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Internal structure of the potato starch granule revealed by chemical gelatinization †

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ABSTRACT

Potato starch was separated into four different size groups. The starch of a uniform granular size with diameters between 30 and 52 μm was chemically gelatinized at the periphery of the granules with aqueous calcium chloride (4 *m*) at 23°C. Chemically gelatinized starch at the periphery was mechanically removed from the granule by using a blender. The gelatinized starch and the remaining granular starch were subjected to iodine titration, gel permeation chromatography, amylopectin branch-chain-length, and phosphorus analyses. The results showed that amylose was more concentrated at the periphery than at the core of the granule. Amylose at the core had larger molecular size than that at the periphery. Amylopectin at the core had longer long B-chains than that at the periphery. Starch phosphate esters were more concentrated at the core than at the periphery.

INTRODUCTION

Native starch granules have a double helical, semi-crystalline structure[1,2]. Within the starch granule, amylopectin molecules are arranged radially in a cluster structure[1,3]. Amylose molecules are dispersed among the amylopectin molecules[4,5]. Biosynthesis of the starch granule is believed to start at the hilum with the amylose and amylopectin molecules growing radially outward[6,7]. As starch granules from maize, potato, pea, and many other varieties mature, the percentages of amylose increase[8,9]. Yun and Matheson[9] reported that amylose molecular size increased with maturation of normal maize starch but decreased with that of ae maize. Branch chain lengths of maize amylopectin vary with maturation and with genotype[9].

Nikuni proposed an ultramolecular or unitary theory of starch granular structure[3]. According to the theory, there is a single molecule in the granule, and the

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molecule breaks down into fragments by the action of digestive enzymes and during preparation and gelatinization. Lineback[2] modified Nikuni's model[3] in order to encompass the molecular sizes of the amylose and amylopectin molecules that had been determined by chemical and physical methods. He also incorporated the concepts of (*a*) double helical structure for the outer chains of amylopectin and (*b*) mixed random coil and single helical conformations for amylose. However, a definite, detailed internal structure for the starch granule is not completely known.

In the present investigation, we have studied the radial distributions of amylose, amylopectin, the intermediate component, and the phosphate ester, and have determined the structures of amylose and amylopectin molecules at different radial locations. Our goal was to obtain a better understanding of the mechanisms of starch swelling, gelatinization, and gel formation. Knowledge of starch granule structure should also provide insights into the mechanism of starch granule biosynthesis.

It is known that aqueous calcium chloride (4 *m*) will chemically gelatinize starch granules starting at the periphery[10,11]. Calcium chloride is known to interact with the hydroxyl groups[12] of starch and other polymers, releasing heat that destroys the tertiary structures of the polymers[13]. At 4 *m* concentration, calcium chloride solution is highly viscous and, therefore, the rate of penetration of the solution into the granule is slow. The slow penetration of the calcium chloride solution enabled us to control the extent of the gelatinization and stop the peripheral gelatinization at designated depths in the granule.

MATERIALS AND METHODS

Normal potato starch was purchased from Sigma Chemical Company (St. Louis, MO). Crystalline *Pseudomonas amylodura* isoamylase was a product of Hayashibara Biochemical Laboratories, Inc. (Okayama, Japan). Bio-Gel P-6 was purchased from Bio-Rad Laboratories (Richmond, CA) and Sepharose CL-2B gels from Pharmacia Inc. (Piscataway, NJ). All other chemicals were reagent grade and used without further purification.

Fractionation of starch.—Normal potato starch was separated into four size groups (i.e., diameters < 20 μm, 20–30 μm, 30–52 μm, and > 52 μm) by using filter cloths of porous sizes 20, 30, and 52 μm (Fisher Scientific, Pittsburgh, PA). Starch (20 g) was wrapped in the filter cloth (20 μm), immersed in 300 mL of distilled water, and manually agitated for 10 min. Starch granules smaller than 20 μm were leached out through the filter. The procedure was repeated several times, until the solution remained clear, indicating that no more starch was being leached out. The suspension of small starch was allowed to stand for 30 min, and the starch was collected by decantation of the supernatant. The remaining starch in the filter cloth was subjected to further separation following the same sizing procedure by sequentially using filter cloths of 30 and 52 μm.

Chemical gelatinization of starch granules.—Potato starch granules of 30- to 52-μm diameter were used for the chemical gelatinization study. The starch (20 g) was suspended in 4 *m* $CaCl_2$ (150 mL) with low speed, magnetic mechanical stirring at 21–22°C for various times. The proportion of starch being chemically gelatinized depended on the length of time of exposure to the $CaCl_2$ solution. At the end of the treatment, the mixture was centrifuged at 3500*g* at −15°C for 20 min. The low temperature stopped the gelatinization process. Immediately after centrifugation, the supernatant was carefully decanted, and the starch was washed twice with distilled water (2 L each time) to remove residual $CaCl_2$.

The treated starch was then resuspended in distilled water (300 mL) and blended with a commercial blender (Hamilton Beach Blender, model 609-4, Hamilton Beach Inc.) for 10 min to separate the gelatinized starch from the ungelatinized starch granules. This was repeated five times with 15-min intervals for cooling to keep the temperature below 50°C. The mixture was then allowed to stand for 30 min. The supernatant with the chemically gelatinized starch was carefully separated from the remaining granular starch. The procedure was repeated one more time with distilled water (300 mL). The remaining granular starch was washed twice with abs EtOH and dried in an air-draft oven at 70°C for 4 h. The supernatant, containing chemically gelatinized starch, was rotary evaporated to remove most of the water, and the residue was treated with abs EtOH (10×, by volume) to precipitate the starch, which was centrifuged at 5000*g* for 30 min. The chemically gelatinized starch was collected, washed twice with abs EtOH, and dried at 70°C for 4 h. The degree of starch chemical gelatinization was calculated by dividing the weight of the chemically gelatinized starch by the total weight of starch recovered [i.e., chemically gelatinized and remaining granular starch (dry starch basis, dsb)].

Determination of amylose content.—Amylose content was determined by potentiometric titration following the procedure of Schoch[14]. The amylose content was calculated by dividing the iodine affinity of the starch by 19.9%, which is the theoretical value of iodine affinity for pure amylose from potato starch.

Gel permeation chromatography (GPC).—Molecular size distributions of the starch molecules were analyzed by GPC following the procedure of Jane and Chen[15]. A column of 2.6 i.d.×80 cm (Pharmacia Inc. Piscataway, NJ) with Sepharose CL-2B gel was used for the analysis. The sample was eluted in an ascending mode with degassed aq 0.025% NaCl. The flow rate was 30 mL/h, and fractions of 4.8 mL each were collected and analyzed by using an AutoAnalyzer II (Technicon Instruments Corp., Elmsford, NY). Total carbohydrate was analyzed by the anthrone–H_2SO_4 method[16], and the amylose-iodine blue value was analyzed following the procedure of Juliano[17].

Isoamylase-debranched amylopectin was analyzed on a Bio-Gel P-6 column (1.5 i.d.×80 cm, Bio-Rad Econo-Column [Bio-Rad Laboratories, Richmond, CA]). The column was run in a descending mode with degassed, deionized, distilled

water. Fractions of 2.3 mL were collected and analyzed for total carbohydrate content by the AutoAnalyzer II.

Analysis of branch chain length of amylopectin.—Potato amylopectin was isolated by using the Sepharose CL-2B column. The fractions were analyzed for total carbohydrate. To avoid contamination of amylose, only the first six fractions under the amylopectin peak were collected, pooled, concentrated, and subjected to branch-chain-length analysis[15].

Amylopectin (12 mg) in water (4 mL) was heated with stirring in a water bath (96°C) for 1 h. After the solution had been cooled to 25°C, 0.5 mL of acetate buffer (pH 3.5 and 0.1 M) and crystalline *Pseudomonas* isoamylase[18] (600 U) were added. The mixture was incubated in a shaking water bath (Bersa-baths, model 236, Fisher Scientific, Pittsburgh, PA) at 40°C and 90 strokes/min for 48 h. The debranched amylopectin was subjected to GPC by Bio-Gel P-6 column chromatography. Fractions (2.3 mL) were analyzed for total carbohydrate. Three fractions at each peak were pooled, concentrated, and subjected to total carbohydrate and reducing value analyses for peak chain-length calculation[15].

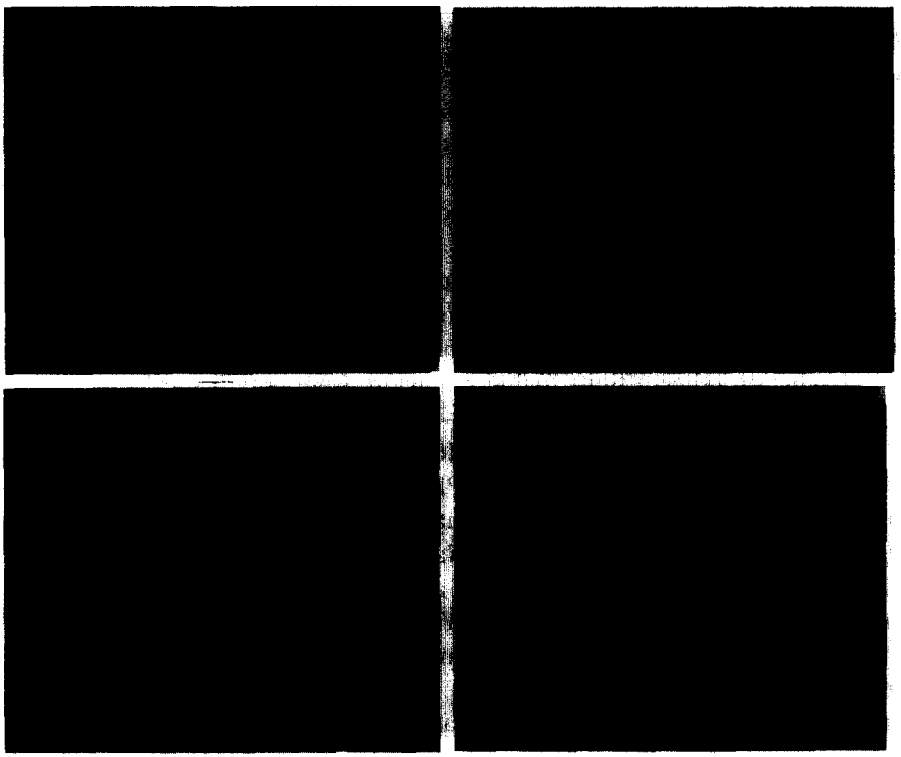

Fig. 1. Scanning electronic micrographs of A, potato starch of diameter 30–52 μm; B, surface structure of the $CaCl_2$-treated remaining granular starch; C, size reduction of the remaining granular starch; D, chemically gelatinized starch. The bar stands for 100 μm.

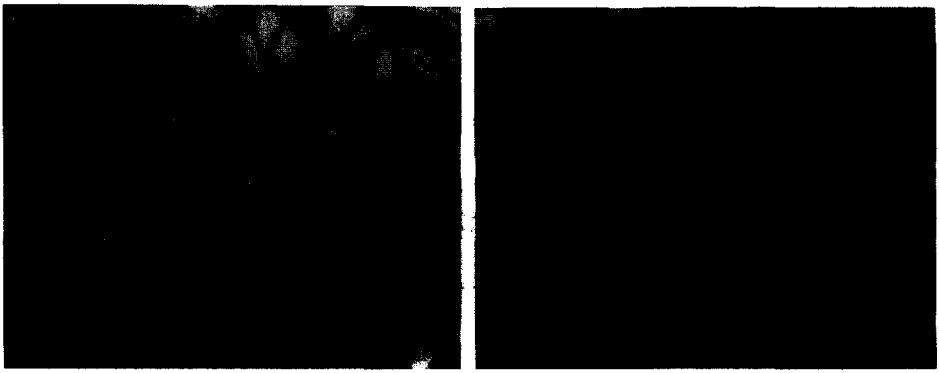

Fig. 2. Polarized light-micrographs of potato starches: A, remaining granular starch; B, native potato starch.

Microscopy.—Scanning electron micrographs were obtained with a Jeol JSM-35 scanning electron microscope (Tokyo, Japan) at 40 kV. Starch samples were dusted on double-adhesive metallic tape and coated with platinum–palladium alloy (60:40).

Polarized light micrographs were obtained with a Nikon Labophot light microscope (Garden City, NY) equipped with a Nikon FX-35 WA camera and HFX-II exposure control system.

Determination of phosphorus content.—The phosphorus content in potato starch was determined following the procedure of Smith and Caruso[19]. Four grams of each starch sample were used for the analysis. Vycor-brand glass crucibles (100 mL) (Fischer Scientific, Pittsburgh, PA) were used for the process.

TABLE I

Amylose contents of potato starch with different granular sizes and of starch at different radial locations

Sample	Amylose content [a,b] (%)
Native potato starch	20.2 ± 0.1
Potato starch (< 20 μm [c])	16.9 ± 0.2
Potato starch (< 30 μm [c])	17.5 ± 0.1
Potato starch (30–52 μm [c])	20.3 ± 0.1
Potato starch (> 52 μm [c])	20.6 ± 0.1
Remaining granular starch after 80% chemical gelatinization	18.8 ± 0.1
Remaining granular starch after 52% chemical gelatinization	19.6 ± 0.1
Chemically gelatinized starch (52% chemical gelatinization)	21.1 ± 0.4
Chemically gelatinized starch (10% chemical gelatinization)	22.0 ± 0.1

[a] The amylose content was calculated by dividing the iodine affinity of the sample by 19.9%. [b] Data reported are the means of three replicates. [c] Diameter.

RESULTS AND DISCUSSION

Native potato starch is composed of widely varying granular sizes, from several to 100 μm in diameter. To achieve a uniform chemical gelatinization of starch granules from the surface, granules of a homogeneous size were used. Native potato starch was separated into four different size groups having diameters of < 20 μm, 20–30 μm, 30–52 μm, and > 52 μm. Starch of diameters between 30

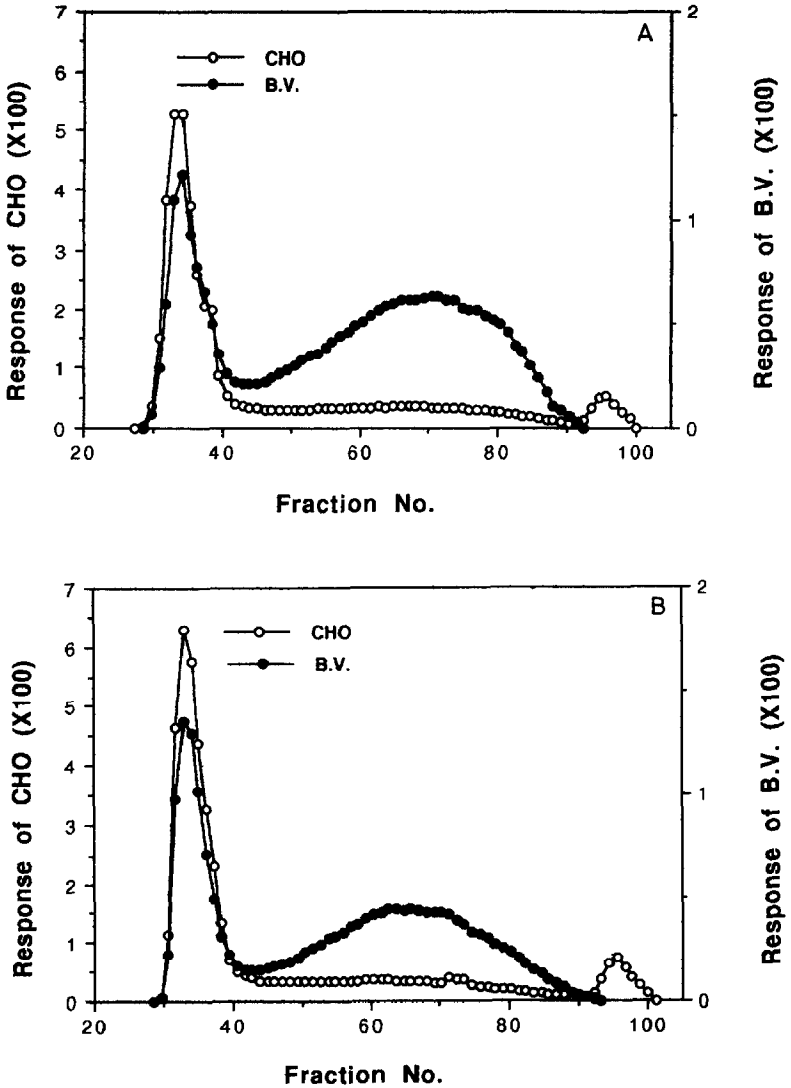

Fig. 3. Sepharose CL-2B column chromatographic profiles of potato starches: A, native potato starch; B, $CaCl_2$-treated native potato starch; C, remaining granular starch after removal of 80% chemically gelatinized starch; D, chemically gelatinized peripheral starch (35%). The profiles were normalized by using the glucose marker as a standard.

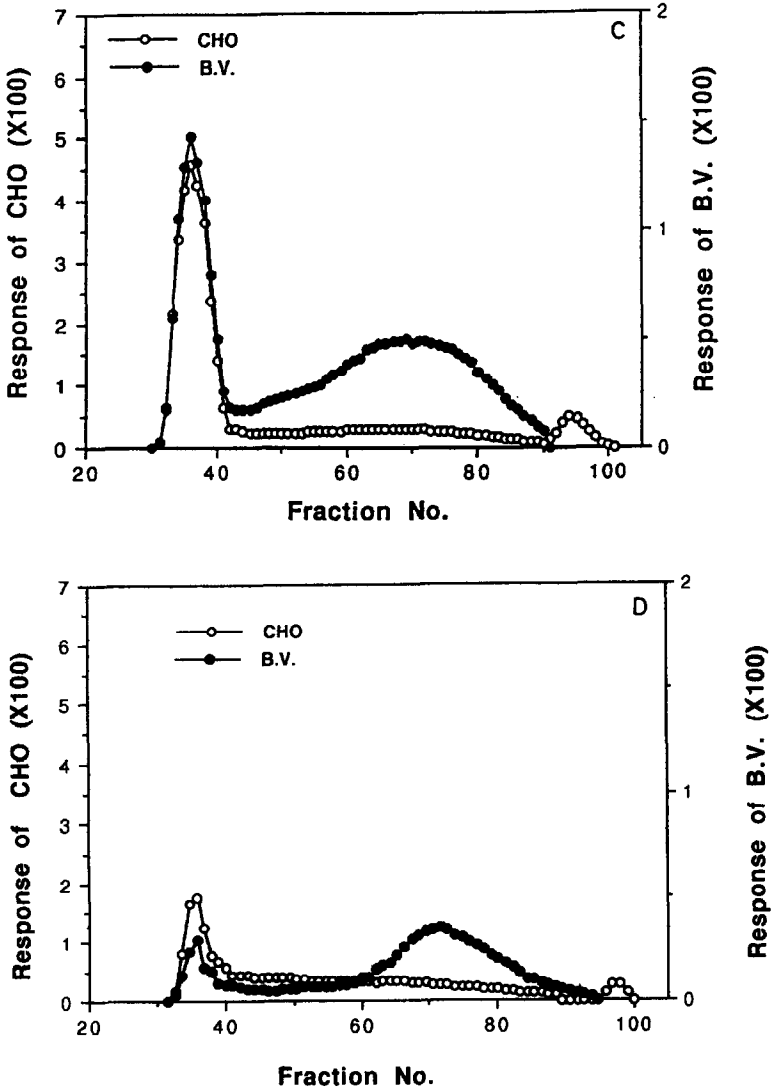

Fig. 3 (continued).

and 52 μm (Fig. 1A) was selected for the $CaCl_2$ treatment. The granules remaining after different degrees of surface gelatinization and the gelatinized starch were subjected to microscopic studies. Scanning electron micrographs of the remaining granular starch showed relatively rough surfaces with reductions in the granular size (Figs. 1B and 1C). The chemically gelatinized starch that was removed from the granules, however, displayed an undefined shape (Fig. 1D). Polarized light micrographs showed that the remaining starch granules partially retained the Maltese Cross (Fig. 2A) compared with native starch (Fig. 2B). The results indicate that the granular structure of the remaining starch was preserved.

Amylose contents of the remaining granular starches, the chemically gelatinized starches, and the starches with different granular sizes are presented in Table I. The results show that the amylose content in native starch increases with increasing size of granule. This coincided with the data for amylose contents in starch of different maturation previously reported[8,9]. The results also show that the remaining granular starch (the starch close to the core of the original granule) had a lower

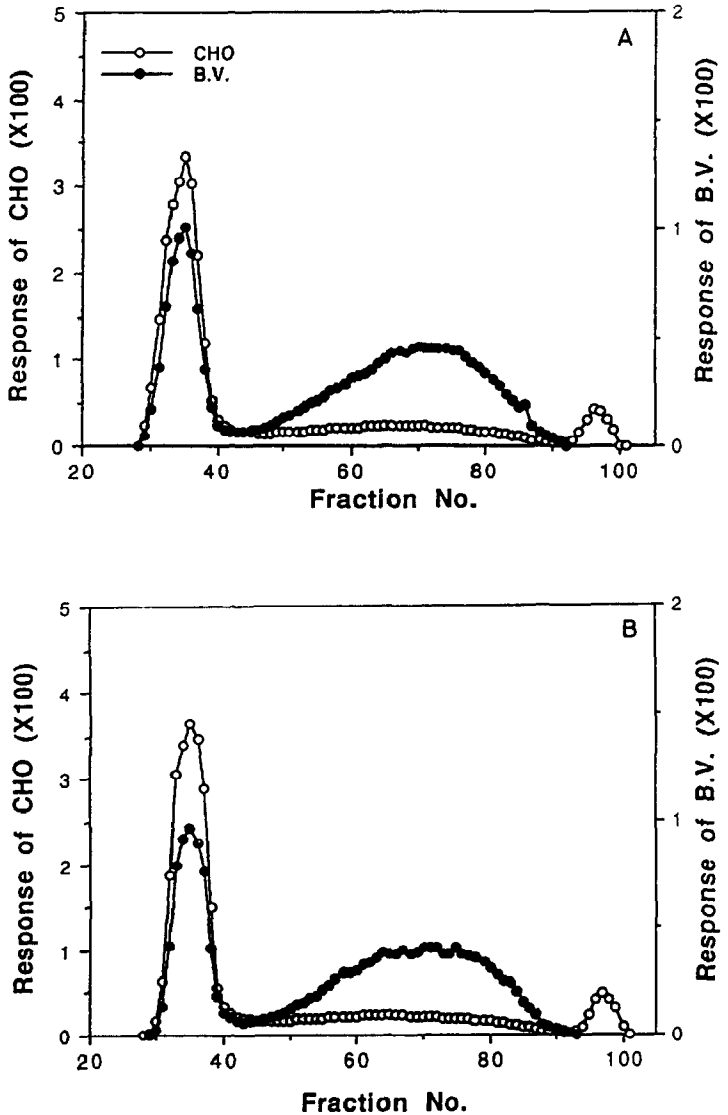

Fig. 4. Sepaharose CL-2B column chromatographic profiles of potato starches of different granular sizes: A, potato starch of diameter < 20 μm; B, potato starch of diameter 20–30 μm; C, potato starch of diameter 30–52 μm; D, potato starch of diameter > 52 μm.

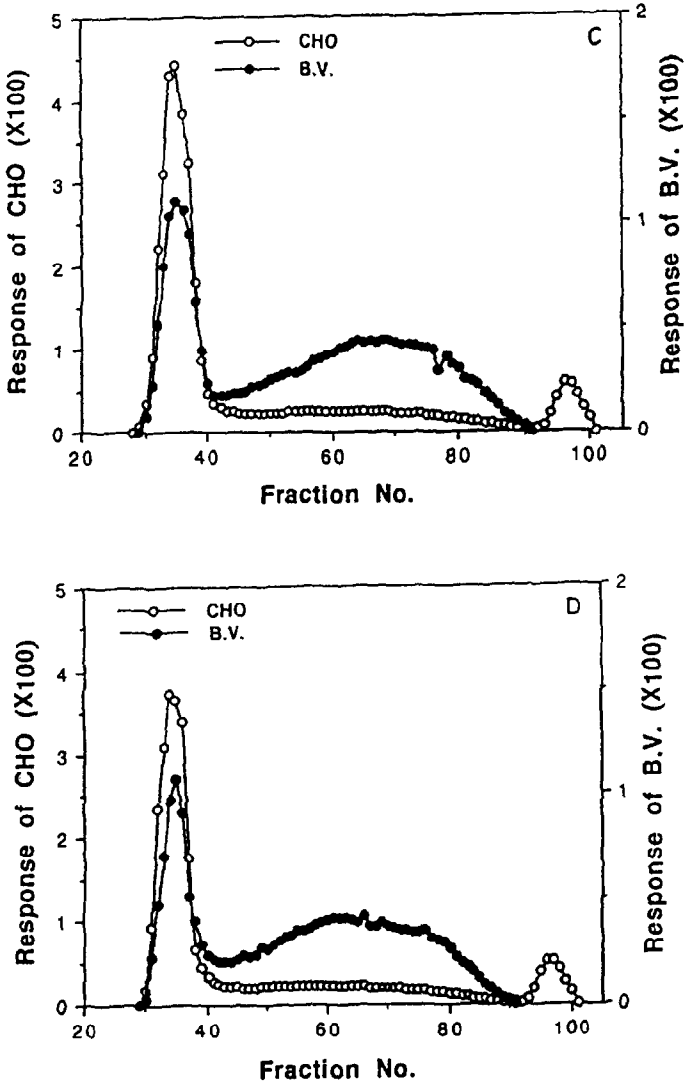

Fig. 4 (continued).

amylose content. The granular starch remaining after the removal of the chemically gelatinized starch (80%, w/w) had 18.8 ± 0.1% amylose, and that after the removal of 52% (w/w) of the chemically gelatinized starch had 19.6 ± 0.1% amylose. In contrast, the chemically gelatinized peripheral starch (10%, w/w) had 22.0 ± 0.1% amylose, and the chemically gelatinized starch (52%, w/w) had 21.1 ± 0.4% amylose, compared with the original granular starch, which had 20.3 ± 0.1% amylose content.

Amylose at different radial locations in the granule differed in molecular size. A GPC profile of native potato starch is presented in Fig. 3A. The profile shows that amylose in the native starch had a broadly distributed molecular size. A GPC profile of native potato starch treated with 4 *m* $CaCl_2$ is shown in Fig. 3B. Molecular degradation was not found in the $CaCl_2$-treated starch.

GPC Profiles of the remaining starch granules (i.e., the granule at the core) showed a significantly greater concentration of large-molecule amylose (Fig. 3C) compared with that of chemically gelatinized (peripheral) starch (Fig. 3D). The profile of the peripheral starch showed no large amylose molecules (Fig. 3D). The GPC profile of the remaining granular starch (Fig. 3C) also showed an intense blue value at the amylopectin peak. The high ratio of blue value to total carbohydrate suggested that the amylopectin at the core of the granule had long branch chains. In contrast, the blue value of the amylopectin at the periphery was significantly weaker, indicating that the branch chain length of the amylopectin at the periphery was shorter. These were later confirmed by branch-chain-length analyses.

In addition, the total carbohydrate profile of the peripheral starch (Fig. 3D) indicated a significant quantity of branched molecules with molecular weights smaller than amylopectin. These intermediate amylopectin molecules had an elution volume similar to amylose, but did not have a significant blue value. The intermediate component was not found in the profile of the remaining starch (Fig. 3C). These results indicated that the intermediate component was primarily located at the periphery, suggesting that the intermediate component could be amylopectin molecules whose biosynthesis had been prematurely terminated.

GPC profiles of potato starch of different granular sizes are shown in Figs. 4A–4D. The profiles showed that the concentration of large-molecule amylose increased with the size of the starch granule. These results suggest that, during the development of starch granules, growth of the amylose molecules continued and that those formed in the early stage (i.e., at the hilum) were fully developed into large molecules. This coincides with the result reported by Yun and Matheson[9] that amylose molecular size of normal maize starch increases with maturation.

Results of branch-chain-length analysis of amylopectins showed that the amylopectin at the core had significantly longer long B-chains compared with that at the periphery (Table II). Coincidentally, granules of small size appeared to have longer long B-chains compared with granules of large size. Their short branch chains (e.g., A-chains and short B-chains), however, were not significantly different. These results suggested that there were more long branch chains at the early stage of starch granule development. When the size of the granule grew larger, amylopectin molecules had less long branch chains and became more highly branched. It is also plausible that the blue staining "cores" found in waxy sorghum[20] could be the iodine complex of long branch chains of amylopectin instead of amylose. The physiological significance of this structure and its biosynthetic mechanism are not known. The peak branch-chain-lengths that we obtained in this study were, in general, slightly shorter than those reported by

TABLE II

Branch chain length of amylopectin debranched with isoamylase [a]

Amylopectin	Branch chain length, dp [b]	
	Long chain	Short chain
Native potato starch	41.2 ± 1.3	13.2 ± 0.3
Potato starch (< 20 μm [c])	44.7 ± 1.3	14.7 ± 0.7
Potato starch (30–52 μm [c])	41.2 ± 1.8	13.2 ± 0.4
Potato starch (> 52 μm [c])	34.0 ± 1.2	13.4 ± 0.2
Remaining granular starch after 80% chemical gelatinization	42.5 ± 1.8	13.1 ± 0.1
Chemically gelatinized starch (20% chemical gelatinization)	32.0 ± 0.8	13.1 ± 0.7

[a] Data reported are the averages of duplicate sample and chemical analyses, except for the long chain of large granules (> 52 μm) (one sample and duplicate chemical analysis). [b] Determined with the three peak fractions; dp, degree of polymerization. [c] Diameter.

Hizukuri[21]. The difference could be attributed to the method of analysis: our data were obtained by using chemical analyses of the total carbohydrate and reducing value of three fractions at the peak, whereas Hizukuri's results were calculated by using low-angle laser-light-scattering and a differential refractometer.

The phosphorus contents of potato starch samples of different granular sizes are presented in Table III. The results show that the phosphorus content decreases with an increase in the granule size. Small granule starch (diameter < 20 μm) contained 0.11 ± 0.01% of phosphorus, whereas large granule starch (diameter > 52 μm) contained 0.065 ± 0.02% of phosphorus. Starch at the core had a greater phosphorus content than that at the periphery (Table III). Both the remaining and the gelatinized starch had smaller contents of phosphorus than the original starch. The difference could be caused by residual Ca^{2+}. Phosphate

TABLE III

Phosphorus contents in potato starch of different size and locations

Sample	Phosphorus content (%) [a]
Native potato	0.081 ± 0.001
Potato starch (< 20 μm [b])	0.11 ± 0.01
Potato starch (20–30 μm [b])	0.096 ± 0.001
Potato starch (30–52 μm [b])	0.079 ± 0.001
Potato starch (> 52 μm [b])	0.065 ± 0.002
Remaining granular starch after after 50% chemical gelatin.	0.077 ± 0.001
Chemically gelatinized starch (80% chemical gelatin.)	0.065 ± 0.001

[a] Data reported are the averages of at least duplicate analyses. [b] Diameter.

derivatives are mainly found on amylopectin instead of on amylose[22]. The difference in the phosphorus content, however, seemed more than the difference in amylopectin content.

In conclusion, we have found that amylose was more concentrated at the periphery of the potato starch granule. Amylose molecules at the core of the potato starch granule were significantly larger than the amylose molecules at the periphery. Amylopectin molecules at the core had longer long B-chains than amylopectin molecules at the periphery. The phosphate was more concentrated at the core than at the periphery of the granule and was of higher content in small granules than in large ones. The intermediate amylopectin component was found concentrated at the periphery of the granule. The results indicated a highly confined starch granule structure. The biosynthesis of starch molecules and the development of starch granules appear to be regulated so as to give the differentially structured granule.

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