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Carbohydrate Polymers 63 (2006) 555–560

Carbohydrate Polymers

www.elsevier.com/locate/carbpol

Short communication

Molecular arrangement in blocklets and starch granule architecture

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Received 4 July 2005; received in revised form 21 September 2005; accepted 6 October 2005
Available online 1 December 2005

Abstract

The main hypotheses and the data regarding the starch granule structure and behaviour were gathered and considered comprehensively in this paper. The starch molecules such as amylopectin, amylose and intermediate materials, the non-starch molecules such as bound phosphates and lipids, and the crystal dimensions etc. their roles were demonstrated in the architectures of blocklet and granular ultrastructure. A normal blocklet is mainly constructed by the crystalline and amorphous lamellaes that are formed with the clusters of amylopectin molecule(s). The reducing terminal of the amylopectins in the blocklets may be toward an equal course. However, the defective blocklet production may be due to the participation of lower branching molecules such as amylose and intermediate materials. From the viewpoint of the physicochemical properties of the starch granules, the blocklet of two types may be arranged into the two formations, heterogeneous shell and homogenous shell. The amylopectin plays main role in blocklet architecture, while the other component is important in contributing to the strength and flexibility of starch granule.

Keywords: Blocklet ultrastructure; Granular ultrastructure; Heterogeneous shell; Homogenous shell

1. Introduction

Starch is a major polysaccharide reserve in green plants, and is lain down in the form of granules. The starch granule has been submitted to structural investigations since the invention of the microscope. Concentric circles can be observed for most starch granules with general optical microscopy. When observed under polarized light, most starch granules show a Maltese cross, as a consequence of their crystallinity (Gallant, Bouchet, & Baldwin, 1997). Native starch granules show three forms of crystalline structures, referred to as the A, B, C type according to X-ray diffractometry, and vary in their crystallinity from 15 to 45% (Zobel, 1988). Thus, crystallinity is not the principal mode of organization of the starch granule polymers. However, the crystallinity plays a critical role in the starch granule architecture and physicochemical characteristics, such as the susceptibility to enzymes and the indissolubility in cold water. The starch granule crystallinity is associated with the amylopectin component.

Since the quantitative separation of amylose and amylopectin was carried out based on the difference of solubility to 1-butanol by Schoch (1942), the procedures to fraction starches were modified by other workers (Adkins & Greenwood, 1969; Klicinec & Thompson, 1998; Lansky, Kooi, & Schoch, 1949; Takeda, Hizukuri, & Juliano, 1986; Wang & White, 1994; Whistler & Doane, 1961; Wilson, Schoch, & Hudson, 1943). An intermediate material, the material that is precipitated with isoamyl alcohol and 1-butanol, but not with 1-butanol alone, is defined. The intermediate materials varied from 4 to 9% in normal and high amylose starches (Adkins & Greenwood, 1969; Klicinec & Thompson, 1998; Whistler & Doane, 1961). In other words, amylopectin is the predominant fraction obtained with alcohol precipitation. However, the amylopectin content is usually higher than its crystalline level in starch granules (Tang, Ando, Watanabe, Takeda, & Mitsunaga, 2000, 2001a,b; Martin & Smith, 1995; Tang, Watanabe, & Mitsunaga 2002a,b).

In answer to this question, Nikuni (1969) proposed a model based on the clustering organization. The representation of the starch layers constituted by groups of clusters made of short chains was an ideal key for further models (French, 1972; Hizukuri, 1986). Hizukuri's model (1986) classified the B chains according to the number of side chain clusters in which they participate. As the most important achievement of the model, the correlation among the chains of different lengths is made clear. The A-type crystallinity contains more abundant and shorter A and B1 chains, and has a ratio of short to long chains of 9–13:1 in molar terms (Hanashiro, Tagawa,

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0144-8617/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2005.10.016

Shibahara, Iwata, & Takeda, 2002; Tang et al., 2001a,b; Tang et al., 2002a), while the B-type crystallinity has more abundant connecting B chains (B2 and B3) and longer A and B1 chains, and a ratio of short to long chains of 2–7:1 (Hanashiro et al., 2002). However, those of the C-type crystallinity are intermediate between the A- and B-types (Hanashiro et al., 2002; Tang et al., 2002a). Furthermore, molecular modeling has shown that the organized molecular structure of glucans with α-(1,4) linkages, having the lowest energy, is based on double helices, regardless of the allomorphic type (A- or B-) (Imberty, Chanzy, Pérez, Buléon, & Tran, 1988). The differences between the two allomorphs relate to the packing of double helices in the crystal unit cell and the quantity of water molecules stabilizing these double helices (Imberty, Chanzy, Pérez, Buléon, & Tran, 1987; Imberty & Pérez, 1988). Although the local structures of the amylopectin molecule, such as the 2D arrangement of the branched chains, the proportion of long chains to short chains, and the 3D arrangement of the double helix chains have been elucidated, a general 3D image of the amylopectin molecule has not yet been stated clearly.

Since the hypothesis of the cluster structure was proposed, the cluster is attracted to explain the starch granule architecture. As a representative, the length of the amylopectin molecules is believed to correspond to the thickness of the growth rings of the starch granule, and is composed of 10–40 clusters (∼15 nm wide) (Jenkins & Donald, 1995; Martin & Smith, 1995). However, the observations of Tang et al. (2002a) are not in agreement with the hypotheses. The thickness of the growth rings may depend on other factors. New evidence now shows that a structure level, termed the ‘blocklet’ exists between the macromolecules and the organization of starch granules (Baker, Miles, & Helbert, 2001; Gallant et al., 1997; Ridout, Parker, Hedley, Bogracheva, & Morris, 2003; Szymoska & Krok, 2003). We gathered the main hypotheses and data regarding the structure and behaviour of starch granules in this paper, and considered comprehensively the architecture of the starch granule.

2. Correlation among blocklet and starch components

The blocklet has a very much asymmetric structure according to atomic force microscopy (AFM), and an axial ratio of 2 or 3:1, with a maximum length of about 130–250 nm for pea starch granules (Ridout et al., 2003), 20–50 nm for potato starch granules (Szymoska & Krok, 2003), and 10–30 nm for corn starch granules (Baker et al., 2001). From the studies, conclusions can been drawn as follows: (1) blocklet structure is similar in shape but differs in size with the plant; (2) in the same plant, most of the blocklets are similar in size although they do range; (3) the blocklet is continuous throughout the granule; (4) the blocklet sizes may not relate to their granular sizes and the thickness of growth or amorphous rings; (5) the blocklet production may have ‘defects’ in the amorphous rings, and is assembled loosely; (6) an interconnecting matrix surrounding groups of blocklets (blocklet complex) exists; (7) the growth rings and amorphous rings are not always continuous structures. However, there is almost no information from the inside of discrete blocklets, because a complete method that isolates the blocklet from starch granules has been not established.

According to the size and distribution of blocklets within starch granules, and the proportion and structural characteristics of the amylopectin molecule, the blocklet is believed to be majorly organized by the crystalline and amorphous lamellae from the amylopectin (Gallant et al., 1997). Amylopectin differs greatly with the plant source, and ranges around 4000–40,000 residues for the number-average degrees of polymerization (DPn), and 180–1800 chains for the average number of chains per molecule (NC) (Takeda, 1993; Tang et al., 2002a; Tang, Mitsunaga, & Kawamura, 2004). Furthermore, the molar-based distributions and DPn of amylopectins from maize, rice, sweet potato and potato, were recently determined by fluorescent labeling of the reducing terminal with gel-permeation HPLC (F-GPC) (Takeda, Shibahara, & Hanashiro, 2003). Resultingly, the amylopectins were found to contain three molecular species, large, medium and small species, and are 13,400–26,500, 4400–8400 and 700–2100 residues for DPn, and are around 630–1260, 200–400 and 40–95 chains for NC, respectively. As for the proportion, the large, medium and small species are 43–63%, 16–28% and 19–38% based on molar amounts, respectively. However, the medium and small species are 8–15% and 1–4% by weight, and the small species in particular are almost negligible. The molecular sizes of large species also do not range within the same starch. The three species are believed to be essentially similar in cluster structure, although differ in the number of clusters per molecule (Takeda et al., 2003). These may be the important molecular foundations of the production of the various blocklets, and the reason why most of blocklets are similar in size for the same plant by AFM. Therefore, finding how many molecules compose a discrete blocklet is the most essential problem.

According to the dimensions of the crystal unit cell containing two double helices (Imberty & Pérez, 1988; Imberty et al., 1988), the cross-sectional area of the crystal unit cell is around 2.1 nm^2 in the A-type structure, and around 3.0 nm^2 in the B-type structure, respectively. Thus, the average volume of two double helices crystallized (contain branch point) is around 19.0 nm^3 for the A-type and 27.0 nm^3 for the B-type, respectively, which is calculated from the cross-sectional area of the crystal unit cell and the size (as 9.0 nm) of the crystalline plus amorphous lamellae determined by small angle X-ray scattering (SAXS) (Jenkins & Donald, 1995; Yuryev, Krivandin, Kiseleva, Wasserman, Genkina, & Fornal, 2004). As for a spheroid in the axial ratio of 2:1 with a maximum length (Ridout et al., 2003), if its short axis diameter is 10–30 nm, their volumes are around 1000–30,000 nm^3, conservatively calculated. Thus, the blocklets should contain around 210–6300 chains for the A-type, and around 140–4400 chains for the B-type, respectively. By way of example, if a potato blocklet is of intermediate size (30 nm in its short axis diameter), it consists of at least six amylopectin molecules with respect to the calculation. Because the large species of potato amylopectin (cv. Eniwa) consists of 16,100 glucose residues, it contains around 730 chains in NC (Hanashiro et al., 2002; Takeda et al., 2003). Thus, the blocklet structure may generally contain several amylopectin molecules. But, when the wideness of an amylopectin cluster is presumed (Jenkins & Donald, 1995; Martin & Smith, 1995), it may be not sufficient for the formation of the blocklet structure, although the clustering concept remains important.

Furthermore, according to the principle of lowest energy, all the chains running through the crystalline and amorphous lamellae should exist in double helices or single helix form except in the neighborhood of branch points and the linear chains of like-amylose, even if it is not crystalline. This is supported by a ^{13}C NMR study from the solid state, in which the level of the helical order in starch granules is often markedly greater than the extent of the crystalline order (Gidley & Bociek, 1985). It is also believed that the chance of helix formation contributes completely during starch biosynthesis (Martin & Smith, 1995; Nakamura, 2002). To crystallize well, the amylopectin molecules in the blocklet structure should be arranged in equal course. The length of the short chains (thickness of crystalline lamellae), the proportion of long chains to short chains, DPn, NC and the dimensions of the crystalline structure, etc. are believed to be objective and repeatable evaluations for amylopectin. The framework of the normal blocklet structure on the basis of the major factors is therefore proposed (Fig. 1).

The longest chains of like-amylose (LC, 200–4000 glucose residues) differ from the connecting B2–B4 chains in the amylopectin molecule, and are the side chains as well as A and B1 (Hizukuri, Takeda, Maruta, & Juliano, 1989; Takeda, 1994; Tang et al., 2001b, 2002a). Normal amylopectins have an LC of ∼13%. The LC may span several blocklets to construct a more durable organization of the granule based on its length. Furthermore, the average thickness of the semi-crystalline growth rings in wheat starch granules increases with increasing amylose content (Yuryev et al., 2004). Generally, amylose seems to be richer in the periphery than in the center of the granules (Hizukuri, 1996). Amylose may disrupt the structural order within the amylopectin crystalline structure (Jenkins & Donald, 1995). Thus, the linear amylose is associated easily with connecting blocklets to the same extent as the LC. However, because the branched amylose and intermediate materials are not elucidated in the location of the starch granule, lower branching molecules are not considered in our model. The defective blocklet produced may be due to these 'defective' molecules in the structure. This is because the proportion of shorter chains able to participate in double helix formation may be smaller (Craig, Lloyd, Tomlinson, Barber, Edwards, & Wang, 1998; Tang et al., 2002a). In addition, because the length of the double helices is not even, the defective structure may form. However, most of the non-branching or lower branching molecules may localize among the blocklets, and serve to connect the blocklets, and contribute to the strength and flexibility of the starch granule. This is because the starch granules with a lower amylose content easily deteriorate physically compared with the normal starch granules (Ridout et al., 2003).

Potato amylopectin contains ∼1000 ppm of bound phosphates, and it has the highest content of those that have been investigated (Hizukuri, 1996). The covalently bound phosphates exist in phosphate ester groups at C-6 or C-3 on the outer chain of amylopectin (Hizukuri, 1996). Thus, the phosphates are believed to exist on the surface of the blocklet to increase the useful hydrophilicity among the blocklets. Lipids are also believed to exist in an amylose-complex and free form in the granule, and are more abundant in cereal starch than in rhizome and legume starches (Hizukuri, 1996). The lipids may contribute to the stability in helical structure and blocklet structure (Gallant,

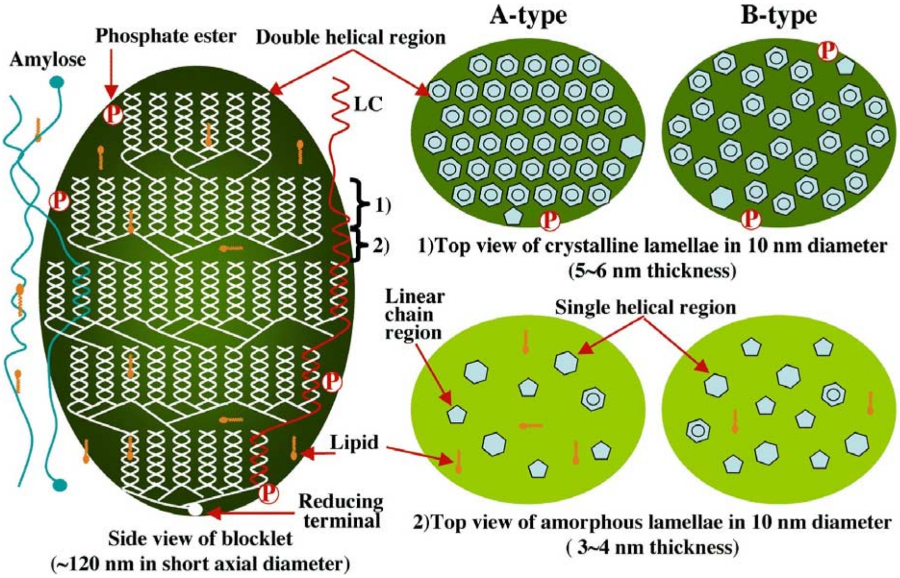

Fig. 1. Scheme of normal blocklet structure in starch granules.

Bouchet, Buléon, & Pérez, 1992; Zobel, 1988). Other components, such as protein have not been elucidated in term of the location of the granules and are not considered in our model.

3. Starch granules and susceptibility to enzymes

According to SEM, TEM, enzyme degradation studies and AFM, although the thickness and hardness of the growth rings differ with the plant starch, the thickness tends to decrease toward the edges of the granules, and the hardness tends to increase (Baker et al., 2001; Gallant et al., 1992; Kimura & Robyt, 1995; Lauro, Forssell, Suortti, Hullenman, & Poutanen, 1999; Ridout, 2003; Szymoska & Krok, 2003; Tang, Yoshida, Watanabe, & Mitsunaga, 1998; Tang et al., 2002a,b; Vasanthan & Bhatty, 1996). There are no discernable growth rings at the centre of the granule near the hilum (Ridout, 2003; Tang et al., 2002a,b). In enzyme degradation studies, the hydrolytic forms of starch granules are similar even if they use glucoamylase, or α- or β-amylase (Gallant et al., 1992; Kimura & Robyt, 1995; Lauro et al., 1999; Tang et al., 1998; Vasanthan & Bhatty, 1996). Thus, the hydrolysis of starch granules does not relate to the characteristics of the enzyme, but rather, the structural characteristics of the granule (Tang et al., 2002a). However, the debranching enzyme (DBE) was an exception (Fig. 2). We observed large starch granules of waxy barley (Hordeum vulgare L. emed., 'Yonezawa No.2' sixth-rowed) with SEM in this paper. The starch granules were treated with β-amylase (2 units/mg starch) and/or isoamylase (14 units/mg starch) for 1 week following the procedure of the previous report (Tang et al., 2002a). The images of SEM (Fig. 2C) of the granules treated with β-amylase and isoamylase were the same as those of our previous report (Tang et al., 2002a,b). In the case of β-amylase alone (Fig. 2B), although the hydrolytic form of the granules was similar to those in Fig. 2C, a like-thread waste was observed, perhaps due to an indisintegrable macromolecular dextrin that subsided together when collecting starch granules with ethanol. However, the granules were not degraded at all in the case of isoamylase alone (Fig. 2A). The results showed that the 1,6-linkages of amylopectin were not revealed on the surface of the granules. This corresponded to the fact that the starch granule grows up from the granule hilum (Martin & Smith, 1995). Thus, it may be important supporting evidence that the reducing terminal of amylopectin molecules in the blocklets is more toward the hilum of the granules.

During β- and iso-amylolysis (Tang et al., 2002a), quinoa starch granules are degraded rapidly from the outer layer, and show a homogeneous organic density observed with TEM. Adzuki starch granules show a homogeneous and highly dense organization, and are strongly resistant to the enzymes but are less resistant at the point where fusion forms a compound granule. Barley starch granules have very specific zones, which rapidly become pitted as well as wheat, rice, corn and cassava starch granules by enzymes, and display a heterogeneous organic density, and a clear network structure when observed with TEM. Potato starch granules are progressively eroded by exo-corrosion with α-amylolysis (Gallant et al., 1992). They do not apparently undergo the formation of surface pores to the same extent as quinoa starch granules (Tang et al., 2002a), but rather strongly resist the enzyme. Amylomaize starch granules appear to be undigestible externally (Gallant et al., 1992). Starch granules that strongly resist enzymes have longer amylopectin side chains, and thicker growth rings, except for wrinkled pea starch granules (Gallant et al., 1992). Although starch granules are variable and complex in their architecture,

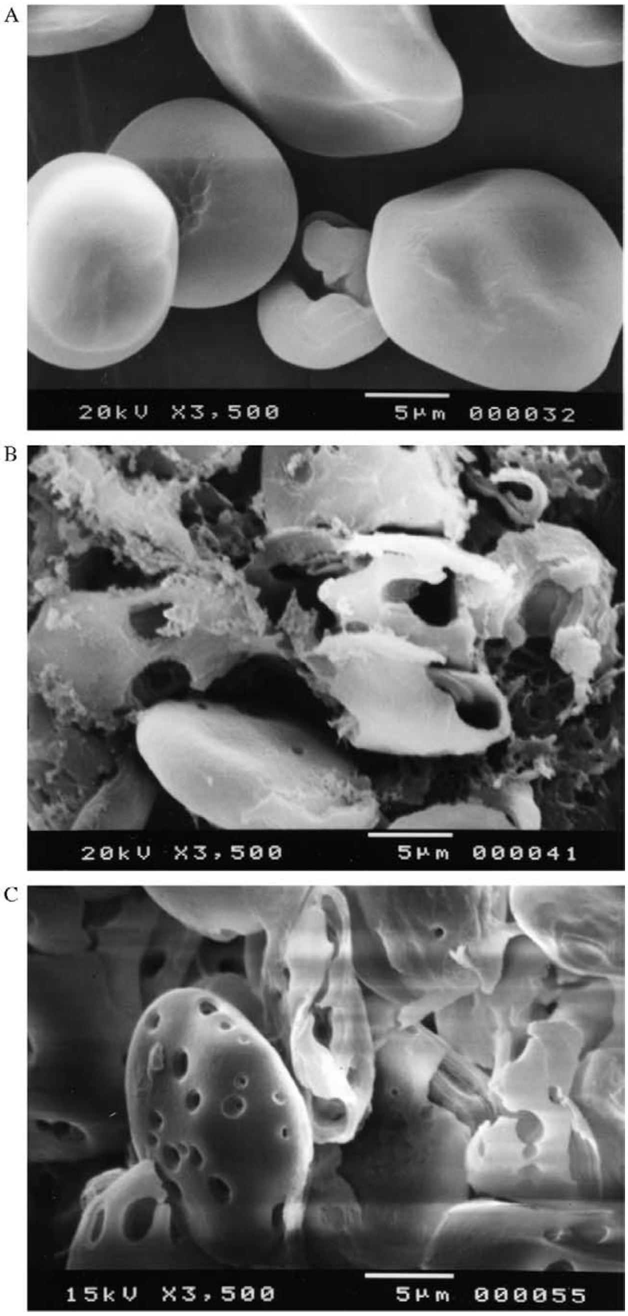

Fig. 2. SEM of waxy barley starch granules with enzymes. A was treated with isoamylase (14 units/mg starch) for 1 week. B was treated with β-amylase (2 units/mg starch) for 1 week. C was treated with isoamylase (14 units/mg starch) and β-amylase (2 units/mg starch) for 1 week.

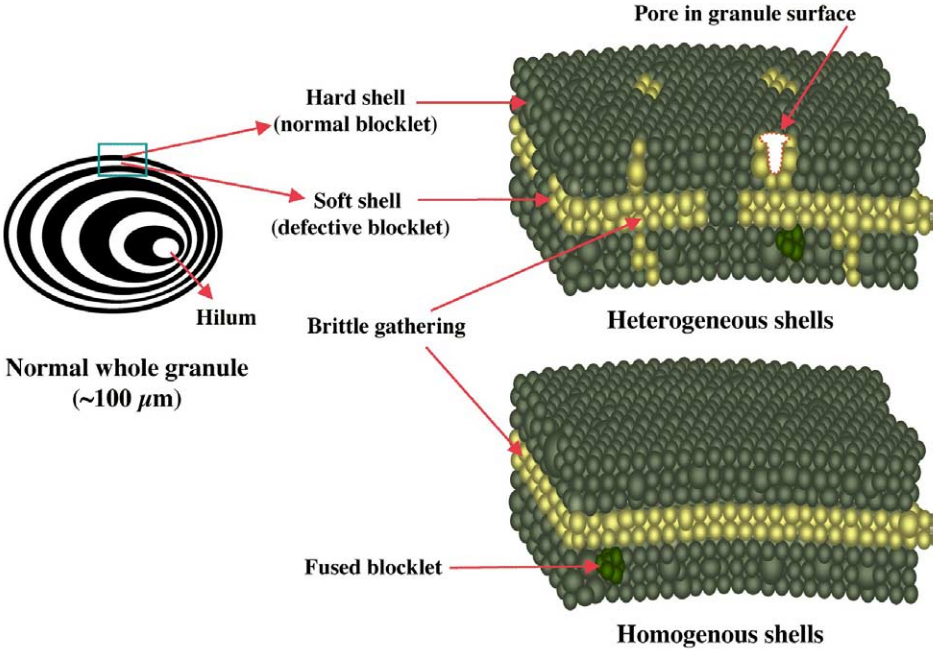

Fig. 3. Scheme of starch granule structures.

they may generally be divided into two types, homogeneity and heterogeneity for the shells of starch granules.

Furthermore, the pores in the surface of the starch granules and channels within the granules were observed with SEM and TEM (Fannon, Hauber, Bemiller, Fannon, Shull, & Bemiller, 1992; Fannon, Shull, & Bemiller, 1993). Perhaps these are accidental occurrences during the preparation and observations of samples because they lack repeatability. Many of us have not observed the existence of the 'pore' and the amorphous 'channel' in the granular structure proposed by Gallant et al. (1997). The 'pore' and 'channel' observed accidentally may be due to zones assembled loosely in 'defective' blocklets.

4. Granular architecture

On the basis of various information and inferences of the starch granules above, we proposed the organization architecture of starch granules in Fig. 3. The semi-crystalline blocklets have generally two types, 'normal' and 'defect' in the same starch, and are basic units that construct starch granules. The normal blocklets construct the hard shells, while the defective blocklets construct the soft shells. The reducing terminal side for all the blocklets is toward the hilum of the granules. It is believed that the surface layer of starch granules consists of the hard shell. However, the starch granules with the heterogeneous shell may be general in nature (Gallant et al., 1992; Kimura & Robyt, 1995; Lauro et al., 1999; Tang et al., 1998; Tang et al., 2002a,b; Vasanthan & Bhatty, 1996). In the heterogeneous shell, both the hard shells and the soft shells are not continuous structures. The normal blocklet zones suspend the stream of the defective blocklets for the soft shells, while the defective blocklet zones suspend the stream of the normal blocklets for the hard shells. Therefore, in the surface of the starch granules, there are many soft zones with the defective blocklets. Because the defective blocklets gather brittlely, the zones are easy to peel off, and form the pore in the surface of starch granules, and weak resistant to the enzymes.

As for the homogeneous shells, the streams of the normal blocklets and the defective blocklets are continuous. There are not the zones that cross mutually in the shells. The shells have uniform resistant to the enzymes. Quinoa, potato and amylomaize starch granules are this type although their resistivities to the enzymes differ (Gallant et al., 1992; Tang et al., 2002a). A special type without the growth rings exists in the granules with the homogeneous shell (Ridout et al., 2003). However, we cannot yet display the organization of the special type, because it is not understood that their blocklets are normal or defective.

5. Conclusion

The microscope has probably been used in investigations of the starch granule since its invention, and it may not be an overstatement to say that starch science would not have developed if there had not been the development of microscopic techniques. Since proposing the clustering concept of amylopectin, researchers have been bothered by the matter of its three-dimensional structure. This article has demonstrated the relation among the various starch molecules and the blocklet, and among the blocklets and the starch granule. The blocklet is a semi-crystalline ultrastructure, and generally consists of several amylopectin molecules. It is thought that the reducing terminal of amylopectin molecules in the blocklets is more toward the same direction, the hilum of the granules. When lower branching molecules that are disadvantage to crystallize are installed in blocklet ultrastructure, it produces the defective blocklet. However, most of lower branching or non-branching molecules may localize among the blocklets, and contribute to the strength and flexibility of the starch granule. The normal and defective blocklets construct the starch granule of two types, the heterogeneous and homogeneous shells. It is expected that the knowledge may contribute to the development of starch science.

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