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Structures of starch granules revealed by chemical erosion method

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Structures of starch granules revealed
by chemical erosion method

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by

James Jingfu Shen

A Thesis Submitted to the
Graduate Faculty in Partial Fulfillment of the
Requirements For the Degree of
MASTER OF SCIENCE

Department: Food Science and Human Nutrition
Major: Food Science and Technology

Signatures have been redacted for privacy

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TABLE OF CONTENTS

	Page
INTRODUCTION	1
LITERATURE REVIEW	3
A. Fine Structure of Amylose	3
B. Fine Structure of Amylopectin	5
1. Branching pattern	6
2. Acid-resistant fraction	11
3. Transmission electron microscopic observation	11
C. Structures of Starch Granules	13
D. Starch Degrading Enzyme	15
1. α -Amylose(EC 3.2.1.1)	17
2. β -Amylose(EC 3.2.1.2)	17
3. Debranching enzyme(EC 3.2.1.41, EC 3.2.1.68)	18
4. Isopululanase(EC 3.2.1.5.7)	18
5. Other enzymes	19
E. Gelatinization	19
F. Structure Studies of Retrograded Starch	20
G. Chemical and Physical Changes During Plant Growth	23
H. Phosphorus in Potato	24
I. Chemical Erosion of Starch Granules	25
MATERIALS AND METHODS	28
A. Materials	28
B. Methods	28
1. Fractionation of starch	28
2. Defatting of starch	29

3. Chemical erosion of starch granules	30
4. Separation of eroded starch	30
5. Determination of amylose content	31
6. Determination of phosphorus content	32
7. Isolation of amylopectin	34
8. Debranching amylopectin	34
9. Total carbohydrate analysis	35
10. Reducing value analysis	35
11. Gel-permeation column chromatography on Sephadex CL-2B	36
12. Gel-permeation column chromatography on Bio-gel P-6	37
13. Light microscopy	37
14. Scanning electron microscopy	38
RESULTS	39
A. Scanning Electron Microscopy	39
B. Distribution of Amylose in Potato Starch Granule	39
C. Distribution of Phosphorus Content in Potato Starch Granule	44
D. Structure Studies of Amylose and Amylopectin	44
E. Branch Chain Length of Amylopectin Isolated from Various Radial Locations in Potato Starch Granules	48
DISCUSSION	62
SUMMARY	70
REFERENCES	72
ACKNOWLEDGMENTS	80

INTRODUCTION

Starch, a major constituent in cereal, can be found in all organs of most higher plants. Starch is produced by photosynthesis in green leaves and stored in seeds, roots and stems. Normally, starch is stored in granular form. In the granules, there are two different types of polysaccharides. The first, amylose, largely composed of long linear chains of (1→4)-linked α -D-glucopyranose, accounts for 20-30% of most starch. The second, amylopectin, consisting of short branch chains with an average degree of polymerization(DP) of 20 and linked into a branched structure, is a major component of normal starch. Structures of amylose and amylopectin have been revealed by the development of enzymatic methods since the late 1960s. It is now generally accepted that amylose is a polysaccharide with few branches, not the absolutely linear polysaccharide as earlier thought. Also, it has been concluded that in amylopectin there is an extremely heterogeneous localization of molecular branch points. This finding has led to acceptance of the "cluster model" structure of amylopectin molecules.

Substantial progress on the fine structures of amylose and amylopectin has been made, but related studies on starch granule and organization of amylose and amylopectin within the

granules have not been so successful (Banks and Muir 1980, Lineback 1986). For example, where and how amylose, fits into the cluster model, is still unclear.

The location of amylose has been studied by using low degree cross-linking reactions (Jane et al. 1992b). These results indicated that amylose is interspersed among amylopectin in corn and potato starch granules.

This study is aimed to investigate how the amylose, amylopectin and phosphate derivatives are distributed radially in a normal starch granule. This information not only is important to the understanding of the granular structure of normal starch but also is useful in studies of biosynthesis of starch and growth of starch granules. Also it will help in the understanding of a time sequence of biosynthesis of amylose and amylopectin.

The objectives of this study are: (1) to study the internal structures of normal starch granules by revealing the radial distribution of amylose, amylopectin and phosphate derivatives in potato starch granules. (2) to develop a novel method to investigate the internal structure of starch granules.

LITERATURE REVIEW

Starch is the main form of energy being stored in plants, and it is found in all organs in the majority of the higher plants, mainly in seeds and tubers or roots (Badenhuizen 1969). Starch is produced in amyloplasts and chloroplasts. Amyloplasts are organelles specialized for starch storage which are developed from proplastids like the chloroplasts. Chloroplast starch is degraded and used as energy during nighttime in the living plant. As a food, starch is of utmost importance, because it provides more than half of the energy intake to the world population. Furthermore, the physical structure changes associated with starch gelatinization play a central role in food processes, e.g., in the baking of bread.

A. Fine Structure of Amylose

The classical chemical studies on amylose in 1940-1950 established that amylose is a linear long chain ($DP > 100$) (1 \rightarrow 4)-linked α -D-glucose molecule (Williams 1968, Banks and Greenwood 1975). Because the samples of amylose were completely hydrolyzed to maltose by a crude β -amylase preparation, the

conclusion was that the molecules were linear. However, Peat et al. (1952) reported that the crystalline sweet potato β -amylase only hydrolyzed approximately 70% of amylose. The nature of the barrier to β -amylase was the subject of much speculation during the next decade. Oxidized glucose residues, ester phosphate groups and side-chains consisting of single β -glucosyl residues were among the suggested barriers. Kjorberg and Manners (1963) reported that pretreatment of potato amylose with yeast isoamylase resulted in a significant increase of the β -amylolysis limit (>90%). This result suggested that the anomalous barrier to β -amylase action in amylose molecules was probably (1 \rightarrow 6)- α -D-glucosidic interchain linkage.

Banks and Greenwood (1966) treated potato and wheat amylose with pullulanase and β -amylase by both successive action and concurrent action. They confirmed the presence of the (1 \rightarrow 6)- α -D-glucosidic interchain and also concluded that the (1 \rightarrow 6)- α -D glucosidic branches were long-chain rather than short-chain.

Recently, Hizukuri et al. (1981) reconfirmed the multibranched structure of amylose. They concluded that these various amylose molecules were composed of 9 to 20 linear (1 \rightarrow 4)- α -D-glucan chains linked by (1 \rightarrow 6)- α -D-glycosidic bonds.

Structures and amounts of branched molecules in rice amylose with low and high affinities for iodine have been reported recently (Takeda et al. 1989a). Rice starch with low

and high affinities for iodine have similar contents of amylose but different affinities of their amylopectins for iodine. Their amyloses have the same affinity for iodine and show no significant differences in molecular size, average chain-length and number of chains (Takada and Hizukuri 1986, Takada et al. 1987a)

Takada and Hizukuri (1987b, 1989b, 1989c, 1990) and Juliano et al. (1987) investigated the structures of rice starches having iodine affinities (g/100g) ranging from 3.69 to 5.27. The rice starches contained similar proportions of amyloses and amylopectins. They reported that iodine affinities of the amylopectin varied in the range of 0.39 to 2.57 because of the variation in chain lengths. The amyloses of the starches were similar to each other in molecular size but contained various proportions of linear and branched molecules (Hizukuri et al. 1989).

B. Fine structure of amylopectin

Amylopectin is the branched component of starch. The D-glucopyranose residues of amylopectin are connected mainly by α -1,6 linkages at the branch points. The average branched-chain length is 20 to 25 glucose units (Manners 1985). The component chains of amylopectin are divided into three categories: 1) the A-chain, short amylose chains unsubstituted

except at the reducing end; 2) B-chains, substituted at one or more C-6-OH groups by A-chains or other B-chains and also substituted at the reducing end; and 3) C-chains, which are substituted at one or more C-6-OH groups but are unsubstituted at the reducing end. There is only one C-chain per amylopectin molecule.

Determination of the ratio of A chain to B chain is one method being used to characterize the structure of amylopectin (Enevoldsen and Juliano 1988). The ratio of A:B chains is calculated from the amount of maltose and maltotriose liberated from β -amylase limit dextrin by pullulanase (Peat et al. 1956, Marshall and Whelan 1974). Manners (1985) summarized the results of previous studies and concluded that the A:B chain ratio of amylopectin lies within the range of 1:1 to 1.5:1.

Banks and Greenwood(1975) determined the molecular weight of various amylopectin samples by a physiochemical method and estimated it to be 10^7 - 10^8 .

Amylopectin structure will be reviewed in three categories: (1) branching pattern, (2) acid-resistant fraction, and (3) electron microscopic observation of waxy maize starch.

1. Branching pattern

Kainuma and French (1970), using porcine pancreatic α -amylase, extensively hydrolyzed defatted waxy maize starch as a

model compound of amylopectin. They determined the structures of single and multiple branched oligosaccharides. Sixty-five percent of branched structures of waxy maize starch amylopectin were isolated as single branched oligosaccharides and thirty-five percent of the branch structures were located in multiply branched oligosaccharides. The results indicate a heterogeneous localization of branch linkages in the amylopectin.

Umeki and Yamamoto (1975) analyzed quantitatively the formation of branch saccharides. Bacterial saccharifying α -amylase was used and the distribution of single branched oligosaccharides and multiple branch oligosaccharides agreed well with the results of Kainuma and French (1970).

The chain length distribution of the starch molecule has been determined by many investigators (Harada et al. 1972, Akai et al. 1975, Mercier 1973, Hizukuri 1986). The chain lengths of long and short chains of amylopectin were analyzed as about DP 45-60, and 15-20 in length, respectively. Hizukuri (1985) and Kobayashi et al. (1985) developed a technique for the fractionation of debranched chain using HPLC. This method shortened the time required for analysis and also provided better separation of the fractions.

Hizukuri and Maehara (1990) reported a new enzymatic method to analyze the fine structure of wheat amylopectin. The B chain was classified into Ba and Bb chains on the basis of

whether A chains were attached (Ba) or whether A chains was not attached (Bb). The number of A chains bound to a Ba chain was determined from the ratio of non-reducing to reducing residues of the stepwise degradation products of the amylopectin with β -amylase, isoamylase, and again with β -amylase. They found that the A:B and the Ba:Bb chain ratios were 1.26:1 and 1.5:1, respectively, indicating that about 40% of the B chains carried no A chains.

Combining the results of structural analysis of the branching pattern of amylopectin and the formation of Nageli amyloextrin by heterogeneous acid hydrolysis (Kainuma and French 1971, 1972), French (1972) proposed a "cluster model" for the amylopectin molecule (Figure 1). The model explained the high viscosity of amylopectin and the possibility of building the high molecular weight amylopectin (10^7 - 10^8).

Robin and co-workers (1974) proposed a similar model of amylopectin (Figure 2). This model is based on the results of a sequential enzymatic treatment of amylopectin and determination of chain length distribution of Lintnerized starch. The results showed the presence of populations of chains having DP 20-25, and 45 with a small proportion of chains having DP 60.

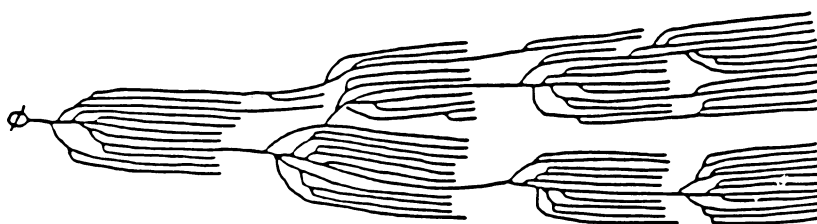
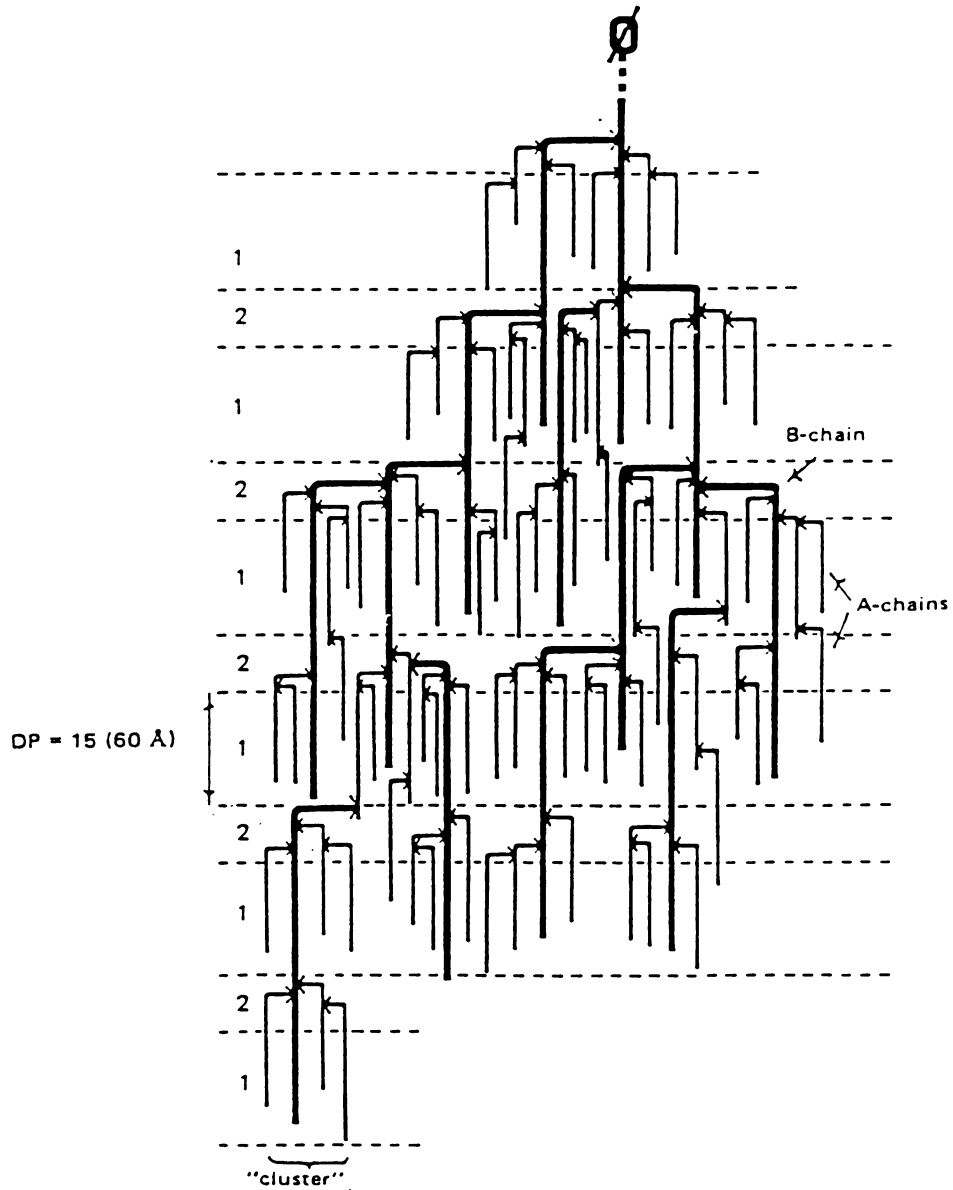


Figure 1. Cluster model for amylopectin molecule (French 1972)



2. Proposed structure for potato amylopectin by Robin et al. (1974). 1=compact area; 2=less compact area, arrows indicate branching points; 0=reducing unit

Manners and Matheson (1981) further developed the cluster model. They claimed the branch points are arranged in "tiers" or clusters and not distributed randomly throughout the macromolecule (Figure 3). Their results supported that the branching pattern in amylopectin is extremely heterogeneous.

2. Acid-resistant fraction

The amorphous region of starch granules readily absorbs aqueous chemical reagents and is degraded by aqueous acid (e.g., 15% H₂SO₄ or 7-10% HCl). The crystalline phase is relatively resistant to such treatment for months. The residual material of starch granules treated extensively (e.g., 3 months with 16% H₂SO₄) is called "Nägeli amyloextrin" (Kainuma and French 1971, Robin et al. 1974). Extensive studies on "Nägeli amyloextrin" (Watanabe and French 1980, Umeki and Kainuma 1978, 1981, Kikumoto and French 1983) showed that a pair of α -(1→4)-linked glucose chains are more stable to acid treatment than single chains and also supported the idea of the double helix model for the crystalline portion of starch.

3. Transmission electron microscopic studies of starch

The method of transmission electron microscopy provides a bridge between the gross structural features seen by optical microscopy or scanning electron microscopy (SEM) and the finer

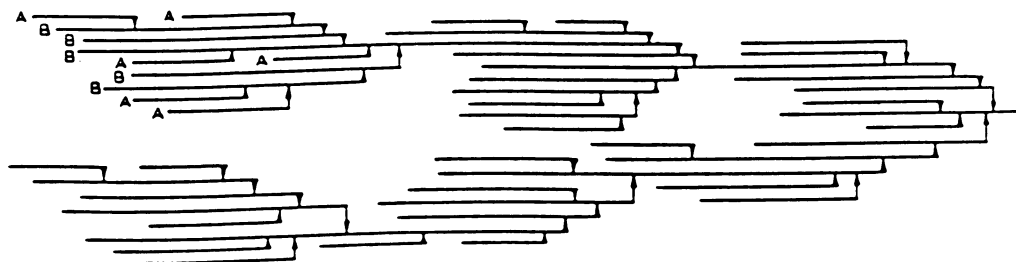


Figure 3. Modified Cluster Model of amylopectin, based on that of French (1972) and Robin et al. (1974). (Manners and Matheson 1981)

structures determined by X-ray diffraction and chemical or enzymatic structural analysis. Early work with thin sections of starch was started by Frey-Wyssling (1953) and Whistler et al. (1955). The results showed that starch has a microgranular structure. Gallant and Guilbot (1969) and Kassenbeck (1978) distinguished the following types of organization: 1) a radial arrangement of the amylose molecule, 2) amylose in an amorphous arrangement, and 3) an arrangement of amylopectin such that the crystallites are in tangential lamellae.

Yamaguchi and co-workers (1979) interpreted the transmission electron microscope data as being consistent with an extended cluster model for amylopectin.

C. Structures of Starch Granules

Very little is known about the arrangement of amylose and amylopectin molecules in the starch granule (Lineback 1986). Nikuni (1969) proposed the "unitary theory" of starch in which all the molecules in a starch granule may well be covalently bound (Figure 4). The molecular weight obtained by this model is much greater than that determined by physicochemical methods. Lineback (1984) proposed the modified Nikuni model (Figure 5). This model incorporates the concept of double helices of outer chains of amylopectin. Amylose could exist in a random coil or helical conformation without binding to an

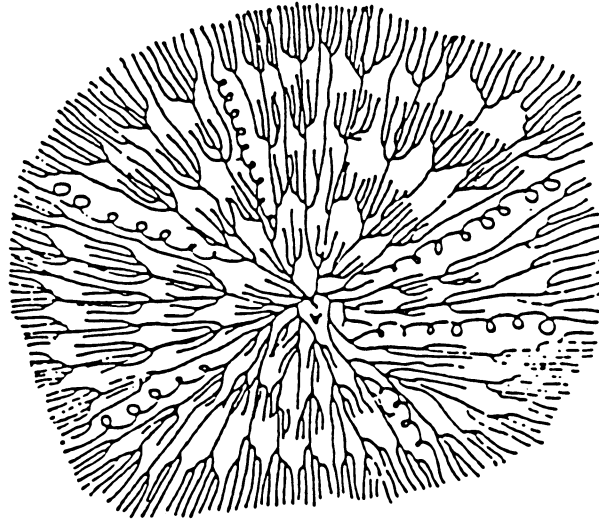


Figure 4. Schematic presentation of starch granules based on "the unitary theory." (Nikuni 1969)

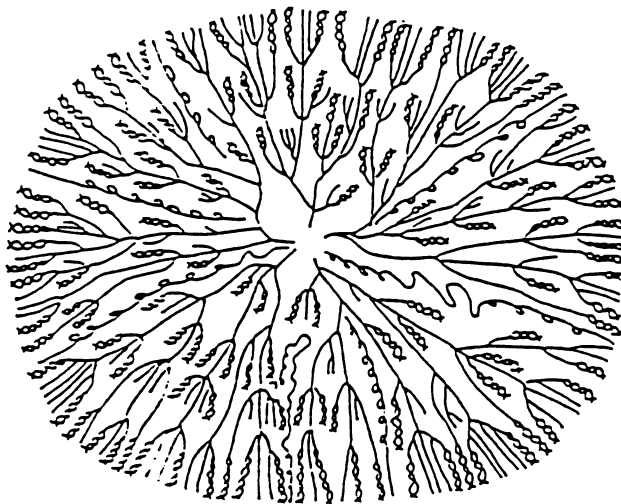


Figure 5. A modified Nikuni model of the starch granule. (Lineback 1984)

amylopectin molecule. The details of conformation of amylose in the granules is not known.

French (1984) proposed a possible arrangement of amylopectin clusters in waxy maize starch granules (Figure 6). This model was based on transmission electron microscopy observations and the results of chemical and biochemical analyses of the branching pattern of amylopectin and also the structure of Nägeli amyloextrin. Since this is the model for waxy maize starch, it is still not clear how amylose molecules are incorporated in this model.

Jane et al. (1992b) studied the location of amylose by using cross-linking reaction with epichlorohydrin. They reported that with low degree of cross-linking, the amylose and amylopectin molecules were cross-linked, and the blue value of the amylopectin peak was increased in the gel permeation chromatography. No cross-linking was found between amylose molecules. Their results were consistent with the view that amylose is interspersed among amylopectin molecules in corn and potato starch granules.

D. Starch Degrading Enzyme

Since the late 1960s, reaction mechanisms of some starch degrading enzymes have been studied extensively. The enzymes most frequently used for starch structural analyses are

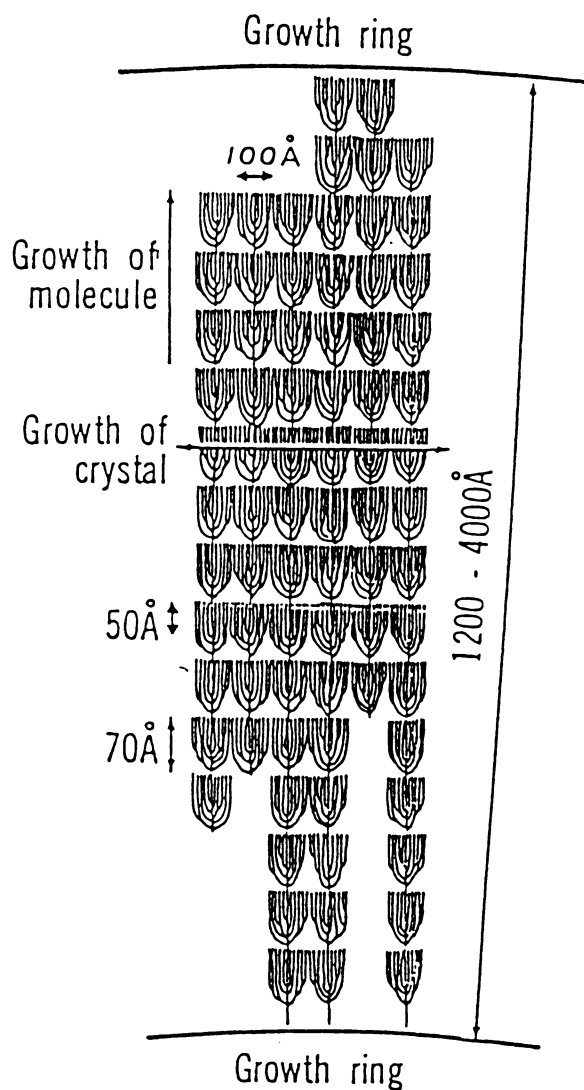


Figure 6. Schematic representation of arrangement of amylopectin molecules within a growth ring. (French 1984)

α -amylase, β -amylase, glucoamylase, pullulanase, isoamylase, and isopullulanase. Their action patterns on poly- or oligosaccharides are fairly well known.

1. α -Amylase (EC 3.2.1.1)

α -Amylase hydrolyzes internal (1 \rightarrow 4)- α -D-glycosidic linkages in starch by partially random action to give a mixture of linear malto-oligosaccharides and branched oligosaccharides called α -limit dextrans. Robyt and French (1970) first determined the frequency distribution of bond cleavage of porcine pancreatic α -amylase (PPA) on malto-oligosaccharides. They reported that the frequency of bond cleavage was highly regulated and the action pattern on short-chain substrates was not random.

Kainuma and French (1969, 1970) synthesized various glucosyl-stubbed malto-oligosaccharides. They showed that the sites of cleavage by the enzyme were extremely restricted by the existence of glucosyl stubs on the linear chain.

Bacterial "saccharifying" α -amylase (Umeki and Yamamoto 1975), salivary α -amylase (Bines and Whelan 1960), and bacterial "liquefying" α -amylase were also used for structural analyses.

2. β -Amylase (EC 3.2.1.2)

The β -amylases attack the next to last glycosidic bond from the non-reducing end of a starch chain to specifically

release β -maltose. The action of the enzyme is blocked by α -D-(1 \rightarrow 6) branch linkages. Amylose should be completely hydrolyzed, whereas only 55% of amylopectin is converted to β -maltose. The remaining 45% is a high-molecular-weight limit dextrin that contains all the branching of the original amylopectin molecule.

Summer and French (1956) reported that the structures of the branched saccharides were resistant to hydrolysis by β -amylase. The cleavage site of some glucosyl-stubbed malto-oligosaccharides was reported by Kainuma and French (1970).

3. Debranching enzymes (EC 3.2.1.41, EC 3.2.1.68)

Pullulanase (EC 3.2.1.41) and *Pseudomonas* isoamylase (EC 3.2.1.68) are the most frequently used debranching enzymes. They selectively hydrolyze the interchain (1 \rightarrow 6)- α -D-glycosidic linkages of branched polysaccharides but have no action on (1 \rightarrow 4)- α -D-glucosidic linkage. Pullulanase cleaves more easily short chain branches such as maltosyl- or maltotriosyl- (Abdullah et al. 1966, Walker 1968) compared with the long-chain branches which *Pseudomonas* isoamylase cleaves very easily.

4. Isopullulanase (EC 3.2.1.5.7)

Isopullulanase reacts on pullulan and liberates oligosaccharides containing α (1 \rightarrow 6) linkage (Sakano et al. 1971,

1972). It reacts specifically on pullulan at the α -(1 \rightarrow 4) linkages present at the reducing-end side of the glucose residue, where the C-6 position is connected to another glucose residue. This enzyme is particularly useful for the determination of the structure of branched oligosaccharides when used in parallel experiments with pullulanase (Umeki and Yamamoto 1975)

5. Other enzymes

Glucan 1,4- α -maltotetrahydrolase (EC 3.2.1.60) (Robyt and Ackerman 1971), glucan 1,4- α -maltohexahydrolase (EC 3.2.1.98) (Kainuma et al 1972, 1975), and other enzymes can also be used for structural studies because of their strict substrate specificity. A combination of enzyme analysis and chromatographic technique has significantly accelerated the structural studies of amylose and amylopectin.

E. Gelatinization

Gelatinization is the process of breaking inter- and intra-molecular hydrogen bonds and hydrophobic interactions within the starch granule (Hari et al. 1989). The gelatinization temperature range is the temperature range over which the swelling of granules and loss of crystalline structures occurs. For example, the gelatinization temperature

range of potato starch is 59-68°C, but that of corn starch is 62-72°C. Enzymatic digestibilities are often used to determine the degree of gelatinization (DG) of starch because the determination is simple, reproducibility is high and the degree of gelatinization can be represented in numerical values easily.

Kainuma et al. (1981) developed the β -amylase-pullulanase process to characterize the digestibility of raw, gelatinized and retrograded starch. This method is particularly useful to determine the change of starch-containing foods during storage.

F. Structural Studies of Retrograded Starch

During storage, gelatinized starch molecules either become gels or precipitate. This phenomenon is defined as "retrogradation". It has been considered simply as a reorganization of the dispersed starch molecule in gelatinized starch, and very little is known about its structure.

Matsukura et al. (1983) studied the structure of retrograded starch. They observed a portion of the retrograded amylopectin molecules being strongly resistant to heterogeneous acid hydrolysis. They proposed the possibility of the formation of inter- or intra-molecular parallel and antiparallel double helices in the retrograded amylopectin. They proposed three domains in retrograded starch based on the

chain length of residual carbohydrate (Figure 7). Domain A is a region that is resistant to acid hydrolysis but is 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. Domain B is considered to be a transition state of gelatinization to retrogradation. It is easily hydrolyzed by acid and enzyme. Domain C is primarily from retrograded amylose molecules which are hydrolyzed by acid but strongly resistant to enzyme action.

Amylose has a tendency to retrograde and is considered primarily responsible for retrogradation of starch. The retrogradation reaction is characterized by a lag period followed by a markedly accelerated phase, then by a slow approach to a limit (Loewus and Briggs 1957). The retrogradation rate of amylose is dependent largely on chain length. A maximum rate of retrogradation 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 were crystalline double helical regions of 10 nm in length interspersed with amorphous regions.

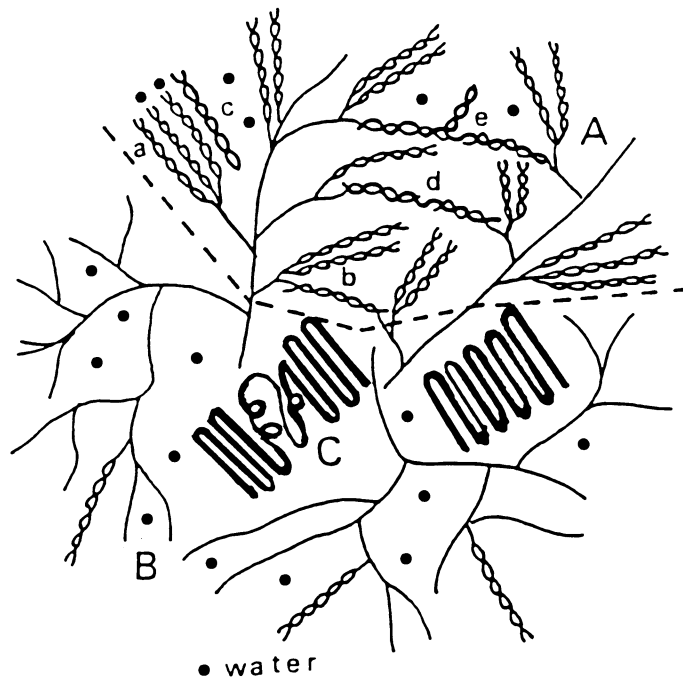


Figure 7. A schematically illustrated structure of retrograded starch. — amylose
 — amylopectin • water (Matsukura et al. 1983)

G. Chemical And Physical Changes During Plant Growth

Asaoka et al. (1984) studied the effects of environmental temperature on the structure, amylose content, and other properties of endosperm starch during the development of rice plants. They found that the amylose content differed with the various growth conditions and the environmental temperature during the first 3 months after heading. The difference is linked closely to amylose biosynthesis in endosperm cells of the rice plant. For the determination of the fine structure of amylopectin, the more detailed experiments using waxy and normal rice plants of near isogenic lines of Japonica cultivar were carried out and it was concluded that the higher environmental temperature (30°C) increased the long B-chain of amylopectin, while decreasing mainly the short B-chains and slightly the A-chains as compared with the lower temperature (20°C) (Asaoka et al. 1985).

Increased amylose concentrations have been observed with increasing age of the tissue from which the starch was isolated for various plant species. For example, Tsai and co-workers (1970) reported an amylose increase from 9% to 27% during 8 to 28 days post pollination. The amylose in potato starch increases from 12% in 0-1 cm tubers to 20% in 15-16 cm tubers

(Geddes et al. 1965). Similar increases in amylose concentration are observed as a function of increasing granule size when granules are from a developing tissue at a single stage of development (Boyer 1976, Geddes 1965).

H. Phosphorus in Potato

Most starches contain small amounts of phosphorus that are either chemically esterified onto the starch molecule or are present as lysophospholipids. Potato starch contains approximately 0.06-0.10% phosphorus (Swinkles 1985). Takeda and Hizukuri (1982) reported that approximately one third of the phosphate groups on potato starch are located in the inner section of the B-chains, and about two thirds of the phosphate groups are located in the A-chains as well as the outer sections of B-chains. The phosphate groups in the potato starch contribute negative charges to the starch molecules. In solution, these negative charges repel each other and cause an increase in the viscosity and thickening power of the potato starch paste (Swinkles 1985). It is also believed that the phosphorus in potato starch contributes to paste clarity, increased viscosity and temperature stability (Osman and Mootse 1958, Osman 1972)

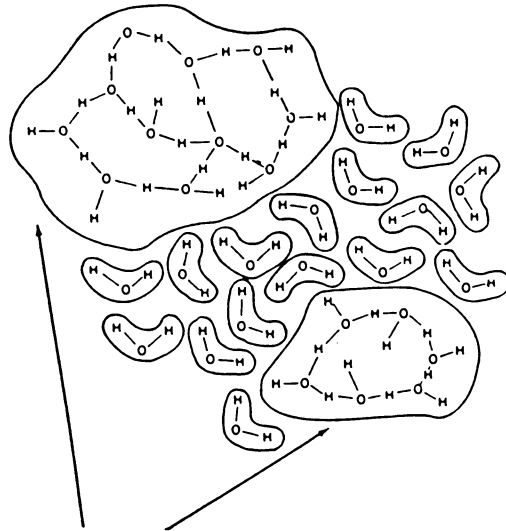
I. Chemical Erosion of Starch Granules

Starch gelatinization is known to be affected by various salts. Evans and Haisman (1982) reported that sodium sulfate substantially increased gelatinization temperature. However, sodium thiocyanate (>2.0 m) was reported to gelatinize starch at room temperature (Sandstedt et al. 1960). Gough and Pybus (1973) showed that increasing the concentration of calcium chloride had an inconsistent effect on starch gelatinization temperature. It was also reported that neutral salts of cations of multi-valent or high charge-density, such as calcium chloride, lithium chloride and aluminum chloride can erode starch granules gradually from the periphery when the concentration is sufficiently high (Gough et al. 1973, Lindqvist 1979, Jane 1986)

Evans and Haisman (1982) found that starch gelatinized with an exothermic enthalpy in the presence of concentrated calcium chloride (>4 m). And also, starch gelatinized with an endothermic enthalpy in the presence of water.

Using DSC, ¹³C-NMR and microscopy, Jane (1986) studied the behavior of starch gelatinization in the presence of various salts. The mechanism of starch gelatinization in salt solution was proposed as: 1) structure-making and -breaking effects of water, and 2) electrostatic interactions between salt and

hydroxyl groups of starch (Figure 8). In general, ions of high charge density increase the structure of water and, thus, stabilize starch granules; ions of low charge density not only break water structure but also interact with hydrophobic portion of starch molecules and, thus, destabilize starch granules. Because of the electronegative property of starch, anions tend to repel starch -OH groups and stabilize starch granules; the repulsion is proportional to the charge density of the anion. Cations, on the other hand, attract starch -OH groups and destabilize starch granules, and the attraction is also proportional to the charge density. For anions, an increase of charge density cause a more structured water and a higher repulsion to starch -OH groups. Both effects tend to stabilized starch granules. For cations, an increase of charge density results in more structured water, which stabilizes starch granules, and a higher attraction to starch -OH groups, which destabilizes starch granules. These two effect conflict with, and tend to cancel, each other. This proposed mechanism explained the phenomenon that anions in the lyotropic series affect the stability of starch granules in a distinct order, but cations in lyotropic series affect the stability of starch in a less clear order which depends on the concentration of the salt.



CLUSTER MODEL OF LIQ. WATER

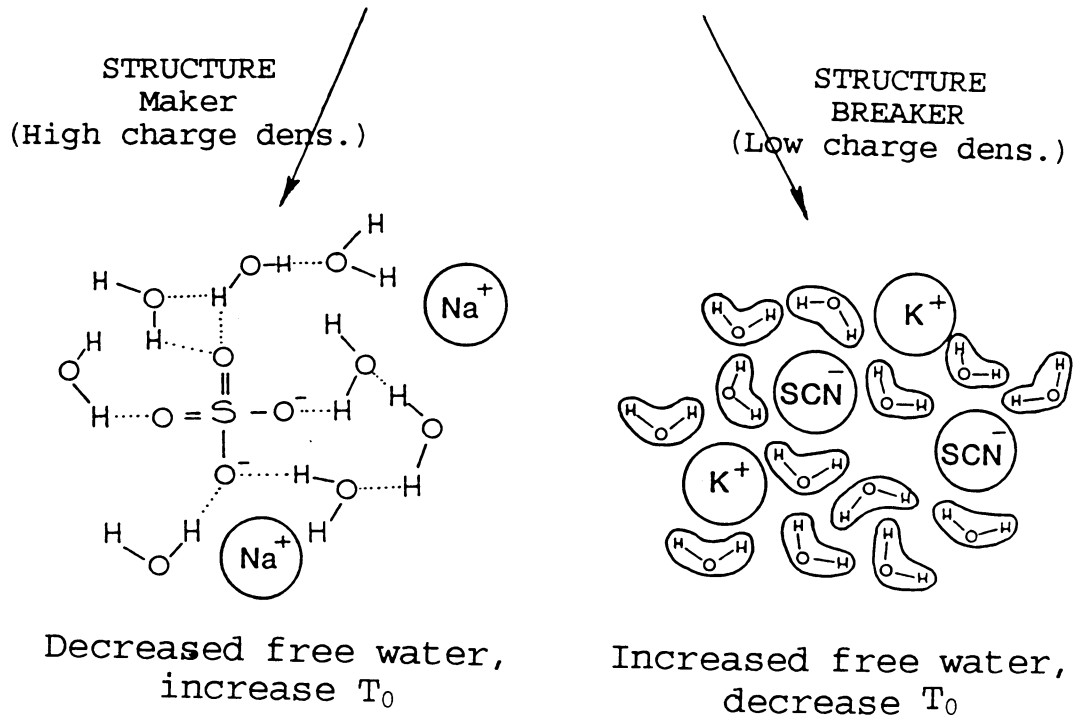


Figure 8. Proposed mechanism of neutral salts affecting starch gelatinization (Jane 1986)

MATERIALS AND METHODS

A. Materials

Normal potato starch was purchased from Sigma Chemical Company (St. Louis, MO). Crystalline *Pseudomonas* isoamylase (EC 3.2.1.68) was a product of Hayashibara Biochemical Laboratories, Inc. (Okayama, Japan). The specific activity was 61,000 units per mg protein. The enzyme was used directly without further treatment. Bio-gel P-6 and Sepharose CL-2B were purchased from Bio-Rad Laboratories (Richmond, CA) and Pharmacia Inc. (Piscataway, NJ), respectively. Other chemicals were all reagent grade and used without further purification.

B. Methods

1. Fractionation of starch

Normal potato starch was fractionated into four fractions according to granular size by using filter cloths. Filter cloths were used in three different sizes: 20, 30, and 52 μm . Potato starch was fractionated into four fractions: small granule (<20 μm), medium granule I (20-30 μm), medium granule II (30-50 μm), and large granule (>52 μm). The procedures of

fractionation are described as following: starch sample (20 g) was wrapped with the filter cloth (20 μm) and tied up. Then the starch sample was immersed in 300 ml distilled water in a 500-ml beaker. After agitation, starch particles smaller than 20 μm were washed out through the filter cloth and entered into the distilled water. After 10 min, starch particles which had passed through the filter cloth were allowed to stand for 30 min, then were collected by decantation from the beaker. Fresh distilled water (300 ml) was used again to repeat the above procedures. When distilled water stayed clear, which indicated that starch particles had stopped passing through filter cloth, the separation was completed. Thus the starch sample was fractionated into two fractions: small granule (<20 μm) and starch granule (>20 μm). Starch particles greater than 20 μm were subjected to further fractionation by the next pore size filter cloths (30 μm). The same procedure was used until starch samples were finally fractionated into four fractions as described above. Medium II fraction (30-52 μm) was used for chemical erosion studies.

2. Defatting of starch

Starch samples were defatted by the method of Schoch (1942). Starches were extracted with 85% methanol for 24 hr in a Soxhlet extractor, washed with 100% methanol twice and then dried in an oven at 70°C.

3. Chemical erosion of starch granules

Potato starch of medium size II was selected for chemical erosion. The optimum concentration of the salt solution was determined to be 4.0 m. A starch sample (20 g) was suspended in 150 ml CaCl₂ (4.0 m) solution with a mechanical stirrer at 21-22°C. Different amounts of starch on the surface of granules were eroded depending on amount of time the granules were suspended in the CaCl₂ solution. After the desired portion of the starch granules was eroded, the mixture was centrifuged at 3500 ×g, at -15°C for 20 min. The low temperature during centrifugation was used to terminate chemical erosion. Supernatant of the sample solution was removed immediately after centrifugation. Partially eroded starch separated by centrifugation was collected and washed twice with 2 L distilled water to remove CaCl₂ from the starch granules. Partially eroded starch was subjected to further separation.

4. Separation of partially eroded starch granules

After the desired portion of starch granules was eroded, the starch sample (20 g) was mixed with 300 ml distilled water and transferred into a blender (Hamilton Beach Blender, model 609-4, Hamilton Beach Inc.). The sample suspension was blended at maximum speed for 10 min, and then allowed to stand for 15 min to keep the temperature of the solution lower than

50°C and also to avoid damage to the motor. The above procedures were repeated five times. The sample solution was allowed to stand for 30 min and the supernatant of the solution with separated chemically gelatinized starch was then collected by decantation from the blender. The residue remaining in the blender was blended again by adding another 300 ml distilled water and the previous procedures were repeated. After chemically gelatinized starch was completely removed from starch granules remaining after erosion, the starch in the blender was washed twice with 100% ethanol, and dried at 70°C for 4 hr and collected as starch granules remaining after erosion. The supernatant collected from the sample solution was evaporated to remove water, and then ten volumes of 100% ethanol was used to precipitate the chemically gelatinized starch completely. The mixture was then centrifuged at 5000 ×g for 30 min and the precipitate was collected as chemically gelatinized starch, washed with 100% ethanol twice, dried at 70°C for 4 hr. The degree of erosion of starch was calculated by dividing the chemically gelatinized starch (on dry basis) by the total weight of starch recovered after chemical erosion (on dry basis).

5. Determination of amylose content

Amylose content was determined by potentiometric titration following the procedure of Schoch (1964). About 100 mg starch

sample (approximately 20 mg amylose) was weighed into a 250 ml beaker which had been tared. Five milliliters of 1.0 N KOH solution was added. The mixture was kept at 4°C for 30 min, and then neutralized with 0.5 N HCl. Ten milliliters of 0.5 N KI solution and sufficient water at 30°C was added to give a total weight of 100.9 g over the weight of the empty beaker. The sample was then titrated with iodine at 30°C with mechanical stirring. A digital pH/mV meter (model 501) (Orion Research Inc., Boston, MA) was used to detect the potentiometric change. The concentration of free iodine of the sample was determined with a standard curve which was constructed by titrating a mixture of KCl (373 mg) and KI (839 mg). The bound iodine was calculated by the difference between the total amount of iodine as titrant and the free iodine. Free iodine (X) was plotted against iodine affinity (Y). The upper linear portion of this curve was extrapolated back to intersect the Y axis. This value was the iodine affinity of the sample. The amylose content was calculated by dividing the iodine affinity of the sample by 19.9% which is the theoretical value of iodine affinity of pure amylose from potato starch.

6. Determination of phosphorus content

Phosphorus content was determined by following the procedure of Smith and Caruso (1964). Starch sample (about 4 g) was weighed accurately into 100-ml vvor dish. Ten

milliliters of 10% zinc acetate solution was added. The sample was evaporated to dryness, heated until thoroughly charred on a hot plate, and then ignited 2 hr in a muffle furnace at 550°C. The sample residue was cooled to room temperature, and wetted by cautious addition of 3 ml of 29% nitric acid. The sample was again evaporated, dehydrated completely, and then returned to the muffle furnace at 550°C for about 30 min. The dish was cooled to room temperature and the sides of the dish were washed down with 10 ml of 29% nitric acid. Then the dish was covered with a watch glass and heated to incipient boiling and held 10 min at that temperature. The solution was then transferred into a 100-ml volumetric flask. Aliquots, 5.0, 10.0, 15.0, 20.0, and 25.0 ml, of standard phosphorus solution containing 0.5, 1.0, 1.5, 2.0, and 2.5 mg of phosphorus, respectively, were pipeted into separate 100-ml volumetric flasks. The 10 ml of 29% nitric acid, 10 ml of 0.25% ammonium vanadate solution, and 10 ml of 5% ammonium molybdate solution were added in the order stated into both sample residue solution and standard solution. Solutions were all diluted with water to volume and allowed to stand 10 min. The absorbance of the sample solution at 460 nm was determined. The phosphorus concentration (mg/100ml) of the sample was read from a standard curve.

$$\% \text{ Phosphorus} = (P \times 100) / \text{sample wt. g} \times 1000$$

in which P = phosphorus content (mg/100 ml) from a standard curve.

7. Isolation of amylopectin

Amylopectin was isolated from potato starch by using Sepharose CL-2B column. Starch samples (15 mg) were precipitated from DMSO solution with 15 volumes of ethanol and then centrifuged at 7000 \times g for 30 min. The precipitant was then suspended in 17 ml water and heated with stirring in a boiling water bath for 30 min. The sample solution was then cooled to room temperature. An aliquot (5 ml) was injected into a 2.6 \times 80 cm column packed with Sepharose CL-2B gel. The sample was eluted in an ascending direction with distilled water containing 0.025% sodium chloride as the eluent. Fractions of 4.8 ml were collected and 15 fractions (from 25 to 40) were checked by using total carbohydrate method (Dubois et al. 1956). Six amylopectin fractions were collected, pooled, and subjected to branch chain length analysis.

8. Debranching amylopectin.

Amylopectin was isolated by Sepharose CL-2B column. Amylopectin solution (containing about 12 mg) was evaporated from 10 ml to 4 ml and then heated with stirring in a boiling water bath for 1 hr. The suspension was cooled to room temperature, and 0.5 ml of acetate buffer (pH 3.5), and 600 U

crystalline *Pseudomonas* isoamylase were added. The mixture was incubated for 48 hr in a shaker bath (Versa-baths, model 236, Fisher Scientific) at 40°C, 90 strokes/min for debranching. The debranched sample was subjected to total carbohydrate and reducing end-groups analyses. The chain length was then calculated by dividing the total carbohydrate by reducing value.

9. Total carbohydrate analysis

Total carbohydrate was analyzed by the method of Dubois et al. (1956). One milliliter of sample solution was mixed with 1 ml 5% phenol solution. Five milliliters of concentrated sulfuric acid was quickly added to generate heat. The solution was mixed well and allowed to stand for 30 min at room temperature. The absorbance at 470 nm was read on a Spectronic 21 spectrophotometer (Milton Roy Company, Rochester, NY). A standard curve was constructed with solutions containing 10, 30, 60, 100 µg of glucose.

10. Reducing value analysis

One milliliter of sample solution was mixed with 0.5 ml of sodium carbonate-sodium hydrogen carbonate buffer containing potassium cyanide (4.8 g of Na₂CO₃, 9.2 g of NaHCO₃, and 0.65 g of KCN/1 of water) and 1 ml of ferricyanide solution (0.5 g/1 of water). The mixture was then heated for exactly 20 min in a

vigorously boiling water bath and cooled to room temperature under running tap-water. Ferric ammonium sulfate solution (2.5 ml) (3 g/l of 50 mM H₂SO₄) was added and the mixture was kept for 20 min at room temperature. Absorbance of the solution was read at 715 nm by using the Spectronic 21 spectrophotometer. A standard curve was made with solution containing 3, 5, 7 µg of glucose.

11. Gel-permeation column chromatography on Sepharose CL-2B

Starch samples were fractionated by gel permeation chromatography, following the procedure of Jane and Chen (1992). A starch sample (50 mg) was precipitated from 15 ml DMSO solution of the sample with 20 volumes of ethanol (100%). After being centrifuged at 7000 ×g for 20 min, the precipitate was resuspended in 15 ml deionized water and heated with stirring in a boiling water bath for 30 min. Then the sample solution was cooled to room temperature and glucose (0.6 mg) was added as a marker. Five milliliters of aliquot was injected into a 2.6 ×80 cm column (Pharmacia Inc., Piscataway, NJ) packed with Sepharose CL-2B gel. The sample was eluted in an ascending direction with 0.025% sodium chloride aqueous solution which had been boiled and degassed. The flow rate was about 30 ml/l. Fractions of 4.8 ml were collected and then analyzed by using an autoanalyzer II (Technicon Instruments Corp., Elmsford, NY). Responses of total carbohydrate and

amylose-iodine blue value were analyzed at 630 nm and 640 nm, respectively. Solutions containing 3, 4, and 5 mg of glucose per 100 ml solution were also measured as standards of total carbohydrate.

12. Gel-Permeation Column Chromatography on Bio-Gel P-6

Debranched sample solution (2 ml) was heated for 10 min in a boiling water bath to terminate the enzyme reaction. The solution was then cooled to room temperature and glucose solution (150 μ g) was added as a marker. The sample solution was then injected into a 1.5 \times 80 cm Bio-Rad Econo-Column (Bio-Rad Laboratories, Richmond, CA) packed with Bio-gel P-6 gel. Samples were eluted in a descending direction with degassed deionized water. Fractions of 2.3 ml were collected and analyzed for total carbohydrate content by the autoanalyzer II. Response of total carbohydrate was determined at 630 nm.

13. Light Microscopy

The proportion of potato starch eroded by CaCl_2 (4.0 m) solution was examined by a Nikon labophot light microscope (Garden City, NY), equipped with a Nikon FX-35 WA camera and HFX-II exposure control system. The solution of the starch sample mixed with CaCl_2 (4.0 m) underwent no special treatment before the degree of erosion was analyzed. One drop of starch

solution was placed on a microslide, fitted with a cover glass, and observed for degree of erosion.

14. Scanning electron microscopy

The surface microstructure of the starch granules remaining after erosion and chemically gelatinized starch was examined at 20 kV on a JEOL JSM-35 scanning electron microscopy (SEM) (Tokyo, Japan) and at 40 kV on a JEOL 1200EX scanning transmission electron microscopic (STEM). The starch sample was attached to 3M metallic tape (3M Co., St. Paul, MN) mounted on individual brass discs. The samples were then sputter coated with platinum/palladium alloy (60/40) in a Polaron SE5100 sputter coater for 4 min and then stored in a desiccator. Micrographs were taken on Polaroid 615 positive/negative film.

RESULTS

A. Scanning Electron Microscopy

Scanning electron micrographs of the starches displayed well separated four size fractions isolated from native potato starch (Figure 9a-d). The sizes among these four fractions are significantly different. The medium granule II (30-52 μm) was used for chemical erosion to ensure an even erosion of starch granules. Surface microstructures of the starch granules remaining after erosion (6% erosion, 20% erosion, 60% erosion) are shown in Figure 10a, 10b, 10c, respectively. Chemically gelatinized starch (52% erosion) was also studied by scanning electron microscope as shown in Figure 10d.

B. Distribution of Amylose in Potato Starch Granule

Amylose contents of potato starch and starch at various radial locations were measured by using the iodine potentiometric titration method and are shown in Table I. The amylose content was calculated by dividing iodine affinity of the sample by 19.9% which is the theoretical value of iodine affinity of pure amylose from potato.

Figure 9. Scanning electron micrographs of four fractions of different size isolated from native potato starch. (a) small granule ($< 20 \mu\text{m}$). (b) medium granule I ($20-30 \mu\text{m}$). (c) medium granule II ($30-52 \mu\text{m}$). (d) large granule ($>52\mu\text{m}$).

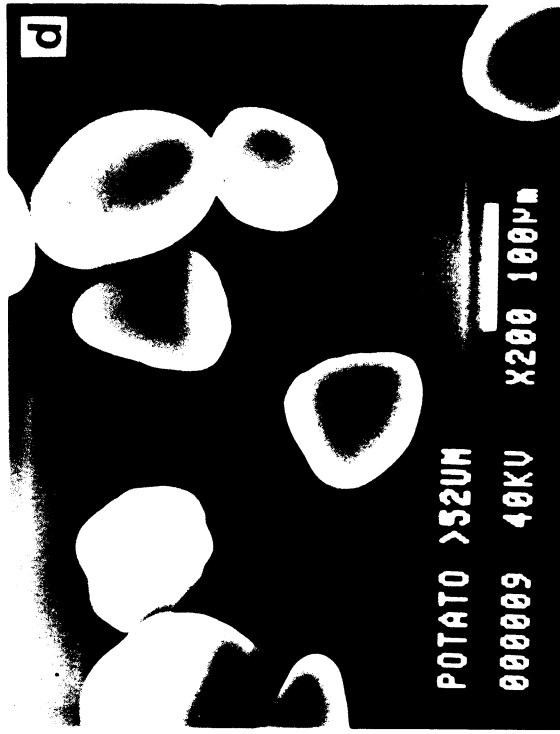
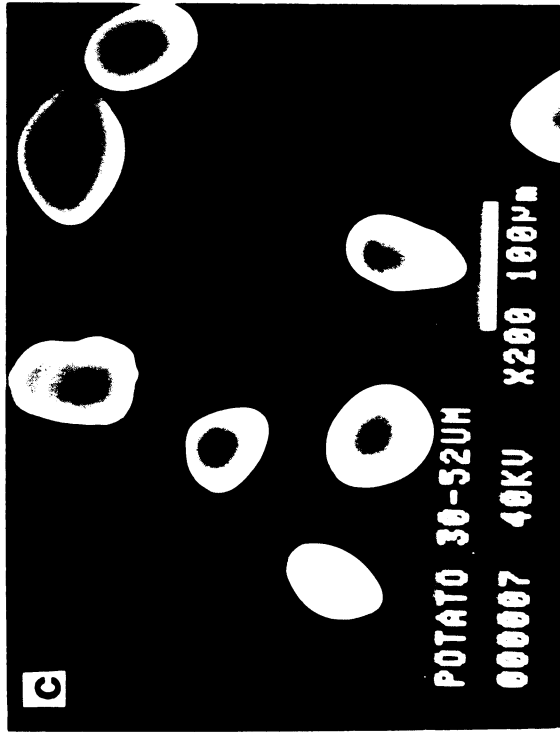
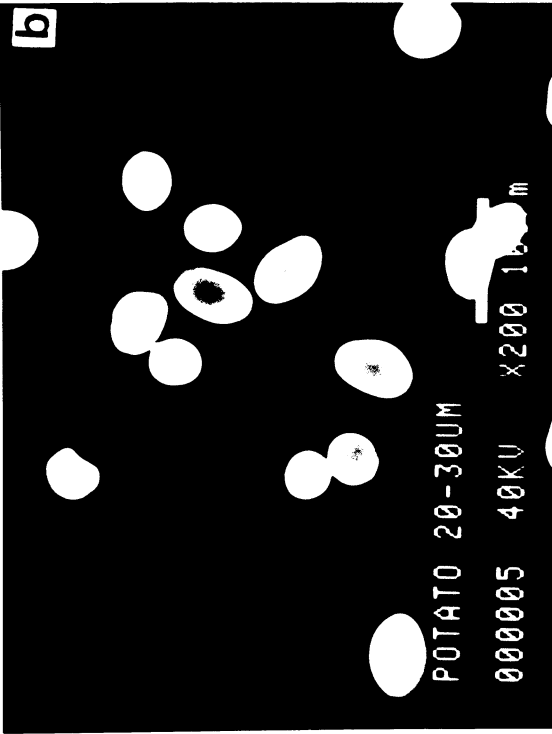
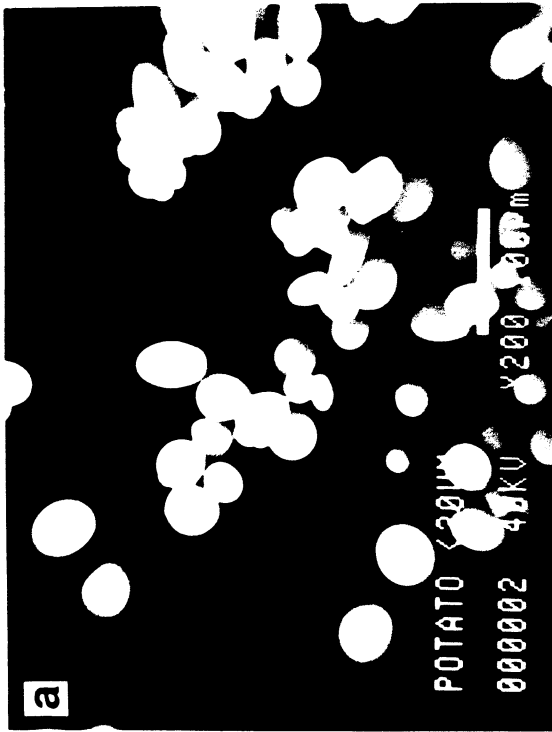


Figure 10. Scanning electron micrographs of potato starch eroded from medium granule II. (a) starch granule remaining after 6% erosion. (b) starch granules remaining after 20% erosion. (c) starch granule remaining after 60% erosion(x1500). (d) chemically gelatinized starch (52% erosion)

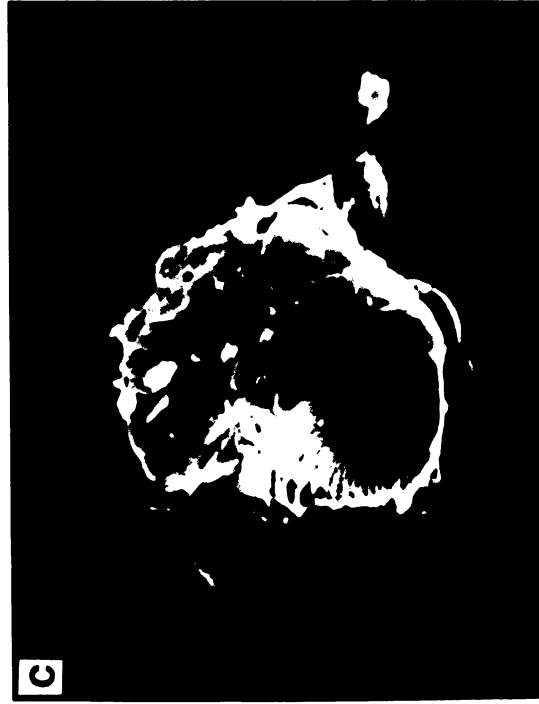
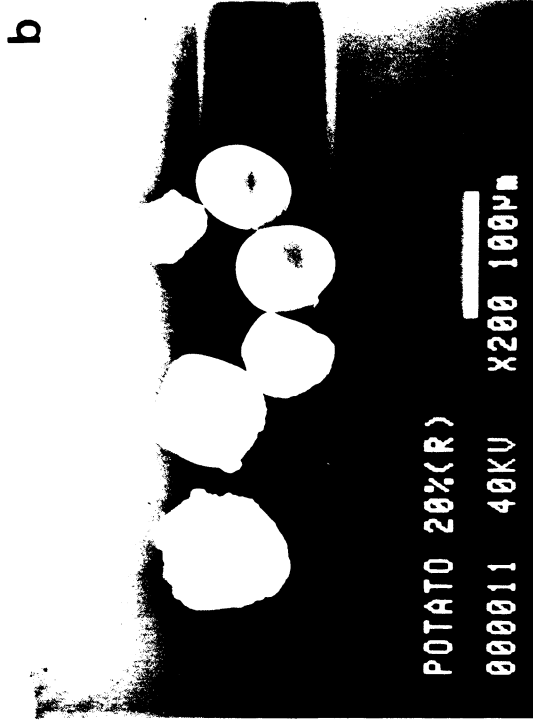


Table I. Amylose contents of potato starch and of various location of the starch

Sample	Amylose Content ^{a,b} (%)
Small granule (<30 μm)	17.5.± 0.1
Starch granule remaining after 80% erosion	18.8 ± 0.1
Starch granule remaining after 52% erosion	19.6 ± 0.1
Native starch	20.2 ± 0.1
Large granule (>52 μm)	20.6 ± 0.1
Chemically gelatinized starch (52% erosion)	21.1 ± 0.4
Chemically gelatinized starch (10% erosion)	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. Distribution of Phosphorus Content in Potato Starch Granule

Phosphorus contents in potato starches of different size and radial locations as determined by the procedure of Smith and Caruso (1964) are presented in Table II. The results indicated that lower phosphorus content was present in the periphery compared with that in the core.

Table II. Phosphorus contents in potato starch of different size and location

Sample	Phosphorus Content (%) ^a
Native potato	0.081 ± 0.001
Small granule (<20 μm)	0.11 ± 0.01
Medium granule I (20-30 μm)	0.096 ± 0.001
Medium granule II (30-52 μm)	0.079 ± 0.001
Large granule (>52 μm)	0.065 ± 0.002
Starch granule remaining after 50% erosion	0.077 ± 0.001
Chemically gelatinized starch (80% erosion)	0.065 ± 0.001

^a Data reported are the averages of at least duplicate analysis.

D. Structure Studies of Amylose and Amylopectin

Molecular sizes of amylose and amylopectin of potato starch at various radial locations were analyzed by gel-permeation column chromatography. Gel permeation profiles of native potato starch and potato starch treated with CaCl₂ (4.0 m) are shown in Figures 11 and 12, respectively. Gel permeation profiles of the starch granules remaining after 80% erosion, which represents the structure at the core of the

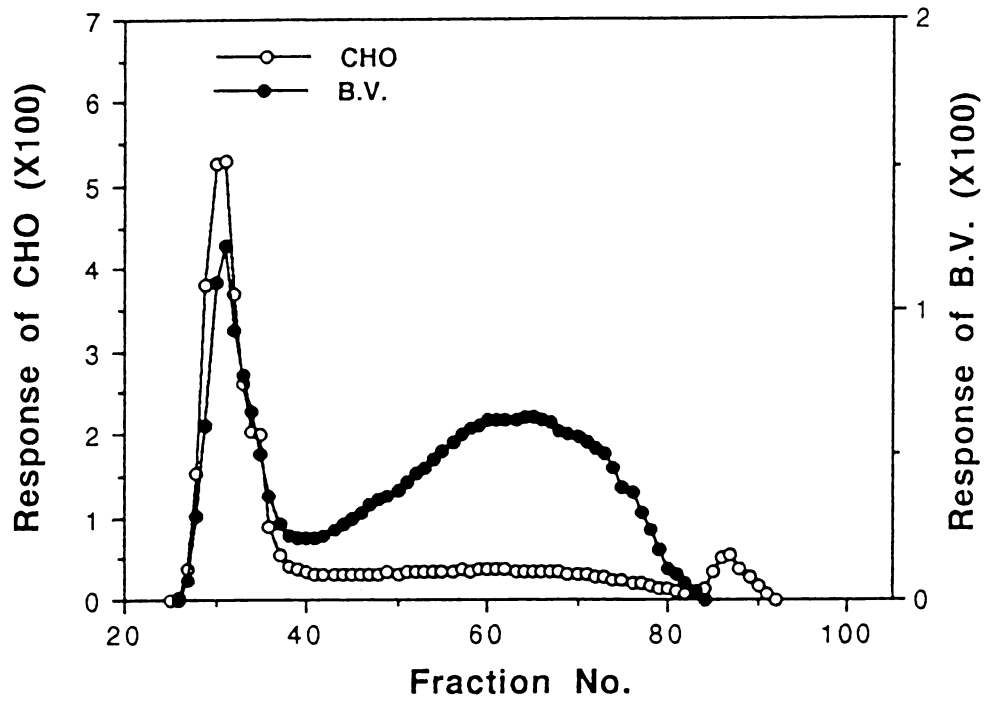


Figure 11. Sepharose CL-2B gel permeation profile of native potato starch. Glucose was used as a marker. CHO=total carbohydrate, B.V. = blue value

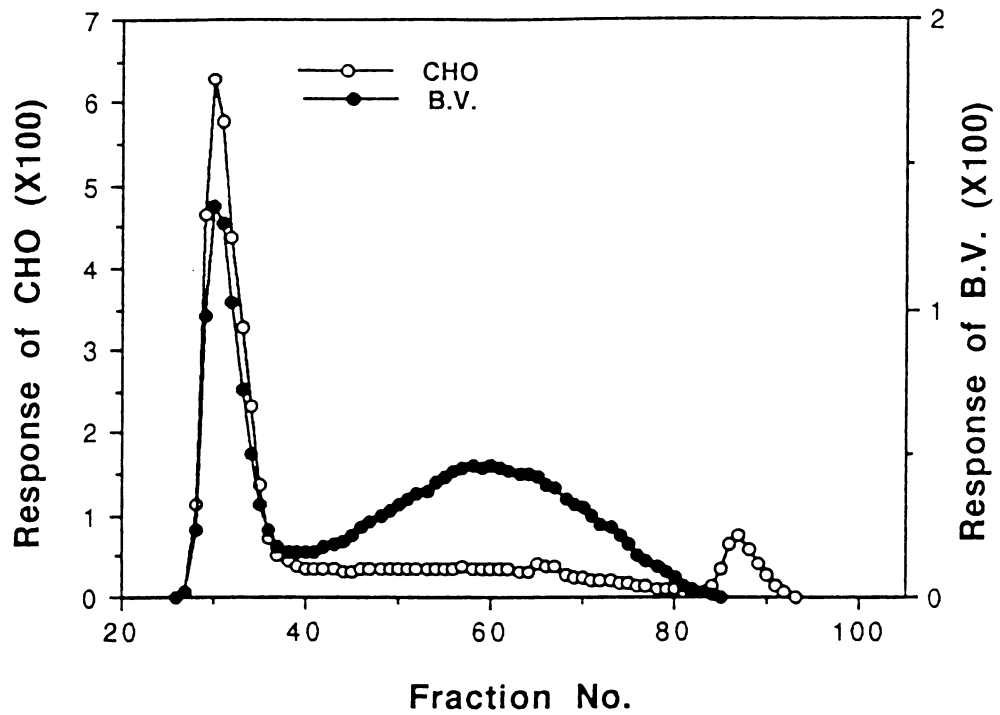


Figure 12. Sepharose CL-2B gel permeation profile of CaCl_2 treated native potato starch. Glucose was used as a marker. CHO=total carbohydrate, B.V.=blue value

starch granule, and of the chemically gelatinized starch (52% and 63% erosion), which represents the structure at the periphery of the starch granule, are presented in Figures 13, 14 and 15. The profiles of potato starch of different granular sizes are shown in Figures 16 to 19. They were used to compare the difference in molecule structure of starches at different stages of maturity and also used as references for the internal structure revealed by chemical erosion of starch granules.

E. Branch Chain Length of Amylopectin isolated from Various Radial Locations in Potato Starch Granules

Gel-permeation profiles of debranched amylopectins isolated from starch granules remaining after 80% erosion and chemically gelatinized starch (20% erosion) are shown in Figures 20 and 21, respectively. Gel-permeation profiles of debranched amylopectins of small granule (<20 μm), medium granule II (30-52 μm), and large granule (>52 μm) are presented in Figures 22, 23, and 24, respectively. The branch chain lengths of the amylopectins are summarized in Table III.

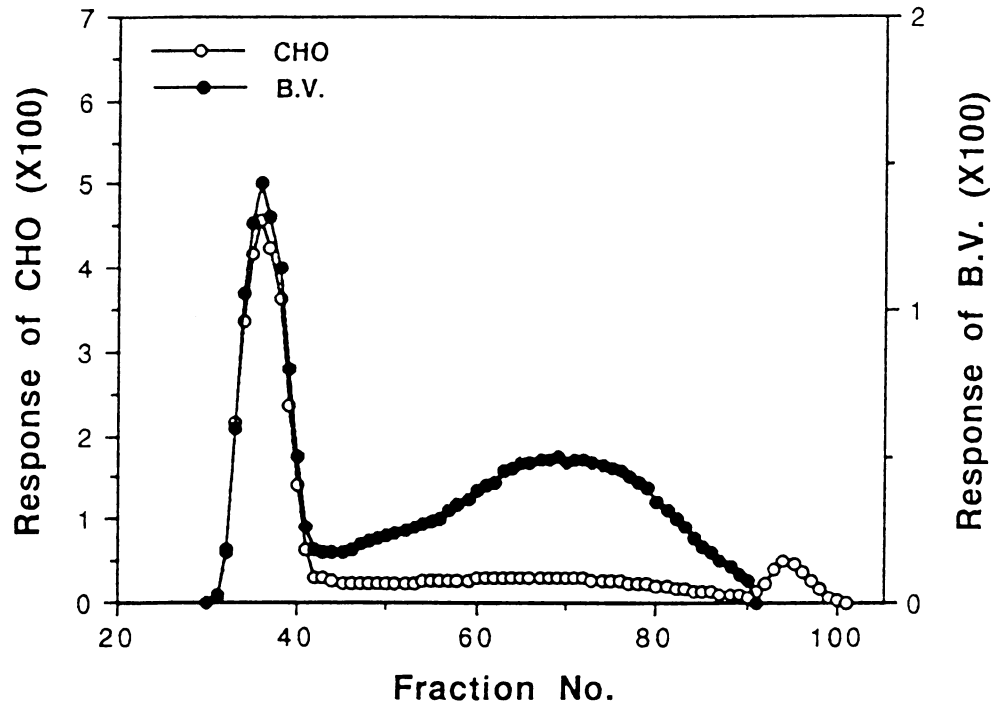


Figure 13. Sepharose CL-2B gel permeation profile of starch granules remaining after 80% erosion eroded from medium size II (30-52 μm) of potato starch. Glucos was used as a marker. CHO=total carbohydrate, B.V.=blue value

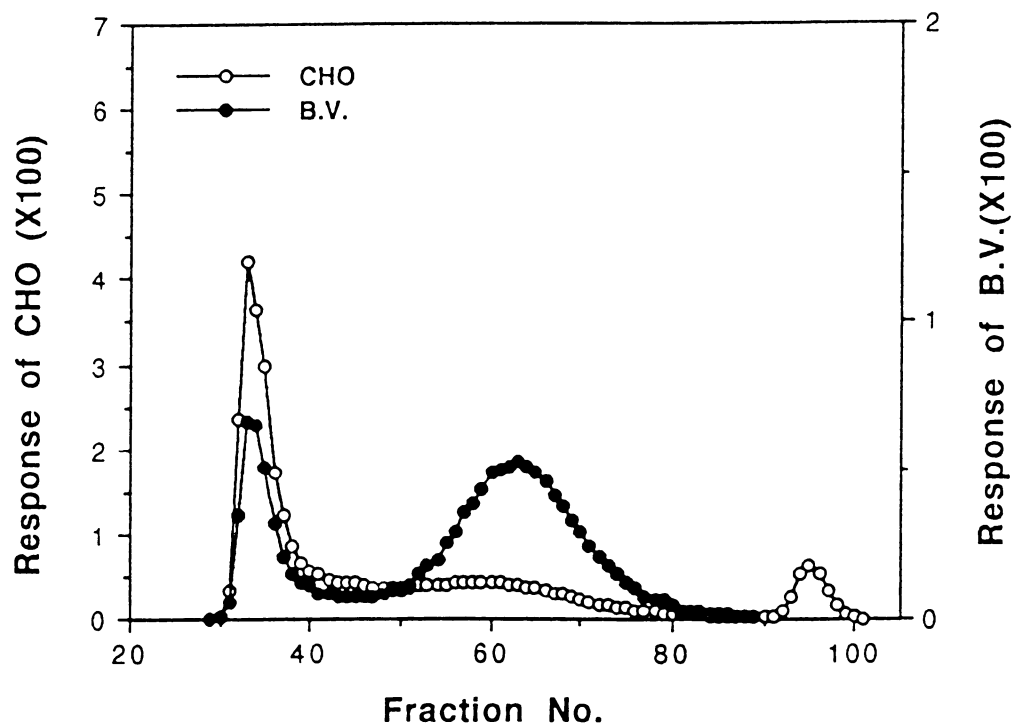


Figure 14. Sepharose CL-2B gel permeation profile of chemically gelatinized starch (52% erosion) from medium size II (30-52 μm) of potato starch. Glucose was used as a marker. CHO=total carbohydrate, B.V.=blue value

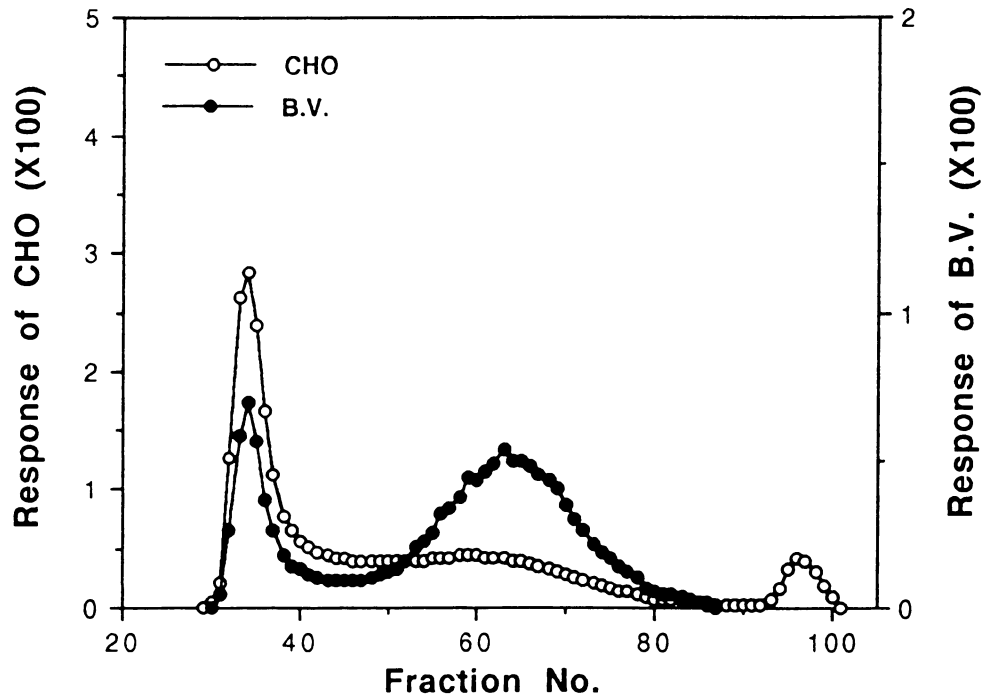
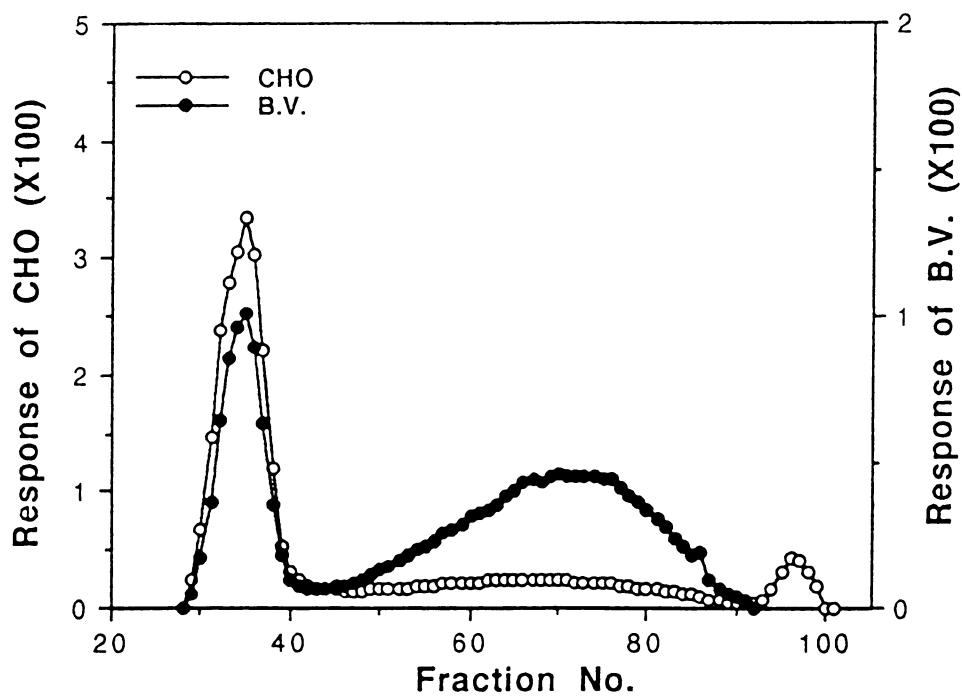


Figure 15. Sepharose CL-2B gel permeation profile of chemically gelatinized starch (63% erosion) from medium size II (30-52 μm) of potato starch. Glucose was used as a marker. CHO=total carbohydrate, B.V.=blue value



16. Sepharose CL-2B gel permeation profile of small granules (<20 μm) of potato starch. Glucose was used as a marker. CHO=total carbohydrate, B.V.=blue value

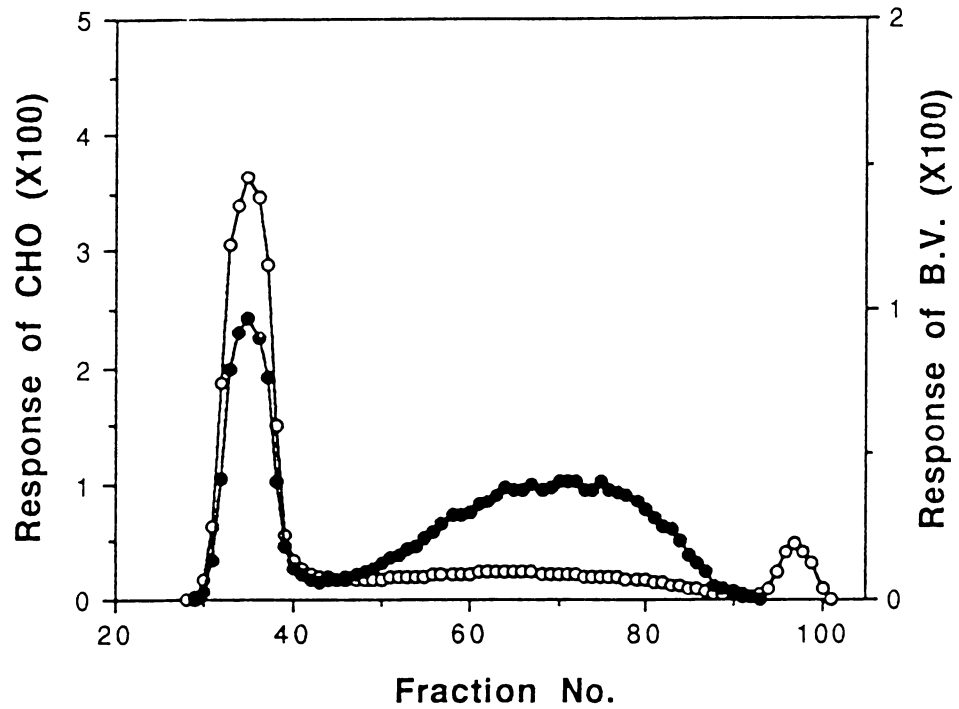
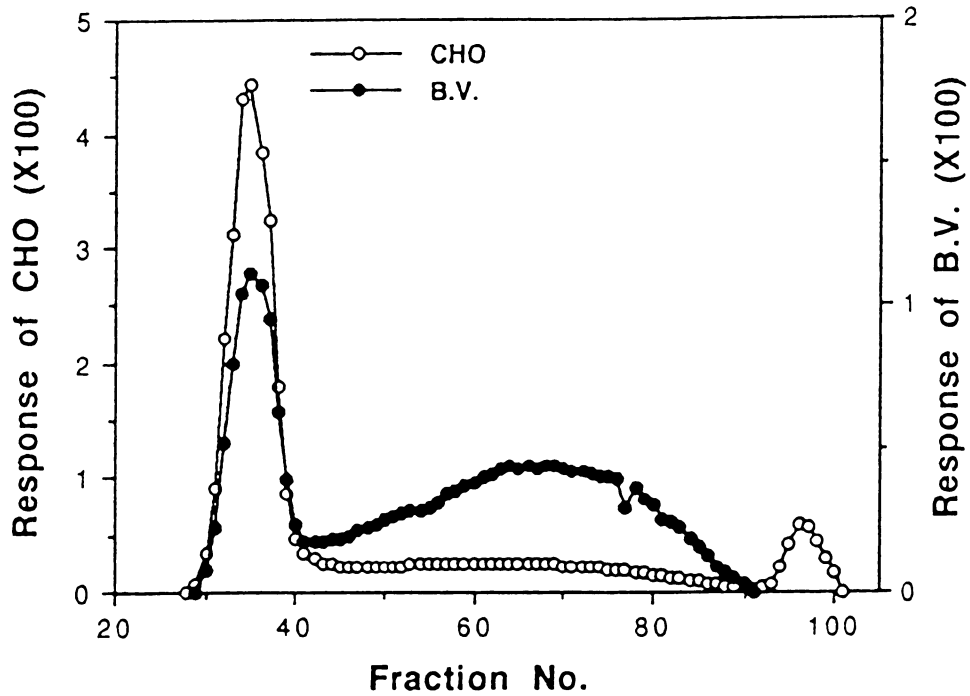


Figure 17. Sepharose CL-2B gel permeation profile of medium size I (20-30 μm) of potato starch. Glucose was used as a marker. CHO=total carbohydrate, B.V.=blue value



18. Sepharose CL-2B gel permeation profile of medium size II (30-52 μm) of potato starch. Glucose was used as a marker. CHO=total carbohydrate, B.V.=blue value

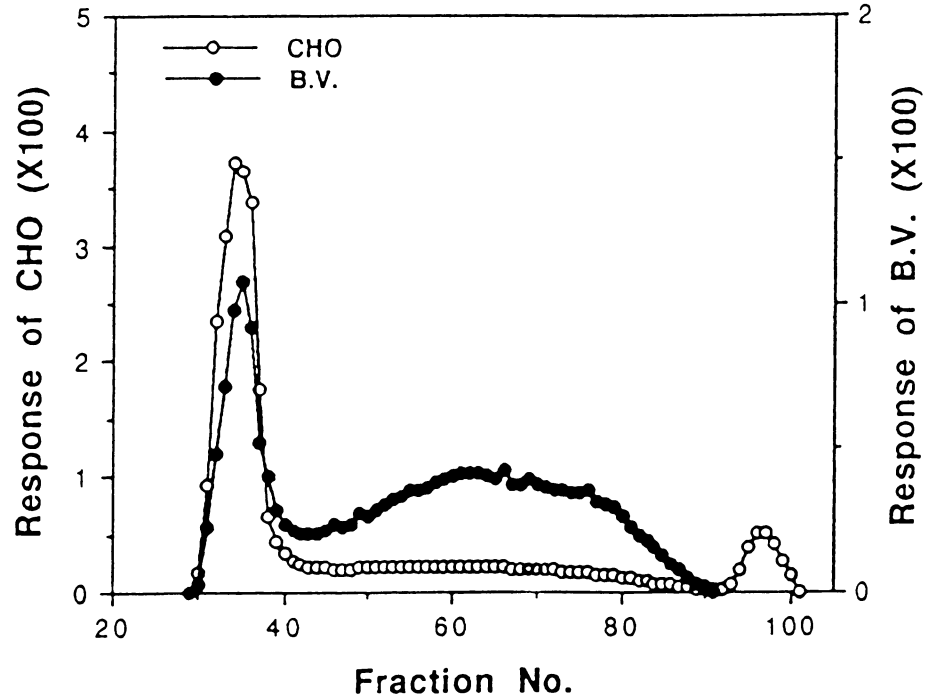


Figure 19. Sepharose CL-2B gel permeation profile of large granule (>52 μm) of potato starch. Glucose was used as a marker. CHO=total carbohydrate, B.V.=blue value

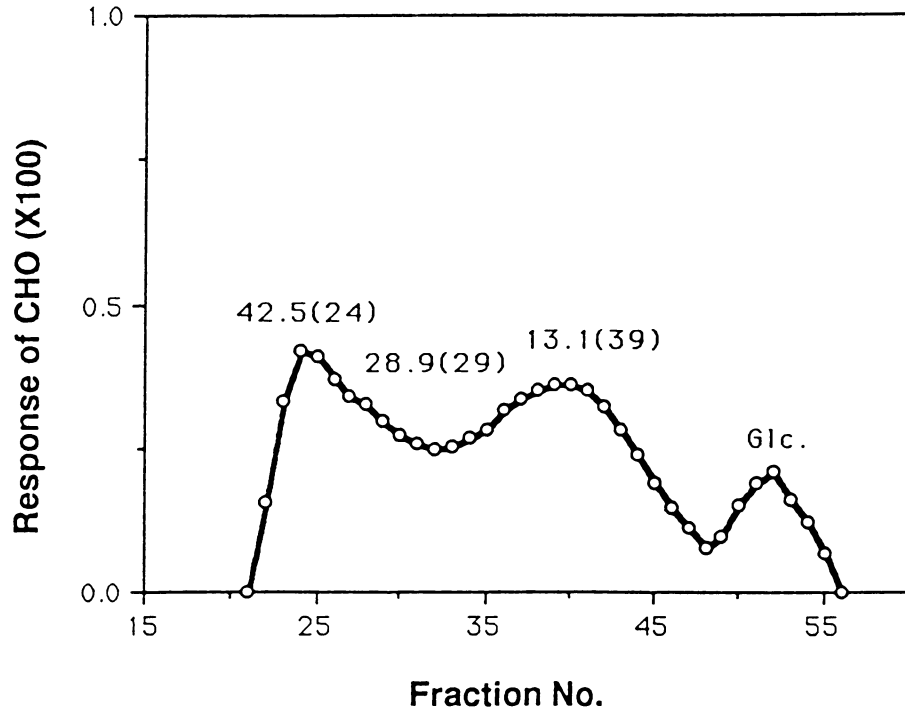


Figure 20. Bio-gel P-6 gel-permeation profiles of amylopectin debranched by isoamylase. Amylopectin was isolated from starch granules remaining after 80% erosion. Glucose (Glc.) was used as a marker. The number at each peak is the average DP of amylopectin in three consecutive peak fractions.

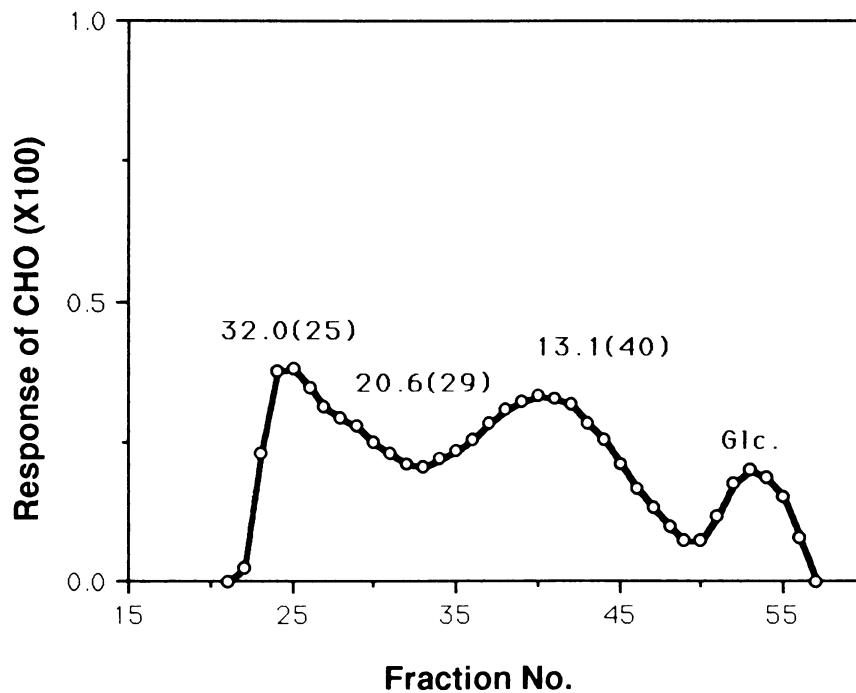


Figure 21. Bio-gel P-6 gel-permeation profiles of amylopectin debranched by isoamylase. Amylopectin was isolated from chemically gelatinized starch (20% erosion). Glucose (Glc.) was used as a marker. The number at each peak is the average DP of amylopectin in three consecutive peak fractions.

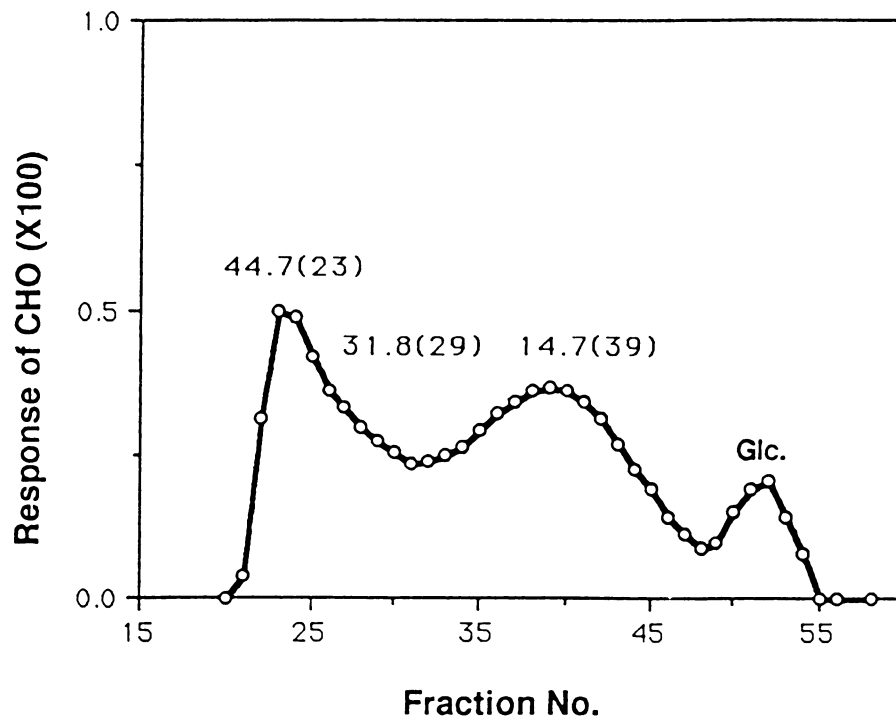


Figure 22. Bio-gel P-6 gel-permeation profiles of amylopectin debranched by isoamylase. Amylopectin was isolated from small granules (<20 μm). Glucose (Glc.) was used as a marker. The number at each peak is the average DP of amylopectin in three consecutive peak fractions.

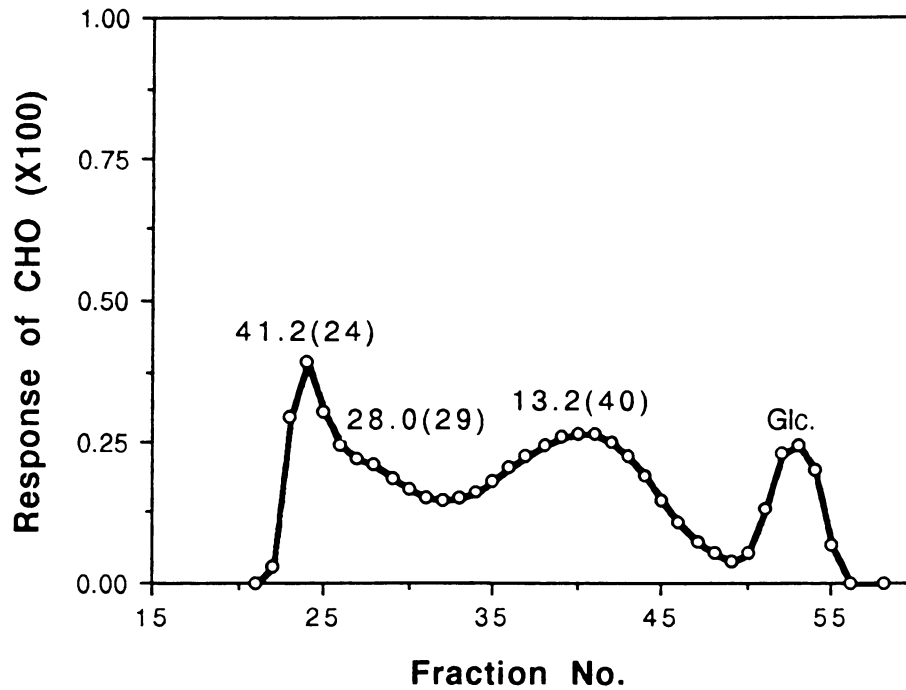


Figure 23. Bio-gel P-6 gel-permeation profiles of amylopectin debranched by isoamylase. Amylopectin was isolated from native potato starch (30-52 μm). Glucose (Glc.) was used as a marker. The number at each peak is the average DP of amylopectin in three consecutive peak fractions.

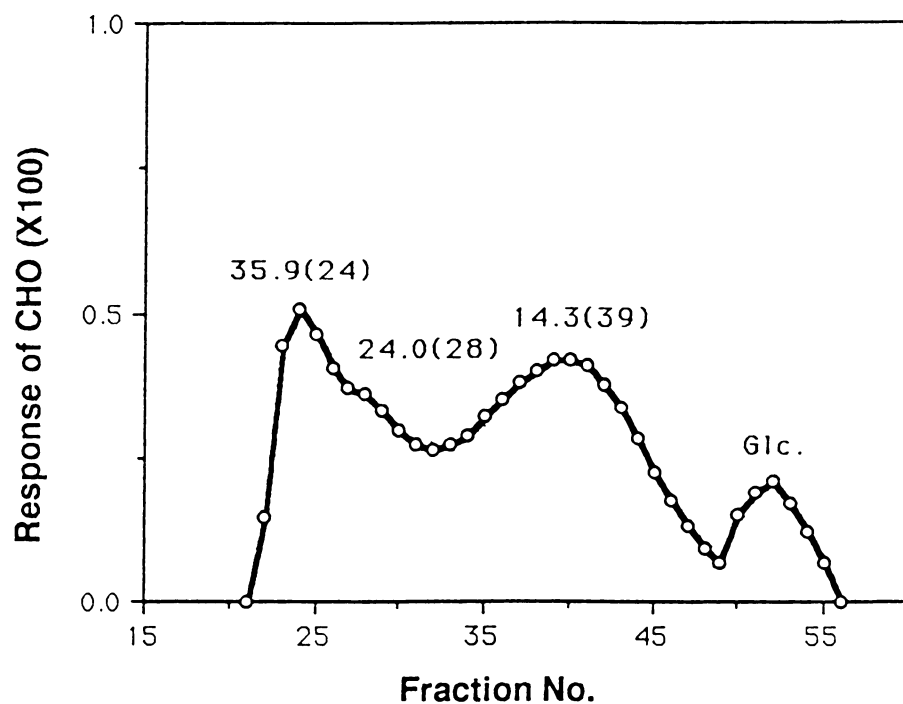


Figure 24. Bio-gel P-6 gel-permeation profiles of amylopectin debranched by isoamylase. Amylopectin was isolated from large granules ($> 52 \mu\text{m}$). Glucose (Glc.) was used as a marker. The number at each peak is the average DP of amylopectin in three consecutive peak fractions.

Table III. Amylopectin branch chain length debranched with isoamylase^a

Amylopectin	Branch Chain Length, DP ^b		
	long chain I	long chain II	Short chain
Starch granule remaining after 80% erosion	42.5 ± 1.8	28.9 ± 1.3	13.1 ± 0.1
Chemically gelatinized starch (20% Erosion)	32.0 ± 0.8	20.6 ± 0.5	13.1 ± 0.7
Small Granule (< 20 μm)	44.7 ± 1.3	31.8 ± 0.8	14.7 ± 0.7
Medium GranuleII (30-52 μm)	41.2 ± 1.8	28.0 ± 1.1	13.2 ± 0.4
Large Granule (> 52 μm)	35.9	24.0 ± 0.4	14.3 ± 0.4

^a Data reported are the averages of duplicate analysis except long chain I of large granule (>52 μm) with one replication.

^b Determined with the three peak fractions, DP = degree of polymerization.

DISCUSSION

It is known that the granule size of potato starch ranges from 10 to 100 μm (Stockham and Fochtman 1977, Allen 1981). Because of the differences in the granule sizes of potato starch, the degree of erosion varied during similar times of chemical erosion. Therefore, it was necessary to use starch of similar granular size for the chemical erosion study. Potato starch was separated into four size fractions and are shown in Figures 9a-d.

Potato starch (medium granule II, 30-50 μm) was used to study the internal structure of normal starch granules. The starch was treated with CaCl_2 solution (4.0 M) and starch molecules at the periphery of the granule were chemically gelatinized. Starch was then separated into two fractions: starch granules remaining after erosion and chemically gelatinized starch. The starch granules remaining after erosion consisted of the starch molecules present at the core of the granule, whereas chemically gelatinized starch consisted of starch molecules present at periphery of the granule.

Starch granules remaining after different degrees of erosion were investigated by using scanning electron microscopy, and the results are shown in Figure 10. starch

granule remaining after 6% erosion (Figure 10a) showed a surface-eroded granule. The surface of the granule was not as smooth as medium granule II (Figure 9c). From the micrograph of the starch granules remaining after 20% erosion (Figure 10b), it was observed that starch granules were no longer intact and the chemically gelatinized starch had been removed from treated starch granules. It also was observed that the size of the starch granules remaining after 20% erosion had a narrow range. The starch granule remaining after 60% erosion is shown in Figure 10c. The result showed a very rough surface of the starch granule and suggested that there might be more binding capacity on the surface of starch granules remaining after erosion. The chemically gelatinized starch was also studied and showed in Figure 10d. The result showed that chemically gelatinized starch had lost its granular structure.

Amylose content of native potato starch was measured as 20.2% (Table I) which was in good agreement with 20.0% reported by Anderson et al. (1955). Results of amylose contents of starch isolated from various radial locations in the granule showed that the starch granules remaining after erosion contained less amylose (18.8% after 80% erosion, 19.7% after 52% erosion) than native starch while the chemically gelatinized starch contained more amylose (21.1% with 52% erosion, 22.0 with 10% erosion) than did native starch. Amylose contents of starch granules remaining after erosion

decreased with an increase in the degree of erosion, while the amylose content of chemically gelatinized starches increased with the increase in the degree of erosion. Amylose contents of starch granules remaining after erosion and of chemically gelatinized starch could deviate by two factors. First, amylose could preferably leach out from the edge of starch granule, and increase the amylose content of chemically gelatinized starch. Second, amylopectin contains long chains which have higher iodine affinity and lead to a higher apparent amylose content. These two factors counteract each other on the amylose contents of treated starch samples. After chemical erosion, it was confirmed that no amylose was leached out from starch granules by analyzing the total carbohydrate in the supernatant of starch-CaCl₂ mixture. In addition, the starch granules remaining after erosion, checked by a polarized light microscope after it was separated from chemically gelatinized starch, showed that the maltose cross birefringence of the starch granules remaining after erosion was retained. Both results suggested that the leaching of amylose from the starch granules remaining after erosion was not likely. Amylose contents of starch granules remaining after erosion and chemically gelatinized starch indicated that there was more amylose located at the periphery of a starch granule. These results were consistent with the amylose content of small (<20 μm) and large (>52 μm) granules. The small granule starch

contained less amylose (17.5%) than the native starch while the large granule showed slightly higher amylose content (20.6%) than native starch. The results were also consistent with the positive correlation between the maturity and the concentration of amylose reported by Boyers et al. (1976). The difference in amylose content between the small granules and native potato starches was greater than that reported in normal barley starch (Macgregor and Morgan 1984).

Phosphorus contents of potato starches of different sizes and locations were analyzed to reveal the distribution of phosphate derivatives in potato starch granules (Table II). Native potato starch and isolated fractions of four sizes of potato starch were analyzed as references. The phosphorus content of native potato starch was 0.081% which agreed well with 0.08% reported by Swinkles (1985). The results of our study showed that starch granules remaining after 50% erosion, had a higher phosphorus content (0.077%) than did chemically gelatinized starch (80% erosion, 0.065%). However, both starch granules remaining after erosion and chemically gelatinized starch showed lower phosphorus contents than present in native potato starch. This might be attributed to the loss of phosphate during the separation of chemically gelatinized starch from starch granules remaining after chemical erosion. It was also found that phosphorus content decreased with the increase of granular size. The results of phosphorus contents

in potato starches of different sizes and location indicated that more phosphate derivatives in potato starch were located at the core rather than at the periphery.

Sepharose CL-2B gel-permeation chromatography was used to study proportions and molecular sizes of amylose, amylopectin and the intermediate components isolated from different radial locations of the granule. Native potato starch was analyzed as reference for comparison (Figure 11). Blue value of the amylopectin peak reflected the branch chain length by increasing with increased branch chain length. Ratios of blue value to total carbohydrate provided information about amylose and the intermediate molecules that existed in the granule. In order to study the effect of CaCl_2 remaining in the starch granule, CaCl_2 -treated native potato starch was analyzed as shown in Figure 12. Starch sample was dissolved in CaCl_2 solution (4.0 M) and washed by 2 L distilled water, exactly the same procedure as used for all erosion studies, but without the further separation of chemically gelatinized starch from starch granules remaining after erosion. In comparing Figure 12 to 11, there was no significant change of the whole pattern of profiles of total carbohydrate and blue value for amylopectin, the intermediate component, and amylose. The results indicated that the effect of CaCl_2 on the chromatographic separation of starch sample was negligible.

The profiles of starch granules remaining after erosion (after 80% erosion), and chemically gelatinized starch (52%, 63% erosion) are presented in Figures 13, 14 and 15. A comparison of these with the profile of native starch (Figure 11), showed that the starch granules remaining after erosion and chemically gelatinized starch presented significantly different elution patterns in their gel-permeation profiles (Figures 13, 14 and 15). In the profile of the starch granules remaining after 80% erosion, the blue value of the amylopectin was extremely high. The profile of the chemically gelatinized starch (52%, 63% erosion) showed that the blue value for the amylopectin peak was very low. The high blue value of the amylopectin peak in the GPC profile of the starch granules remaining after erosion (Figures 13, 14 and 15) suggested that amylopectin at the core had long branch chains, whereas the amylopectin at the periphery had short branch chains. These results were later confirmed by chain length analysis of amylopectin (Table III).

The profile of chemically gelatinized starch (Figure 14 and 15) showed that the blue value of the amylose peak was shifted toward medium size molecular weight with a narrower size distribution compared with those in the profiles of native and granular starches remaining after erosion (Figure 13). In the mean time, the total carbohydrate peak was much greater at the region between the peaks of amylopectin and amylose

(fraction number 40 to 50) with very little blue value, compared with the profile of the starch granules remaining after erosion. We also observed that after fraction 75 (Figure 13), both blue value and total carbohydrate decreased rapidly, indicating that very little of the small molecular sized amylose was present at the periphery of the granule. This suggested that amylose at the periphery had an intermediate molecular size and that a great amount of the intermediate component was present at the periphery.

For reference, the four different size fractions of potato starch were also studied by Sepharose CL-2B gel-permeation chromatography as shown in Figures 16 to 19. Comparing these four profiles, we found that the blue value had appeared at higher molecular weight as the granule size of the starch sample increased. The results indicated that the large granules contain amylose of large molecular weight. It is plausible that in a large granule, amylose molecules get a longer growing period to develop into large molecular size. This was consistent with the amylose isolated from the core of a granule, which was synthesized earlier during the development of the granule and was allowed longer period to mature, had large molecules that were not found at the periphery of the granule.

To reveal the differences in branch chain lengths of amylopectins isolated from various locations, purified

amylopectin samples (by Sepharose CL-2B column) were debranched by using isoamylase and analyzed by Bio-gel P-6 gel-permeation chromatography. The profiles of amylopectins isolated from starch granule after 80% erosion, chemically gelatinized starch (20% erosion), small granule (<20 μm), medium granule II (30-50 μm), and large granule (>52 μm) shown in Figures 20 to 24. The results (Table III) confirmed that amylopectin at the core of the granule had longer long chain I (DP at peak =42.5) and long chain II (DP at peak =28.9) than did the amylopectin at the periphery (long chain I DP at peak =32.0, long chain II DP at peak =20.6). Long chain I and II of small granules (long chain I DP at peak =44.7, long chain II DP at peak =31.8) were also greater than that of large granules (long chain I DP at peak =35.9, long chain II DP at peak =24.0). The result did not show a great difference between A chains of amylopectin according to the location.

SUMMARY

Granular structure of potato starch was studied by a chemical erosion method. Starch with uniform particle sizes were obtained from native potato starch by screening starch through macroporous filter cloths with selected mesh sizes. The starch was then treated with CaCl_2 solution (4.0 M) for different periods of time at 21-22°C to achieve varying extents of surface erosion. The treated starch was then removed, washed and separated by mechanical shearing into two fractions: starch granules remaining after erosion and chemically gelatinized starch, respectively, representing starch molecule components at the core and at the periphery of the granule.

Scanning electron micrographs of screen-separated starch showed a narrow distribution of granular size. SEM studies showed that the surface of starch granules remaining after erosion was rough, and the chemically gelatinized starch had lost its granular structure. Polarized light microscopy showed that the maltose cross birefringence was retained in the starch granules remaining after erosion.

Amylose contents of starch isolated from various radial locations in the granule showed that the starch granules remaining after erosion contained less amylose (18.8% after 80%

erosion, 19.6% after 52% erosion) than chemically gelatinized starch (21.1% with 52% erosion, 21.9% with 10% erosion). The results indicated that amylose was more concentrated at the periphery of a potato starch granule. Phosphorus contents in potato starch of different sizes and locations showed that more phosphate derivatives were at the core rather than at the periphery of the granule.

Sepharose CL-2B gel-permeation chromatography was used to study proportions and molecular sizes of amylose, amylopectin and the intermediate components in the starch isolated from different radial locations of the granule. The results suggested that amylopectin at the core had long branch chains, whereas the amylopectin at the periphery had short branch chains. Amylose at the periphery had an intermediate molecular size, and a significant amount of the intermediate component was present at the periphery.

Branch chain length of amylopectin analyzed by using isoamylase and Bio-gel P-6 column confirmed that amylopectin at the core of the granule had longer long chain I (DP at peak = 42.5) compared with the amylopectin at the periphery (long chain I DP at peak = 32.0). The results did not show any significant difference between short chain of amylopectin according to the location.

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