Modeling Bovine Serum Albumin Binding of Flavor Compounds (Alcohols, Aldehydes, Esters, and Ketones) as a Function of Molecular Properties

Y. TAN AND K.J. SIEBERT

ABSTRACT: Interactions between bovine serum albumin (BSA) and 4 classes of flavor compounds (alcohols, esters, aldehydes, and ketones) in aqueous solution were investigated using solid phase microextraction (SPME) of sample headspace and gas chromatography. Alcohols did not bind significantly to BSA. The binding of fixed amounts of individual flavor compounds with each functional group to increasing amounts of BSA was modeled as a function of several descriptors using partial least squares regression (PLSR). Ester binding was modeled as a function of the number of carbon atoms in a molecule and its boiling point ($R^2 = 0.954$). Aldehyde binding was modeled as a function of the number of hydrogen atoms and boiling point ($R^2 = 0.922$). Ketone binding was modeled as a function of the numbers of carbon atoms, length of the longer hydrocarbon chain, and degree of branching ($R^2 = 0.961$).

Keywords: headspace analysis, hydrophobic bonding, partial least squares regression, protein

Introduction

It is well known that some of the constituents of a food matrix interact with others to depress the perceptions of certain aromas (Druaux and Voilley 1997; Guichard 2002). This flavor binding or partitioning is demonstrated by carbohydrates, lipids, and proteins. Proteins have the potential to interact with flavor compounds in a variety of ways due to the considerable variation in their amino acid side chain structures. These include ionic bonding, hydrogen bonding, and hydrophobic bonding. Essentially, all of these types of interactions are noncovalent and thus reversible. So flavor compounds bound by proteins under 1 set of conditions can be released under others (in the mouth, for example). Different proteins have different proportions of amino acids of particular side chain types, leading to distinctly different interactions. Steric factors (particularly protein 3 dimensional structure) can also play a significant role. Albumins, for example, typically are folded with an interior nonpolar region (Saito and others 1993). This is where lipids are sequestered, for example, when they are transported in the blood stream by albumins.

In at least some cases, there is evidence that a protein can itself be modified when it binds to another compound (Damodaran and Kinsella 1980). Not surprisingly, the environmental conditions (solution pH, temperature, and so on) also impact the extent of binding (Druaux and Voilley 1997; Andriot and others 1999; Seuvre and others 2002).

Numerous studies have compared the binding of a range of flavor compounds to particular proteins. Patterns have been seen both between compounds with different functional groups and within a range of compounds with the same functional group. Within homologous series, increasing binding with increasing chain length has typically been observed. This has been seen with aldehydes (Franzen and Kinsella 1974; Weel and others 2003), ketones (Franzen and Kinsella 1974; Andriot and others 2000), and esters (Landy and others 1995; Pelletier and others 1998; Reiners and others 2000), and it was attributed to hydrophobic bonding. Others have concluded that hydrogen bonding as well as hydrophobic bonding is involved (Tromelin and Guichard 2003). There is also evidence that steric factors impact interaction, as some stereoisomers are bound to quite different extents than their counterparts (Reiners and others 2000). Tromelin and Guichard (2003) constructed QSAR models of binding of several different compound classes (esters, furans, phenols, pyrazines, and terpenes) as a function of the relative spatial locations of hydrogen bond acceptor and hydrophobic features; these ranged in strength from $R^2 = 0.527$ for all 35 compounds to $R^2 = 0.729$ through 0.996 for subsets of the data.

Some of the common methods used to study binding of flavor compounds to proteins have included headspace gas chromatography (Franzen and Kinsella 1974; Kinsella 1989; Philippe and others 2003; Heng and others 2004), NMR (Jung and others 2002; Lubke and others 2002; Tromelin and Guichard 2006), fluorescence (Damodaran and Kinsella 1980; Dufour and Haertle 1990; Relkin and others 2001), equilibrium dialysis (Beyeler and Solms 1974; Druaux and others 1995; Fares and others 1998), and liquid chromatography (Pelletier and others 1998; Andriot and others 1999; Jouenne and Crouzet 2000).

The binding of flavor compounds to a number of specific proteins has been investigated. The protein most studied in this context is beta-lactoglobulin (Andriot and others 2000; Guichard 2000; Jouenne and Crouzet 2000; Reiners and others 2000; Seuvre and others 2002; Tromelin and Guichard 2006). Bovine serum albumin (BSA) and casein have also received considerable

MS 20070450 Submitted 6/12/2007, Accepted 9/26/2007 *Author Tan is with the Research, Development & Engineering Dept., Philip Morris, Richmond, VA 23234, U.S.A. Author Siebert is with the Dept. of Food Science & Technology, Cornell Univ., Geneva, NY 14456-0462, U.S.A. Direct inquiries to author Siebert (E-mail: kjs3@cornell.edu).*

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attention (Damodaran and Kinsella 1980; Chobpattana and others 2002; Guichard 2002). A few studies have included several different proteins (Franzen and Kinsella 1974; Jasinski and Kilara 1985; Kinsella 1989; Chobpattana and others 2002; Heng and others 2004).

A number of publications have described relationships between the extent of flavor compound binding to a protein and flavor compound physicochemical or thermodynamic properties (Damodaran and Kinsella 1980; Druaux and others 1995; Landy and others 1995; Druaux and Voilley 1997; Philippe and others 2003).

A previous study indicated that it was possible to model flavor thresholds of compounds with single functional groups in terms of simple molecular parameters such as numbers of carbon and hydrogen atoms, number of double bonds, degree of branching, and so on (Tan and Siebert 2004). It was of interest to see if a similar approach could be used to model the binding of flavor compounds to a well-characterized protein. BSA is known to occur in milk, cheese, and meat and has been much studied.

BSA is a globular protein that contains 583 amino acids and has a molecular weight of 66432. It has an isoelectric point of 5.47, and very small net charge between pH 5.5 and pH 9. BSA contains 14 mole% basic amino acids, 17 mole% acidic amino acids, 32 mole% hydrophobic amino acids, and 25.7% hydrophilic amino acids. It is organized into 3 lipid binding domains. It is predominantly alpha-helical (67%) with turns and extended or flexible regions between domains but no beta-sheets (much of these data are from http://www.friedli.com/research/PhD/chapter5a.html).

Table 1—Results of preliminary experiment on interactions between alcohols and BSA.

	Headspace area counts	Headspace area counts
Alcohol	without BSA	with 0.5 mL BSA
Methanol	2299	2616
1-propanol	3045	3005
1-butanol	11498	11527
2-pentanol	25594	25502
1-hexanol	122431	124924

Table 2—Values of compound properties selected for modeling interactions between esters and BSA.

Esters	C_NO[a]	BP[b]
Butyl propionate	7	147
Amyl acetate	7	149
Methyl hexanoate	7	150
Butyl butyrate	8	166
Ethyl hexanoate	8	167
Hexyl acetate	8	172
Ethyl heptanoate	9	187
Octyl acetate	10	211

[a]C_NO: the number of carbon atoms.
[b]BP: boiling point.

Materials and Methods

Materials

Test flavor compounds (alcohols, see Table 1; esters, see Table 2; aldehydes, see Table 4; and ketones, see Table 6) were obtained from ACROS (Morris Plains, N.J., U.S.A.). Bovine serum albumin (Fraction V, approximately 99%, prepared from pasteurized serum by heat treatment and organic solvent precipitation) was obtained from Sigma (St. Louis, Mo., U.S.A.). Vials (10 mL serum-type with crimp closures) and serum caps with 20 mm black Viton septa were obtained from Supelco (Bellefonte, Pa., U.S.A.).

Methodology

BSA was dissolved in pH 7.0 buffer (0.02 mM Na_2HPO_4 to 0.15 M NaCl) at 650 mg/18 mL (36 mg/mL). Each flavor compound was prepared as a stock solution of 10 μL/L buffer. Ten-milliliter vials containing 5 mL of liquid were prepared as follows: A 40-μL aliquot of a single flavor compound stock solution was added to each vial. A volume (0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, or 2.0 mL) of bovine serum albumin solution was added to each vial. The balance of the liquid volume of each vial was made up with the buffer (this resulted in flavor compound concentrations of 0.080 μL/L and BSA concentrations ranging from 0 to 14.4 mg/mL). The vials were closed by crimping on a serum cap. The 10 vials were then placed in a temperature controlled (30 °C) Varian model CP-8200 SPME autosampler mounted on a Hewlett-Packard Model 5890 Series II gas chromatograph (GC) equipped for single-column capillary use with a flame ionization detector. Both the GC and the autosampler were connected to a computer (Gateway P4, 1.8 GHz), which controlled the SPME autosampler using the Varian Star Workstation version 6.0 program and the GC operation and data acquisition using the Agilent ChemStation program. The time allowed for equilibration of a flavor compound between the liquid and headspace phase was 45 min. A 100 μm polydimethylsiloxane (red) SPME fiber (Supelco) was then exposed in the vial headspace for 4.5 min. The introduction into the GC was accomplished by exposing the fiber in the injector for 1.5 min at a temperature of 250 °C. Injection was splitless, with 1.5 min purge valve delay. The GC column was Hewlett-Packard HP-1 (cross linked methyl silicone) on a fused silica capillary (25 m × 0.32 mm × 1.05 μm film thickness). The temperature program used for all 4 classes of flavor compounds had an initial 3 min hold at 40 °C, followed by a linear ramp at 12 °C min to 240 °C, which was held for 3 min before returning to the starting temperature. Quantitation was done by peak integration and reported as area counts.

Table 3—Best-fit PLSR models (highest R^2cv) relating BSA-ester interactions to ester molecular properties for each volume of BSA solution.

Volume added	R^2	R^2cv	Components[a]	Intercept	C_NO	BP
0.2	0.663	0.216	2	−0.754	−0.492	0.0284
0.4	0.815	0.321	2	−1.27	−0.729	0.0430
0.6	0.903	0.769	2	−1.23	−0.416	0.0282
0.8	0.933	0.824	2	−1.42	−0.561	0.0365
1.0	0.956	0.872	2	−1.32	−0.346	0.0261
1.2	**0.954**	**0.891**	2	−1.10	−0.466	0.0309
1.4	0.930	0.817	2	−1.26	−0.492	0.0331
1.6	0.805	0.855	1	−1.05	0.0883	0.00444
2.0	0.906	0.866	1	−1.10	0.0942	0.00468

[a]Components: The number of PLSR components (latent variables) used in the best-fit PLSR model.

Data analysis

Attempts were made to model the binding behaviors of flavor compounds of single compound classes (alcohols, aldehydes, esters, ketones) to BSA as a function of molecular properties using partial least squares regression (PLSR) with the SCAN (Software for Chemometric Analysis) Release 1.1 Computer Program (MINITAB, State College, Pa., U.S.A.). The data were expressed as the fraction by which the headspace count decreased (presumably the proportion bound) for each volume of protein solution added:

$$\text{Fraction bound} = 1 - (\text{counts with BSA})/(\text{counts with no BSA})$$

Separate models were constructed for additions of each volume (0.2 through 2.0 mL) of BSA solution. In each case, a number of the molecular descriptors [the number of carbon atoms (C_NO), the number of hydrogen atoms (H_NO), molecular weight (MW), the number of carbon–carbon double bonds (CC_NO), the number of conjugated double bonds, the number of carbon atoms connected to 3 or more nonhydrogen atoms (3C_NO, a way to represent branching), the number of carbon atoms on the alcohol side of an ester (AC_NO), the number of carbon atoms in the longer chain adjacent to a ketone function (LC_NO), melting point (MP), boiling point (BP) and density] were used to model the binding. Models with various combinations of these molecular descriptors were constructed. Cross-validation was performed with the leave one out method. The magnitudes of the cross-validated multiple correlation coefficients (R^2cv) obtained were evaluated to find the combination of molecular properties that produced the highest R^2cv.

Results and Discussion

Preliminary testing was carried out to establish the times required for equilibration between the gas and liquid phases in the sample vials and for exposure of the SPME fiber in the headspace. The results were used to select the conditions specified in Materials and Methods.

Table 4—Values of compound properties selected for modeling interactions between aldehydes and BSA.

Aldehyde	H_NO[a]	BP[b]
Benzaldehyde	6	179
Hexanal	12	131
Heptanal	14	153
E-2-nonenal	16	57
Octanal	16	171
E-2-decenal	18	79
Decanal	20	208
Undecanal	22	112

[a]H_NO: the number of hydrogen atoms.
[b]BP: boiling point.

In empirical modeling with multiple predictor (x) variables, the multiple correlation coefficient (R^2) is taken as an indicator of how well the model produced fits the data used to obtain it (this is considered to represent the proportion of the variance in the data set that is explained by the model). A more conservative estimate is provided by the cross-validated R^2 (R^2cv), which is considered to provide a more realistic assessment of how well the model obtained would predict with new data (Massart and others 1997). Cross-validation is a method of internal validation in which one (or several) of the samples at a time is systematically removed from the data set and a model fit without it is used to predict its value for the dependent variable. This is compared with the actual result. The removed sample is returned to the data set, another sample (or group of samples) is removed, and the process is repeated until each sample has been omitted once. A calculation (R^2cv) is then made of the performance of the model, based on the extent of agreement between the predicted and observed values.

In general, models with fewer meaningful predictors are stronger (higher R^2cv) than those with more predictors. Non-useful predictors contribute noise and uncertainty (including error in fitting additional coefficients) and tend to increase R^2 but do not strengthen the predictive ability of a model. For this reason, it is usually beneficial to test models with various combinations of predictors to arrive at the strongest one (Massart and others 1997). R^2cv values can be compared to select the best predictive model. This approach was used in this study.

Fixed amounts of each test flavor compound were dissolved in buffer with increasing concentrations of BSA. When interaction between a flavor compound and the protein occurred, the free concentration of flavor compound in solution decreased, and this resulted in a decrease in the flavor compound concentration in the headspace of a sealed vial. Larger additions of protein should produce larger and thus more readily measured changes; since the measurement error should be similar throughout, larger additions should produce better signal to noise ratios.

Partial least squares regression computes principal components of the predictor variables that both explain variance in the predictor variables and are maximally useful for modeling the dependent variable. PLSR then models the response as a function of these (Vandeginste and others 1998). As a result, there can be any integer number of components up to the number of predictors used. Each of these is typically tested by cross-validation, and the optimum number of components is that with the highest R^2cv; this often will not correspond to the model with the highest R^2. Since the PLSR components are each linear functions of all the predictors used, it is readily possible to express the result as an equation describing the dependent variable as a linear function of the original predictor variables.

Table 5—Best-fit PLSR models (highest R^2cv) relating BSA–aldehyde interactions to ester molecular properties for each volume of BSA solution.

Volume added	R^2	R^2cv	Components[a]	Intercept	H_NO	BP
0.2	0.835	0.343	2	−0.572	0.0574	0.000659
0.4	0.869	0.521	2	−0.525	0.0625	0.000371
0.6	0.848	0.604	2	−0.524	0.0537	0.00115
0.8	0.863	0.629	2	−0.454	0.0625	0.000244
1.0	0.788	0.432	2	−0.370	0.0511	0.000995
1.2	0.922	0.822	2	−0.293	0.0534	0.000800
1.4	0.909	0.785	2	−0.447	0.0558	0.00156
1.6	**0.922**	**0.845**	2	−0.132	0.0491	0.000452
2.0	0.898	0.774	2	−0.227	0.0479	0.00136

[a]Components: The number of PLSR components (latent variables) used in the best-fit PLSR model.

As with any empirical models, interpolation can be used, but extrapolation is not valid. In other words, predictions can be made for the responses of other compounds that have values of predictor variables within the range encompassed by the data used to construct the model. However, predictions for compounds that are outside this range are invalid.

Alcohol binding to BSA

A preliminary study with alcohols was carried out with a single BSA addition rate (0.5 mL). The results obtained are shown in Table 1. It can be seen that there were only small differences in the headspace area counts with or without 0.5 mL added BSA solution, indicating that there was essentially no interaction between the alcohols and BSA. This presumably occurred because most of the alcohols are fairly hydrophilic, while the binding nature of BSA is largely hydrophobic. Similar behavior was previously seen with soy protein isolates (Zhou and Cadwallader 2006) and was attributed to competition with water for the binding sites.

Table 6—Values of compound properties selected for modeling interactions between ketones and BSA.

Ketone	C_NO[a]	LC_NO[b]	3C_NO[c]
4-Heptanone	7	3	1
2-Heptanone	7	5	1
3-Octanone	8	5	1
5-Methyl-3-heptanone	8	5	2
2-Octanone	8	6	1
Acetophenone	8	6	2
2,6-Dimethyl-4-heptanone	9	4	3
2-Nonanone	9	7	1
2-Decanone	10	8	1

[a]C_NO: the number of carbon atoms.
[b]LC_NO: the number of carbon atoms at the side of longer chain.
[c]3C_NO: the number of carbon atoms connecting to ≥ 3 non-hydrogen atoms.

Ester binding to BSA

Several of the results obtained with BSA additions to esters are shown in Figure 1. It can be seen that, in general, as the number of ester carbon atoms increased, the interaction between the flavor compound and protein became stronger (the free ester concentration in the headspace declined to a greater extent and the steepness of the decline increased). Presumably, the decline in the headspace is proportional to the amount of the ester bound to BSA. The amount bound would reduce the free ester concentration in solution and, due to partitioning between headspace and liquid phase, would cause the decline in headspace concentration.

Various combinations of predictors were examined for their utility in modeling ester binding by BSA. The molecular descriptors ultimately chosen for the esters were the number of carbon atoms and the boiling point, as indicated in Table 2. Models were constructed for each addition volume of BSA used. Because 2 descriptors were used, in each case PLSR models were constructed with both 1 and 2 components. The results in Table 3 show the R^2cv values for the models with each BSA addition rate. It is clear that even with only 2 predictors the models were strong, indicating that it is possible to predict the extent of binding of different esters to BSA as a function of these ester properties. Not surprisingly, the model is weaker with lower BSA solution volumes added. That is to be expected due to the smaller change in response, which tends to make results less precise (poorer signal to noise ratio). For the larger 2 volumes added (1.6 mL and 2.0 mL), the best-fit (highest R^2cv) model had only 1 latent variable. This suggests that ester binding to BSA can be accounted for by a single fundamental property, although the strongest model (see Figure 2), which had $R^2\text{cv} = 0.891$ and $R^2 = 0.954$, was produced with 2 components at 1.2 mL BSA solution added:

$$\text{Fraction Bound} = -1.095 - 0.465\ \ 8 \bullet \text{C\_NO} + 0.03087 \bullet \text{BP}$$

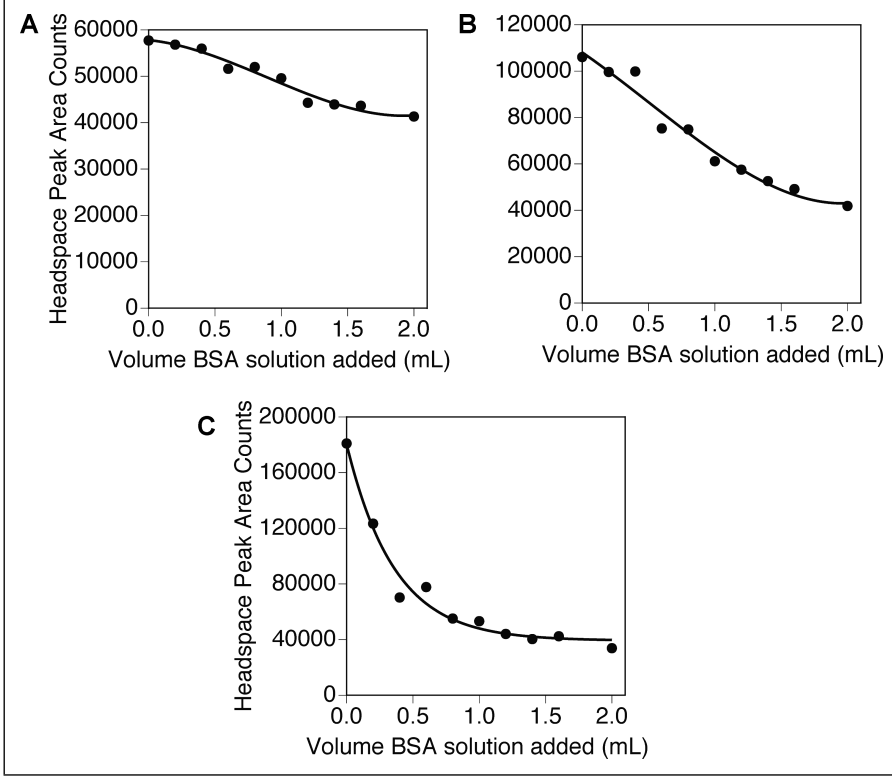

Figure 1—GC response to addition of various volumes of BSA solution to (A) amyl acetate, (B) ethyl heptanoate, and (C) octyl acetate.

S: Sensory & Food Quality

The order of the samples in the data set used to obtain the best fit was several times randomized followed by PLSR modeling; this yielded identical results in each case, demonstrating a stable model. With the 2 component models, the number of carbon atoms (C_NO) had a negative effect on binding, while the boiling point had a positive effect. With the 1-component models, both predictors had positive signs. Many regression procedures produce statistics that aid in comparing the relative predictive importance of each independent variable. In the case of the PLSR routine in SCAN, this is expressed as the "predictor importance." Since the magnitude (absolute value) of the predictor importance value of the boiling point (3.53) was higher than that of C_NO (–2.61) in the strongest model, the boiling point contributed somewhat more to the binding than C_NO. Considering the positive correlation between the sizes of molecules and their boiling points, that is, smaller molecules generally have lower boiling points while larger molecules have higher boiling points, the BSA binding of esters is overall positively correlated with the chain length of the esters. This agrees with the previous reports that the interaction between esters and BSA is largely hydrophobic (Landy and others 1995; Pelletier and others 1998; Philippe and others 2003). However, since principal components, such as those constructed during PLSR, are orthogonal to one another (at right angles in multidimensional space) and thus uncorrelated, 2 fundamentally different molecular properties are involved in the best-fit model (each a different function of both BP and C_NO).

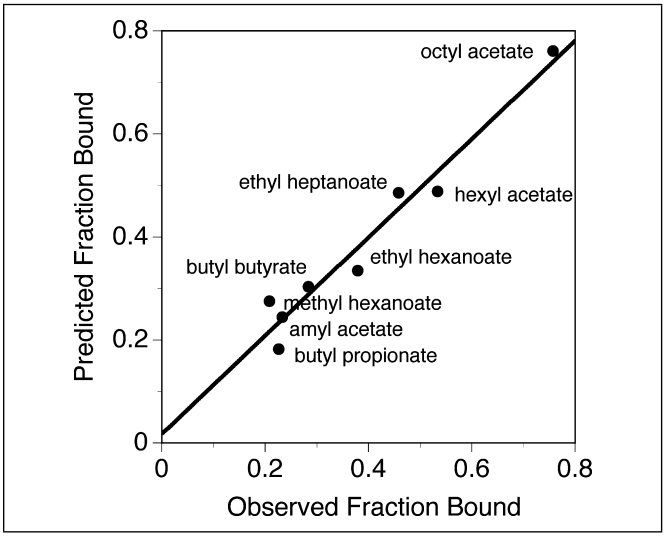

Figure 2—Best-fit PLSR model of ester binding to BSA as a function of the number of carbon atoms in the molecule and the boiling point (R^2 = 0.954, R^2cv = 0.891).

Aldehyde binding to BSA

Some of the results obtained with BSA additions to aldehydes are shown in Figure 3. It is clear that, in general, the steepness of the initial decline of the curves increases along with an increase in the carbon number of the aldehydes; this indicates stronger binding to the protein. The monounsaturated *E*-2-decenal response (Figure 3A) largely resembles that seen with the saturated compound with the same carbon number, decanal (Figure 3B). The aromatic benzaldehyde (Figure 3C), on the other hand, is bound much more weakly than the saturated compound with the same carbon number, heptanal (Figure 3D). Presumably, its aromatic nature makes benzaldehyde slightly more polar and the interaction with protein is weaker than with more nonpolar compounds. Another possibility is that its greater molecular width (due to the ring) does not fit as well into the hydrophobic region of the BSA molecule.

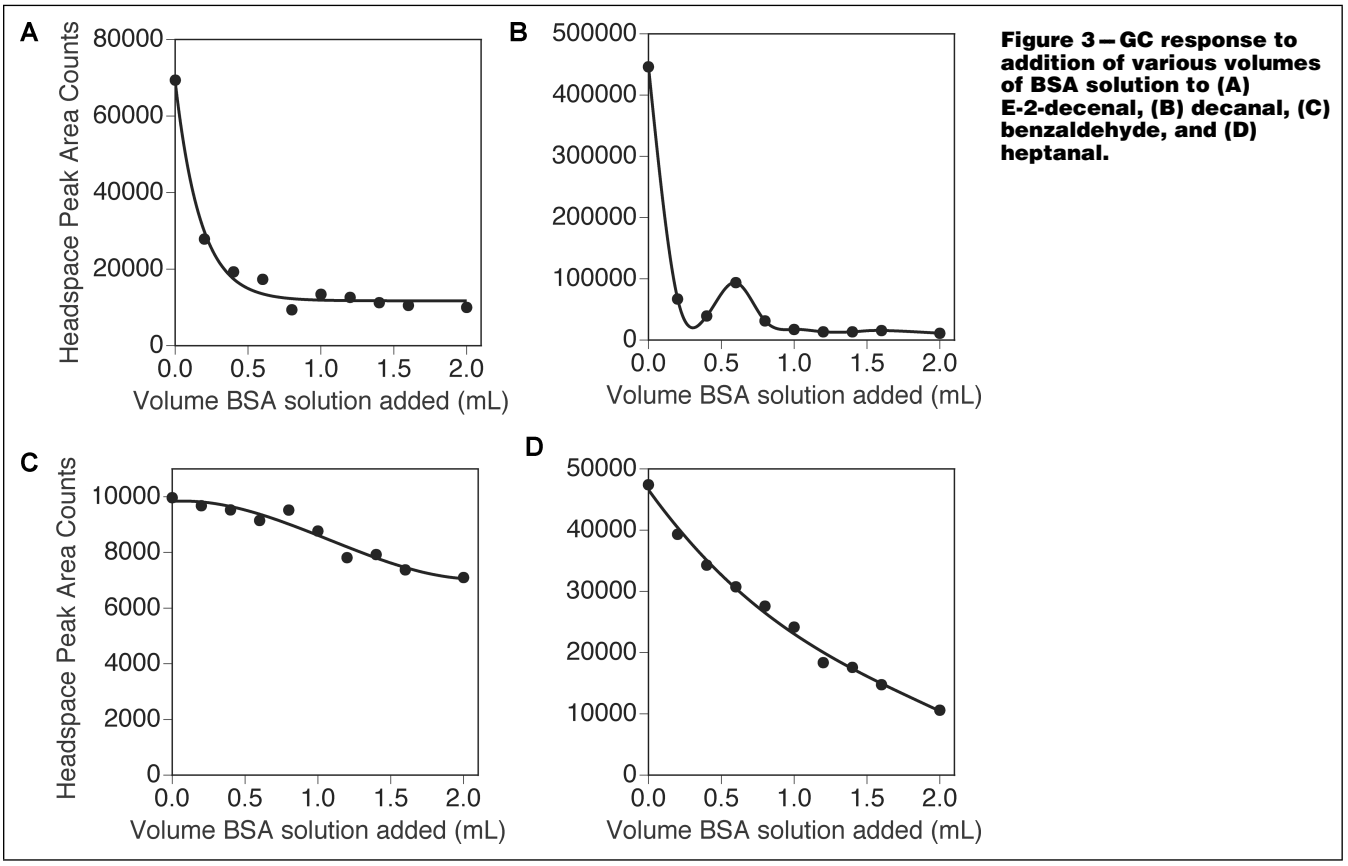

Figure 3—GC response to addition of various volumes of BSA solution to (A) E-2-decenal, (B) decanal, (C) benzaldehyde, and (D) heptanal.

BSA binding to aldehydes was best modeled with 2 molecular properties: the number of hydrogen atoms (H_NO) and the boiling point, as shown in Table 4. The PLSR results are presented in Table 5. The models are fairly strong (having reasonably high R^2cv), indicating that it is readily possible to predict the extent of binding of different aldehydes to BSA as a function of these 2 aldehyde molecular properties. Again, the models were somewhat weaker with lower volumes of BSA solution. At each volume added, the best-fit (highest R^2cv) PLSR model had 2 components, suggesting that both H_NO and boiling point are important in the prediction. The strongest model (see Figure 4), which had R^2cv = 0.845 and R^2 = 0.922, was produced with 2 components (at 1.6 mL added):

$$\text{Fraction Bound} = -0.132 + 0.0491 \bullet \text{H\_NO} + 0.000452 \bullet \text{BP}$$

The order of the samples in the data set used to obtain the best fit was several times randomized followed by PLSR modeling; this yielded identical results in each case, demonstrating a stable model. The regression coefficients for each volume added are shown in Table 5. It is clear that both H_NO and boiling point had positive effects on the binding. Considering the general positive correlation between the chain length of organic molecules and both H_NO and the boiling point, the interactions between aldehydes and BSA are likely to be hydrophobic. Longer chain aldehydes have previously been reported to bind better to whey proteins (Weel and others 2003). The magnitudes of the predictor importance values of the molecular properties indicated that H_NO (0.977) contributed substantially more to the binding than did boiling point (0.093). But the fact that the strongest model had 2 PLSR components indicates that 2 fundamental properties influence the binding.

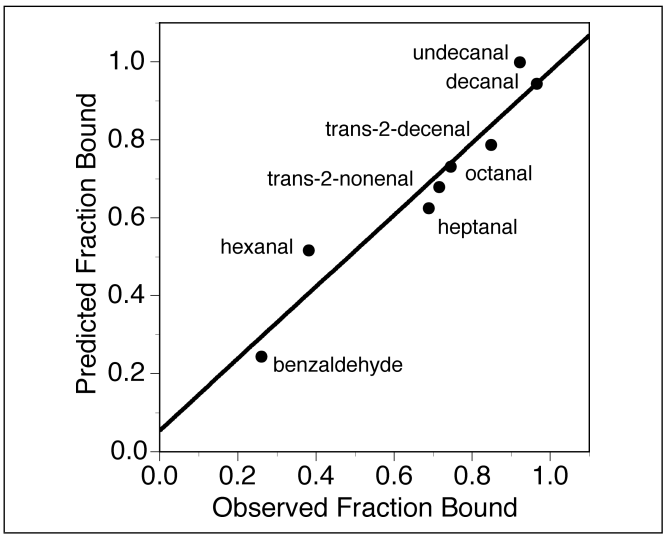

Figure 4 — Best-fit PLSR model of aldehyde binding to BSA as a function of hydrogen atoms in the molecule and the boiling point (R^2 = 0.922, R^2cv = 0.845).

Ketone binding to BSA

Some of the results obtained with BSA additions to ketones are shown in Figure 5. A pattern of stronger binding with increasing chain length similar to that seen with aldehydes was exhibited, but ketones generally bound to a weaker extent than aldehydes with the same carbon number. Perhaps the location of the somewhat polar and/or sterically larger functional group more toward the center of the compound interferes with a long nonpolar chain fitting

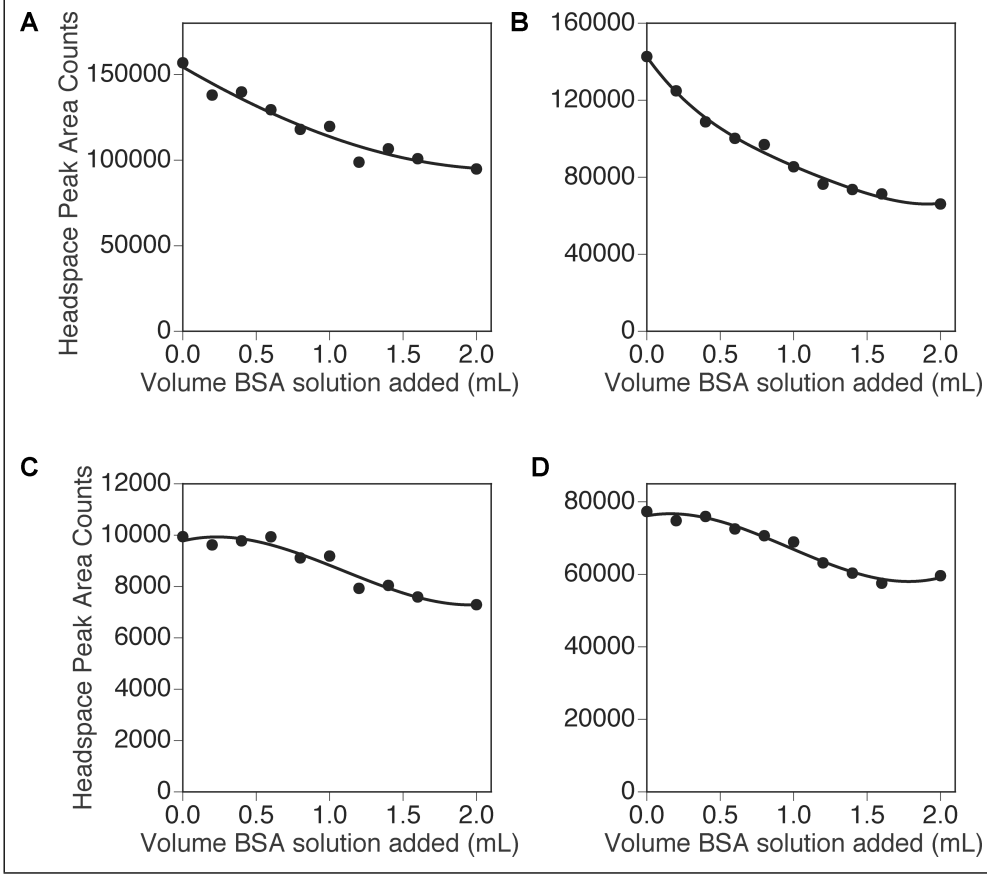

Figure 5 — GC response to addition of various volumes of BSA solution to (A) 2,6-dimethyl-4-heptanone, (B) 2-nonanone, (C) acetophenone, and (D) 3-octanone.

Table 7—Best-fit PLSR models (highest R^2cv) relating BSA–ketone interactions to ester molecular properties for each volume of BSA solution.

Volume added	R^2	R^2cv	Components[a]	Intercept	C_NO	LC_NO	3C_NO
0.2	0.896	0.598	3	−0.523	0.0807	−0.00323	−0.0239
0.4	0.893	0.584	3	−0.971	0.151	−0.00874	−0.0765
0.6	0.920	0.717	3	−1.21	0.204	−0.0279	−0.119
0.8	0.902	0.601	3	−1.18	0.201	−0.0292	−0.0867
1.0	0.857	0.574	3	−0.955	0.191	−0.0389	−0.122
1.2	0.942	0.815	3	−1.12	0.187	−0.00736	−0.0620
1.4	0.964	0.881	3	−1.17	0.197	−0.00572	−0.0904
1.6	0.962	0.840	3	−1.07	0.186	−0.00051	−0.0869
2.0	**0.961**	**0.888**	3	−1.09	0.175	0.00961	−0.0476

[a]Components: The number of PLSR components (latent variables) used in the best-fit PLSR model.

into a nonpolar groove in the protein. However, it appears that the branched ketones (see, for example, 2,6-dimethyl-4-heptanone in Figure 5A) bound to BSA only slightly more weakly than straight chain ketones with the same number of carbons (see 2-nonanone, Figure 5B). The aromatic acetophenone (Figure 5C), with 8 carbons, appears to have bound with a pattern similar to the aliphatic 3-octanone (Figure 5D), although perhaps not as strongly as with 2-octanone (not shown).

Table 6 shows the 3 molecular properties chosen for modeling the binding of ketones to BSA: the number of carbon atoms (C_NO), the number of carbon atoms in the longer chain adjacent to the ketone functional group (LC_NO), and the number of carbon atoms connected to 3 or 4 non-hydrogen atoms (3C_NO), which represents the degree of branching.

Table 7 presents the results of PLSR modeling of the interactions between ketones and BSA. In this case, the R^2 varied little with the protein solution addition volume, but R^2cv, in general, tended to increase and reached a maximum with 2.0 mL BSA solution added. The number of PLSR components used in the best-fit model with each volume added was three, suggesting that all 3 molecular descriptors are important in the prediction and that 3 fundamental properties are needed to model ketone binding to BSA. The strongest model (see Figure 6), which had R^2cv = 0.888 and R^2 = 0.961, was produced with 3 components (at 2.0 mL added):

$$\text{Fraction Bound} = -1.09 + 0.175 \bullet \text{C\_NO} + 0.00961 \bullet \text{LC\_NO} - 0.0476 \bullet \text{3C\_NO}$$

The order of the samples in the data set used to obtain the best fit was several times randomized followed by PLSR modeling; this yielded identical results in each case, demonstrating a stable model. The regression coefficients of the molecular descriptors for each volume added are shown in Table 7. At each volume added, the positive sign of the regression coefficient of C_NO indicates the binding of ketone to BSA increases with increasing C_NO or chain length. This suggests that the interaction between ketones and BSA is likely to be hydrophobic. That is in agreement with a previous study in which binding of methyl ketones to beta-lactoglobulin was considered to be largely hydrophobic (Andriot and others 2000). The negative signs for the regression coefficients of 3C_NO indicate that the binding of ketone to BSA decreases with increasing branching. The effect of LC_NO was positive for the strongest model (at 2.0 mL volume), although it was negative at all other added volumes. This is peculiar and does not seem reasonable, particularly since the model for 1.4 mL added is almost equally strong, yet with the opposite sign for LC_NO. According to the predictor importance values of the molecular properties, C_NO (0.936) contributed much more to the binding than LC_NO (0.0798) or 3C_NO (–0.190).

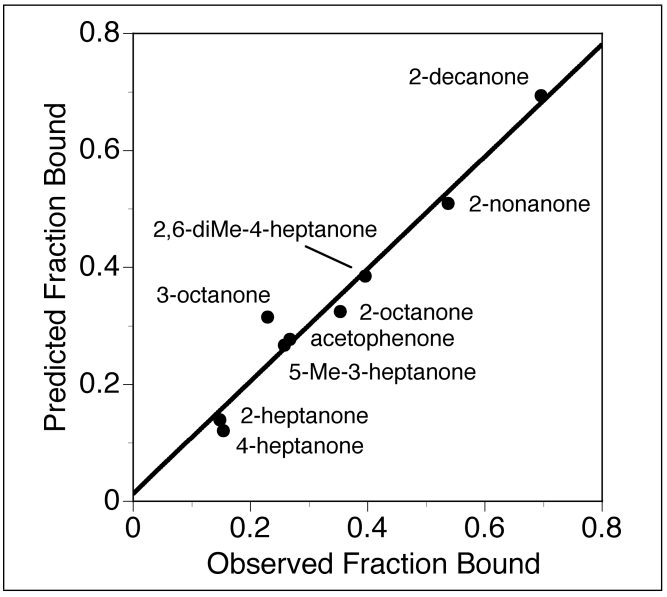

Figure 6—Best-fit PLSR model of ketone binding to BSA as a function of the number of carbon atoms in the molecule, the number of carbon atoms in the longer chain adjacent to the ketone function and the degree of branching (R^2 = 0.961, R^2cv = 0.888).

Conclusions

Clearly, relatively strong binding models that encompassed significant differences in structure were developed from simple, easily determined molecular features. In modeling of BSA binding to both esters and aldehydes, boiling point was used to predict the degree of binding, while C_NO was used for modeling of BSA binding to both esters and ketones. Considering the general positive correlation between the chain length of molecules and boiling points, it appears that the interactions between BSA and these 3 classes of flavor compounds are primarily hydrophobic. These 2 predictors showed opposite direction effects in the case of esters, although the boiling point, which had greater predictive importance, had a positive sign. In the case of modeling BSA binding to ketones, 3C_NO was used to denote the degree of branching and LC_NO was used to denote the position of the ketone functional group in the molecule. Branched structure makes the binding of ketones to BSA weaker. Neither the esters nor the aldehydes used in this study encompassed branched compounds.

Compared to the Tromelin and Guichard (2003) models, those described here are similar in explaining power (R^2) and far simpler and easier to employ to make predictions for other members of the modeled classes of compounds.

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References

Andriot I, Marin I, Feron G, Relkin P, Guichard E. 1999. Binding of benzaldehyde by beta-lactoglobulin, by static headspace and high performance liquid chromatography in different physico-chemical conditions. Lait 79:577–86.

Andriot I, Harrison M, Fournier N, Guichard E. 2000. Interactions between methyl ketones and beta-lactoglobulin: sensory analysis, headspace analysis, and mathematical modeling. J Agric Food Chem 48:4246–51.

Beyeler M, Solms J. 1974. Interaction of flavor model compounds with soy protein and bovine serum albumin. Lebensm Wiss Technol 7:217–9.

Chobpattana W, Jeon IJ, Smith JS, Loughin TM. 2002. Mechanisms of interaction between vanillin and milk proteins in model systems. J Food Sci 67:973–7.

Damodaran S, Kinsella JE. 1980. Flavour protein interactions. Binding of carbonyls to bovine serum albumin: thermodynamic and conformational effects. J Agric Food Chem 28:567–71.

Druaux C, Voilley A. 1997. Effect of food composition and microstructure on volatile flavour release. Trends Food Sci Technol 8:364–8.

Druaux C, Lubbers S, Charpentier C, Voilley A. 1995. Effects of physico-chemical parameters of a model wine on the binding of gamma-decalactone on bovine serum albumin. Food Chem 53:203–7.

Dufour E, Haertle T. 1990. Binding affinities of beta-ionone and related flavor compounds to beta-lactoglobulin: effects of chemical modifications. J Agric Food Chem 38:1691–5.

Fares K, Landy P, Guilard R, Voilley A. 1998. Physicochemical interactions between aroma compounds and milk proteins: effect of water and protein modification. J Dairy Sci 81:82–91.

Franzen KL, Kinsella JE. 1974. Parameters affecting the binding of volatile flavor compounds in model food systems. I Proteins. J Agric Food Chem 22:675–8.

Guichard E. 2000. Interaction of food matrix with small ligands influencing flavour and texture. Food Res Int 33:187–90.

Guichard E. 2002. Interactions between flavor compounds and food ingredients and their influence on flavor perception. Food Rev Int 18:49–70.

Heng L, van Koningsveld GA, Gruppen H, van Boekel MAJS, Vincken JP, Roozen JP, Voragen AGJ. 2004. Protein-flavour interactions in relation to development of novel protein foods. Trends Food Sci Technol 15:217–24.

Jasinski E, Kilara A. 1985. Flavor binding by whey proteins. Milchwissenschaft 40:596–99.

Jouenne E, Crouzet J. 2000. Determination of apparent binding constants for aroma compounds with beta-lactoglobulin by dynamic coupled column liquid chromatography. J Agric Food Chem 48:5396–400.

Jung D-M, de Ropp JS, Ebeler SE. 2002. Application of pulsed field gradient NMR techniques for investigating binding of flavor compounds to macromolecules. J Agric Food Chem 50:4262–9.

Kinsella JE. 1989. Flavor perception and binding to food components. In: Min DB, Smouse TH, editors, Flavour chemistry of lipid foods. Champaign, Ill.: American Oil Chemists' Society. p 376–403.

Landy P, Druaux C, Voilley A. 1995. Retention of aroma compounds by proteins in aqueous solution. Food Chem 54:387–92.

Lubke M, Guichard E, Tromelin A, Quere JLl. 2002. Nuclear magnetic resonance spectroscopic study of beta-lactoglobulin interactions with two flavor compounds, gamma-decalactone and beta-ionone. J Agric Food Chem 50: 7094–9.

Massart DL, Vandeginste BGM, Buydens LMC, De Jong S, Lewi PJ, Smeyers-Verbeke J. 1997. Handbook of chemometrics and qualimetrics: Part A, Vol. 20A. Amsterdam: Elsevier Science.

Pelletier E, Sostmann K, Guichard E. 1998. Measurement of interactions between beta-lactoglobulin and flavor compounds (esters, acids, and pyrazines) by affinity and exclusion size chromatography. J Agric Food Chem 46:1506–9.

Philippe E, Seuvre AM, Colas B, Langendorff V, Schippa C, Voilley A. 2003. Behavior of flavor compounds in model food systems: a thermodynamic study. J Agric Food Chem 51:1393–8.

Reiners J, Nicklaus S, Guichard E. 2000. Interactions between beta-lactoglobulin and flavour compounds of different chemical classes. Impact of the protein on the odour perception of vanillin and eugenol. Lait 80:347–60.

Relkin P, Molle D, Marin I. 2001. Non-covalent binding of benzaldehyde to beta-lactoglobulin: characterisation by spectrofluorimetry and electrospray ionisation mass-spectrometry. J Dairy Res 68:151–5.

Saito M, Chikuni K, Monma M, Shimizu M. 1993. Emulsifying and oil-binding properties of bovine serum albumin and its enzymatic hydrolyzate. Biosci Biotech Biochem 57:952–6.

Seuvre AM, Espinosa-Diaz MA, Voilley A. 2002. Retention of aroma compounds by beta-lactoglobulin in different conditions. Food Chem 77:421–9.

Tan Y, Siebert KJ. 2004. Quantitative structure-activity relationship modeling of alcohol, ester, aldehyde and ketone flavor thresholds in beer from molecular features. J Agric Food Chem 52:3057–64.

Tromelin A, Guichard E. 2003. Use of catalyst in a 3D-QSAR study of the interactions between flavor compounds and beta-lactoglobulin. J Agric Food Chem 51:1977–83.

Tromelin A, Guichard E. 2006. Interaction between flavour compounds and beta-lactoglobulin: approach by NMR and 2D/3D-QSAR studies of ligands. Flavor Fragrance J 21:13–24.

Vandeginste BGM, Massart DL, Buydens LMC, De Jong S, Lewi PJ, Smeyers-Verbeke J. 1998. Handbook of chemometrics and qualimetrics: Part B, Vol. 20B. Amsterdam: Elsevier Science.

Weel KGC, Boelrijk AEM, Burger JJ, Claassen NE, Gruppen H, Voragen AGJ, Smit G. 2003. Effect of whey protein on the in vivo release of aldehydes. J Agric Food Chem 51:4746–52.

Zhou Q, Cadwallader K. 2006. Effect of flavor compound chemical structure and environmental relative humidity on the binding of volatile flavour compounds to dehydrated soy protein isolates. J Agric Food Chem 54:1838–43.