Internal structure of the maize starch granule revealed by chemical surface gelatinization

by

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INTRODUCTION

Starch is a polysaccharide produced by photosynthesis in most higher plants. It is found in many sources such as cereal grains (seeds), tapioca and potato (roots and tubers), sago (stems) and banana (fruits). Normally, starch is stored in a granular form.

In the starch granule, there are mainly two types of polysaccharides: amylose and amylopectin. Most starches contain 20-30% of amylose.

The fine structure of amylose and amylopectin have been well revealed since the early 1950's. Amylose is a basically linear polysaccharide consisting of α-1,4 linked D-glucopyranose units with a few branches. Amylopectin is a highly branched molecule composed of short linear chains linked by α-1,6 linkages.

In the native starch granule, amylopectin molecules are arranged radially and the branch chains are in a cluster structure (French, 1984). Amylose molecules are interspersed among amylopectin molecules (Jane et al., 1992, Kasemsuwan and Jane, 1994). The outer chains of amylopectin molecules are in double helical structure. Amylose molecules exit as random coils and single helices (Morrison, 1988; Morrison et al., 1993).

Studies of the internal structure of the starch granule, for example, how amylose molecules and branch chains of amylopectin molecules are distributed within the granule, is at a beginning stage. Jane and Shen (1993) studied the distribution of amylose, amylopectin branch chains and phosphate esters in the potato starch granules by using a chemical gelatinization method. Their results showed that amylose was more concentrated at the periphery than at the core of the granule; amylopectin at the core had longer long B-chains than at the periphery of the granule and phosphate ester was more concentrated at the core than at the periphery of the granule. The authors suggested that amylose increases with the development of the starch granule, and that amylopectin has a longer branch chain length at the early stage of the development of the starch granule. The internal granular structure from other starch sources has not been studied since the research work of Jane and Shen (1993).

The objectives of this study were: (1) to study the internal structure of the maize starch granule by using a chemical gelatinization method; (2) to investigate the surface structure of remaining granules partially gelatinized by different salt solutions; and (3) to develop a chemical gelatinization method using different salt solutions and modifying the separation method to obtain starch samples without molecular degradation.

Thesis organization

This thesis consists of two papers which will be submitted to the journal of Carbohydrate Research. These two papers entitled "Internal structure of the normal maize starch granule revealed by chemical surface gelatinization" and "Internal structure of the waxy maize starch granule revealed by chemical surface gelatinization" follow the format of the journal of Carbohydrate Research. The papers are preceded by a literature review and followed by general conclusions

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LITERATURE REVIEW

Starches are widely used in food and other industries. Various properties of starches from different botanical sources depend on the molecular structures of starch polysaccharides and other minor substances such as lipids and protein and the organization of these molecules in the starch granule.

Structures of starch molecules have been revealed rapidly with the development of enzymatic and instrumental studies. The details of the internal structure of the starch granule remains a challenge. Studies on the granular structure are aimed at how amylose and amylopectin molecules and other non-starch substances are distributed within the starch granule. It is important to the understanding of the starch granule development and provides information to the study of the starch biosynthesis.

Components of starch polysaccharides

Amylose, amylopectin and a small amount of intermediate material are present in the starch granule. A great diversity of composition of these components exists among starches of different species. Most normal starches contain 20-30% of amylose, whereas high-amylose starch contain 40-70% of amylose. Waxy starches contain nearly no amylose (Greenwood and Thomson,

1962; Shannon and Greenwood, 1984). The intermediate component has a structure and molecular size between those of amylose and amylopectin. Generally, there is 5-10% of intermediate fraction in most starches (Erlander and French. 1958).

Fine structure of amylose

The studies on amylose until 1950's established that amylose was a linear long chain composed of α-1,4 linked D-glucopyranose units. With the development of enzymatic studies, however, it was found that amylose was not absolutely linear. Peat et al. (1952) reported that about 70% of amylose was hydrolyzed to maltose by a crystalline sweet potato β-amylase.

Kjorberg and Manners (1963) suggested that the barrier to β-amylase was probably α-1,6 linkage. They pretreated potato amylose with yeast isoamylase and obtained a significant increase of β-amylolysis limit. Banks and Greenwood (1966) confirmed this result by treating potato and wheat amylose with pullulanase and β-amylase. They also concluded that the α-1,6-D-glucosidic branches were long chains rahther than short chains.

Hizukuri et al. (1981) reconfirmed the multibranched structure of amylose. They concluded that various amylose molecules were composed of 9 to 20 linear α-1,6-D-glucan chains linked by α-1,6 linkages. Amylose of potato starch has a DP of about 6000. Takeda et al.(1989) reported that amylose of high-

amylose maize starches had a DP of about 700. In general, cereal amylose appears to be smaller than amylose from other sources.

Amylose exists as a random coil in a neutral aqueous solution or forms complexes when interacting with certain organic compounds such as butyl alcohol, dimethyl sulfoxide (DMSO), fatty acids, surfactants and iodine, etc.. This type of complex is called amylose-V complex (Jane and Robyt, 1984; Morrison, 1988). X-ray diffraction pattern indicates that the complex is in a helical structure in which a complex agent is enclosed in the amylose molecule (Rundle and French, 1943; Osman et al., 1961; Hoover and Haziyev, 1981). The formation of an amylose-V complex with butyl alcohol is most commonly used for the separation of amylose from amylopectin (Schoch, 1964). The amylose-iodine complex gives a blue color which has a maximum absorbance at 650 nm. The formation of this amylose-iodine complex is used for the determination of amylose qualitatively and quantitatively. The blue color also reflects the relative chain length amylopectin (Bailey and Whelan, 1961).

Fine structure of amylopectin

Amylopectin is the major component of most starches and is comprised of highly branched macromolceules. Branch chains of amylopectin containing, on average, 20-25 α-1,4 linked D-

glucopyranose units are linked by α-1,6 linkages (Manners, 1985).

The branch chains of an amylopectin molecule are divided into three categories: A-chains, short linear chains which are unsubstituted except at the reducing end; B-chains, which are substituted at one or more C-6-OH groups by A-chains or other B- and C-chains, which are substituted at more C-6-OH groups but not the reducing end. There is only one C-chain in each amylopectin molecule.

The ratio of A-chain to B-chain indicates the multiplicity of amylopectin component chains. This ratio is determined by the amount of maltose and maltotriose liberated from β-amylase limit dextrin by pullulanase (Peat et al., 1956). Manners (1985) summarized the previous studies and concluded that the ratio of A-chain to B-chains was in a range of 1.1:1 to 1.5:1. The average branch chain length was reported as 20-25, and the molecular size for an entire amylopectin was DP 10^4-10^5 (Hizukuri et al., 1983; Manners, 1985; Takeda et al., 1988).

Models of the organization of branch chains in an amylopectin molecule have been proposed and developed since early times. Meyer and Bernfeld (1940) proposed the "ideal Meyer model". This model introduced the concept of a multiple branching feature. It was thought that all branch linkages could be isolated. The homogeneity of amylopectin molecules was later challenged by Kainuma and French (1970). They

extensively hydrolyzed a defatted waxy maize starch as a model of amylopectin by using a porcine pancreatic α-amylase and found that only 65% of the branch points were isolated. Therefore, they suggested a heterogeneous location of a branch linkage in amylopectin molecules. A cluster model was introduced during the last 20 years. French (1972) proposed a "cluster model" (Figure 1) in accord with the macromolecule as revealed by X-ray diffraction and the relative resistance to attack of acid and amylotic enzymes (Kainuma and French, 1971 and 1972).

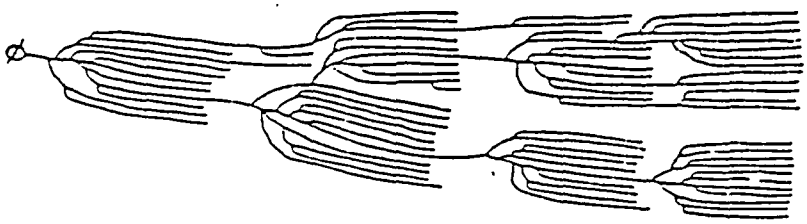

Figure 1. Cluster model for amylopectin (French, 1972)

Robin et al. (1974) studied the sequential degradation of amylopectin and proposed a similar model (Figure 2) in which A-chains and B-chains were defined. This model showed the presence of population of chains having a chain length of DP 15-20 which were highly ordered in clusters. Manners and Matheson (1981) further developed the cluster model based on

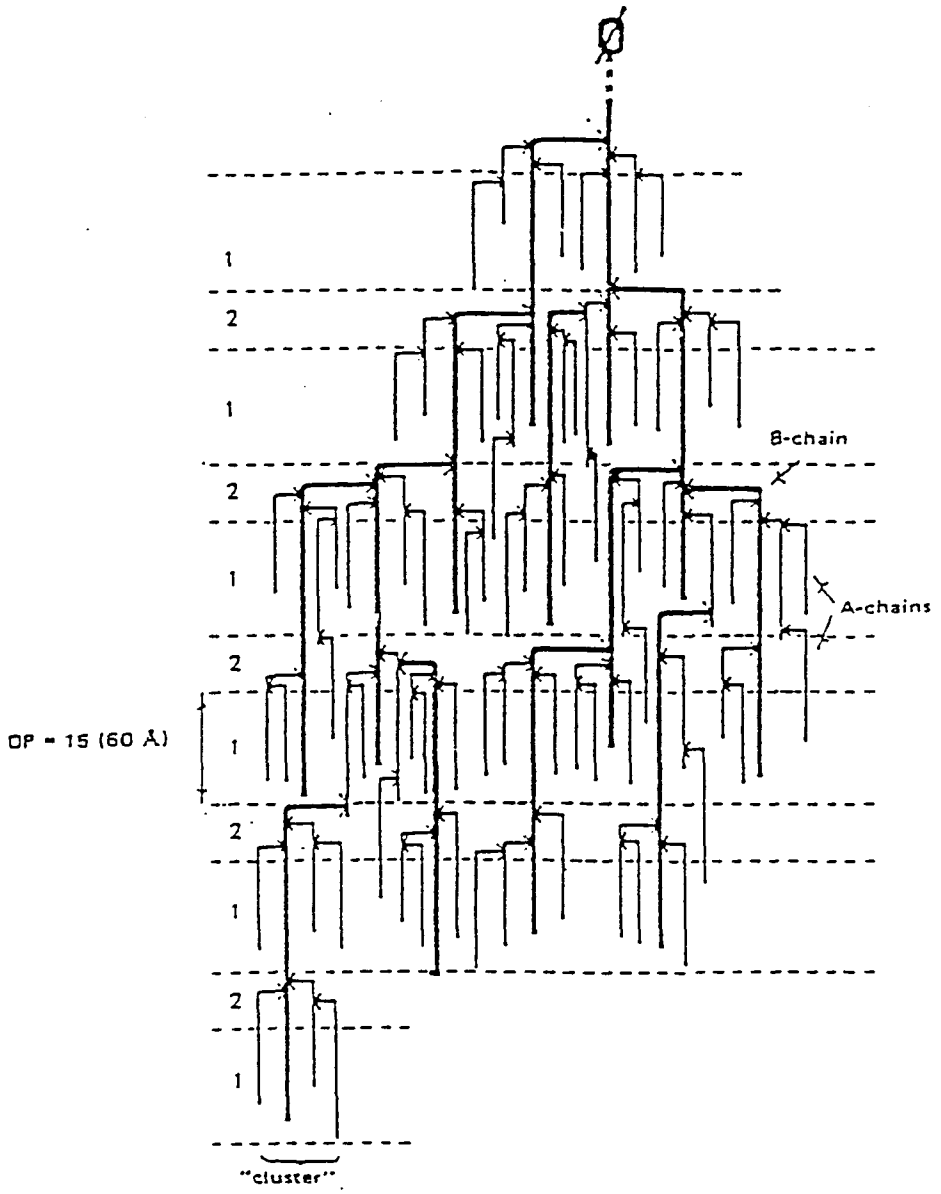

Figure 2. Proposed structure for potato amylopectin by Robin et al. (1974) 1: compact area; 2: less compact area; Arrow: branching point; and Ø: reducing unit

the ratio of A- to B-chains. They indicated that the branch points were arranged in "tiers" or clusters not distributed randomly throughout the macromolecule (Figure 3).

Since the discovery of debranching enzymes, the distribution of the branch chain lengths of amylopectin has been studied by using HPLC (Harada et al., 1972; Akai et al., 1975; Mercier, 1973; Kainuma et al., 1978; Ikawa, 1980; Hizukuri, 1985). The DP is 45-60 for long chains and is 15-20 for short chains. In the last decade, Hizukuri (1986)) analyzed the distribution of potato amylopectin and waxy rice starch by using gel-permeation chromatography. One fraction of A-chains and four fractions of B-chains were separated. It was concluded that the A-chain had DP of 12-16 and that three fractions of B-chains (B1, B2 and B3) had DP of 20-24, 42-48 and 69-75, respectively. The chain length of potato amylopectin is longer than that of most starches, except high-amylose starches. The arrangement of A-chains to B-chains in the amylopectin molecule was proposed as shown in Figure 4. One percent of chains, as fraction B4, could even extend into four clusters.

Intermediate fraction

In the starch granule, there is a branched component called the "intermediate fraction" whose molecular structure and size are between those of amylose and amylopectin.

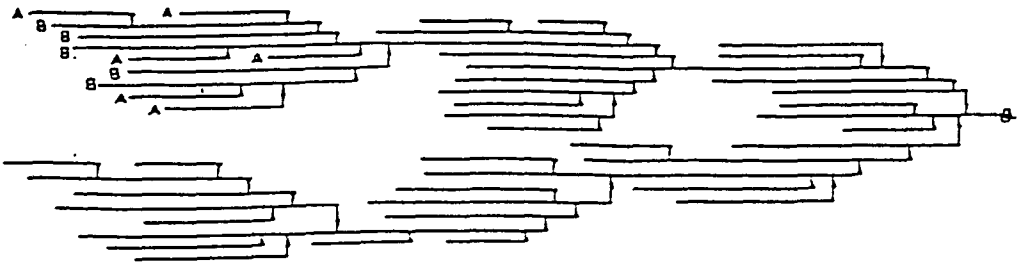

Figure 3. Modified cluster model of amylopectin, based on that of French (1972) and Robin et al. (1974). (Manners and Matheson, 1981)

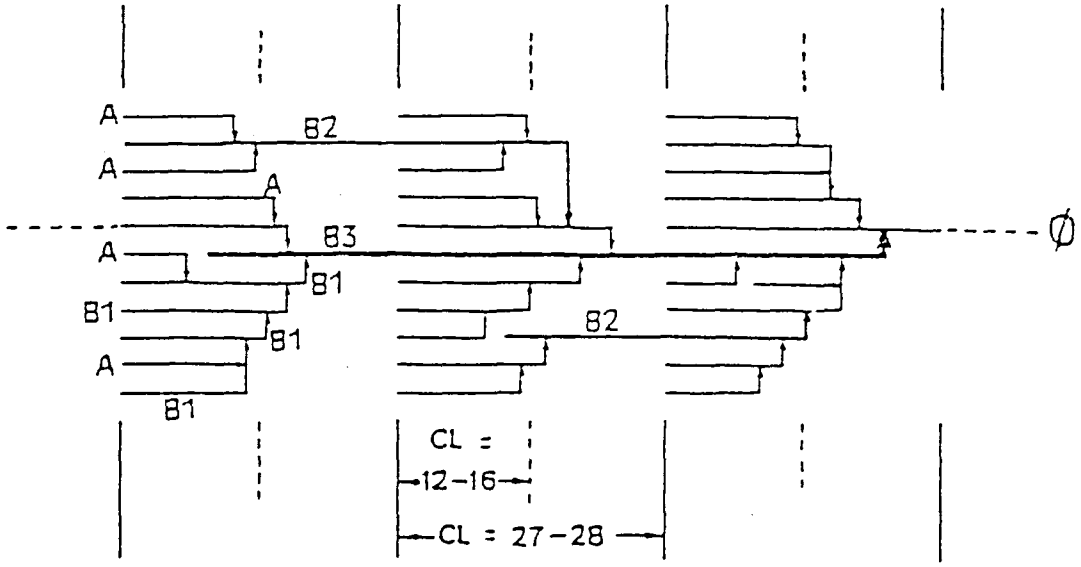

Figure 4. Cluster model of amylopectin proposed by Hizukuri (1986)

Generally, there is 5-7% of intermediate fraction in most cereal and tuber starches (Banks and Greenwood, 1975).

Whistler and Doane (1961) isolated the intermediate fraction from maize starches by precipitating with 2-nitropropane. They found that high-amylose maize starch contains a great amount of intermediate fraction (8.7% in *du* su_2 type) and normal maize starch contains a lower amount of lower intermediate fraction (4.5% in normal commercial maize starch).

The structure of the intermediate fraction is not completely known. Whistler and Doane (1961) reported that all intermediate fractions from different starches gave a deep-blue color with iodine. The maximum absorbance for the iodine solution of the intermediate fraction was at 588-600 nm, which was between the values for amylopectin (540 nm) and amylose (650 nm) (Swanson, 1948). The intermediate fraction was also reported to have a molecular size similar to that of amylopectin but have a longer branch chain length (Takeda et al., 1986, Wang and White, 1994). Jane and Shen (1993) studied the molecular distribution of intermediate fractions in potato starch granules. They suggested that the intermediate fraction could be an amylopectin molecule whose biosynthesis is prematurely terminated.

Structure of the starch granule

The structure of the starch granule is dependent on the biosynthesis of starch molecules in amyloplasts and the genetic controlled growth condition. Polarized light microscopy, scanning electron microscope (SEM), transmission electron microscopy (TEM), X-ray diffraction and other instrumental methods have indicated that the starch molecules are arranged radially in the granule; the outer chains of amylopectin molecules exhibit a double helical structure and the native starch granule is present as a semi-crystalline structure. The internal structure, however, is not completely known.

Growth rings of starch granules can be observed under an optical microscope (Frey-Wyssling, 1953). French (1972) considered that these rings represent concentric shells on layers of alternating high and low refractive index, density, crystallinity and resistance or susceptibility to chemical and enzymatic attack. Yamaguchi et al. (1979) suggested that the ring may be the association of linear chains from adjacent molecules which form crystalline regions and other less densely packed regions which are amorphous and susceptible to acid and enzymatic hydrolysis.

The semi-crystallinity of the starch granule is also shown by treating the granule with 16% sulfuric acid for 3 months. The residual material is called "Nägeli amylodextrin" (Kainuma and French, 1971, 1972; Robin et al., 1974). In the

starch granule, the amorphous regions are readily degraded by aqueous acid, and the crystalline regions are relatively resistant to such treatment. Acid treatment of waxy maize starch granules gives a highly crystalline residue. Umeki and Kainuma (1981) studied the Nägeli amylodextrin of waxy maize starch in detail. They found that a pair of α-1,4-linked glucan chains were more resistant to acid treatment than was a single chain; and the crystalline regions were packed with a number of pairs of intertwining linear chains of approximately 14 glucose residues. These evidences supported the theory of a double helical structure for the crystalline regions of starch suggested by the study of X-ray diffraction, reaction with iodine, and the space filling model of Kainuma and French (1971).

Gallant and Bulpin (1969) and Kassenbeck (1978) studied starch granules by using advanced staining techniques and concluded the following organizations in starch granules: amylose molecules are radially arranged in amorphous regions and amylopectin molecules were arranged in tangential lamellae to form crystalline regions.

Nikuni (1969) proposed the "unitary theory" of starch in which all molecules in a starch granule may well be covalently bound (Figure 5). Both amylose and amylopectin molecules are incorporated with the appearance of centric ring structure in this model. Lineback (1984) proposed a modified model, in

which the concept of double helices of the outer chains of amylopectin molecules was included (Figure 6). Amylose molecules exist in random coils and single helical structures in this model.

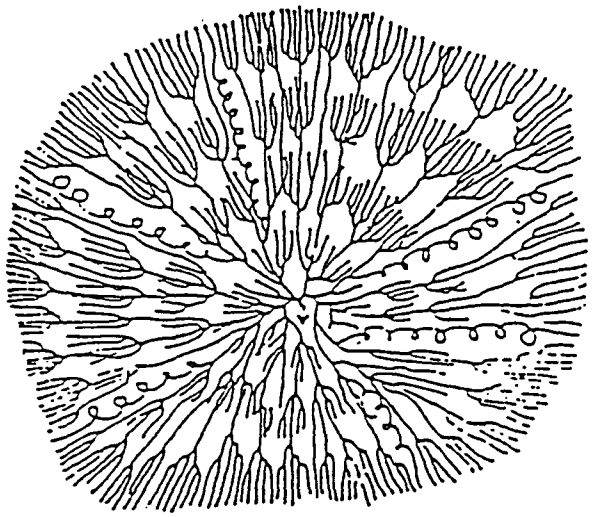

Figure 5. Schematic proposal of the starch granule based on "the unitary theory" (Nikuni, 1969)

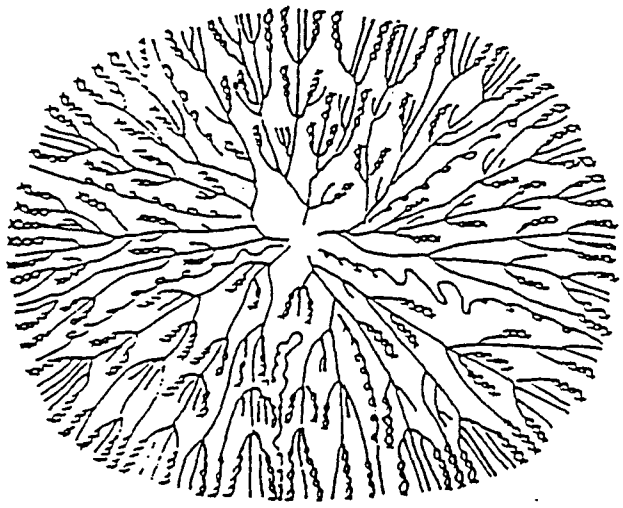

Figure 6. Modified model of the starch granule based on Nikuni's model (Lineback, 1984)

Jane et al. (1992) studied the location of amylose by a low degree cross-linking reaction with epichlorohydrin. They found that the blue value of amylopectin was increased in gel permeation chromatography, and after debranching, cross-linked amylopectin displayed amylose chains. The results indicated that amylose and amylopectin molecules were cross-linked after the treatment. Hence, Jane and her coworkers suggested that amylose molecules are interspersed among amylopectin molecules. This result was recently confirmed by Kasemsuwan and Jane (1994) by using cross-linking resealed by phosphorous-31 NMR.

The radial distributions of amylose, amylopectin, and phosphate ester in the potato starch granule were studied by Jane and Shen (1993) by a chemical gelatinization method. Results showed that amylose was more concentrated at the periphery of the granule; amylopectin at the core had longer long B-chains; and phosphate ester was more concentrated at the core of the granule. They suggested that amylose grows with the development of the starch granule and that amylopectin at the early stage of growth of the starch granule has a longer long B-chain length.

Gelatinization and retrogradation of starch granules

Gelatinization and retrogradation are two remarkable characteristics of the starch granule. They are the results of the interaction between starch polysaccharides and water

molecules and the change of starch conformation when treating starch granules with water at various temperatures.

Gelatinization

Gelatinization is the process of breaking inter- and intra-hydrogen bonds and hydrophobic interactions with the addition of water within the granule. When starch granules are progressively heated with water from room temperature to about 60°C, the physical changes include slight granule swelling and loss of properties of birefringence. If the temperature continues to increase, starch granules will be dispersed in water and become soluble to form a colloidal sol.

Generally, gelatinization of starch granule happens over a range of temperatures which differ from specie to specie. Main methods for the determination of gelatinization temperature are: loss of birefringence, increases of optical transmission and increase of viscosity. Recently, differential scanning calorimetry (DSC) has been widely used to determine the temperature of gelatinization.

Properties of gelatinization depend on the structure of starch molecules, amylose content and other components in the starch granule such as sugar, pH, salt, etc. For example, potato starch has a lower gelatinization temperature (59°-68°C) than maize starch (62°-72°C). High amylose maize starch granules only swell to a limited degree at 100°C and do not gelatinize until 125°C (Greenwood, 1976). Sugar increases

gelatinization temperature and extends the range (Osman, 1972). A study using ^{13}C-NMR suggested that the interaction of sugar-starch during heating occurs before the onset temperature of starch gelatinization (Hansen et al., 1989). Salt have complicated effects on the starch gelatinization. The mechanism will be discussed in the later part of this review.

Retrogradation

Retrogradation is the process that allows gelatinized starch molecules to become gels or to crystallize and precipitate during standing. It is considered an undesired change of solution or paste in many uses. Changes of starch retrogradation include formation of crystalline structure, decrease in light transmission and loss of the ability to form a blue complex with iodine (Collison, 1968).

The rate of retrogradation depends on starch varieties, amylose content, temperature, etc. For example, normal cereal starches appear to retrograde more readily than tuber starches. The rate increases with the increase of starch concentration and is greatly enhanced by a temperature at about 0°-5°C (French, 1975).

The structure of retrograded starch molecules was studied by Matsukura et al.(1983) Figure 7 schematically illustrates the structure. Amylose chains and component chains of amylopectin form parallel and antiparallel double helical structure. These helical structures as shown in "a", "b", "c",

"d" and "e" are formed by: interaction of amylose molecules; interaction of component chains of amylopectin; and interaction of amylose molecules and component chains of amylopectin. There are three domains: domain A, which is primarily from retrograded amylopectin, is regions that are resistant to acid hydrolysis but more susceptible to enzyme attack; domain B is a slightly retrograded structure of gelatinized starch, in which amylopectin molecules are still well-hydrated and well-dispersed; and domain C is primarily from retrograded amylose molecules which are hydrolyzed by acid but resistant to enzyme action.

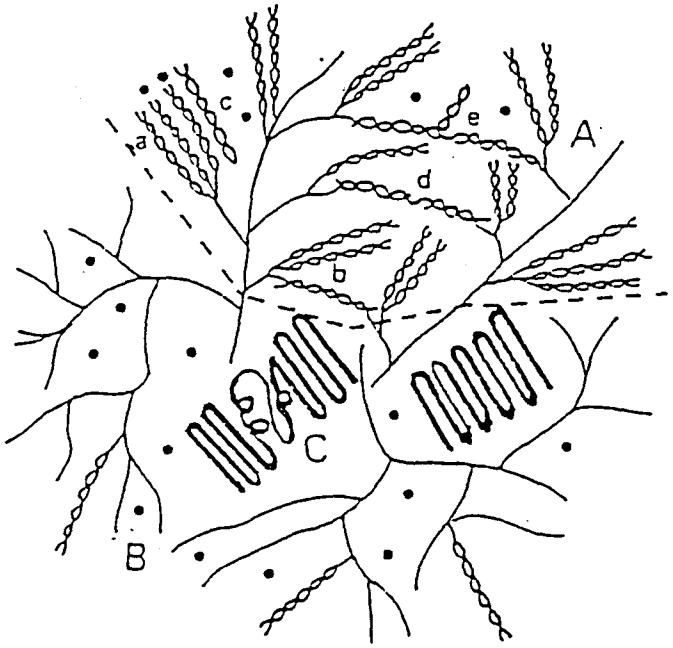

Figure 7. Schematically illustrated structure of retrograded starch ▬▬▬: amylose, ———: amylopectin, ·: water molecule (Matsukura et al., 1983)

Amylose is considered primary responsible for starch retrogradation. The rate of retrogradation is related to the molecular size of amylose. A maximum rate was found at DP of 80-100 (Pfannemuller et al., 1971., Gidley and Bulpin 1989).

Jane and Robyt (1984) proposed a structure of retrograded amylose in which there are double helical structures with a length of 10 nm. These crystalline regions are interspersed among amorphous regions.

Chemical gelatinization of starch granule

Studies have shown that starch gelatinization can be affected by various salts. For example, Na_2SO_4 retards gelatinization (Evans and Hainsman, 1982), and KSCN gelatinize starch granules readily at room temperature (Sandatedt et al., 1960). DSC analysis shows that starch gelatinizes with an exothermic change in the presence of concentrated (>4m) $CaCl_2$ (Evans and Hainsman, 1982) whereas gelatinization of starch in water is an endothermic change.

The mechanism of the gelatinization of starch in salt solution depends on several factors. The effects of salt are related to the model of water molecule and the hydrogen bonds forming between starch and water molecules.

It is known that a model of liquid water is composed of mixture of hydrogen-bound clusters and unbound free-water molecules. Ions of high charge-density such as Li^+ have strong

electrostatic interaction with water molecules. These ions known as "structure makers" reduce the fraction of free-water molecules (Paqutte and Joliceur, 1977) and increase the viscosity of the solution; ions of low charge-density called "structure breakers" such as SCN^-, K^+ increase the free-water fraction and decrease the viscosity of the solution.

The presence of free water is believed to promote hydrogen bonds between -OH groups of starch and water molecules. Those bonds replace the hydrogen bonds between starch chains, facilitating the dissociating of starch chains in crystalline regions and suppressing the melting temperature.

Jane (1993b) observed that gelatinization pattern of maize starch granules in KI and KSCN solution began at the hilum and this pattern remained unchanged with salt concentration; however, the gelatinization pattern in $CaCl_2$ and LiCl solution changed with the salt concentration, it began at the periphery at a higher concentration (e.g. >14m for LiCl and >2.5m for $CaCl_2$).

Study of ^{13}C-NMR and microscopic analysis suggested that KSCN and KI solution have low viscosity and are easy to diffuse into the granule; an increase of free-water fraction in KSCN and KI solution make them better hydrating agents and the low charge-density ions interactions with starch induce a single helical conformation, facilitating the dissociation of starch molecules in the granule (Jane 1993a, Jane 1993b).

The other type of salt (LiCl and $CaCl_2$) has high charge-density cations. A low salt concentration solution retards the starch gelatinization temperature. However, when salt concentration is higher, there are other factors affecting starch gelatinization besides the high charge-density. ^{13}C-NMR, DSC and microscopic analysis indicated that dipole-metal interaction becomes dominant at a high concentration. At an intermediate concentration (1-2m $CaCl_2$ and 4-12m LiCl), starch granules can be gelatinized promptly at room temperature. At a very high salt concentration, the diffusion of cations (e.g. Li^+) into the starch granule is hindered by its charge and high viscosity. Heat is then generated by the dipole-metal interaction on the surface of the granule. This heat in turn melts nearby starch crystalline region inward. At a higher concentration, the penetration of salt into granules is slower.

According to this theory, starch granules can be chemically gelatinized from the periphery to the hilum in different degrees by controlling the salt concentration and time available for the gelatinization. Starch granules, therefore, can be "eroded" in different desired degrees. So-called "surface gelatinization" is carried on by treating granules with salt solution at a desired degree.

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INTERNAL STRUCTURE OF THE NORMAL MAIZE STARCH GRANULE REVEALED BY CHEMICAL SURFACE GELATINIZATION

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Abstract

Normal maize starch was separated into two fractions: large granules with diameters above 5μm and small granules with diameters less than 5μm. The large granules were surface-gelatinized by treating them with an aqueous LiCl solution (13M) at 22-23°C. Surface-gelatinized remaining granules were obtained by mechanical blending, and surface-gelatinized starch was obtained by grinding using a mortar and a pestle. Starches of different granular sizes and radial locations were subjected to scanning electronic microscopy, iodine titration, gel permeation chromatography and amylopectin branch-chain length analysis. Results showed that the remaining granules had a rough surface with a lamella structure. Amylose was more concentrated at the periphery than at the core of the granule. Amylopectin had longer B2-chains at the core than at the

periphery of the granule. Greater portions of B2-chains were concentrated at the core than at the periphery of the granule.

Introduction

The granule is a storage form of starches biosynthesized in most higher plants. In the starch granule, there are mainly two types of polysaccharides: amylose and amylopectin. Amylose is a primarily linear molecule with a few branches; and amylopectin is a highly branched molecule whose outer branch chains form helical and cluster structures[1-4].

A unitary theory was proposed by Nikuni to explore the organization of amylose and amylopectin molecules within the starch granule[5]. In this model, there was a single molecule in the starch granule, and the molecular weight was much greater than that determined by chemical and physical methods. Lineback proposed a modified model in which amylose and amylopectin molecules were separated, and the concept of double helical structure was incorporated[6]. Amylose molecules existed in random coil and single helical conformation[6].

Recent studies indicated that amylose molecules are randomly interspersed among amylopectin molecules[7,8] rather than located in bundles[4,9]. The distributions of amylose and branch chains of amylopectin were studied in the potato starch granule by using a chemical surface gelatinization method[10]. It was found that amylose was more concentrated at the periphery than

at the core of the granule; and amylopectin had longer long B-chains at the core than at the periphery of the granule.

Certain sorts of salt solutions were found to promote starch gelatinization at room temperature[11-15]. $CaCl_2$ and LiCl solutions at a high concentration (e.g. 4 m and 14 m, respectively) gelatinize native starch granules gradually beginning at the periphery[16] of the granule. These salt cations are considered to interact with the hydroxyl groups of starch molecules, releasing heat that melts nearby starch crystalline regions inwardly[16,17]. The slow penetration of a highly viscous salt solution into starch granules was used to control the gelatinization of potato starch granules[10].

The objectives of this study were to: study the internal structure of normal maize starch by using a method of chemical surface gelatinization; to investigate the surface structure of the remaining granules partially gelatinized by a LiCl and a $CaCl_2$ solutions; and to develop chemical gelatinization methods by using different salt solutions and modifying the separation method to obtain starch samples without molecular degradation.

Materials and methods

Normal maize starch was a gift of American Maize Products Company (Hammond, IN). Sepharose CL-2B gel was purchased from Pharmacia Inc. (Piscataway, NJ). Bio-gel P-6 from Bio-Rad Laboratories (Richmond, CA). Isoamylase (EC3.2.1.68, crystal,

from *Pseudomonas* amyloderamosa, 59,000 units/mg protein) was a product of Hayashibara Biochemical Laboratories, Inc. (Kayama, Japan). Other chemicals were all reagent grade and used without further purification.

Fractionation of native starch granules

Two fractions of native granular normal maize starch, large and small size, were obtained by using a nylon filter cloth with a porous size of 5 μm according to the following procedure:

About 20 g of native starch granules were wrapped in the nylon filter cloth, tied up, immersed in 300 ml of distilled water and agitated. Starch granules smaller than 5 μm in diameter were filtered out and large granules were left in the cloth. After a 10 minutes agitation, the cloth bag was immersed and agitated in another 300 ml of fresh water and the procedure was repeated for several times until there were no more small granules filtered out. Both large and small granules were dried at 40°C. The large granules were subjected to chemical treatment and analysis. The small granules were used for comparison analysis.

Defatting of granular starch

Native fractionated large and small granular starch were defatted by extracting with a mixture of methanol and water (85% v/v) following a general method of Schoch[18].

Chemical surface gelatinization

Large native granules (20 g) of normal maize starch were suspended in 150 ml of a 13 M LiCl solution and stirred at 22-23°C for different periods of time. A desired extent of surface-gelatinization was determined by observing the treated sample using a Nikon labophot light microscope (Garden City, NY). The reaction was stopped by mixing the suspension with 1200 ml of 4°C distilled water. The mixture was then centrifuged at 3200 X g for 15 minutes and washed twice with 1800 ml of water.

Large granules were also surface-gelatinized in a 4M $CaCl_2$ solution for comparison analysis of the surface structural of starch granule. Starch granules (20 g) were suspended in 150 ml of a 4M $CaCl_2$ solution and stirred at 22-23°C for 1 hour. Same methods described above were used for stopping the reaction and washing the partially $CaCl_2$-gelatinized starch granules.

Separation of the gelatinized starch from the remaining granules

The partially gelatinized starch granules were treated in different ways to obtain starches remaining granules, which resemble the core of the starch granule (with higher degrees of surface gelatinization) and surface-gelatinized starch, which resembles the periphery of starch granule (with lower degrees of surface gelatinization).

To isolate the remaining granules, the partally surface-gelatinized starch granules were suspended in 120 ml of distilled water and blended by using a Hamilton Beach Blender (Model 609-4, Hamilton Beach Inc.) at about 22,000 rpm for 10 minutes and then filtered. Another 120 ml of water was added to the precipitate and blended. This procedure was repeated for 3-5 times until the supernatant was clear. The remaining granules were washed with 100% ethanol and dried at 40°C over 8 hours. The supernatant containing the gelatinized starch was collected and concentrated by evaporating to a smaller volume under vacuum at a temperature around 30°C. The gelatinized starch was then precipitated with 2 volumes of 95% ethanol, washed with 100% ethanol and dried at 40°C over 8 hours.

To collect the gelatinized peripheral starch, the partially surface-gelatinized granules were suspended in 80 ml of distilled water and ground gently using a mortar and a pestle. The mixture was then filtered. The precipitate was resuspended in another 80 ml of water and ground. This procedure was repeated for 5-7 times until the supernatant was then clear. The remaining granules were washed with 100% ethanol and dried at 40°C over 8 hours. The supernatant containing gelatinized peripheral starch was collected and concentrated to a smaller volume by evaporating under vacuume at a temperature around 30°C. The gelatinized starch was precipitated with 2 volumes of 95% ethanol, washed with 100% ethanol and then dried at 40°C over 8 hours.

The degree (in percentage) of surface gelatinization was calculated according to the following equation:

$$\% \text{ of gelatinization} = \frac{\text{Gelatinized starch (g)}}{\text{Gelatinized starch (g) + Remaining starch (g)}}$$

Scanning electronic microscopy (SEM)

The granular sizes of native starch granules and the surface structures of chemical treated starch granules were analyzed by scanning electronic microscopy (SEM) following the procedure of Jane and Shen[10].

Effect of blending on granular and gelatinized starch

Three types of samples were used for the study of the effects of mechanical blending on the starch molecular structure: large granules without mechanical treatment, large granules subjected to machanical blending and chemical gelatinized starch of large granular size subjected to mechanical blending. The mechanical blending was conducted at 22,000 rpm for 10 minutes; and this procedure was repeated for four times with 30 minutes intervals to prevent rising temperature. The chemically gelatinized starch was prepared by treating 20 g of large granular starch with a LiCl solution (8M) for 10 minutes. The paste was washed twice with 1800 ml of water, mixed with 120 ml of water and then subjected to the same mechanical blending. The blended gelatinized starch was

precipitated with 240 ml of 95% ethanol, washed with 100% ethanol and dried at 40°C ovre 8 hours.

Determination of amylose content

Amylose content was determined by iodine potentiometric titration following the procedure of Schoch[19]. The amylose content was calculated by dividing the iodine affinity of the starch by 19.0%, which is the theoretical value of iodine affinity of pure amylose from maize starch.

Gel permeation chromatography (GPC) by using a Sepharose CL-2B column

Molecular size distributions of starches of different granular sizes and radial locations were analyzed by GPC using a Sepharose CL-2B column following the procedure of Jane and Chen[20]. About 5 mg of starch (5 ml) aqueous solution was injected to a 2.6 X 85 cm column packed with Sepharose CL-2B gel (Pharmacia Inc., Piscateway, NJ). The column was eluted with the solution containing 25 mM NaCl and 10 mM NaOH in an ascending direction. The flow rate was 0.5 ml/min. Fractions of 4.8 ml were collected. Total carbohydrate and blue value of each fraction were analyzed by using an autoanalyzer II (Technicon Instruments Corp., Elmsford, NY) at 630 nm and 640 nm, respectively. The relative ratio of total carbohydrate to blue value at the amylopectin peak of each sample was calculated by dividing the ratio of the blue value (B.V) to the total carbohydrate (CHO) at the amylopectin (Ap) peak by the

ratio of the total carbohydrate to the blue value at the amylose (Am) peak. It was expressed as the following equation:

$$\text{Ratio} = \frac{\text{B.V/CHO (Ap)}}{\text{B.V/CHO (Am)}}$$

Isolation of amylopectin and amylopectin Branch chain length analysis

Amylopectin was isolated from normal maize starch of different granular sizes and radial locations following the procedure of Jane and Shen[10]. Fractions 31 to 37 were collected from the Sepharose CL-2B column. The solutions were concentrated by evaporating to 4 ml under vacuum at a temperature around 30°C and heated in a boiling water bath for 1 hour. The suspensions were then cooled to room temperature. Acetate buffer (0.5 ml) was added to the mixture and the pH was adjusted to 3.5. Crystalline *Pseudomonas* isoamylase (600U) was added. The mixtures were incubated for 48 hours in a shaker at a rate of 100 strokes/min at 40°C. The reaction was stopped by adjusting the pH to 6 and heating in a boiling water bath for 10 minutes. Sodium azide (0.02%) was added as a preservative. The concentration of the debranched amylopectin solutions was about 2 mg/ml.

The molecular size distributions of isoamylase-debranched amylopectin molecules were analyzed by GPC using a Bio-gel P-6 column following the procedure of Jane and Chen[20]. One ml of the solution of debranched amylopectin was injected into a 1.5 X

90 cm column packed with Bio-gel P-6 gel (Bio-Rad Laboratories, Richmond, CA) and eluted with deionized water in a descending direction. Fractions of 2.3 ml were collected and subjected to the the total carbohydrate analysis by using an AutoAnalyzer II (Technicon Instruments Corp., Elmsford, NY) at 630 nm. There were three peaks (I, II and III) as shown in profiles. The sum of total carbohydrate of each peak component and its percentage were calculated. The ratio of the total carbohydrate of peak II to that of peak III of each sample was thus obtained. Branch chain lengths of amylopectin were determined following the procedure of Jane and Shen[10]. According to the profiles of GPC Bio-gel P-6, fractions of the whole peak component (peak II and III) in the profiles were collected for average chain length analysis, and the three fractions that contained the highest total carbohydrate at each peak in the profiles were collected for peak chain length analysis. The chain length was calculated by dividing the total carbohydrate (μg/ml) by the reducing value (μg/ml). The total carbohydrate was read in the profiles based on a standard curve in a range of 7 μg/ml to 60 μg/ml of glucose. The reducing value was determined following the procedure of Jane and Chen[20].

Analysis of debranched amylopectin by high-performance anion-exchange chromatography (HPAEC) on a Dionex system

The distribution of branch chains of amylopectin was analyzed by HPAEC-PAD (high-performance anion-exchange

chromatograohy-pauled amperometric detection) on a Dionex system following the procedure of Wong and Jane[21]. DP of each peak on the profiles was identified on the basis of the elution time and the area counted as homologues. HPAEC-PAD profiles were normalized by dividing the area of each peak by the largest area in the same profile.

Results and discussion

Starch granules were fractionated to obtain uniform sizes so that the surface gelatinization could take place evenly. The large granules were chosen for the chemical treatment. Small granules were considered to be starch developed at an early stage, whereas large granules were more mature. The remaining granules after the removal of the surface-gelatinized starch had structures resembling to the core of the starch granule, particularly at a high degree of surface gelatinization; whereas the surface-gelatinized starch had a structure close to the periphery of the starch granule, particularly at a low degree of surface gelatinization.

After fractionation, the weight ratio of large granules to small granules (w/w) was 9:1. 34%, 65% and 84% LiCl-gelatinized remaining granules and 8% and 27% LiCl-gelatinized starch were used for structural analysis. These samples were based on one replicate.

The granular sizes of fractionated native granules are shown in Fig. 1-A and Fig. 2-B. The surface structures of LiCl-gelatinized remaining granules are shown in Fig. 1-C and Fig. 1-D. These remaining granules have been "eroded" and left a rough surface. The remaining granules with a higher degree of surface gelatinization (84%) had a lamellar structure. It has been reported that both high concentration $CaCl_2$ and LiCl solutions gelatinized starch granule beginning at the periphery[16]. Observation by the optical microscope indicated that starch granules were surface-gelatinized more evenly in a 13M LiCl solution than in a 4M $CaCl_2$ solution. The $CaCl_2$-gelatinized remaining granules shown in Fig. 2-A displayed a less sharply-separated granular structure. Gelatinized starch shown in Fig.2-B displayed no more granular form.

It was questioned whether mechanical blending would have degraded the starch molecules. The effect was studied by examining the molecular size distribution using a GPC Sepharose CL-2B column shown in Fig. 3. The similar profiles of native granular starch without blending (Fig. 3-A) and after blending (Fig. 3-B) indicated that starch molecules remained intact after blending. The profile of gelatinized starch after blending was changed (Fig. 3-C). The profile showed degradation of amylopectin molecules. This result demonstrated that gelatinized starch molecules were degraded after mechanical blending. This result showed that mechanical blending at a high shear rate caused degradation of gelatinized

starch but did not affect the starch molecules in granule. Therefore, remaining granular starch can be prepared by blending to remove the surface gelatinized starch; and gelatinized starch must be prepared by a gentle separation such as grinding with a mortar and a pestle.

The amylose content analysis (Table I) showed that large granules and 8% gelatinized starch had high amylose contents (27.8% for large granules and 35.0% for gelatinized starch), and small granules and surface gelatinized remaining granules had lower amylose contents (22.8% for small granules and 23.8% for 84% remaining granules). The remaining granules retained maltese cross when viewed under a polarized-light microscope[10], which indicated that the retention of crystalline structure of the granule. Therefore, it is not likely to have significant leaching of amylose. Remaining granules after removal of a high degree of surface gelatinized starch (e.g. 84%) resemble the core of the granule, whereas the gelatinized starch of a low degree of gelatinization (e.g. 8%) resembles the peripheral starch of the granule. The results of amylose contents analysis showed that amylose was more concentrated at the periphery than at core of the normal maize starch granule. It is consistent with the results obtained from potato starch granules[10]. It is also in an agreement with previous studies that amylose increasing with the maturity of the starch granule[22-24].

Molecular size distributions of starches of different granular sizes and radial locations are shown in profiles of GPC Sepharose CL-2B column (Fig. 4). Surface-gelatinized starch (8%) showed a unique profile which indicated that more intermediate component in the periphery of the granule. There were differences among the relative ratio of blue value to total carbohydrate at the amylopectin peak. Large ratios indicate that the amylopectin had long branch chain length. The result of relative ratios shown in Table II indicated a tendency that amylopectin has a longer chain length in small and remaining granules and a shorter chain length in large granules and gelatinized peripheral starch. This result was consistent with the branch chain length analysis in potato starch granules[10] Amylopectin in potato starch granule has longer long B-chains at the core of the granule. These chain-length distributions were confirmed by using chemical analysis.

Distributions of branch chains of amylopectins isolated from starches of different granular sizes and radial locations are shown in Fig. 5. According to Hizukuri's study[25], the peak marked I in the profile is assigned as very long B-chains (B3 and longer) and contaminate of incompletely-debranched residues, peak II and peak III represent long B-chains (B2-chains) and mixture of short B-chains (B1-chains) and A-chains, respectively. The branch chain length of peak II and peak III and the ratio (in weight) of peak II to peak III are shown in Table III. The peak chain length and the average chain length

showed an agreement. It was found that large granules and gelatinized starch (8%) had shorter B2-chain lenths, and small granules and surface-gelatinized remaining granules had longer B-2chain lenths. There was no significant difference in chain lengths of Bl-chains and A-chains. This result confirmed the information given by GPC Sepharose CL-2B profiles that amylopectin had longer branch chains at the corethan at the periphery od the granule. It also gave an agreement with the chain length analysis in patato starch granule[10], that is, amylopectin has lobger long B-chains at the early stage of the growth of the granule. In Table III, it was also that small granules and remaining granules had higher ratios and large granules and gelatinized starch had lower ratios. This results indicated that greater portions of lobger chains (B2-chains) were concentrated at the core than at the periphery of the granule.

HPAEC-PAD analysis gave molecular size distributions of debranched amylopectin in a range of DP 6 to 60 (Fig. 6). There is one major peak in a range of DP 10 to 20. This component may correspond to short chains (Bl-chains and A-chains) in the profiles of GPC Bio-P6. The slight differences among the HPAEC-PAD profiles are shown in normalized curves (Fig. 7). For untreated starches (Fig. 7-A), small granules had more longer chain above DP 20. No significant difference was found in surface-gelatinized remaining granules (Fig. 7-B). In Fig. 7-C, it appears that surface-gelatinized starch had

less longer branch chains. These results indicated the same tendency shown in weight ratios of peak II to peak III.

In conclusion, the surface gelatinization of normal maize starch granules in the 13M LiCl solution can be controlled to produce remaining granules in different extents. The remaining granules can be obtained by mechanical blending, the gelatinized starch should be separated by a gentle method, such as grinding with a mortar and a pestle. SEM showed that the remaining granules had a rough surface with a lamella structure. Amylose and intermediate component were more concentrated at the periphery than at the core of the granule. The amylopectin branch chain length analysis showed that amylopectin had longer B2-chains at the core than at the periphery of the granule. The distribution of amylopectin branch chains indicated that a greater portion of B2-chains were concentrated at the core than at the periphery of the granule.

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Figures Legends

Fig. 1 Scanning electronic micrographs of normal maize starches of native fractionated granules and chemical treated granular: A. large size (>5 μm in diameter); B. small size (<5 μm in diameter); C. 65% LiCl surface-gelatinized remaining granule; D. 84% LiCl surface-gelatinized remaining granule.

Fig. 2 Scanning electronic micrographs of normal maize starches of chemical treated granular and gelatinized starch: A. 24% $CaCl_2$ surface-gelatinized remaining granules, B. 8% LiCl surface-gelatinized starch.

Fig. 3 Gel permeation chromatographic profiles of normal maize starch by using a Sepharose CL-2B column: A. large granular starch; B. large granular starch after mechanical blending; C. gelatinized starch of large granular size after mechanical blending.

Fig. 4 Gel permeation chromatographic profiles of normal maize starches by using a Sepharose CL-2B column: A. large granules (>5 μm in diameter); B. small granules (<5 μm in diameter); C. 34% LiCl surface-gelatinized remaining granules; D. 65% LiCl surface-gelatinized remaining granules; E. 84% LiCl surface-gelatinized remaining granules; F. 8% LiCl surface-gelatinized starch; G. 27% LiCl surface-gelatinized starch.

Fig. 5 Gel permeation chromatographic profiles of normal maize starch by using a Bio-gel P-6 column: A. large granules (>5 μm in diameter); B. small granules (<5 μm in diameter); C. 34% LiCl surface-gelatinized remaining granules; D. 65% LiCl surface-gelatinized remaining granules; E. 84% LiCl surface-gelatinized remaining granules; F. 8% LiCl surface-gelatinized starch; G. 27% LiCl surface-gelatinized starch.

Fig. 6 HPAEC-PAD profiles of normal maize starch analyzed by a Dionex system: A. large granules (>5 μm in diameter); B. small granules (<5 μm in diameter); C. 34% LiCl surface-gelatinized remaining granules; D. 65% LiCl surface-

gelatinized remaining granules; E. 84% LiCl surface-gelatinized remaining granules; F. 8% LiCl surface-gelatinized starch; G. 27% LiCl surface-gelatinized starch.

Fig. 7 Normalized distribution of relative amount of amylopectin debranched chains of versus DP of normal maize starch: A. large (>5 μm in diameter) and small granules (<5 μm in diameter); B. 34%, 65% and 84% LiCl surface-gelatinized remaining granule; C. 8% and 27% LiCl surface-gelatinized starch.

Table I. Amylose contents of normal maize starches of different granular sizes of radial locations

Sample	Amylose content[a] (%)
Large granules (>5μm[b])	27.8 ± 0.4
Small granules (<5μm[b])	22.8 ± 0.1
34% SGRG[c]	25.5 ± 0.0
65% SGRG[c]	24.4 ± 0.4
84% SGRG[c]	23.2 ± 0.4
8% SGS[d]	35.0 ± 0.5
27% SGS[d]	28.3 ± 0.4

[a] The data are averages of two measurements with standard deviation.

[b] In diameter

[c] Surface-gelatinized remaining granules

[d] Surface-gelatinized starch

Table II. Relative ratios of the blue value to the total carbohydrate a amylopectin peaks of normal maize starch of different granular sizes and radial locations

Sample	Ratio[a]
Large granules (>5μm)	0.085 ± 0.000
Small granules (>5μm)	0.095 ± 0.005
34% remaining granules	0.086 ± 0.002
65% remaining granules	0.086 ± 0.006
84% remaining granules	0.105 ± 0.005

a. The data are averages of two replicates.

Table III. Branch chain lengths of debranched amylopectin isolated from normal maize starches of different granular sizes and radial locations

Sample	B-2 chain[a] (DP)		B-1 and A-chain[a] (DP)		Ratio of II/III[b]
	Peak	Average	Peak	Average	
LG[c]	35.2 ± 0.6	34.0 ± 1.6	10.6 ± 0.1	11.2 ± 0.1	0.298 ± 0.002
SG[d]	37.4 ± 0.9*	39.2 ± 0.0*	11.3 ± 0.1	10.6 ± 0.2	0.232 ± 0.003*
34% SGRG[e]	33.5 ± 1.6	33.8 ± 1.0	9.8 ± 0.2	12.3 ± 0.3	0.296 ± 0.001
65% SGRG[e]	36.8 ± 1.2	35.4 ± 0.3	11.5 ± 0.7	10.0 ± 0.0	0.316 ± 0.006*
84% SGRG[e]	37.4 ± 0.4*	37.7 ± 0.0*	10.4 ± 0.8	12.8 ± 0.2	0.314 ± 0.007*
8% SGS[f]	30.9 ± 0.4*	31.6 ± 1.0*	10.0 ± 0.4	10.2 ± 0.9	0.277 ± 0.003*
27% SGS[f]	34.0 ± 0.9	32.9 ± 0.6	10.1 ± 0.4	10.9 ± 0.1	0.303 ± 0.001

[a] The data are averages of three measurements with standard deviation.

[b] The data are averages of two measurements with standard deviation.

[c] Large granules with the diameters more than 5 μm

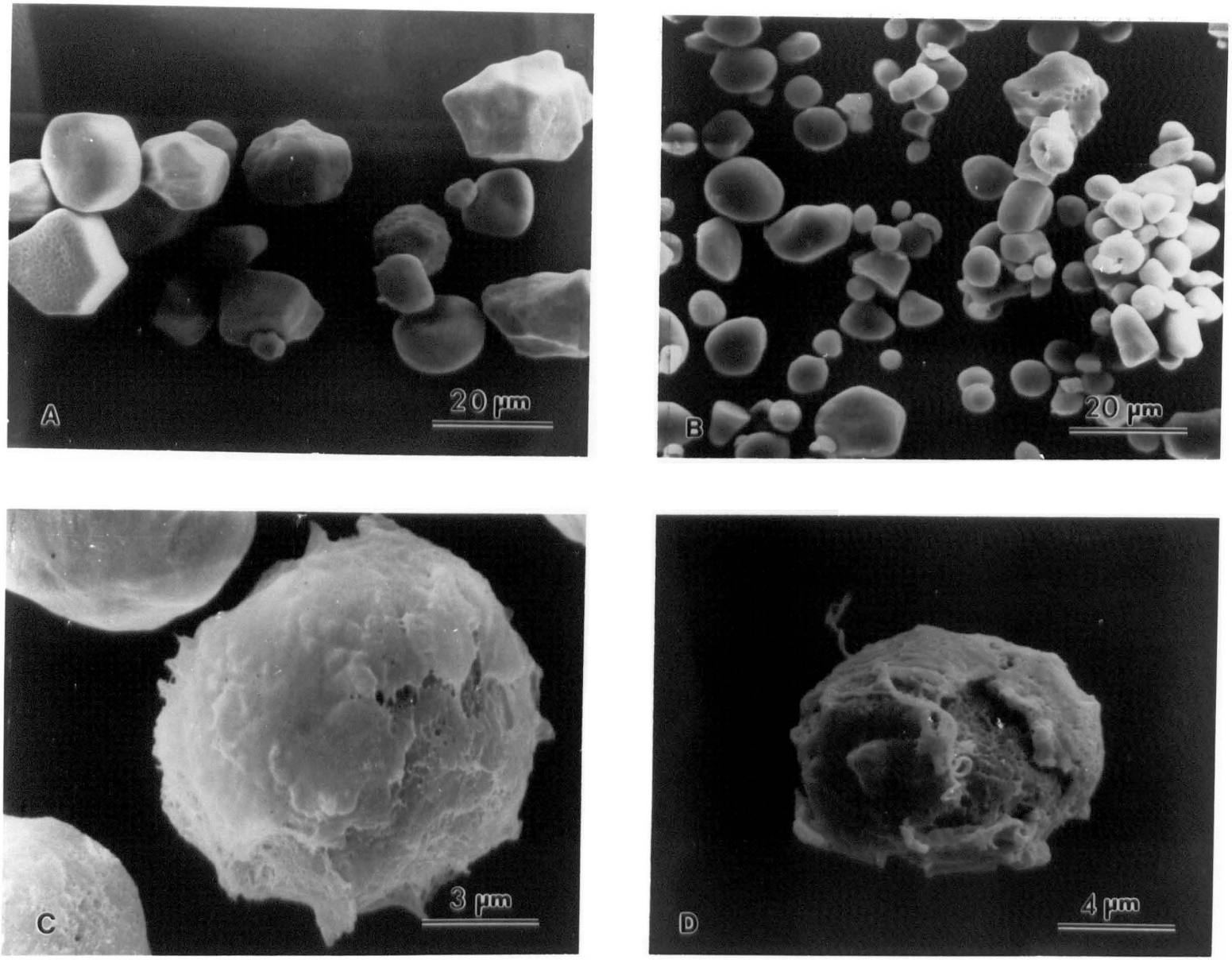
[d] Small granules with the diameters less than 5 μm

[e] Surface-gelatinized remaining granules

[f] Surface-gelatinized starch

* Data are significantly different compared to large granular starch at ($P<0.05$).

Fig. 1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B
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C
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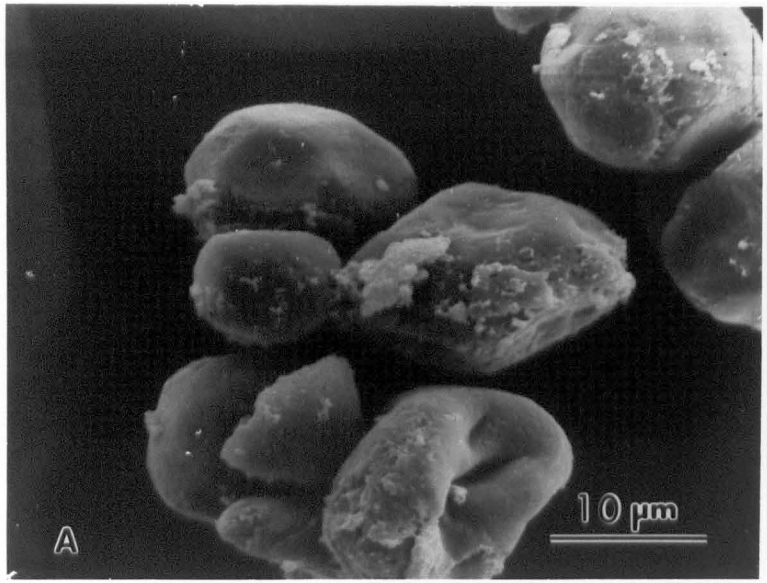
D
4 µm

Fig. 2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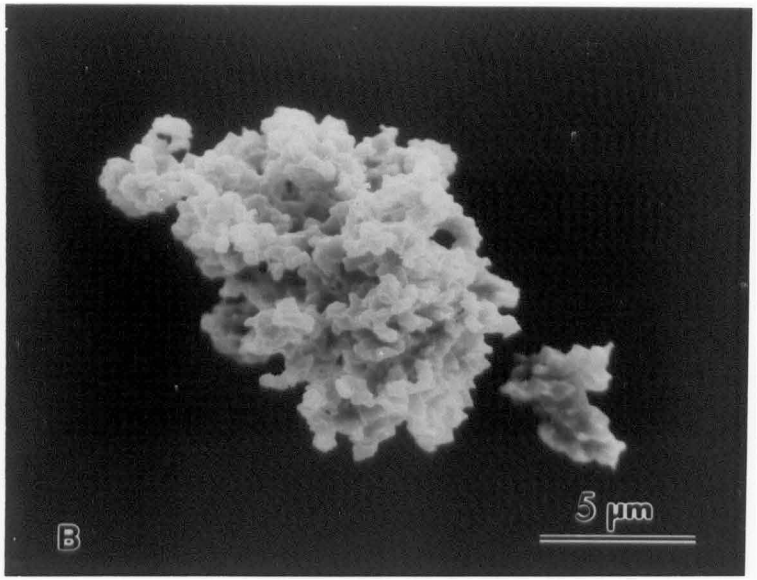
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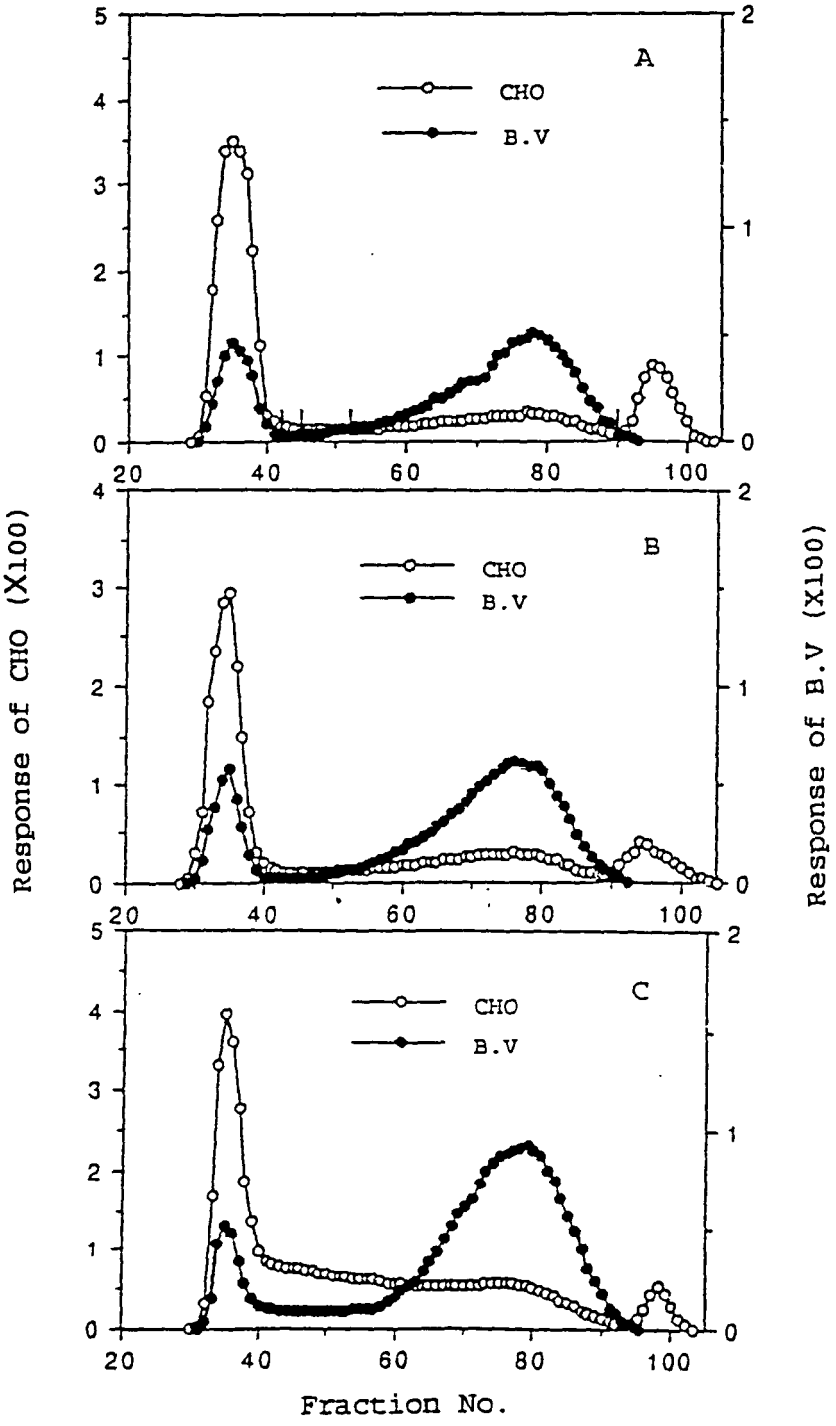
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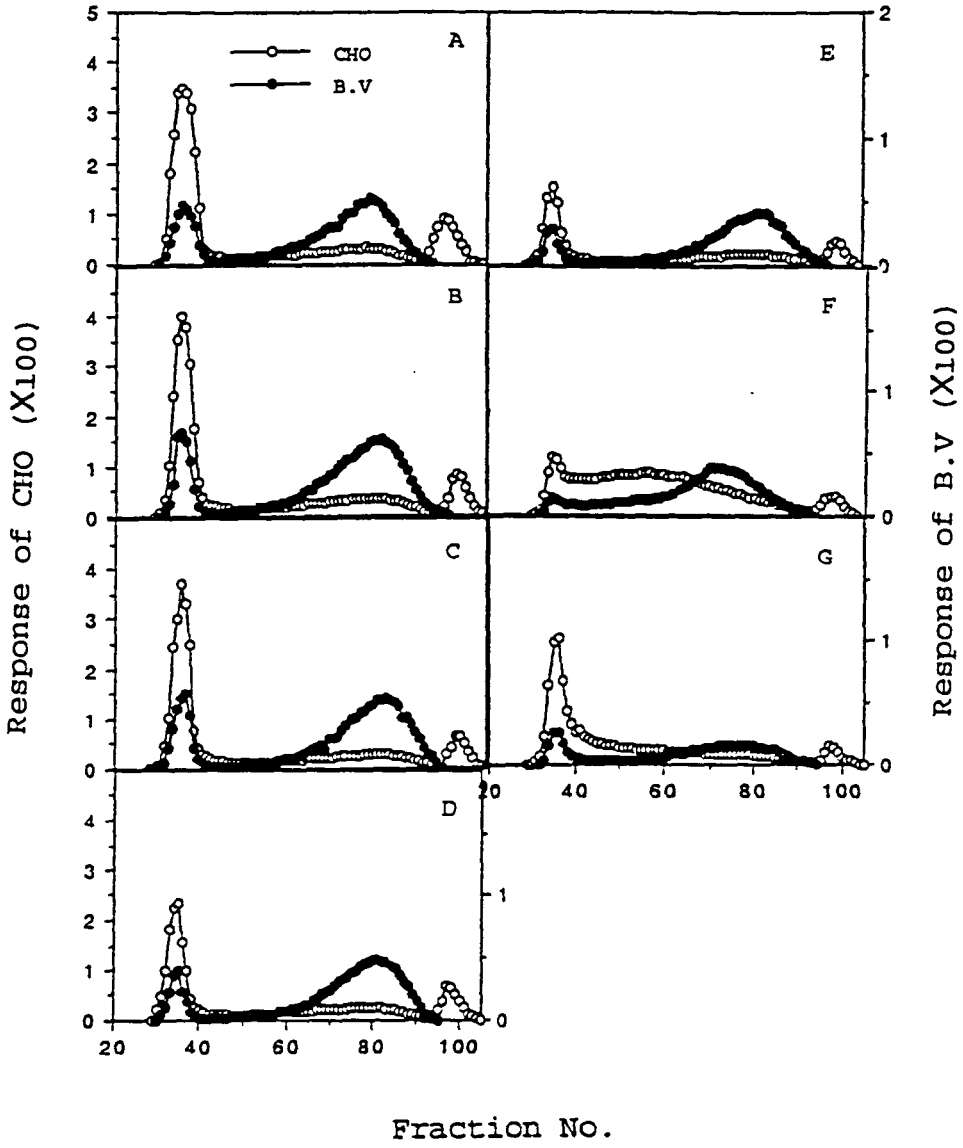
Response of B.V (X100)
Fraction No.

Fig. 3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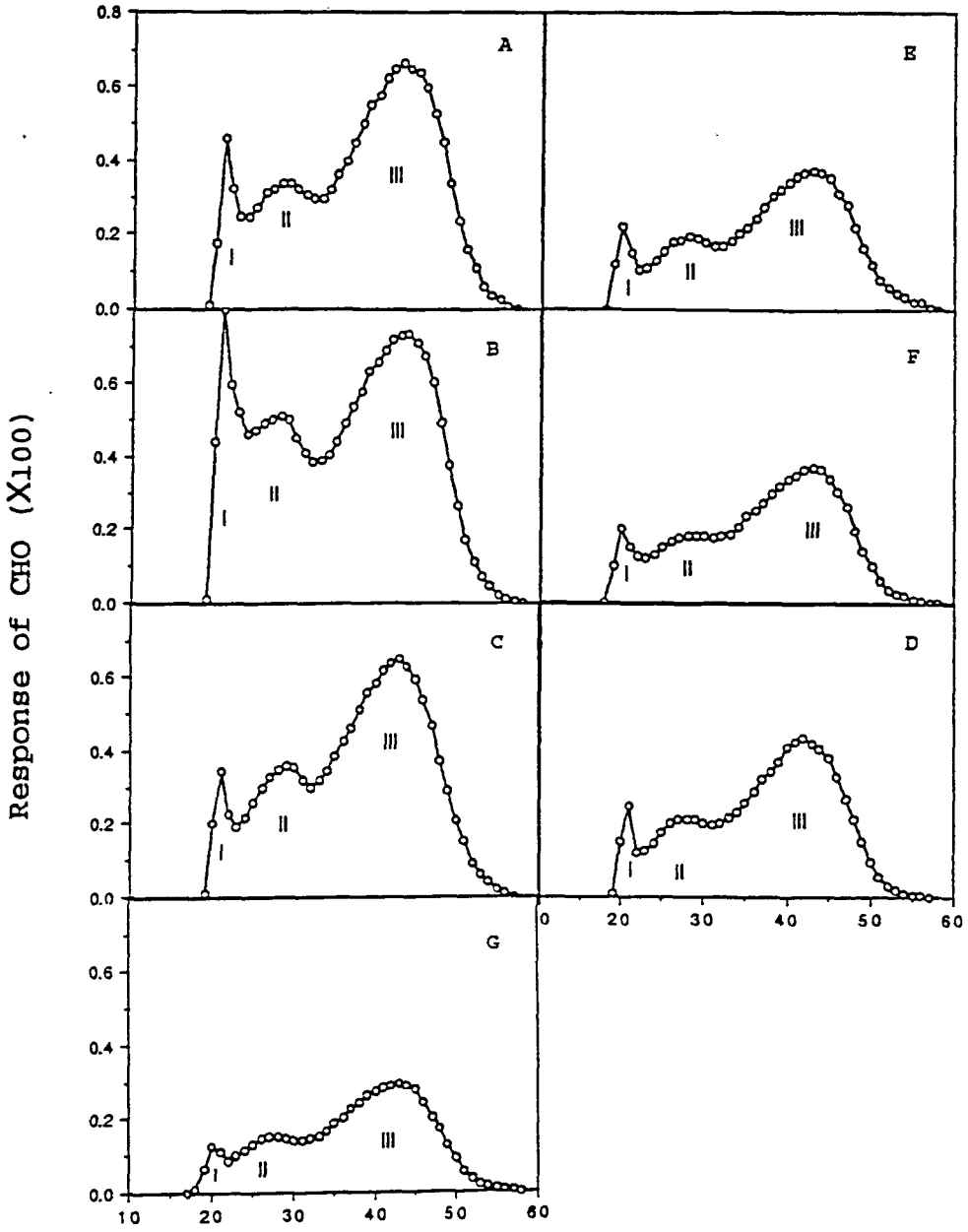
Fraction No.

Fig. 4


Response of CHO (X100)
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III
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20
30
40
50
60
Fraction No.

Fig. 5

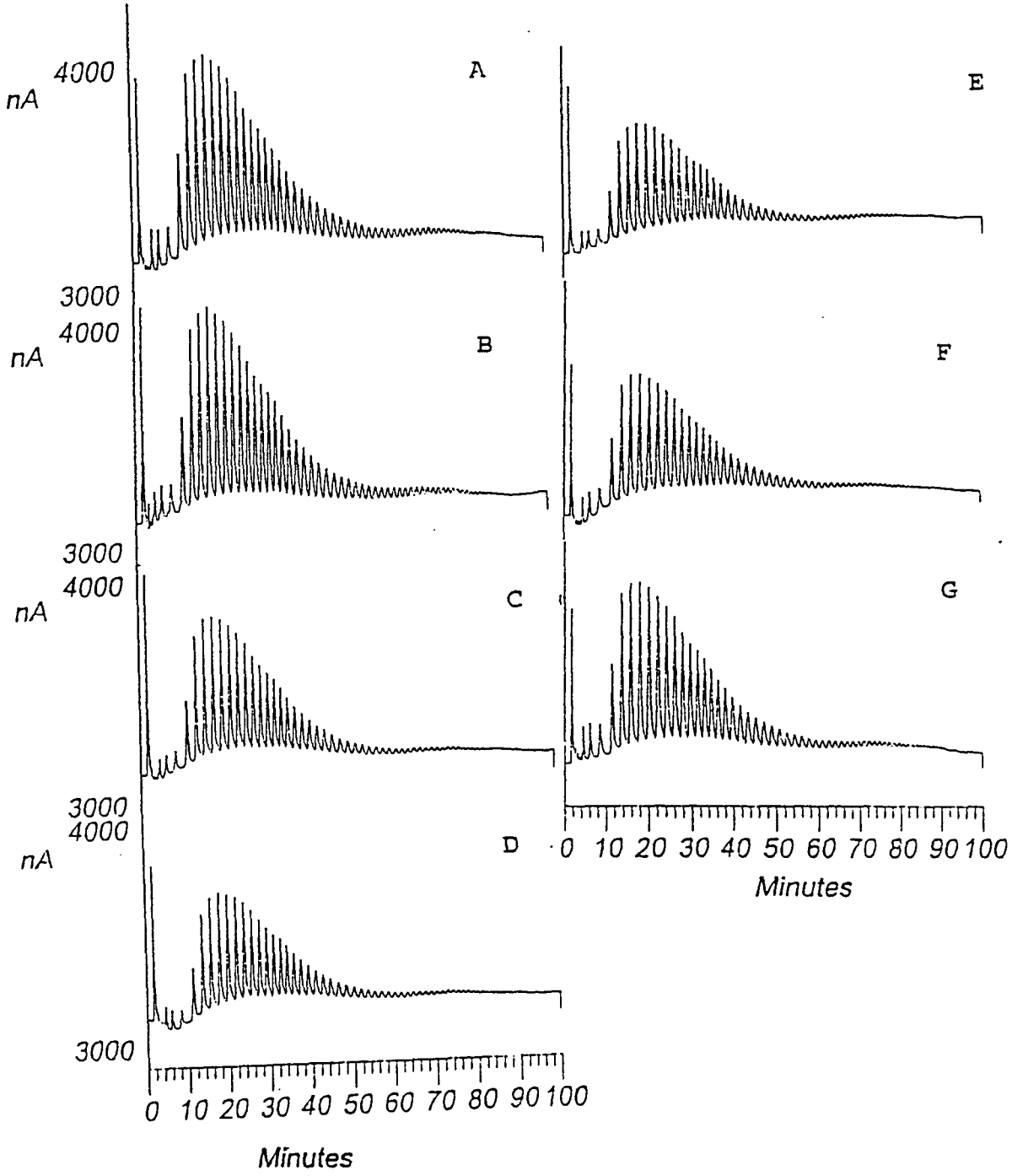

Fig. 6

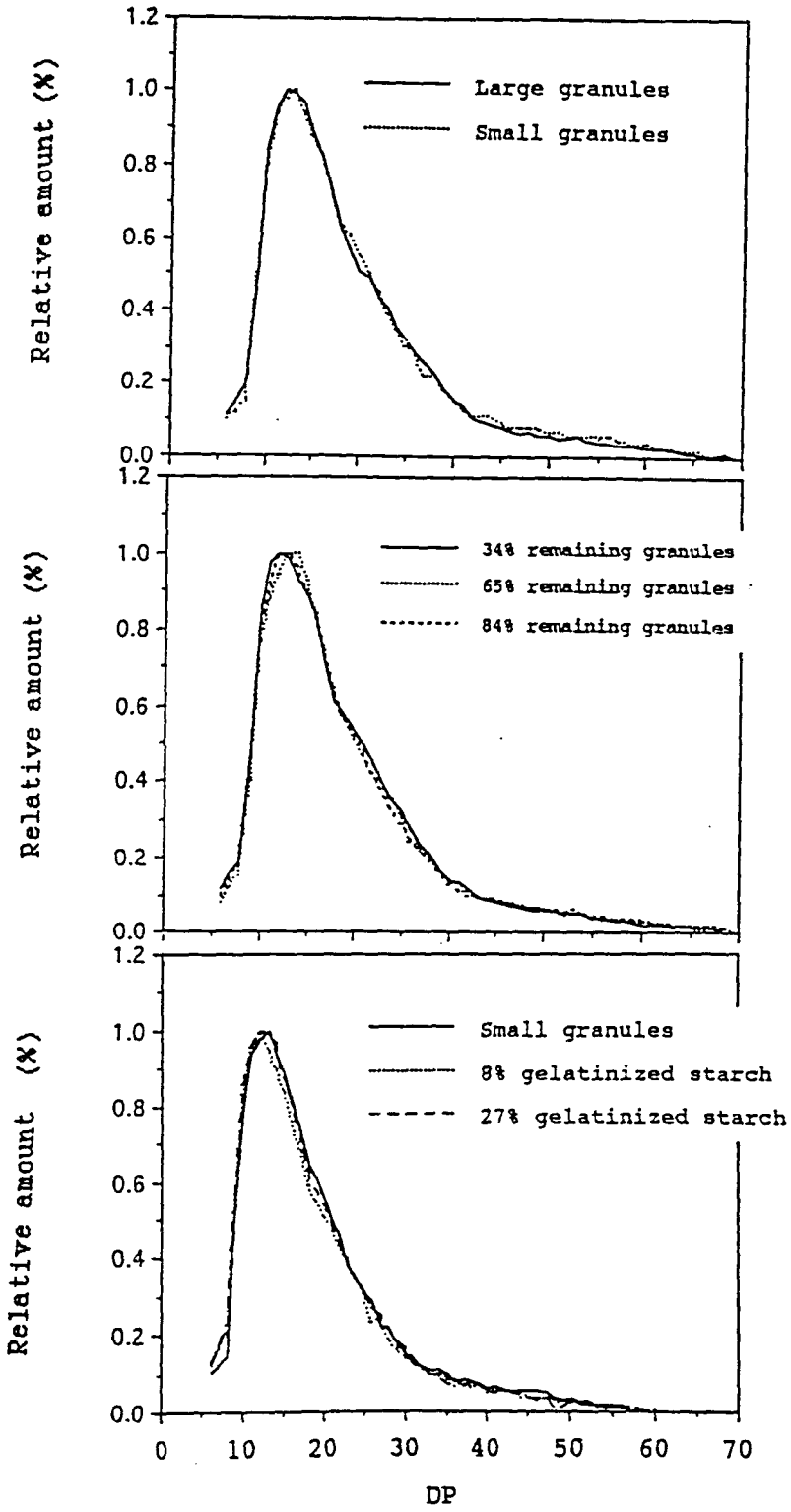
Relative amount (%)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Large granules
Small granules
Relative amount (%)
34% remaining granules
65% remaining granules
84% remaining granules
Relative amount (%)
Small granules
8% gelatinized starch
27% gelatinized starch
0
10
20
30
40
50
60
70
DP

Fig. 7

INTERNAL STRUCTURE OF THE WAXY MAIZE STARCH GRANULE REVEALED BY CHEMICAL SURFACE GELATINIZATION

A Paper to be Submitted to the Journal of Carbohydrate Research

Dora D. Pan and Jay-Lin Jane

Abstract

Waxy maize starch was separated into two fractions: large granules with diameters more than 5 μm and small granules with diameters less than 5 μm. The large granules were surface gelatinized by treating them with an aqueous LiCl solution (13M) at 22-23°C. Remaining granules were obtained by mechanical blending to remove the surface-gelatinized starch, and gelatinized peripheral starch was obtained by grinding with a mortar and a pestle. Starches of different granular size and radial locations were subjected to scanning electronic microscopy, gel permeation chromatography, and amylopectin branching chain length analysis. Results showed that remaining granules had a rough surface. Amylopectin had longer B2-chains at the core than that at the periphery of the granule. Greater

portions of B2-chains were concentrated at the core than at the periphery of the granule.

Introduction

The fine structures of amylose and amylopectin have been well revealed with the development of enzymatic and instrumental analysis[1-4]. The organization of amylose and amylopectin molecules in the granule, however, is not completely known. The model proposed by Lineback describes the arrangement of amylose and amylopectin molecules[5]. The location of amylose molecules were found to be interspersed among amylopectin molecules[6,7]. The distributions of amylose and branch chains of amylopectin were studied by Jane and Shen in the potato starch granule[8]. It was found that amylose was more concentrated at the periphery than at the core of the granule; amylopectin had longer long B-chains at the core than at the periphery of the granule. Recent results of our study in the normal maize starch granule gave an agreement with that obtained from the potato starch granule[9]. The result, in addition, indicated that more longer B-chains (B2-chains) were concentrated at the core than at the periphery of the granule.

Waxy maize starch is a mutant of normal maize starch[10-12]. It contains almost 100% of amylopectin[13]. The internal structure of the waxy maize starch granule was used as a model of the organization of amylopectin molecules[14]. Our goal in

this study was to study the internal structure of the waxy maize starch granule by a chemical surface-gelatinization method[8,9].

Materials and Methods

Waxy maize starch was a gift of American Maize Products Company (Hammond, IN). Sepharose CL-2B gel was purchased from Pharmacia Inc. (Piscataway, NJ). Bio-gel P-6 was purchased from Bio-Rad Laboratories (Richmond, CA). Isoamylase (EC3.2.1.68, crystal, from *Pseudomonas* amyloderamosa, 59,000 units/mg protein) was a product of Hayashibara Biochemical Laboratories, Inc. (Kayama, Japan). Other chemicals were all reagent grade and used without further purification.

Fractionation of native starch granules

Two fractions of native granular starch, large and small size, were obtained by using a nylon filter cloth in porous size of 5 μm as previously described by Pan and Jane[10].

Defatting of granular starch

Native, fractionated large and small granular starch were defatted by extracting with mixture of methanol and water (85% v/v) following the general method of Schoch[15].

Chemical surface gelatinization

Large granules (10 g) of waxy maize starch were suspended in 150 ml of a 13 M LiCl solution and stirred at 22-23°C for different periods of time. The desired extent of surface gelatinization was determined using a Nikon labophot light microscope (Garden City, NY). The reaction was stopped by mixing the suspension with 1200 ml of 4°C distilled water. The mixture was then centrifuged at 3200 X g for 15 minutes and washed twice with 1800 ml of water.

Separation of the gelatinized starch from remaining granules

Gelatinized starch was separated following the method previously described by Pan and Jane[10].

Scanning electronic microscopy (SEM)

The granular size and surface structure of native granules, surface-gelatinized remaining granules and gelatinized peripheral starch were analyzed by using a scanning electronic microscope (SEM) following the procedure of Jane and Shen[8].

Gel permeation chromatography (GPC) by using a Sepharose CL-2B column

Molecular size distributions of starches of different granular sizes and radial locations were analyzed by GPC with a Sepharose CL-2B column following the procedure of Jane and Chen[16] , Pan and Jane[10].

Analysis of amylopectin branch chain length

Amylopectin of waxy maize starch was prepared by precipitating 1 ml of DMSO solution (10 mg starch) with 2 volumes of ethanol. After washing with methanol, the starch was redissolving in 4 ml of water. The amylopectin solution was heated in a boiling water bath for 1 hour and then cooled to room temperature. Acetate buffer (0.5 ml) was added to the solution, and the pH was adjusted to 3.5. Crystalline *Pseudomons* isoamylase (600U) was added. The mixture was incubated in a shaker at a rate of 100 strokes/min at 40°C for 48 hours. The enzyme reaction was stopped following the procedure of Pan and Jane[9]. The molecular size distributions of amylopectin branch chains were analyzed by GPC using a Bio-gel P-6 column following the procedure of Pan and Jane[9]. The branch chain lengths of the amylopectin was caculated by dividing the total carbohydrat (μg/ml) by the reducing value (μg/ml). The total carbohydrate was determined following the procedure of Dubois et al.[17]. The reducing value was determined following the procedure of Jane and Chen[16].

Analysis of debranched amylopectin by high-performance anion-exchange chromatography (HPAEC) on a Dionex system

Debranched amylopectin of waxy maize starch of different granular sizes and radial locations were analyzed by HPAEC with pulsed amperomertic detector (HPAEC-PAD) following the

procedure of Wong and Jane[18]. DP of each peak on profiles was identified on the basis of the elution time and the area counted as homologues. HPAEC-PAD profiles were normalized by dividing the area of each peak by the largest area in the same profile.

Results and discussion

Waxy maize starch has a similar granular size and size distribution as normal maize starch[13]. The starch was fractionated to two groups; each group consisted of granules of a uniform size so that surface gelatinization could take place evenly. Large granules were subjected to LiCl solution treatment and small granules were used for comparison analysis.

The weight ratio of large and small granules (w/w) was 9:1 after fractionation. Remaining granules with degrees of surface gelatinization of 25% and 80% and gelatinized peripheral starch with a degree of surface gelatinization of 13% were used for structural analysis. These samples were based on one replicate.

The granular sizes of fractionated large and small granules are shown in Fig. 1-A and Fig. 1-B, respectively. The surface structures of the remaining granules after 25% and 80% surface gelatinization are shown in Fig. 1-C and Fig. 1-D, respectively. The treated starch granules showed a rough surface structure. The surface gelatinization of waxy maize

starch was found more difficult than that of normal maize starch. The paste viscosity of waxy maize starch is known higher than that of normal maize starch[13]. This property resulted in the difficulty of the penetration of salt cations into the waxy maize starch granules.

GPC Sepharose CL-2B profiles of waxy maize starches of different granular sizes and radial locations are shown in Fig. 2. No amylose fraction was detected. Ratios of blue value to total carbohydrate, as indications of extent of amylopectin branch chain lengths, were obtained by experiments under the same condition and shown in Table I. Results showed that the high ratios in small and surface-gelatinized remaining granules indicated longer branch chains, and low ratios in large granules and surface-gelatinized starch indicated shorter branch chains. This result was confirmed later in branch chain length analysis.

Molecular distributions of amylopectin of waxy maize starches of different granular sizes and radial locations are shown in Fig. 3. According to Hizukuri's model[19], three peaks marked in profiles (I, II and III) are identified as very long B-chains (B3-chains or longer chains), long B-chains (B2-chains) and the mixture of short B-chains (B1-chains) and A-chains, respectively. The ratio of peak II to peak III as shown in Table II indicated the relative amount of long chains and short chains. The data showed that large granules and surface-gelatinized starch had lower ratios, and small and

surface-gelatinized remaining granules had higher ratios, indicating that greater portions of longer branch chains were concentrated at the core than at the periphery of the granule. This result gave an agreement with that obtained in normal maize starch granule[9].

The branch chain lengths of waxy maize starches of different granular sizes and radial locations are shown in Table II. There was not significant difference between peak chain lengths and average chain lengths. Small and surface-gelatinized remaining granules had longer B2-chain lengths, whereas large granules and surface-gelatinized starch had shorter B2-chain length. No significant difference was found in B1- and A-chain length among samples. This result, consistent with that obtained from normal maize starch, indicated that amylopectin of waxy maize starch had longer B2-chains at the core than at the periphery of the granule. These results confirmed that obtained from potato starch granule, which indicated that amylopectin had a larger molecular size at the early stage of the growth of the granule.

HPAEC-PAD profiles (Fig. 4) and normalized curves (Fig. 5) showed the distributions of isoamylase-debranched amylopectin branch chains in a range of DP 6 to 60. The profiles are similar to that of normal maize starch. The normalized curves showed slight difference among the samples. Fig. 5-A showed that large and small granules had a similar distribution in most DP range. Large granules had slightly

less amount of branch chains in a range of DP 30 to 60. In Fig. 5-B, 25% and 80% surface-gelatinized remaining granules had a similar distribution. However, 13% surface-gelatinized starch had lower amount of long branch chains. This result is in agreement with the ratio of long chains to short chains obtained by using GPC in a Bio-gel P-6 column (Table II), indicating that greater portions of longer branch chains were concentrated at the core than at the periphery of the granule.

In conclusion, waxy maize starch granules were partially gelatinized in 13M LiCl solution, but the gelatinization was more difficult than that of normal maize starch granules. The surface-gelatinized remaining granules had a rough surface. The amylopectin of waxy maize starch had longer B2-chains at the core than at the periphery of the granule. Greater portions of B2-chains were concentrated at the core than at the periphery of the granule. The internal structure of waxy maize starch granule was found to be consistent with the amylopectin distribution in normal maize starch granule. Amylopectin had larger molecular size at the early stage of the development of the granule.

Acknowledgment

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Figure legends

Fig. 1 Scanning electronic micrographs of waxy maize starches of native fractionated granules and chemical treated granular and gelatinized starch: A. large size (>5 μm in diameter); B. small size (<5 μm in diameter); C. 25% LiCl surface-gelatinized remaining granule; D. 80% LiCl surface-gelatinized remaining granule.

Fig. 2 Gel permeation chromatographic profiles of waxy maize starch by using a Sepharose CL-2B column: A. large granules (>5 μm in diameter); B. small granules (<5 μm in diameter); C. 25% LiCl surface-gelatinized remaining granules; D. 80%

LiCl surface-gelatinized remaining granules; E. 13% LiCl surface-gelatinized starch.

Fig. 3 Gel-permeation chromatographic profiles of waxy maize starch by using a Bio-gel P-6 column: A. large granules (>5 μm in diameter); B. small granules (<5 μm in diameter); C. 25% LiCl surface-gelatinized remaining granules; D. 80% LiCl surface-gelatinized remaining granules; E. 13% LiCl surface-gelatinized starch.

Fig. 4 HPAEC-PAD profiles of waxy maize starch analyzed by a Dionex system: A. large granules (>5 μm in diameter); B. small granules (<5 μm in diameter); C. 25% LiCl surface-gelatinized remaining granules; D. 80% LiCl surface-gelatinized remaining granules; E. 13% LiCl surface-gelatinized starch.

Fig. 5 Normalized distribution of relative amount of amylopectin debranched chains of versus DP of waxy maize starch: A. large and small granules; B. 25%, 80% LiCl surface gelatinized remaining granules and 13% LiCl surface gelatinized starch.

Table I. Ratios of the blue value to the total carbohydrate at amylopectin peaks of waxy maize starches of differnet granular sizes and radial locations

Sample	Ratio[a]
Large granules (>5μm[b])	0.046 ± 0.006
Small granules (>5μm[b])	0.052 ± 0.005
25% SGRG[c]	0.042 ± 0.008
80% SGRG[c]	0.054 ± 0.005
13% sSGS[d]	0.041 ± 0.004

[a] The data are averages of two replicates.
[b] In dimeter
[c] Surface-geltinized remaining granules
[d] Surface-gelatinized starch

Table II. Branch chain lengths of debranched amylopectin of waxy maize starches of different granular sizes and radial locations

Sample	B-2 chain[a] (DP)		B-1 and A-chain[a] (DP)		Ratio of II/III[b]
	Peak	Average	Peak	Average	
LG[c]	30.2 ± 0.8	29.9 ± 1.6	11.4 ± 0.4	11.2 ± 0.5	0.321 ± 0.006
SG[d]	34.8 ± 1.0*	33.7 ± 1.4*	12.3 ± 0.2	10.4 ± 1.2	0.350 ± 0.005*
25% SGRG[e]	29.8 ± 1.0	29.1 ± 0.2	11.7 ± 0.2	11.5 ± 0.2	0.342 ± 0.010*
80% SGRG[e]	31.9 ± 1.8	32.8 ± 0.0*	11.7 ± 0.2	10.2 ± 0.2	0.340 ± 0.002*
13% SGS[f]	27.8 ± 0.4	29.0 ± 0.8	10.7 ± 0.2	10.2 ± 0.2	0.302 ± 0.008*

[a] The data are averages of three measurements with standard sedivation.

[b] The data are averages of two measurements with standard sedivation.

[c] Large granules with the diameters more than 5 μm

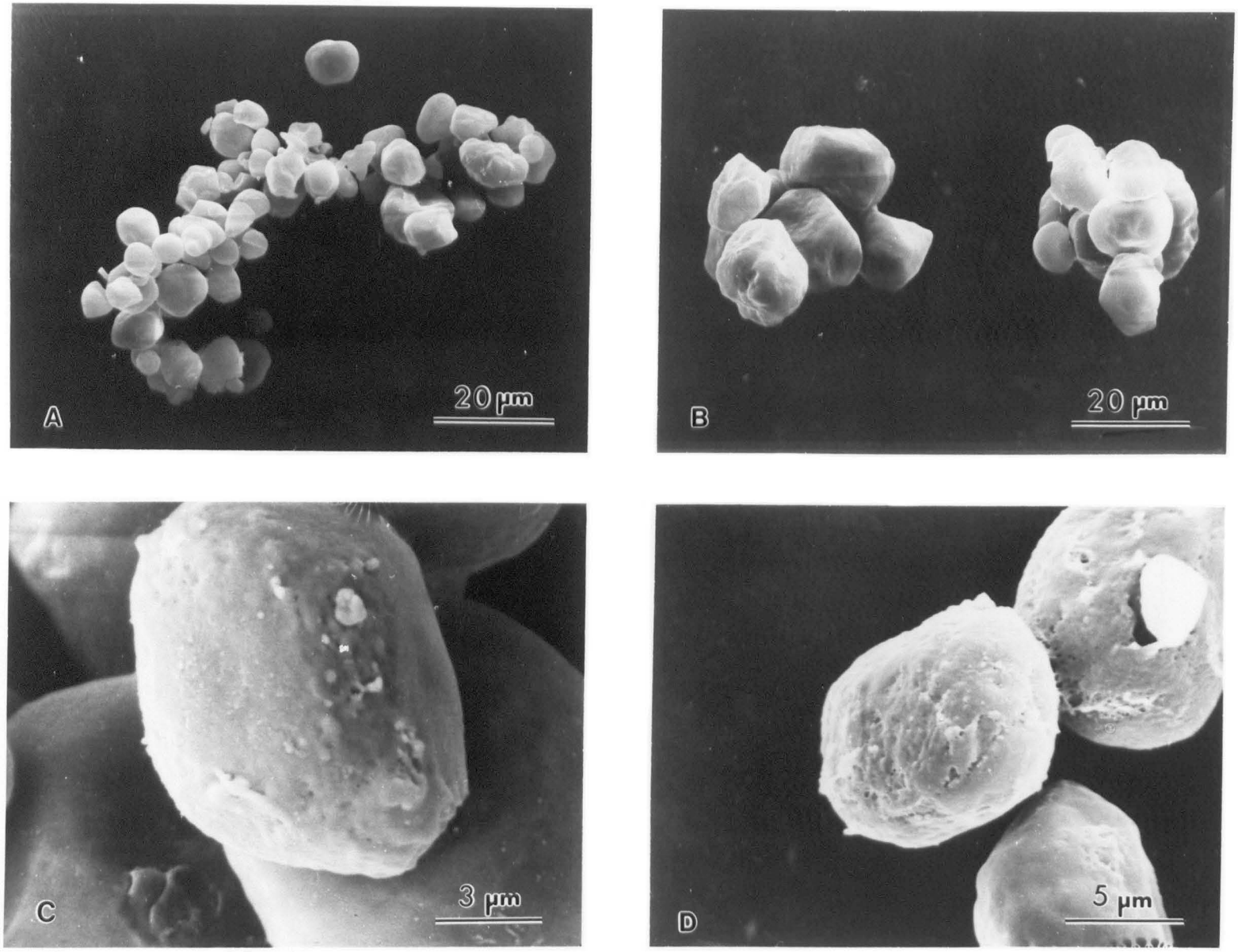
[d] Small granules with the diameters less than 5 μm

[e] Surface gelatinized remaining granules

[f] Surface gelatinized starch

* Data are significantly different compared to large granular starch at ($P<0.05$).

Fig. 1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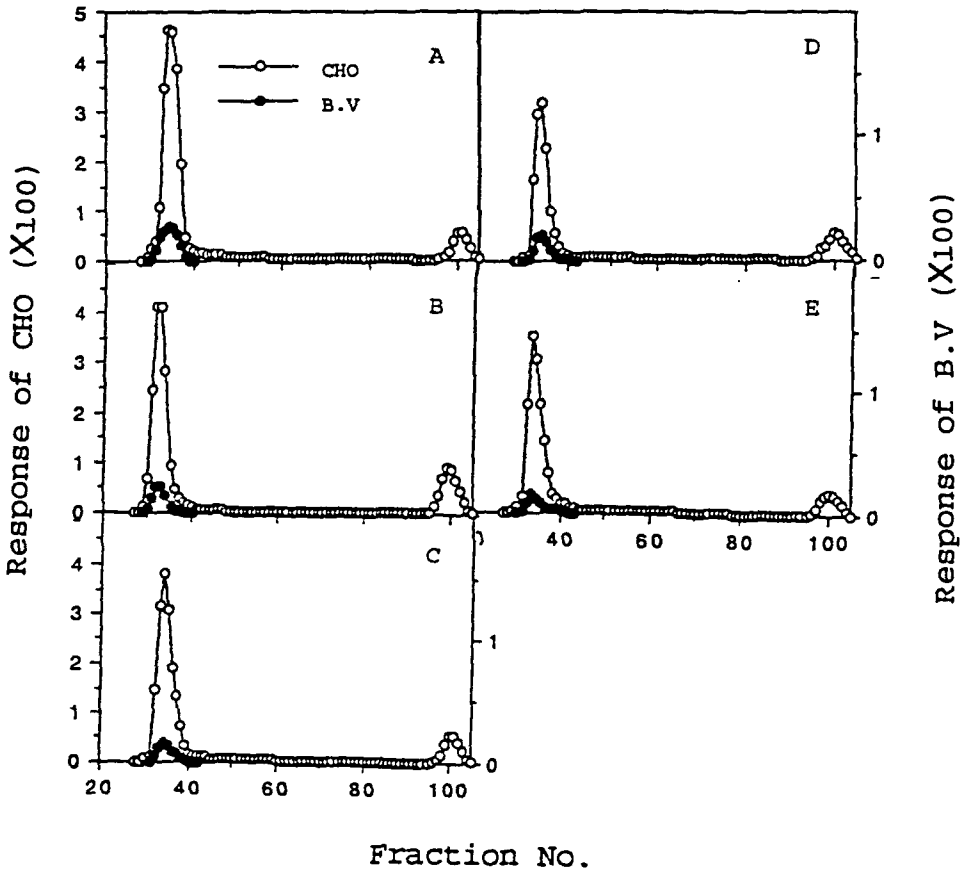
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Fig. 2

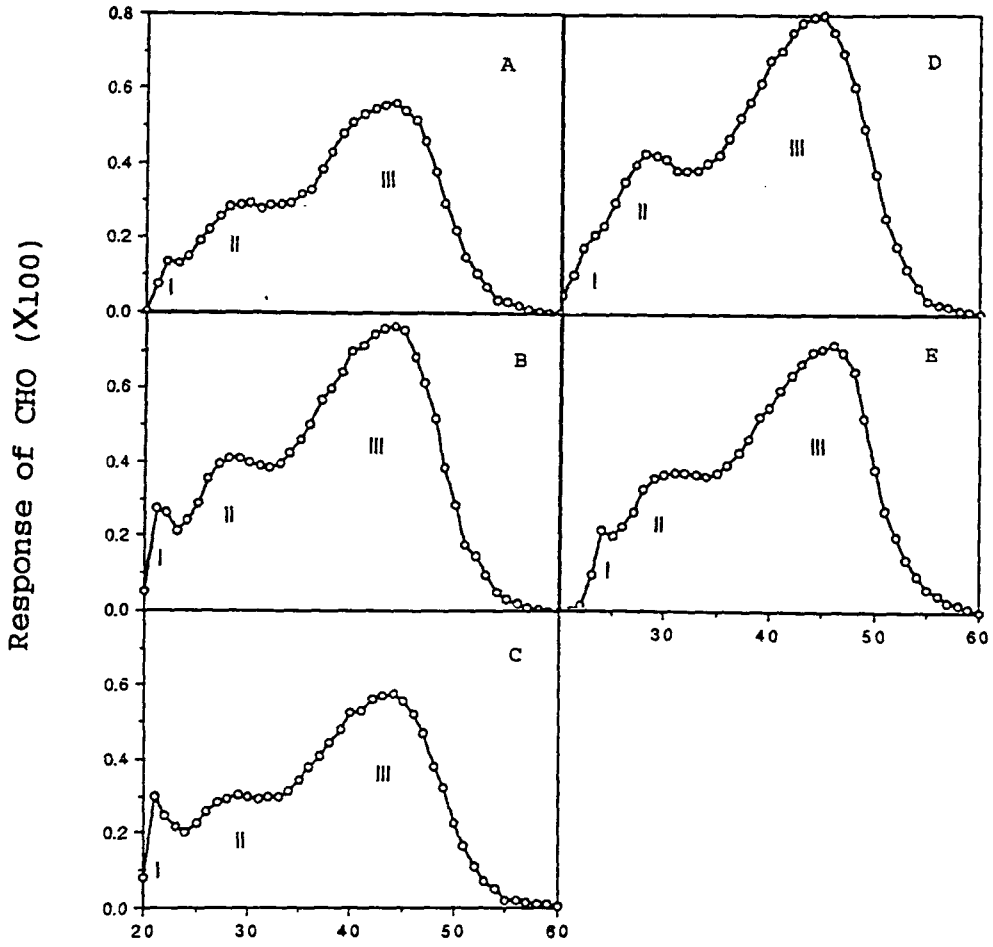
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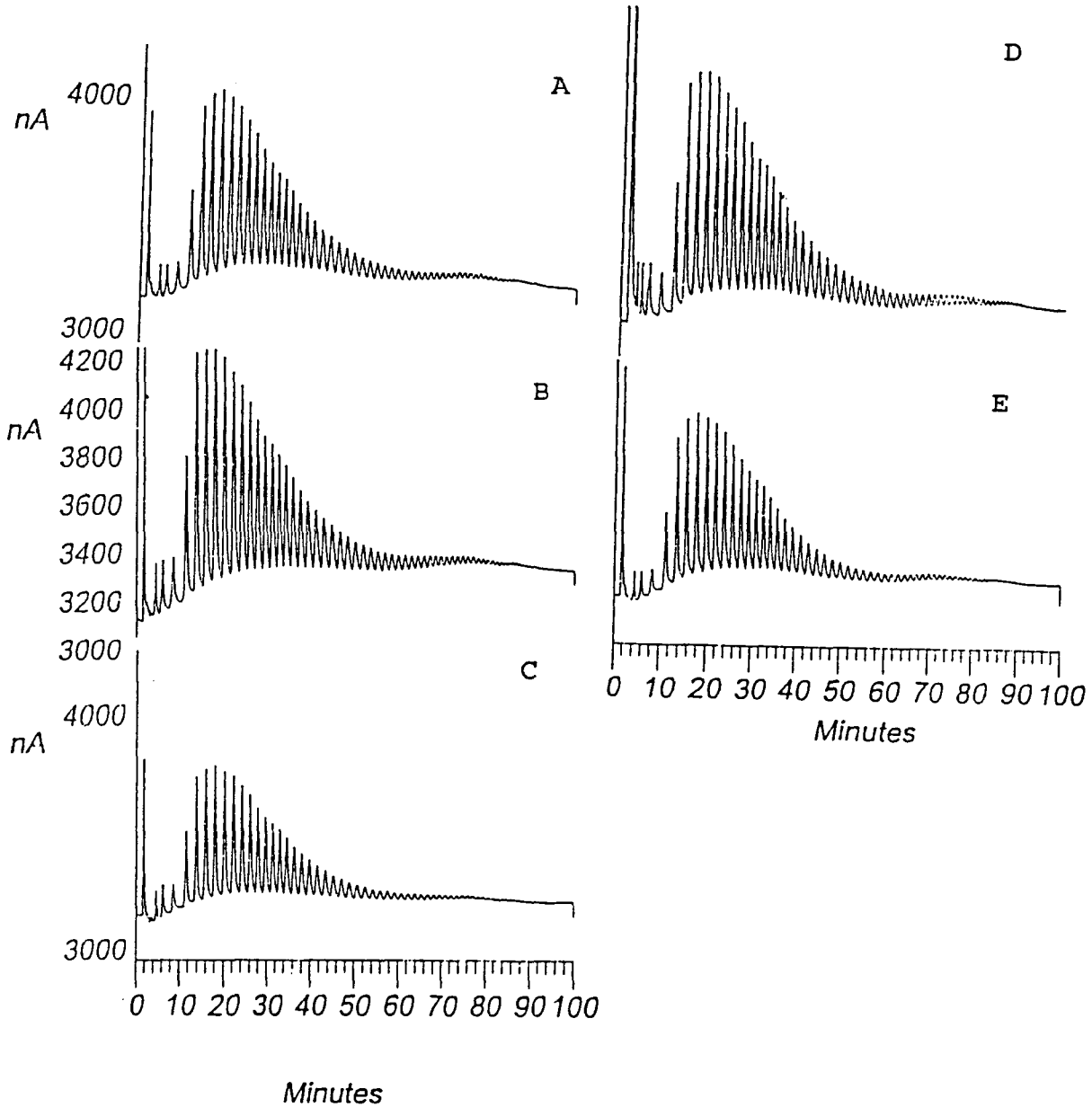
Fig. 3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Minutes

Fig. 4

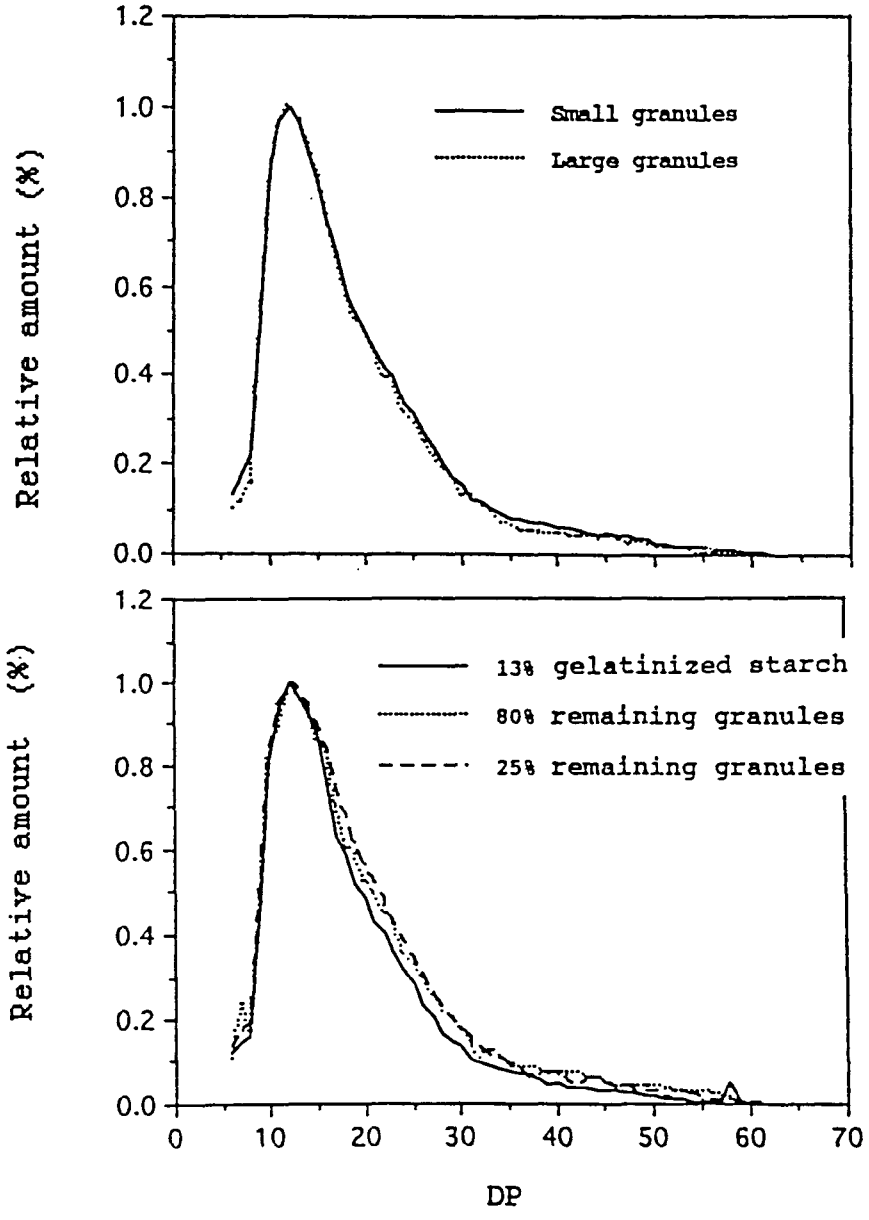
Relative amount (%)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Small granules
Large granules
Relative amount (%)
13% gelatinized starch
80% remaining granules
25% remaining granules
0
10
20
30
40
50
60
70
DP

Fig. 5

GENERAL CONCLUSIONS

The internal structure of normal and waxy maize starch granules were studied by using a chemical surface gelatinization method. The analysis of starches of different granular sizes and radial locations included scanning electronic microscopy, amylopectin titration, gel permeation chromatography and branch chain length determination. Results showed that maize starch granules had a radial distribution of amylose molecules and amylopectin branch chains. Amylose was more concentrated at the periphery than at the core of the granule. Amylopectin had longer long B-chains (B2-chains) at the core than at the periphery of the granule. More longer long B-chains (B2-chains) were concentrated at the core than at the periphery of the granule. This distribution showed an agreement with that in tube potato starch granule, indicating a highly confined starch granular structure. Amylose increases with the development of the granule. Amylopectin has a larger molecular size at the early stage of the growth of the granule.

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