by acetic acid bacteria are indeed common forms of spoilage in draught beer in partly filled kegs.[129] Baker *et al*[130] were the first carry out a thorough investigation on microorganisms associated with the type of beer spoilage known as ropiness. The causative organisms were identified to be *Acetobacter viscosum* and *A capsulatum*[131] (now reassigned to the genus *Gluconobacter*[133]) and now known to produce dextran from (beer) maltodextrins with their dextran dextrinase activity.

These 'beer spoiler' strains and their dextran dextrinase enzyme are now also being studied intensively as useful biocatalysts to produce (oligo)dextrans from maltodextrins.[92,93,95–100]

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REFERENCES

1 Scheibler C, Investigation on the nature of the gelatinous excretion (so-called frog's spawn) which is observed in production of beet-sugar juices. *Z Dtsch Zucker-Ind* **24**:309–335 (1874).
2 Pasteur L, On the viscous fermentation and the butyrous fermentation. *Bull Soc Chim Fr* 30–31 (1861).
3 Van Tieghem P, On sugar-mill gum. *Ann Sci Nature Bot Biol Veg* 7:180–203 (1878).
4 Leathers TD, Dextran, in *Biopolymers. Vol. 5. Polysaccharides I: Polysaccharides from Prokaryotes*, ed by Vandamme EJ, De Baets S and Steinbüchel A. Wiley-VCH, Weinheim, pp 299–321 (2002).
5 Hehre EJ, Natural synthesis of low molecular weight (clinical type) dextran by a *Streptococcus* strain. *J Biol Chem* **222**:739–750 (1956).
6 Chludzinski AM, Germaine GR and Schachtele CF, Purification and properties of dextransucrase from *Streptococcus mutans*. *J Bacteriol* **118**:1–7 (1974).
7 Sidebotham RL, Dextrans. *Adv Carbohydr Chem Biochem* **30**:371–444 (1974).
8 Robyt JF, Dextran, in *Encyclopaedia of Polymer Science*, Vol 4, ed by Kroschwitz JI. Wiley-VCH, New York, pp 753–767 (1985).
9 Takagi K, Ioroi R, Uchimura T, Kozaki M and Komagata K, Purification and some properties of dextransucrase from *Streptococcus bovis* 148. *J Ferment Bioeng* 77:551–553 (1994).
10 Hehre EJ and Hamilton DM, Bacterial conversion of dextrin into a polysaccharide with the serological properties of dextran. *Proc Soc Exp Biol Med* **71**:336–339 (1949).
11 Barker SA, Bourne EJ, Bruce GT and Stacey M, Immunopolysaccharides. Part XI. Structure of an *Acetobacter capsulatum* dextran. *J ChemSoc* **4**:4414–4416 (1958).
12 Ruckel ER and Schuerch C, Chemical synthesis of a dextran model, poly-α-(1,6)-anhydro-D-glucopyranose, in *Biopolymers*, Vol 5, ed by Goodman M. Wiley, Chichester, pp 515–523 (1967).

13 Jeanes A, Haynes WC, Wilham CA, Rankin JC, Melvin EH, Austin MJ, Cluskey JE, Fisher BE, Tsuchiya HM and Rist CE. Characterization and classification of dextrans from ninety-six strains of bacteria. *J Am Chem Soc* **76**:5041–5052 (1954).
14 Lindberg B and Svensson S, Structural studies on dextran from *Leuconostoc mesenteroides* NRRL B-512. *Acta Chem Scand* **22**:1907–1912 (1968).
15 Robyt JF and Walseth TF, Production, purification and properties of dextransucrase from *Leuconostoc mesenteroides* NRRL B-512F. *Carbohydr Res* **68**:95–111 (1979).
16 Alsop RM, Industrial production of dextrans, in *Microbial Polysaccharides*, ed by Bushell ME. Elsevier, Amsterdam, pp 1–44 (1983).
17 Guggenheim B and Schroeder HE, Biochemical and morphological aspects of extracellular polysaccharides produced by cariogenic streptococci. *Helv Odontol Acta* **11**:131–152 (1967).
18 Quirasco M, Lopez-Munguia A, Remaud-Simeon M, Monsan P and Farres A. Induction and transcription studies of the dextransucrase gene in *Leuconostoc mesenteroides* NRRL B-512F. *Appl Environ Microbiol* **65**:5504–5509 (1999).
19 Kim D and Robyt JF, Properties of *Leuconostoc mesenteroides* B-512FMC constitutive dextransucrase. *Enzyme Microb Technol* **16**:1010–1015 (1994).
20 Mizutani N, Yamada M, Takayama K and Shoda M, Constitutive mutants for dextransucrase from *Leuconostoc mesenteroides* NRRL B-512F. *J Ferment Bioeng* 77:248–251 (1994).
21 Kitaoka M and Robyt JF, Use of a microtiter plate screening method for obtaining *Leuconostoc mesenteroides* mutants constitutive for glucansucrase. *Enzyme Microb Technol* **22**:527–531 (1998).
22 Ryu HJ, Kim D, Kim DW, Moon YY and Robyt JF, Cloning of a dextransucrase gene (*fmcmds*) from a constitutive dextransucrase hyper-producing *Leuconostoc mesenteroides* B-512 FMCM developed using VUV. *Biotechnol Lett* **22**:421–425 (2000).
23 Paul F, Auriol D, Oriol E and Monsan P, Production and purification of dextransucrase from *Leuconostoc mesenteroides* NRRL B-512(F). *Ann N Y Acad Sci* **434**:267–270 (1984).
24 Miller AW, Eklund SH and Robyt JF, Milligram to gram scale purification and characterization of dextransucrase from *Leuconostoc mesenteroides* B-512F. *Carbohydr Res* **147**:119–133 (1986).
25 Fu D and Robyt JF, A facile purification of *Leuconostoc mesenteroides* B-512FM dextransucrase. *Prep Biochem* **20**:93–106 (1990).
26 Kobayashi M and Matsuda K, Characterization of the multiple forms and main component of dextransucrase from *Leuconostoc mesenteroides* NRRL B-512F. *Biochim Biophys Acta* **614**:46–62 (1980).
27 Willemot RM, Monsan P and Durand G, Effects of dextran on the activity and stability of dextransucrase from *Leuconostoc mesenteroides*. *Ann N Y Acad Sci* **542**:169–172 (1988).
28 Robyt JF, Kim D and Yu L, Mechanism of dextran activation of dextransucrase. *Carbohydr Res* **266**:293–299 (1995).
29 Robyt JF and Eklund SH, Stereochemistry involved in the mechanism of action of dextransucrase in the synthesis of dextran and the formation of acceptor products. *Bioorg Chem* **11**:115–132 (1982).
30 Robyt JF, Mechanism and action of glucansucrases, in *Enzymes for Carbohydrate Engineering*, ed by Park KH, Robyt JF and Choi YD. Elsevier, Amsterdam, pp 1–21 (1996).
31 Robyt JF, Kimble BK and Walseth TF, The mechanism of dextransucrase action: direction of dextran biosynthesis. *Arch Biochem Biophys* **165**:634–640 (1974).
32 Robyt JF and Martin PJ, Mechanism of synthesis of D-glucans by D-glucosyltransferases from *Streptococcus mutans* 6715. *Carbohydr Res* **113**:301–315 (1983).
33 Luzio GA, Parnaik VK and Mayer RM, A D-glucosylated form of dextransucrase: Demonstration of partial reactions. *Carbohydr Res* **121**:269–278 (1983).
34 Ebert KH and Schenk G, Mechanisms of biopolymer growth: the formation of dextran and levan. *Adv Enzymol* **30**:179–219 (1968).
35 Robyt JF and Walseth TF, The mechanism of acceptor reactions of *Leuconostoc mesenteroides* B-512F dextransucrase. *Carbohydr Res* **61**:433–445 (1978).
36 Robyt JF and Eklund SH, Relative, quantitative effects of acceptors in the reaction of *Leuconostoc mesenteroides* B-512F dextransucrase. *Carbohydr Res* **121**:279–286 (1983).
37 Fu D and Robyt JF, Acceptor reactions of maltodextrins with *Leuconostoc mesenteroides* B-512FM dextransucrase. *Arch Biochem Biophys* **283**:379–387 (1990).
38 Su D and Robyt JF, Control of the synthesis of dextran and acceptor-reactions by *Leuconostoc mesenteroides* B-512FM dextransucrase. *Carbohydr Res* **248**:339–348 (1993).
39 Pereira AM, Costa FAA, Rodrigues MI and Maugeri F, *In vitro* synthesis of oligosaccharides by acceptor reaction of dextransucrase from *Leuconostoc mesenteroides*. *Biotechnol Lett* **20**:397–401 (1998).
40 Smiley KL, Slodki ME, Boundy JA and Plattner RD, A simplified method for preparing linear isomalto-oligosaccharides. *Carbohydr Res* **108**:279–283 (1982).
41 Dols M, Remaud-Simeon M, Willemot RM, Vignon MR and Monsan PF, Structural characterization of the maltose acceptor-products synthesised by *Leuconostoc mesenteroides* NRRL B-1299 dextransucrase. *Carbohydr Res* **305**:549–559 (1997).
42 Dols M, Remaud-Simeon M, Willemot RM, Demuth B, Jördening HJ, Buchholz K and Monsan P, Kinetic modeling of oligosaccharide synthesis catalyzed by *Leuconostoc mesenteroides* NRRL B-1299 dextransucrase. *Biotechnol Bioeng* **63**:308–315 (1999).
43 Tsuchiya HM, Hellman NN, Koepsell HJ, Gorman J, Stringer CS, Rogovin SP, Bogard MO, Bryant G, Feger VH, Hoffman CA, Senti FR and Jackson RW, Factors affecting molecular weight of enzymatically synthesized dextran. *J Am Chem Soc* **77**:2412–2419 (1955).
44 Oriol E, Paul F, Monsan P, Heyraud A and Rinaudo M, Transfer reaction of glucosyl residues to maltose and purified oligosaccharides using highly active *Leuconostoc mesenteroides* NRRL B-512F dextransucrase. *Anne N Y Acad Sci* **501**:210–215 (1987).
45 Paul F, Oriol E, Auriol D and Monsan P, Acceptor reaction of a highly purified dextransucrase with maltose and oligosaccharides. Application to the synthesis of controlled-molecular-weight dextrans. *Carbohydr Res* **149**:433–441 (1986).
46 Kim D and Day DF, A new process for the production of clinical dextran by mixed-culture fermentation of *Lipomyces starkeyi* and *Leuconostoc mesenteroides*. *Enzyme Microb Technol* **16**:844–848 (1994).
47 Robyt JF and Taniguchi H, The mechanism of dextransucrase action: biosynthesis of branch linkages by acceptor reactions with dextran. *Arch Biochem Biophys* **174**:129–135 (1976).
48 Bozonnet S, Dols-Laffargue M, Fabre E, Pizzut S, Remaud-Simeon M, Monsan P and Willemot RM, Molecular characterization of DSR-E, an alpha-1,2 linkage-synthesizing dextransucrase with two catalytic domains. *J Bacteriol* **184**:5753–5761 (2002).
49 Fabre E, Bozonnet S, Arcache A, Willemot RM, Vignon M, Monsan P and Remaud-Simeon M, Role of the two catalytic domains of DSR-E dextransucrase and their involvement in the formation of highly alpha-1,2 branched dextran. *J Bacteriol* **187**:296–303 (2005).
50 Remaud-Simeon M, Willemot RM, Sarcabal P, de Montalk GP and Monsan P, Glucansucrases: molecular engineering and oligosaccharide synthesis. *J Mol Catal B* **10**:117–128 (2000).
51 Devulapalle KS, Goodman SD, Gao Q, Hemsley A and Mooser G, Knowledge-based model of a glucosyltransferase from the oral bacterial group of mutant streptococci. *Protein Sci* **6**:2489–2493 (1997).

52 Davies G and Henrissat B, Structures and mechanisms of glycosyl hydrolases. *Structure* **3**:853–859 (1995).
53 Devulapalle KS and Mooser G, Subsite specificity of the active site of glucosyltransferases from *Streptococcus sobrinus*. *J Biol Chem* **269**:11 967–11 971 (1994).
54 Mooser G, Shur D, Lyou M and Watanabe C, Kinetic studies on dextransucrase from the cariogenic oral bacterium *Streptococcus mutans*. *J Biol Chem* **260**:6907–6915 (1985).
55 Mooser G, Hefta SA, Paxton RJ, Shively JE and Lee TD, Isolation and sequence of an active-site peptide containing a catalytic aspartic acid from two *Streptococcus sobrinus* alpha-glucosyltransferases. *J Biol Chem* **266**:8916–8922 (1991).
56 Albenne C, Skov LK, Mirza O, Gajhede M, Feller G, D'Amico S, André G, Potocki-Véronèse G, van der Veen BA, Monsan P and Remaud-Simeon M, Molecular basis of the amylose-like polymer formation catalyzed by *Neisseria polysaccharea* amylosucrase. *J Biol Chem* **279**:726–734 (2004).
57 Sabatie J, Choplin L, Moan M, Doublier JL, Paul F and Monsan P, The effect of synthesis temperature on the structure of dextran NRRL B512F. *Carbohydr Polym* **9**:87–101 (1988).
58 Yusef HH, El-Aassar SA and Fathy SMF, Optimization of culture conditions for the production of dextran by *Leuconostoc mesenteroides*. *Adv Food Sci* **19**:152–158 (1997).
59 Tanriseven A and Robyt JF, Interpretation of dextransucrase inhibition at high sucrose concentrations. *Carbohydr Res* **245**:97–104 (1993).
60 Holzapfel WH and Schillinger U, The genus *Leuconostoc*, in *The Prokaryotes*, Vol. 2, ed by Balows A, Trupper HG, Dworkin M, Harder W and Schleifer KH. Springer, New York, pp 1508–1534 (1992).
61 El-Sayed AHMM, Abdul-Wahid K and Coughlin RW, Investigation of production of dextran and dextransucrase by *Leuconostoc mesenteroides* immobilized within porous stainless steel. *Biotechnol Bioeng* **40**:617–624 (1992).
62 Alcalde M, Plou FJ, Gomez de Segura A, Remaud-Simeon M, Willemot RM, Monsan P and Ballesteros A, Immobilization of native and dextran-free dextransucrase from *Leuconostoc mesenteroides* NRRL B-512 F for the synthesis of glucooligosaccharides. *Biotechnol Tech* **13**:749–755 (1999).
63 Tanriseven A and Dogan S, Production of isomaltooligosaccharides using dextransucrase immobilized in alginate fibres. *Process Biochem* **37**:1111–1115 (2002).
64 Monsan P and Paul F, Enzymatic synthesis of oligosaccharides. *FEMS Microbiol Rev* **16**:187–192 (1995).
65 Jeanes A, Haynes WC, Wilham CA, Rankin JC, Melvin EH, Austin MJ, Cluskey JE, Fisher BE, Tsuchiya HM and Rist CE, Characterization and classification of dextrans from ninety-six strains of bacteria. *J Am Chem Soc* **76**:5041–5052 (1954).
66 Remaud-Simeon M, Lopez-Munguia A, Pelenc V, Paul F and Monsan P, Production and use of glucosyltransferases from *Leuconostoc mesenteroides* NRRL B-1299 for the synthesis of oligosaccharides containing alpha-(1 → 2) linkages. *Appl Biochem Biotechnol* **44**:101–117 (1994).
67 Remaud M, Paul F and Monsan P, Characterization of α-(1 → 3) branched oligosaccharides synthesized by acceptor reaction with the extracellular glucosyltransferases from *L. mesenteroides* NRRL B-742. *J Carbohydr Chem* **11**:359–378 (1992).
68 Jeanes A, Wilham CA and Miers JC, Preparation and characterization of dextran from *Leuconostoc mesenteroides*. *J Biol Chem* **176**:603–615 (1947).
69 Wilham CA, Alexander BH and Jeanes A, Heterogeneity in dextran preparations. *Arch Biochem Biophys* **59**:61–75 (1955).
70 Harris PJ, Henry RJ, Blakeney AB and Stone BA, An improved procedure for the methylation analysis of oligosaccharides and polysaccharides. *Carbohydr Res* **127**:59–73 (1984).
71 Mountzouris KC, Gilmour SG, Jay AJ and Rastall RA, A study of dextran production from maltodextrin by cell suspensions of *Gluconobacter oxydans* NCIB 4943. *J Appl Microbiol* **87**:546–556 (1999).
72 Sawai T, Tohyama T and Natsume T, Hydrolysis of fourteen native dextrans by *Arthrobacter* isomaltodextranase and correlation with dextran structure. *Carbohydr Res* **66**:195–205 (1978).
73 Cheetham NWH, Fiala-Beer E and Walker GJ, Dextran structural details from high-field proton NMR spectroscopy. *Carbohydre Polym* **14**:149–158 (1991).
74 Sims IM, Thomson A, Hubl U, Larsen NG and Furneaux RH, Characterisation of polysaccharides synthesised by *Gluconobacter oxydans* NCIMB 4943. *Carbohydr Polyms* **45**:285–292 (2001).
75 Covacevich MT and Richards GN, Frequency and distribution of branching in a dextran: an enzymic method. *Carbohydr Res* **54**:311–315 (1977).
76 Ammeraal RN, Delgado GA, Tenbarge FL and Friedman RB, High-performance anion-exchange chromatography with pulsed amperometric detection of linear and branched glucose oligosaccharides. *Carbohydr Res* **215**:179–192 (1991).
77 Larm O, Lindberg B and Svensson S, Studies on the length of the side-chains of the dextran elaborated by *Leuconostoc mesenteroides* NRRL B-512. *Carbohydr Res* **20**:39–48 (1971).
78 Taylor C, Cheetham NWH and Walker GJ, Application of high-performance liquid chromatography to a study of branching in dextrans. *Carbohydr Res* **137**:1–12 (1985).
79 Kobayashi M and Matsuda K, Action of a glucoamylase on dextrans as an endodextranase. *Agric Biol Chem* **42**:181–183 (1978).
80 Manners DJ, Masson AJ and Sturgeon RJ, An enzymic method for the determination of the degree of polymerisation of glucans. *Carbohydr Res* **17**:109–114 (1971).
81 Challa G, Polymeerchemie, in *Prisma Technica 52*. Het Spectrum, Utrecht, pp 101–118 (1973).
82 Alsop RM, Byrne GA, Done JN, Earl IE and Gibbs R, Quality assurance in clinical dextran manufacture by molecular weight characterisation. *Process Biochem* **12**:15–35 (1977).
83 Senti FR, Hellman NN, Ludwig NH, Babcock GE, Tobin R, Glass CA and Lamberts BL, Viscosity, sedimentation and light-scattering properties of fractions of an acid-hydrolyzed dextran. *J Polym Sci* **17**:527–546 (1955).
84 Veljkovic VB and Lazic ML, Studies on dextran fermentation broth rheology. *Enzyme Microb Technol* **10**:686–688 (1988).
85 Gasciolli V, Choplin L, Paul F and Monsan P, Viscous properties and molecular characterization of enzymatically size-controlled oligodextrans in aqueous solutions. *J Biotechnol* **19**:193–202 (1991).
86 Sun SF and Wong E, Modified method of universal calibration for the determination of molecular weight and molecular-weight distribution. *J Chromatogr* **208**:253–259 (1981).
87 Coté GL, Alternan, in *Biopolymers. Vol. 5. Polysaccharides I: Polysaccharides from Prokaryotes*, ed by Vandamme EJ, De Baets S and Steinbüchel A. Wiley-VCH, Weinheim, pp 299–321 (2002).
88 Raemakers MHM and Vandamme EJ, Production of alternansucrase by *Leuconostoc mesenteroides* NRRL B-1335 in batch fermentation with controlled pH and dissolved oxygen. *J Chem Technol Biotechnol* **69**:470–478 (1997).
89 Smith MR, Zahnley JC, Wong RY, Lundin RE and Ahlgren JA. A mutant strain of *Leuconostoc mesenteroides* B-1355 producing a glucosyltransferase synthesizing α(1,2) glucosidic linkages. *J Ind Microbiol Biotechnol* **21**:37–45 (1998).
90 Côté GL, Ahlgren JA and Smith MR, Some structural features of an insoluble α-D-glucan from a mutant strain of *Leuconostoc mesenteroides* NRRL B-1355. *J Ind Microbiol Biotechnol* **23**:656–660 (1999).
91 Hehre EJ. The biological synthesis of dextran from dextrins. *J Biol Chem* **192**:161–174 (1951).
92 Yamamoto K, Yoshikawa K and Okada S. Structure of dextran synthesised by dextrin dextranase from *Acetobacter capsulatus* ATCC 11 894. *Biosci Biotechnol Biochem* **57**:1450–1453 (1993).

93 Suzuki M, Unno T and Okada G, Functional characteristics of a bacterial dextrin dextranase from *Acetobacter capsulatum* ATCC 11 894. *J Appl Glycosci* **48**:143–151 (2001).

94 Morin A, Screening of polysaccharide-producing microorganisms, factors influencing the production and recovery of microbial polysaccharides, in *Polysaccharides. Structural Diversity and Functional Versatility*, ed by Dumitriu S. Marcel Dekker, New York, pp 275–296 (1998).

95 Naessens M and Vandamme EJ, Transglucosylation and hydrolysis activitiy of *Gluconobacter oxydans* dextran dextrinase with several donor and acceptor substrates, in *Biorelated Polymers: Sustainable Polymer Science and Technology*, ed by Chiellini E, Gil H, Braunegg G, Burchert J, Gatenholm P and van der Zee M. Kluwer, Dordrecht, pp 195–203 (2001).

96 Naessens M, Vercauteren R and Vandamme EJ, Relationship between intra- and extracellular dextran dextrinase from *Gluconobacter oxydans* ATCC 11 894. *Med Fac Landbouw Univ Gent* **67**:41–44 (2002).

97 Naessens M, Dextran dextrinase of *Gluconobacter oxydans*: production and characterisation. PhD Thesis, Faculty of Bioscience Engineering, Ghent University (2003).

98 Naessens M and Vandamme EJ, Multiple forms of microbial enzymes. *Biotechnol Lett* **25**:1119–1124 (2003).

99 Naessens M, Vercauteren R and Vandamme EJ, Three factor response surface optimisation of the production of intracellular dextran dextrinase by *Gluconobacter oxydans*. *Process Biochem* **39**:1299–1304 (2004).

100 Naessens M, Soetaert W and Vandamme EJ. Dextrandextrinase and dextran of *Gluconobacter oxydans*. *J Ind Microbiol Biotechnol* **32**:in press (2005).

101 De Belder AN, Medical applications of dextran and its derivatives, in *Polysaccharides in Medicinal Applications*, ed by Dumitriu S. Marcel Dekker, New York, pp 505–523 (1996).

102 Hedin H and Richter W, Pathomechanism of dextran-induced anaphylactoid/anaphylactic reactions in man. *Int Arch Allergy Appl Immunol* **68**:122 (1982).

103 Anonymous, Dextran: 50 years—still going strong. *Agro-Food-Industry Hi-Tech* **March/April**:10–11 (1997).

104 Blanshard JMV and Mitchell JR, *Polysaccharides in Food*. Butterworth, London, pp 253–254 (1979).

105 Ahsan N, Intravenous infusion of total dose iron is superior to oral iron in treatment of anemia in peritoneal dialysis patients: a single center comparative study. *J Am Soc Nephrol* **9**:664–668 (1998).

106 Auerbach M, Witt D, Toler W, Fierstein M, Lerner R and Ballard H, Clinical use of the total dose intravenous infusion of iron dextran. *J Lab Clin Med* **111**:566–570 (1988).

107 Piret J, Lamontagne J, Bestman-Smith J, Roy S, Gourde P, Desormeaux A, Omar RF, Juhasz J and Bergeron MG, *In vitro* and *in vivo* evaluations of sodium lauryl sulfate and dextran sulfate as microbicides against herpes simplex and human deficiency viruses. *J Clin Microbiol* **38**:110–119 (2000).

108 Ueno R and Kuno S, Anti-HIV synergism between dextran sulfate and zidovudine. *Lancet* **3**:796–797 (1987).

109 James JS, Dextran sulfate: new promising antiviral. *AIDS Treat News (electronic journal)* **50**:(1988).

110 Vandamme EJ, Renard CEFG, Arnaut FRJ, Vekemans NMF and Tossut PPA, Process for obtaining improved structure build-up of baked products. US Patent 6 399 119 (2002).

111 Food SCO, *Opinion of the Scientific Committee on Food on a Dextran Preparation, Produced Using Leuconostoc mesenteroides, Saccharomyces cerevisiae and Lactobacillus spp, as a Novel Food Ingredient in Bakery Products*. European Commission, Health and Consumer Protection Directorate-General, Brussels (2000).

112 Vandamme EJ, Renard CEFG, Arnaut FRJ, Vekemans NMF and Tossut PPA, Process for obtaining improved structure build-up of baked products. European Patent, EP 0 790 003A1 (1997).

113 Murphy PT and Whistler RL, Dextrans, in *Industrial Gums. Polysaccharides and Their Derivatives*, 2nd edn, ed by Whistler RL and BeMiller JN. Academic Press, New York, pp 513–542 (1973).

114 Giffard PM, Simpson CL, Milward CP and Jacques NA, Molecular characterization of a cluster of at least two glucosyltransferase genes in *Streptococcus salivarius* ATCC 25 975. *J Gen Microbiol* **137**:2577–2593 (1991).

115 Guggenheim B and Newbrun E, Extracellular glucosyltransferase activity of an HS strain of *Streptococcus mutans*. *Helv Odontol Acta* **13**:84–97 (1969).

116 Newbrun E, Extracellular polysaccharides synthesized by glucosyltransferases of oral *Streptococci*. *Caries Res* **6**:132–147 (1972).

117 Hamada S and Slade HD, Synthesis and binding of glucosyltransferase and *in vitro* adherence of *Streptococcus mutans* grown in a synthetic medium. *Arch Oral Biol* **24**:399–402 (1979).

118 Ugarte MA and Rodriguez P, Presence of an extracellular glycosyltransferase in human dental plaque. *Int J Biochem* **23**:719–726 (1991).

119 Leach SA and Hayes ML, A possible correlation between specific bacterial enzyme activities, dental plaque formation and cariogenicity. *Caries Res* **2**:38–46 (1968).

120 Keniry JS, Lee JB and Mahoney VC, Improvements in the dextran assay of cane sugar materials. *Int Sugar J* **17**:230–233 (1969).

121 Roberts EJ, A quantitative method for dextran analysis. *Int Sugar J* **85**:10–13 (1983).

122 Robinson RK, Batt CA and Patel PD, *Encyclopedia of Food Microbiology*, Vol 2. Academic Press, London, pp 1191–1194 (2000).

123 Clarke MA, Dextran in sugar factories: causes and control. *Sugar y Azucar* **November**:22–34 (1997).

124 DeStefano RP and Irey MS, Measuring dextran in raw sugars—historical perspective and state of the art. *J Am Sugar Cane Technol* **6**:112–120 (1986).

125 Brown CF and Inkerman PA, Specific method for quantitative measurement of the total dextran content of raw sugar. *J Agric Food Chem* **40**:227–233 (1992).

126 Lonvaud-Funel A, *Leuconostoc*, in *Encyclopedia of Food Microbiology*, Vol 2, ed by Robinson RK, Batt CA and Patel PD. Academic Press, London, pp 1191–1194 (2000).

127 Harisson J, Webb TJB and Martin PA, The rapid detection of brewery spoilage micro-organisms. *J Inst Brew* **80**:390–398 (1974).

128 Compagnion D, Hygiène en bouteillerie: sources potentielles d'infection. *Cerevisia and Biotechnology* **3**:59–61 (1991).

129 Haikara A, Beer spoilage organisms: occurence and detection with particular reference to a new genus *Pectinatus*. PhD Thesis, Faculty of Agriculture and Forestry, University of Helsinki (1984).

130 Baker JL, Day FE and Hulton HFE, A study of microorganisms causing ropiness in beer and wort. *J Inst Brew* **18**:651–672 (1912).

131 Shimwell JL, A study of ropiness in beer. *J Inst Brew* **53**:280–294 (1947).

132 De Clerck J, Filage, in *Cours de Brasserie*, 2nd edn, Vol. 1, ed by De Clerck J. Chaire Jean De Clerck, Louvain-la-Neuve, pp 871–872 (1984).

133 De Ley J and Frateur J, The status of the generic name *Gluconobacter*. *Int J Syst Bacteriol* **20**:83–95 (1970).