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Ghosts of B-type Wheat Starch Granules in Concentrated KI/I_2 Solution

When B-type (2.0–8.0 μm) wheat starch granules containing various amounts (2.1–25%) of amylose were treated with 25% KI/10% I_2 solution, low-amylose (below 10% amylose) B-type wheat starch granules changed to the ghost form. It is known that A-type (25–35 μm) wheat starch granules change to the ghost form and show a typical double structure (a black-brown central portion and red-brown surrounding portion), however, the B-type wheat starch ghosts did not show the same double structure but rather a simple (red-brown portion) sack form. The relative ghost areas in the B-type wheat starch granules were highly correlated to the amylose content (%), which was similar to the results of A-type starch granules. This suggests that the amylose molecule in B-type starch contributes to the structural stability of the starch granule.

Keywords: Waxy B-type wheat starch granules, Ghost of starch granule; A- and B-type starch granules, comparison

1 Introduction

It is known that waxy wheat starch granules change to the ghost form in concentrated KI/ I_2 solution (25% KI/10% I_2 solution), but nonwaxy wheat starch granules do not show the ghost form in the same treatment, which suggests that the amylose molecule plays an important role in the structural stability of the starch granule [1]. This ghost phenomenon of the waxy wheat starch granule in concentrated KI/ I_2 solution has also been observed in waxy barley, rice, maize, proso millet, and foxtail millet starch granules [2]. The importance of the role of the amylose molecule in the stability of barley and rice starch granules was reported by *Yasui* et al. [3] and *Seguchi* et al. [4], respectively. However, waxy wheat starch granules have a peculiar nature, showing a remarkable double structure in the ghost, that is, a black-brown central portion and red-brown surrounding portion [1]. *Hayashi* et al. [5] showed that the amylose molecules were mainly present in the central portion of the ghost, as indicated by the data of determination of amylose content and column chromatography profiles, and that the ghost central portion is more rigid in the concentrated KI/I_2 solution than the surrounding portion. *Seguchi* and *Kanenaga* [6] opened normal wheat starch granules from the equatorial groove by Remazol brilliant blue staining and washing with 1% sodium dodecyl sulfate solution containing 1% 2-mercaptoethanol at room temperature, and showed the presence of double structures. The double structures of the barley starch granule were also reported by *Vasanthan* et al. [7]. These results suggest that there are two synthetic systems in the starch granule, that is, one for the inner region and the other for the outer region, and that the inner region is synthesized first, followed by the outer region [8]. Two types of starch are present in wheat, that is, A-type (25–35 μm) and B-type (2.0–8.0 μm) starch granules. The large A-type granules, which are predominant in wheat starch (75%), have a disk shape, whereas the small B-type granules (25%) display spherical and irregular shapes. The B-type granules have a higher gelatinization temperature than the A-type granules [9]. We reported the ghost structure of waxy wheat starch granules in concentrated KI/I_2 solution [5], which was mainly present as A-type granules. However, the ghost structure of the B-type waxy wheat starch granules was not yet known. In this paper wheat starch granules containing various amounts (2.1–25%) of amylose were treated with 25% KI/10% I_2 solution, and the relationship between the relative ghost areas in B-type wheat starch granules and the amylose content (%) was examined. We suggest that the amylose molecule in B-type wheat starch granules also contribute to the structural stability of the wheat starch granule.

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2 Materials and Methods

2.1 Wheat samples

Samples of 12 different wheat types, containing various amounts of amylose were used in this experiment (Tab. 1). The wheat was grown at the National Agriculture Research Center (NARC) in Japan in 1995 and 1996, and harvested grains were stored at 4°C until used.

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Tab. 1. Amylose (%)[a] and relative ghost areas[b] in various wheat starch.

Wheat	Amylose [%][a]	Relative ghost areas[b]		*L* value
		A-type granules	B-type granules	
Noring 61 (self)	25.0 ± 0.9	1.52 ± 0.05	1.50 ± 0.23	39.6
Noring 61/Tanikei H1881	22.1 ± 1.5	1.62 ± 0.11	1.69 ± 0.10	34.7
Tanikei H1881/Norin 61	17.5 ± 2.3	1.38 ± 0.09	1.52 ± 0.07	31.7
Tanikei A6099 (self)	12.4 ± 0.9	1.99 ± 0.06	1.36 ± 0.24	51.8
Tanikei A6099/Tanikei A6599-4	8.7 ± 0.6	2.71 ± 0.13	2.73 ± 0.00	63.0
Tanikei A6099/Tanikei H1881	8.5 ± 0.6	3.67 ± 0.51	1.90 ± 0.17	39.1
Tanikei A6599-4/Tanikei A6099	6.3 ± 0.4	3.03 ± 0.40	3.20 ± 0.12	72.0
Tanikei H1881/Tanikei A6099	5.0 ± 0.4	4.81 ± 0.11	2.46 ± 0.21	32.4
Tanikei A6599-4 (self)	3.7 ± 0.4	7.83 ± 0.75	3.47 ± 0.69	76.2
Tanikei A6599-4/Tanikei H1881	3.1 ± 0.2	5.18 ± 0.36	4.10 ± 0.19	50.6
Tanikei H1881/Tanikei A6599-4	2.7 ± 0.1	5.99 ± 0.19	5.29 ± 1.12	72.7
Tanikei H1881 (self)	2.2 ± 0.6	6.42 ± 0.67	7.31 ± 0.67	52.2

Values represent average and standard deviation.
[a] Amylose content (%) in wheat starch.
[b] Relative ghost areas in wheat starch.

2.2 Amylose content of wheat starch

A sample of wheat seeds was ground manually using a test grain crusher. The ground meal was sifted with a testing-sieve (150-μm openings) manually and the wheat flour that passed through the sieve was used for the analysis of apparent amylose content. The apparent amylose content of the wheat starch samples was determined colorimetrically using a Technicon Autoanalyzer, as described by *Kuroda* et al. [10]. The regression curve was derived using potato amylose as a standard. When the value of 100 mg wheat flour measured colorimetrically corresponded to that of *A* mg potato amylose, the apparent amylose content of the wheat flour was defined as *A*%.

2.3 Ghost of B-type wheat starch granule by concentrated KI/I_2 solution

Wheat starch granules were isolated from the wheat grains as follows: a seed was cut into halves on a glass slide and the center of the grain was scraped with a razor to collect starch granules. B-type starch granules could be observed with A-type starch granules, and concentrated KI/I_2 (25% KI/10% I_2) solution was added to the starch granules under a microscope (Olympus BX 50–34-DIC, Tokyo, Japan). The concentrated KI/I_2 solution was prepared as follows; KI (25 g) was dissolved in 10% I_2 solution and finally filled up to 100 mL with the same solution. Microphotographs of the B-type starch ghosts were taken as described by *Seguchi* et al. [1].

2.4 Determination of the relative ghost areas

To obtain the relative ghost areas in a B-type starch granule, ten granules of concentrated KI/I_2-treated B-type starch granules and untreated granules, respectively, were selected and cut off from each microphotograph, and the area (cm^2) of each granule was measured from the images. The relative ghost areas of the B-type starch granules were calculated as follows and averaged: area (cm^2) of a concentrated KI/I_2-treated starch granule: area (cm^2) of the untreated starch granule = relative ghost areas. The standard deviation of the relative ghost areas has been determined by separate experiments on different sets of 10 granules in the same sample.

2.5 Determination of *L* value (lightness) of B-type starch granules

Microphotographs of concentrated KI/I_2 treated B-type starch granules were taken and ten granules per sample were cut off from a complete microscopic image in order to eliminate the effect of A-granules which were also present in the image and the *L* values (lightness) were measured with a Color Meter NE 2000 (Nippon Denshoku Kogyo Co., Ltd., Tokyo, Japan). Values were averaged.

3 Results and Discussion

Fig. 1 shows the 25% KI/10% I_2-treated B-type wheat starch granules that contained 22.1, 8.5 and 2.2% of amylose. When 22.1% amylose-containing B-type starch

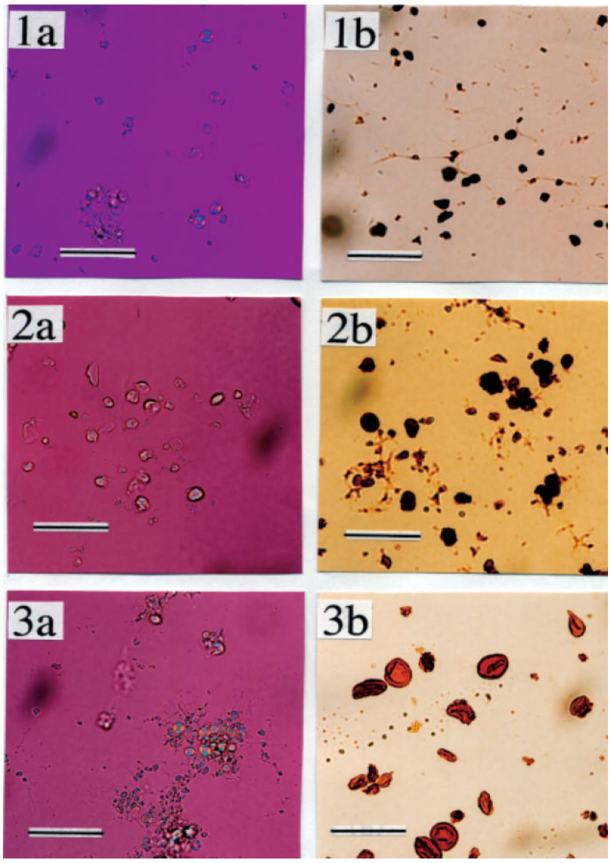

Fig. 1. B-type wheat starch granules containing 22.1% (Norin 61/Tanikei H1881), (Fig. 1–1), 8.50% (Tanikei A 6099/Tanikei H 1881) (Fig. 1–2), and 2.2% amylose (Tanikei H1881 (self) (Fig. 1–3). Untreated (a) and treated (b) with 25% KI/10% I_2 solution. Scale 20 μm.

granules (Norin 61/Tanikei H1881, Tab. 1) were treated with the concentrated KI/I_2 solution, the granules stained with a deep black-brown color (*L* value 34.7), and did not show any ghost form (Fig. 1–1). The *L* value indicated the concentration of amylose in a starch granule, that is, a higher value showed a greater lightness of starch due to a higher amylopectin content and less amylose/I_2 complexes. Fig. 1–2 shows that 8.5% amylose-containing B-type starch granules (Tanikei A6099/Tanikei H1881, Tab. 1) showed a slight ghost form, and with a black-brown color, and *L* value of 39.1. In the 2.2% amylose-containing B-type starch granules (Tanikei H1881(self), Tab. 1) a remarkable ghost form was observed, with a light brown color, and *L* value of 52.2 (Fig. 1–3). It was reported by *Seguchi* et al. [1] that waxy wheat starch granules (A-type) showed a remarkable double structure of black-brown central and red-brown surrounding portions, however, this was not observed in B-type waxy wheat starch granules. Twelve kinds of B-type starch granules containing various amounts (2.1~25.0%) of amylose were treated with concentrated KI/I_2 solution, and the relative ghost areas were measured (Tab. 1 and Fig. 2). Fig. 2 indicates that a decrease in the amylose content increased the relative ghost areas of the B-type starch granules, and that the relative ghost areas increased sharply at 5–6% of amylose. When the amylose content was extrapolated to 0%, a relative ghost areas of 7.00–8.00 was measured. The correlation coefficient (*r*) between amylose content and the relative ghost areas of the B-type starch granules was -0.7273, which suggests that the amylose molecule in B-type starch contributes to the structural stability of the starch granule. It was reported by *Hayashi* et al. [5]

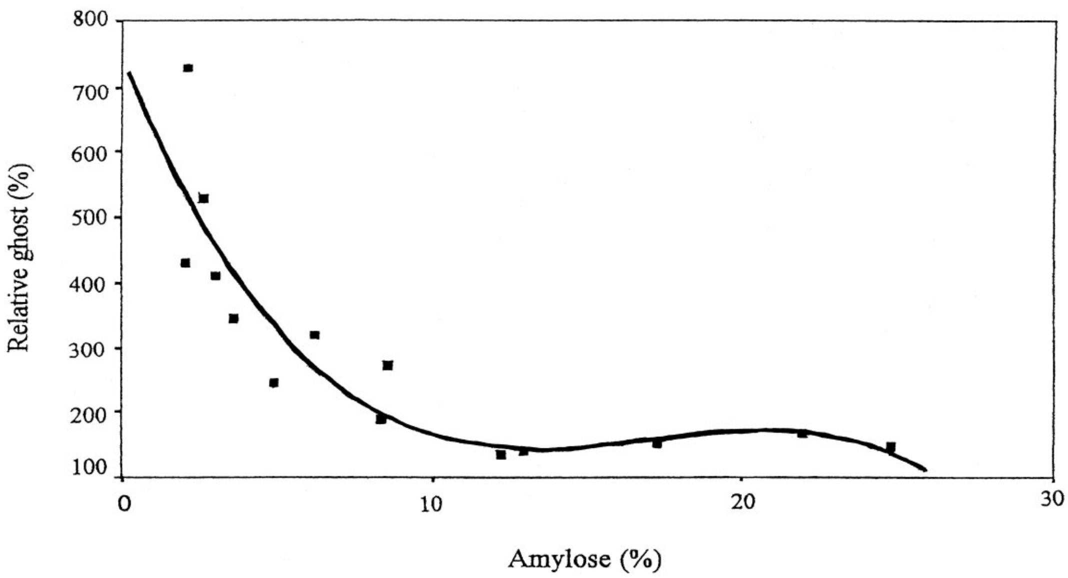

Fig. 2. Relationship between amylose content (%) and the relative ghost areas in B-type wheat starch granules.

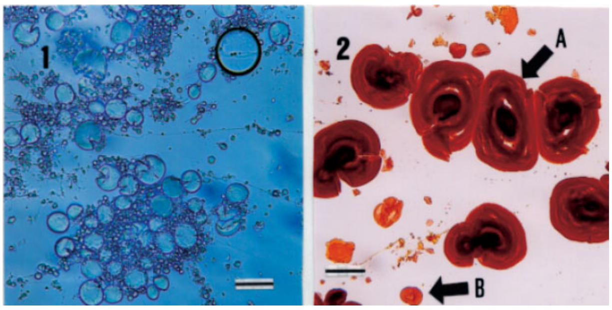

Fig. 3. Microphotographs of A (25–35 μm) and B (2.0–8.0 μm) -type wheat starch granules (Tanikei H1881 (self) (3–1), and the double structure of A ghost form (mark A) and simple sack form of B ghost (mark B) (Fig. 3–2). Scale 20 μm.

that A-type wheat starch granules showed a high correlation coefficient (*r*=-0.8380) between the relative ghost areas and amylose content in various wheat starches, and that the amylose molecule in A-type starch granules could act as a stabilizer in the granules. Although both A- and B-type waxy starch granules in the same wheat strain (Tanikei H1881 (self), Tab. 1 and Fig. 3–1) showed the ghost form in concentrated KI/I_2 solution, A- and B-type starch granules showed the double structure and a simple sack form, respectively (Fig. 3–2), which indicates that the mechanism of biosynthesis of B-type starch granule may be different from that of A-type starch granules. However, the correlation coefficient (*r*) between the relative ghost areas of A- and B-type starch granules in the same wheat strain (Tab. 1) was 0.7600, so the biosynthesis of both starch granules could be controlled by the same gene system. It was suggested that waxy wheat strains could have a synthesizing ability to produce amylopectin, and a minor amylose content was present in the A-type granules, however, in the B-type granule-biosynthesis system the ability to produce amylose was completely lost. This was supported by the fact that the amylose content in waxy rice starch granules showing a simple sack form such as wheat B-type starch granules was 0% [4].

4 Conclusions

B-type waxy wheat starch granules changed to the ghost form when mixed with 25% KI/10% I_2 solution, which was similar to A-type waxy wheat starch granules. However, the B-type wheat starch ghost did not have the double structure that was observed in the A-type wheat starch granules in the same wheat strain.

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