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STRUCTURE OF STARCH GRANULES

Abstract

Starch is produced in a semicrystalline granule form by higher plants for energy storage. The granule size and granule shape of starch differ with the botanical source. The diameter of starch granule varies from submicron to more than a hundred microns. The shape of the granules include spherical, oval, disk, polygonal, elongated, and kidney. Starch consists of amylopectin, a highly branched molecule, and amylose, primarily a linear molecule with few branches. Biosynthesis of starch is originated at the hilum, and the starch granule development is by apposition. The amylose content of starch granules increases with the maturity and the size of the starch granule, and amylose is found more concentrated at the periphery of the granule. The branch chain length of amylopectin, however, decreases as the granule size increases. Amylopectin molecules at the hilum consist of exceedingly long branches which are loosely packed with little crystallinity. These long branches are susceptible to iodine to develop a blue core in the granule. The outer chains of amylopectin are in a double helical crystalline structure. Starches which consist of amylopectin with longer branch chains (such as potato and high-amylose maize starches) display the B-type X-ray diffraction pattern, whereas those with shorter branch chains (such as wheat, rice, and maize) display the A-pattern. Starches with branch chain length in between (such as tapioca and banana) display the C-pattern.

Amylose in the granule is dispersed among amylopectin. This is evident as amylose molecules are cross-linked to amylopectin, whereas amylose molecules are not found cross-linked among themselves. The molecular size of amylose increases with the increase of the granular size. Most amylose in the starch granule is present in a free form not complexed with lipids; however, about 21% amylose in non-waxy barley starch is present as lysophospholipid complex.

^{31}P-NMR studies have shown that phospholipids are present in all the normal cereal starches investigated but not in tuber, root, and legume starches. With few exceptions (e.g., du waxy maize starch), most waxy starches donot contain phospholipids. Phosphate derivatives are primarily on amylopectin. Studies conducted by using DSC, X-ray, chemical analysis, and ^{31}P-NMR of Naegeli dextrins showed that a substantial proportion of phosphate derivatives were located within the crystalline region of amylopectin and were protected from exhaustive acid hydrolysis.

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Starch is produced by green plants for energy storage and is synthesized in a granular form. Biosynthesis of starch granules takes place primarily in the amyloplast. The biosynthesis of the granule is initiated at the hilum, and the starch granule grows by apposition [1]. Starch granules are densely packed with semi-crystalline structures and have a density of about 1.5 g/cm^3 [2]. Because of this stable semi-crystalline structure, starch granules are not soluble in water. Without gelatinization, starch can absorb water and swell up to 30% of its dry weight. The swelling process is reversible upon drying. The starch granules are effectively stored in seeds, roots, tubers, stems, and leaves. Grain seeds, such as maize kernels, contain up to 75% starch. The stored starch granules can be hydrolyzed by hydrolytic enzymes to glucose, and the glucose is utilized to generate energy during germination and whenever energy is needed. In the granular form, starch can be easily isolated by gravity sedimentation, centrifugation, and filtration, and can be subjected to various chemical, physical, and enzymatic modifications with subsequent washing and processing. Consequently, starch is produced as one of the most economical commodity products.

Starch is a biopolymer and consists of two major components: amylopectin and amylose. Amylopectin is a highly branched molecule, with α 1-4 linked D-glucose backbones and α 1-6 linked branches. Amylose is primarily a linear molecule with α 1-4 linked glucose units. Some amylose molecules, particularly those with large molecular weight, may have up to 10 or more branches [3]. Amylose and amylopectin have different properties. For example, amylose has a high tendency to retrograde and produce tough gels and strong films, whereas, amylopectin, in an aqueous dispersion, is more stable and produces soft gels and weak films. Entanglements between amylose and amylopectin, particularly with the presence of lipids or phospholipids, have been demonstrated to significantly affect the pasting temperature, paste viscosity, stability, and clarity, as well as the retrogradation process.

Other minor components found in starch granules include the intermediate components which have structures in between amylose and amylopectin, starch lipids (including phospholipids), and phosphate monoester derivatives. Phytoglycogen is found in certain varieties of starch, such as the sugary mutant of maize starch. Some of the minor components, such as phosphate monoester derivatives and phospholipids [4], [5], although at low concentrations, can drastically affect the properties of starch pastes and gels. Phosphate monoester derivatives, carrying negative charges at the neutral pH, are found in many starches. Potato starch, consisting of about 0.09% phosphate derivatives, displays extremely high paste viscosity and clarity and a low gelatinization temperature. The unique properties of potato starch are attributed to the charge repelling between the covalently attached phosphate monoester groups.

An understanding of the internal organization of starch granules is crucial for scientists and engineers to optimize reaction conditions for starch chemical, physical, and enzymatic modifications. The knowledge of the internal organization can help us understand the functionalities and the transformation behaviors of starch and improve the properties and stability of starch products. This knowledge will also help biochemists reveal the mechanism by which starch granules are developed during biosynthesis. In this paper, some recent advances in the understanding of the structure of starch granules are reviewed.

Morphology of starch granules

Starches isolated from different botanical sources display characteristic granular morphology [6]. Starch granules have various shapes, including spherical, oval, polygonal, disk, elongated, and kidney shapes. Normal and waxy maize starches are spherical and polygonal in shape. Immature sweet corn starch has a multi-modal size distribution, but starch granules are not found in the mature sweet corn. High-amylose maize starches have elongated and curved rod-shaped granules in addition to polygonal and spherical granules; some granules also have granular appendages. It is not known whether the elongated shape is correlated with the amylose content. Potato starch has oval and spherical shapes. Wheat, triticali, barley, and rye starches have bimodal size distributions. The large (A) granules have a disk shape, whereas the small (B) granules have a spherical shape. Sorghum starch also has a bimodal size distribution, but the shapes are different: large granules of sorghum starch have polygonal and spherical, instead of disk, shapes. Diffenbachia starch has an elongated submarine shape. Shoti starch has a disk shape with sharp edges. Almost all the legume starches have a characteristic indentation on granules of bean-like shapes.

Diameters of starch granules vary from submicron, such as amaranth and small pigweed, to more than one-hundred microns, such as canna starch [6]. Other starches, such as small wheat granules have diameters of 2-3 microns; large wheat granules, 22-36 microns; potato, 15-75 microns; maize, 5-20 microns; rice, 3-8 microns; and legume starches, 10-45 microns [6].

Most starch granules are produced individually in separate amyloplasts; however, some starches, such as rice and oats, have several starch granules produced simultaneously in a single amyloplast. These starches are known as compound starch. The compound starch has granules tightly packed together, which are relatively difficult to separate. The shapes of the compound starch granules are mostly polyhedral, possibly as a result of space constraints during the development of starch granules.

Small-particle starch can be prepared by treating common granular starch with acid to hydrolyze and remove the glucan at the amorphous region [7]. After the acid treat-

ment, the crystallites of the starch granule are detached. With mild attrition, the starch granules break into small particles. The size of the small-particle starch can be in submicrons and is dependent on the conditions of the acid hydrolysis; the more extensive acid hydrolysis, the smaller particle sizes are produced. The small-particle starch displays irregular shapes with strong birefringence when viewed under a polarized light-microscope. The small-particle starch produced from normal maize starch also displays an enhanced A-type X-ray crystallinity.

Structures and locations of amylose in the starch granule

Amylose is easily leached out from swollen granules at a temperature slightly above the gelatinization. Amylose also does not contribute to the total crystallinity of starch granules. All the results indicate that amylose is present in the amorphous region of the granule. Recent studies conducted by Morrison and co-workers [8] by using solid ^{13}C-NMR have shown that up to 21% of amylose in the granule is complexed with lipids at a single helical conformation, and the remaining amylose is in a random coil conformation free of lipids.

Two hypotheses were proposed regarding where and how amylose was located in the granule relative to amylopectin. One was that amylose must be present in a separate compartment from amylopectin; thus, amylose was not susceptible to branching enzyme reactions. The other was that amylose was dispersed among amylopectin; thus, the two molecules are intertwined to hold the integrity of the granule. To test if amylose molecules are present in close proximity in the granule, cross-linking reactions of starch granules were conducted by using various cross-linking reagents of different molecular chain length [9], [10].

Epichlorohydrin (ECH), adipic/acetic anhydrides, and phosphoxy chloride were used at low concentrations (e.g., 0.013% to 0.13%, ECH/starch=w/w), to cross-link native granular starch [9]. The cross-linked starch was then subjected to gel permeation column chromatography to analyze if amylose molecules were cross-linked among themselves which would increase the molecular weight. Results, however, showed that amylose was cross-linked onto amylopectin and co-eluted with amylopectin at the void volume, as indicated by the increase in blue-value of the amylopectin peak. The molecular size of the amylose peak did not increase after cross-linking, indicating that amylose molecules were not cross-linked among themselves. Analyses of the phosphoxy chloride cross-linked starch, by using ^{31}P-NMR, showed that the amylose isolated from the cross-linked starch had only phosphate monoester derivatives but no phosphate diesters (cross-link) [10]. The monoester derivatives of the amylose are responsible for the resistance of the amylose isolated from cross-linked starch to enzyme hydrolysis (Table 1). All these

results suggest that amylose is located adjacent to or intertwined with amylopectin but not in close proximity with other amylose molecules.

Studies of amylose biosynthesis have demonstrated that amylose is synthesized by granular-bound starch synthase, whereas amylopectin is synthesized by soluble starch synthase [11]. Because amylose is synthesized by the granular-bound enzyme, the amylose molecule is likely confined in the granule and has little opportunity to form double helices with other starch molecules to form branches. This biosynthetic pathway also excludes the hypothesis that amylose molecules are separated from amylopectin.

Table 1

Percentage of β-amylolysis of amyloses isolated from native and cross-linked granular corn starch with and without prior isoamylase treatment[abc]

Treatment	Native Starch	Cross-linked[d] Starch
β-amylase (30 units)	64.6%	53.7%
β-amylase (90 units)	62.8%	54.8%
Isoamylase (328 units) β-amylase (30 units)	85.1%	56.7%

[a] Calculated on the basis of total carbohydrate present in maltose peak separated by the Sephadex G-25 column.

[b] Amylose was isolated with the Sepharose CL-2B column.

[c] Amylose (15 mg) in 15 ml acetate buffer solution (pH5, 20mM), conditions are given in Material and Methods.

[d] Cross-linked with 0.25% ECH (ECH/starch = w/w).

Amylose contents of starch granules increase with the maturity and the increase of the granule size (Table 2). Studies conducted by using surface gelatinization of starch granules have shown that amylose molecules isolated from the core of the granule have substantially larger molecular sizes than those at the periphery [12]. These results are consistent with results of starch granules of different granular sizes and maturities. The structural feature of having a large concentration of small-molecular-weight amylose present at the periphery coincides with the phenomenon that small-molecular amylose leach out from the granule soon after starch gelatinization.

Structures and locations of amylopectin in the granule

Amylopectin is a highly branched molecule. There are three types of branch chains. A-chains are linked with other chains (B- or C-) by the reducing ends through α 1-6 bonds, but A-chains do not carry other chains. B-chains are similarly linked to another B-

chain or C-chain, but B-chains also carry A-chains or other B-chains at the C-6 of the glucose unit. Each amylopectin molecule has only one C-chain which carries the sole reducing end of the molecule.

Table 2

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

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

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

Branch chain length of amylopectin varies with the origin and maturity of the starch and the location of molecules in the granule. Hizukuri and co-workers [13] surveyed a wide variety of starch and reported average chain lengths of the starches. It is known that amylopectin makes up the crystalline structure of starch, whereas amylose is in the amorphous form. Starches isolated from potato and high-amylose maize, which comprise long branches, display the B-type x-ray pattern. Other starches isolated from normal and waxy maize and wheat, which comprise short branches, display the A-type pattern. Starches isolated from banana and tapioca, which have average branch chain length in between the A and B starches, display the C-pattern.

Nikuni [14] and French [15] independently proposed the cluster model of amylopectin, in which the branch points are located in clusters and the branch chains are present in double helical crystalline structure. The structure of the amylopectin molecule consists of alternating crystalline and amorphous regions. Kassenbeck [16] studied enzymatically treated starch granules and reported a repeating distance of 70 Å. Yamaguchi

et al. [17] examined wet meshed and acid hydrolyzed waxy maize starch by using transmission electron microscopy and reported a repeating distance of 70±10 Å and lamellae thickness of about 50 Å. The authors proposed that alternating crystalline and amorphous regions of 50 Å and 20 Å, respectively, are arranged in the amylopectin structure of waxy maize starch.

Blanshard et al. [18] observed a Bragg peak at approximately 100 Å by using small-angle neutron scattering studies of starch granules. The Bragg peak disappeared on gelatinization. Recent studies of amylopectin structural periodicity by using small angle X-ray scattering have shown a constant repeating distance of between 8.7 and 9.2 nm for the six starch varieties examined [19]. Results obtained from these two scattering studies show larger repeating distances than those reported by Kassenbeck and by Yamaguchi et al. Blanshard et al. [18] attributed the difference to the drying process of starch samples required for the electron microscopy. There may be shrinkage of starch structures during the drying.

This consistent distance of about 9 or 10 nm coincides with the distances of amylose double helical crystallites and the lamellar distance of amylose single helical complex formed during the biosynthesis, about 10 nm [20]. Whether the repeating distance of amylopectin is controlled by the size of double helix is of great interest to study. Further studies may reveal the mechanism of starch biosynthesis and granule development.

To investigate the structures of amylopectin molecules at different radial locations (e.g., the core and the periphery) of the granule, amylopectin in various starch fractions, separated by surface gelatinization using neutral salt solutions, was isolated and analyzed [12]. Results showed that amylopectin isolated from the core, close to the hilum, has substantially longer long-B branches compared with the amylopectin molecules isolated from other parts of the granule. The amylopectin isolated from the periphery of the granule has the shortest long-B chains (Table 3). These results indicate that the branching frequency increases at the periphery. When stained with iodine, the very long amylopectin branch chains at the hilum results in the dark blue core of waxy barley and waxy potato starch granules.

Starches of the B-type X-ray pattern, such as potato and high-amylose maize, are more resistant to enzyme hydrolysis [21]. Whereas those starches of the A-type are more susceptible to enzyme hydrolysis [21]. The B-type X-ray diffraction corresponds to an orthogonal unit cell packing of starch double helices, which consists of an open channel at the center. The A-type starch has a hexagonal unit cell packing [2]. It is puzzling of how such small differences in the average chain lengths of amylopectins can affect the packing of A- and B-types of starch and resulting in substantially different enzyme digestibility. For example, normal maize amylopectin (DP 19.5) and sweet potato amylopectin (DP 20.4) display the A-type pattern, whereas potato amylopectin (DP 22.4) and

Table 3

Amylopectin branch chain length debranched with isoamylase[a]

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

[a] Data reported are the averages of duplicate sample and chemical analyses except long chain of large granule (>52 μm) with one sample replication and duplicate chemical analysis.

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

[c] Diameter

Tulip (DP 20.9) display B-type pattern [13].

Recent studies conducted in our lab by using Naegeli dextrins of normal maize and potato starches [22] showed that after hydrolysis with 16% sulfuric acid for one, two and three months at 22°C, normal maize Naegeli dextrins consisted of substantially more branches than their potato counterparts. In the early stage of the acid hydrolysis, normal maize starch had a higher degradation rate than potato counter parts. X-ray differential patterns of potato Naegeli dextrins showed increases in reflection peak intensity as the hydrolysis progressed, whereas those of normal maize showed decreases in amorphous background but no significant increase in peak intensity. The results suggested that potato amylopectin had more branches located in the amorphous regions which were more susceptible to acid hydrolysis. Normal maize amylopectin, however, had branch points scattered in both the amorphous and the crystalline regions. After extensive acid hydrolysis, normal maize Naegeli dextrins consisted substantial branches compared with potato counterparts. With the highly clustered branch points, potato amylopectin branch chains could form double helices of less interruption, which are more resistant to enzyme hydrolysis. In addition, the double helices could be arranged into a more perfect crystalline structures after acid hydrolysis which removed the molecular constraint caused by branch structures. With the presence of scattered branch points within the crystallin regions, normal maize amylopectin had less perfect double helical structures. The double

helices of the normal maize starch, containing branches, were more susceptible to enzyme attack.

Phosphorus structures and locations in the granule

Phosphorus in starch is mainly present in two forms: phosphate-monoesters and phospholipids [4, 5]. The two constituents have opposite effects on starch paste properties. Phosphate monoesters, present in potato and other starches, increase the paste clarity and paste viscosity, whereas, phospholipids, found in cereal normal starches, such as wheat, rice, and maize, decrease the paste clarity and viscosity. ^{31}P-NMR spectroscopy has been developed as a useful method to determine the structures and contents of phosphorus in starch [23, 24].

Starch phosphate monoesters in native starches, such as potato and rice, are primarily found in amylopectin [4, 5]; only a trace is found in amylose. About 61% phosphate monoester in potato starch is on the C-6, 38% on the C-3, and possibly 1% on C-2 of the glucose unit. Whereas 80-90% phosphate-monoester in waxy rice starch is on C-6 of the glucose unit [5]. Takeda and Hizukuri [25] reported that potato amylopectin contains one phosphate monoester per 317 glucose units, equivalent to one phosphate in 13 branch chains. The phosphate groups are present in long branch chains (long B-chains, DP about 42) and located more than nine glucosyl residues away from branch points.

Recent studies of our research group showed that Naegeli dextrins of potato starch consisted of a substantial amount of phosphorus (65% remained after 3 months hydrolysis). ^{31}P-NMR spectra showed the structure of the phosphorus in the potato Naegeli dextrins remained the same as that of native potato starch with and additional residual peak of glucose-6-phosphate, a product of acid hydrolysis. This result showed that phosphate-monoester derivatives were present in the crystalline region of potato starch and were protected from acid hydrolysis. The phosphorus content of potato starch was also found inversely proportional to the crystallinity of the starch, which was consistent with that potato starch gelatinization enthalpy-changes decrease with the increase of phosphate content [26].

REFERENCES

[1] Badenhuizen N.P., Dutton R.W.: Protoplasma, **XLVII**, 1956, 156-163.
[2] Sarko A., Wu H.C.H.: Starch/Staerke, **30**, 1978, 73.
[3] Hizukuri S., Takeda Y., Yasuda M., Suzuki A.: Carbohydr. Res., **94**, 1981, 205.
[4] Schoch T.J.: J. Amer. Chem. Soc., **64**, 1942, 2954.
[5] Hizukuri S., Tabata S., Nikuni Z.: Staerke, **22**, 1970, 338.
[6] Jane J., Kasemsuwan T., Leas S., Zobel H.F., Robyt J.F.: Starch/Staerke, **46**, 1994, 121.
[7] Jane J., Shen L., Wang L., Maningat C.C.: Cereal Chem., **69**, 1992, 280.
[8] Morrison W.R., Tester R.F., Snape C.E., Law R., Gidley M.J.: Cereal Chem., **70**, 1993, 385.

[9] Jane J., Xu A., Rodosovljivic M., Seib P.A.: Cereal Chem., **69**, 1992, 405.
[10] Kasemsuwan T., Jane J.: Cereal Chem., **71**, 1994, 282.
[11] Shannon J.C., Garwood D.L., in: Starch: Chemistry and Technology. 2nd Ed. Eds. Whistler, R. L., BeMiller J.N., Paschall E.F., Academic Press. Orlando, FL, 1984, p. 25.
[12] Jane J., Shen J.J.: Carbohydr. Res., **247**, 1993, 279.
[13] Hizukuri S., Kaneko T., Takeda, Y.: Bioch. Biophysica Acta, **760**, 1983, 188.
[14] Nikuni Z.: Starch/Staerke, **30**, 1978, 105-111.
[15] French D.J.: Jpn. Soc. Starch Sci., **19**, 1972, 8-25.
[16] Kassenbeck V.P.: Starch/Staerke, **30**, 1978, 40.
[17] Yamaguchi M., Kainuma K., French D.: J. Ultrastruc. Res., **69**, 1979, 249.
[18] Blanshard J.M.V., in: Chemistry and Physics of Baking, Blanshard, J. M. V. ed.; Royal Soc. of Chem., London, 1986, p. 1.
[19] Jenkins P.J., Cameron R.E., Donald A.M.: Starch/Staerke, **45**, 1993, 417.
[20] Jane J., Robyt J.F.: Carbohydr. Res., **132**, 1984, 105.
[21] Fuwa H., Takaya T., Sugimoto Y., in: Mechanisms of saccharide polymerization and depolymerization. Ed. Marshall, J.J., Academic Press, New York, NY, 1980, p. 73.
[22] Kainuma K., French D.: Biopolymers, **11**, 1972, 2241.
[23] Lim S.-T., Kasemsuwan T., Jane J.: Cereal Chem., **71**, 1994, 488.
[24] Kasemsuwan T., Jane J.: Cereal Chem. In revision.
[25] Takeda Y., Hizukuri S.: Carbohydr. Res., **102**, 1982, 321.
[26] Muhrbeck P., Eliasson A.-C.: J. Sci. Food Agric., **55**, 1991, 13.

STRUKTURA GAŁECZEK SKROBIOWYCH

Streszczenie

Omówiono strukturę polisacharydów skrobiowych, morfologię gałeczek skrobiowych, rozmieszczenie amylozy i amylopektyny w gałeczkach oraz postać i umiejscowienie w nich fosforu.