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Microscopy of starch: evidence of a new level of granule organization

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Considerable information on starch granule structure may be gathered from a review of published data. Evidence from a range of different (predominantly microscopic) techniques is compared and discussed, allowing the presence of a level of starch granule organization between that of the amylopectin lamellae and the large 'growth rings' to be deduced. This structural level of the granule involves the organization of the amylopectin lamellae into effectively spherical 'blocklets' which range in diameter from 20 to 500 nm depending on starch botanical type and their location in the granule. The presence of short, radial 'channels' of amorphous material within starch granules from some starch varieties is confirmed. The organization and structure of the crystalline and amorphous amylopectin lamellae is also discussed. Consideration of the information regarding starch granule structure and organization to date has significant implications on the internal architecture of the starch granule, and it is evident that the presence of the blockets and amorphous channels play a role in both the resistance of starch to enzymic attack and the structure of the semi-crystalline shells. © 1997 Elsevier Science Ltd

INTRODUCTION

The starch granule has been submitted to structural investigations from the invention of the microscope. A range of microscopic techniques have been applied to this field, giving rise to a large number of antagonistic speculations. To understand why so many contradictions arose, we must remember that the interpretation of images is sometimes difficult. Microscopy provides images in two dimensions (2D) whereas in reality the objects, the starch granules, are in 3D. The significance and quality of an image depends on various parameters, of which the resolution and the depth of field are of particular importance (Gallant & Sterling, 1976). Furthermore, many kinds of chemical or physicochemical events may occur during the preparation and observation of samples, leading to imaging artifacts (Gallant & Guilbot, 1971). The resolution (which determines the separating power of two adjacent points according to Abbe's formula) is about 0.2 μm for an optical system, e.g. the light microscope; the resolution is at least 10 times better with the scanning electron microscope (SEM) and 1000 times better with the transmission electron microscope (TEM). However, when image contrast is poor (or even non-existent) due to bad conditions of sample preparation, even the best microscope cannot render images containing useable information. This explains why the same structures have often been interpreted so differently.

In this paper we will review the main hypotheses regarding starch granule structure and behaviour, and attempt to clarify some of the areas where conflicting hypotheses exist. Furthermore, as a result of this review of both old and new literature, evidence of a new level of starch granule structural organization ('the blocklet concept') will be presented.

INTRODUCTION TO STARCH GRANULE STRUCTURAL FEATURES

As a consequence of their crystallinity, most starch granules show a Maltese cross when observed under polarized light. Furthermore, using an additional $\lambda/4$ filter, polarized light reveals the positive birefringency of the starch granules which theoretically indicates a radial orientation of the principal axis of the crystallites (Gallant, 1974; Gallant *et al.*, 1992).

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According to Zobel (1988), native starch granules have a crystallinity varying from 15 to 45%. Thus, crystallinity is not the principal mode of organization of the starch granule polymers. As a consequence, starch granules are a mosaic of hard and soft material. At the lowest level of structure, the granule appears to be made up of alternating semi-cystalline and crystalline shells which are between 120 and 400 nm thick (Yamaguchi *et al.*, 1979; French, 1984). Little is definitely known regarding the organization of starch polymers in semi-crystalline shells, with the bulk of research to date being concentrated on crystalline shells. It is evident, however, from solid state ^{13}C NMR studies (Gidley & Bociek, 1985) that the level of helical order in starch granules is often significantly greater than the extent of crystalline order. Consequently, it appears that much of the amylopectin in semi-crystalline shells is in the double helical form, although it is not crystalline.

Based on X-ray diffraction from crystalline shells, cereal starches (and also small starch granules of some tropical tubers) give an A-crystalline pattern (monoclinic lattice) and thus possess densely packed crystallites (Imberty *et al.*, 1987). Potato starch and certain other tropical tuber starches which are morphologically similar with respect to their granule shapes and sizes, as well as some amylose-rich starch granules (amylomaize, barley and wrinkled pea) have a B-crystalline pattern (hexagonal lattice). They, therefore, contain much more water than type A starches (Imberty & Pérez, 1988; Gallant *et al.*, 1992). Starch granules from other tropical tubers and seeds as well as most legume starches possess the C pattern (Gallant *et al.*, 1992). Finally, due to amylose complexation, a V pattern (often associated with the A, B or C patterns) can appear after gelatinization, although such patterns are also reported to exist in native starches (Gallant *et al.*, 1992).

Considerable evidence now exists that the crystalline shells consist of alternating amorphous and crystalline 'lamellae' which are approximately 9–10 nm thick (Kassenbeck, 1975, 1978; Oostergetel & van Bruggen, 1989; Jenkins *et al.*, 1993). These lamellae are believed to represent the crystalline (side chain clusters) and amorphous regions (branching regions) of the amylopectin molecules according to the models of Robin *et al.* (1974) and French (1984). It has been estimated by Manners (1989) that 80–90% of the total number of chains in an amylopectin molecule are involved in forming the side chain clusters, whilst the remaining 10–20% of chains form the inter-cluster connections. Detailed knowledge regarding the structure, organization and arrangement of the lamellae and their constituent amylopectin (and/or amylose) polymers has begun to be accumulated (Hizukuri, 1986; Manners, 1989; Oostergetel & van Bruggen, 1989) but is still limited.

With the introduction of improved analytical and microscopic techniques, the different levels of starch granule organization have begun to be visualized. The choice of microscope for high resolution visualisation of starch granule structure largely depends on the type of information required, i.e. surface or internal. Surface information may be acquired using either scanning electron microscopy (SEM) or the newly developed atomic force microscopy (AFM). Internal information regarding architectural organization at high resolution requires the use of transmission electron microscopy (TEM).

The structural evidence regarding the starch granule acquired to date from each technique will be discussed below, commencing with granule structural evidence acquired via SEM from enzymic degradation studies.

STARCH GRANULE SUSCEPTIBILITY TO α-AMYLOLYSIS

SEM investigations of starch were started around the year 1970, and were mainly devoted to surface imaging. For 10 years, research was particularly focused on the behaviour of the starch granules subjected to hydrolysis. During bacterial or pancreatic α-amylolysis, the soft (less crystalline) parts of the starch granules are more easily digested than the hard (more crystalline) parts. The different susceptibilities of the starch granules (Evers & McDermott, 1970; Gallant *et al.*, 1972, 1973, 1992; Gallant & Guilbot, 1973; Gallant, 1974; Fuwa *et al.*, 1978, 1979; Duprat *et al.*, 1980; Gallant & Bouchet, 1986) depend on the botanical origin, giving evidence of several structural features. These have been classified by the intensity and the manner by which the granules are eroded and/or internally corroded (Gallant *et al.*, 1973). Lenticular wheat, barley and rye starches, as well as cassava starch granules have very specific zones which rapidly become pitted. Pits enlarge and canals of endo-corrosion sink into the granules. The pits are then randomly formed all over the granule surface and the canals enlarge and merge. Maize and rice starch show random pitting, with the pits enlarging through the granules at each subsequent layer. With waxy maize, deep pitting is present in association with tangential digestion which cuts out the softer parts of each layer. With amylomaize (which appears undigestible externally), SEM is unable to visualize endo-corrosion except for the random formation of small protuberances, each containing a pore on the top. Hydrolysis which progresses inside the granule via these pores can therefore only be studied using TEM. Potato starch granules, which are considered as resistant starch, are slowly but progressively eroded by exo-corrosion, without the apparent formation of surface pores. More recently, however, α-amylase from a new source (*Aspergillus fumigatus*) has been shown to have a different pattern of degradation, in that it forms pores at the surface of the granules even with resistant starches (Planchot *et al.*, 1995).

THE BLOCKLET CONCEPT, A FORGOTTEN ORDER OF CRYSTALLINITY

Before 1960, many authors such as Hanson and Katz (1934), Badenhuizen (1936), Whistler and Turner (1955), Nikuni and Hizukuri (1957), Heyn (1959) and Buttrose (1960) were in agreement with the hypothesis that starch granules were composed of crystalline units embedded in amorphous material. Such a concept was not new, and can be traced back to the prescence of Nägeli (1858) who, although not possessing the resolving power of modern-day microscopes, intuitively developed the idea of a micellar starch granule structure consisting of organized (crystalline) regions of starch material surrounded and held together by less-organized material. The concept was developed principally, however, by Badenhuizen (1937) who following the initial work of Hanson and Katz (1934), demonstrated the presence of natural resistant units of material in chemically degraded starch via the use of a micromanipulator under the light microscope. He consequently described these resistant blocks as 'blöckchen Strüktur', from which the term 'blocklet concept' is derived.

As detailed by Sterling (1968), the blocklet concept was opposed by the fibrillar concept of starch granule structure. The two concepts, which were deemed mutually exclusive, were hotly debated, although at the time the question regarding the true structure of the starch granule could not be resolved.

In 1969, however, following the discovery of new biochemical methods allowing sequential chain counting and determination of chain lengths, Nikuni proposed a model based on clustering organization (Nikuni, 1969). This was a new concept, and was immediately used by scientists to improve their representation of the amylopectin molecule. The scheme designed by Nikuni represents only one reducing group at the hilum, and thus only one amylopectin macromolecule for the whole granule. This is now known to be incorrect. However, the representation of the starch layers constituted by groups of clusters made of short chains was an ideal key for further models (French, 1984; Lineback, 1986). These models of amylopectin structure, which are perhaps closest to the fibrillar concept, dominated thinking, and for more than 10 years the blocklets scheme was largely forgotten.

During this time, however, substantial evidence in favour of a 'blocklet' organization within starch granules was reported. This evidence arises from a range of techniques and is discussed below.

At high magnification under the SEM, using a very low primary electron beam current (10^{-13} A), the residual starch granules (after being treated by α-amylases) appear to be composed of small, more or less spherical 'blocklets' stacked on top of each other (Fig. 1a and b). Such structures are very similar to the blocklet structures described by Badenhuizen (1936). SEM examinations (Gallant, 1974; Duprat *et al.*, 1980; Gallant *et al.*, 1992) reveal, however, that the diameter of the blocklets is different in the alternate hard and soft layers. In the semi-crystalline shells of wheat starch, the blocklet diameter is small (25 nm), whereas in the hard, crystalline shells the blocklet diameter is larger, 80–120 nm (Fig. 1a); blocklets have somewhat the same size distributions in maize and in the centre of potato starch granules. However, in potato starch which shows the B-crystalline pattern, much larger blocklets (200–500 nm) are stacked one on top of another at the granule surface to a depth of about 10 microns (Fig. 1b).

These observations of the 'blocklet' structure of starch are strongly supported by the recent atomic force microscopy (AFM) study performed by Baldwin (1995). Small protrusions of *ca* 10–50 nm were observed at the surface of native wheat starch granules (Fig. 2a) whereas larger, more or less spherical protrusions (200–500 nm) were evident at the surface of native potato starch granules (Fig. 2b). Taking into account the sizes of these structures, it becomes evident that 'blocklets'

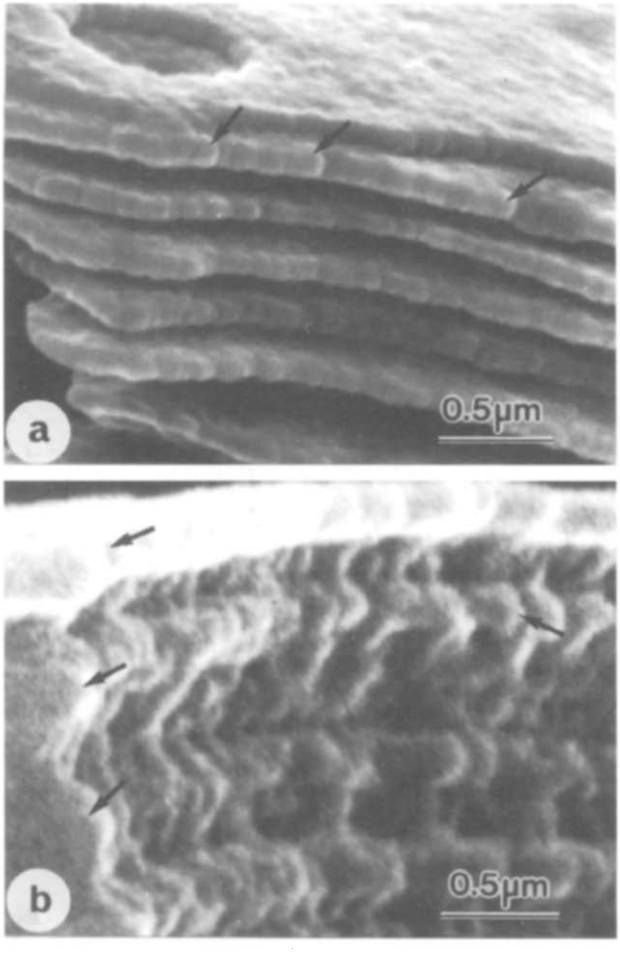

Fig. 1. Scanning electron micrographs of residual starch granules after partial pancreatic α-amylase hydrolysis: (a) wheat; (b) potato. Blocklets are shown by arrows. Scale bar = 0.5 μm. (From Gallant *et al.*, 1992.)

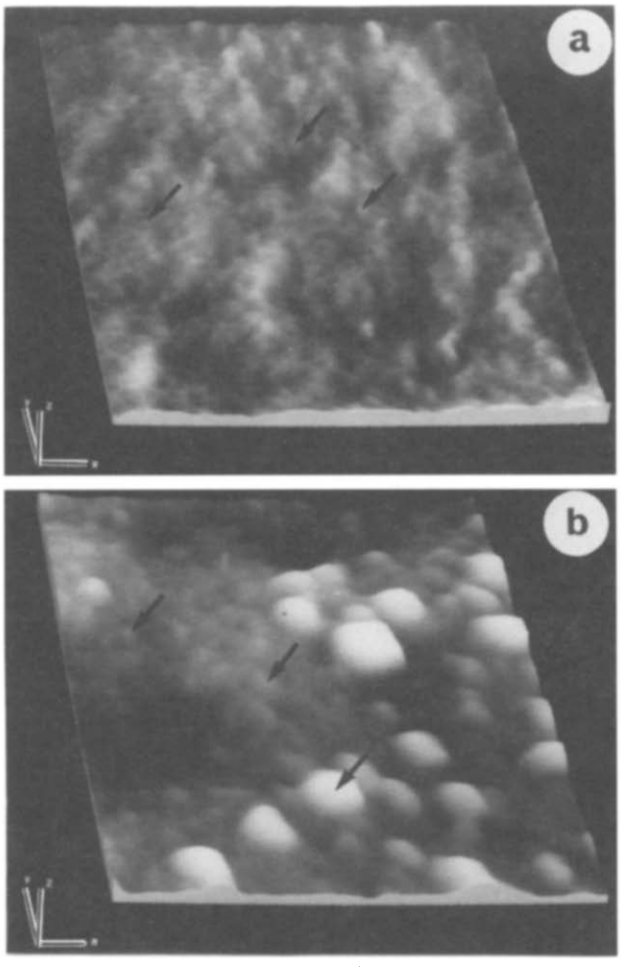

Fig. 2. Atomic force microscopy images of native starch granule surfaces: (a) wheat; (b) potato. Surface protrusions are shown by the arrows. The x and y scan sizes are 1×1 μm, respectively. The z height scales are 52.6 nm in (a) and 84.5 nm in (b). (From Baldwin, 1995.)

and 'protrusions' are the same structures. Furthermore, the AFM observation of structures of similar sizes to those observed by SEM and TEM lends considerable credibility to the SEM and TEM observation of 'blocklets' within starch granules due to the minimal sample preparation required for atomic force microscopy of starch granule surfaces (Baldwin *et al.*, 1996).

We shall now consider TEM evidence of granule structure to date, and discuss how this and recent crystallographic evidence of starch polymer organization fits in relation to the 'blocklet concept'.

THE CLUSTERING CONCEPT, THE SUB-MICROSCOPIC STRUCTURE OF THE BLOCKLETS

Studies using TEM [which has a very high resolving power, theoretically around the size of the glucose unit (4–5 Å)] started with Whistler and Turner (1955) and Whistler and Thornburg (1957), and were followed by Buttrose's work (1960). In these studies, structural differentiations of starch granules were obtained following either enzymic or acidic pre-treatment.

At that time, however, contrasting methods were not available, and therefore the deduced structure of the starch granule derived from these studies on ultrathin sections was far from accurate and the details were poor. To improve the quality of the results, different staining procedures were developed between 1965 and 1975 which are based on the reactivity of the chemical groups in the starch polysaccharides with heavy atoms (Fig. 3). The PATAg reaction (periodic acid, thiosemicarbazid, silver) was found to be particularly useful. This staining method is derived from the PAS (periodic acid–Schiff) light microscopy stain for starch but has been adapted for electron microscopy. α-Glycols at C_2–C_3 of the anhydro-glucose units are oxidized to dialdehyde groups and then are able via thiosemicarbazide to fix mono-valent heavy metal ions such as silver (Gallant & Guilbot, 1969a, b; Gallant, 1974).

The originality of the PATAg method of starch granule sample preparation for TEM observation is due to the exclusive reaction on bulk samples (i.e. before embedding and sectioning) in order to differentiate the kinetics of oxidation between the amorphous and crystalline parts of the starch granule (Fig. 4). When oxidation of the starch is performed to a low degree, the silver ions are unable to react at the crystalline regions of the granule, and thus bind preferentially to the starch polymers in the amorphous regions of the granule. As a consequence, the amorphous regions of the granule appear dark in the resulting TEM images (due to the presence of the silver ions), whilst crystalline regions appear lighter. This is noted here since it is the inverse of the normal situation in TEM of (untreated) starch (Mussulman & Wagoner, 1968), where a darker region

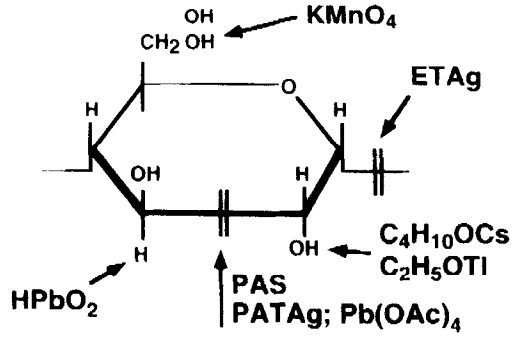

Fig. 3. Several contrasting methods are available for TEM (after Gallant, 1974). Each functional group of the anhydroglucose unit may be reacted with ions of heavy metals: protons are revealed with plumbite; hydroxyls with alkaline alcoholates of cesium or thallium; the secondary alcohol is oxidized by permanganate in acidic medium; α-glycols at C_2–C_3 may be transformed to dialdehyde by periodic acid (PAS or PATAg) or by lead tetraacetate. A similar reaction (ETAg) may be obtained following use of a hydrolase according to Joseleau & Ruel (1985).

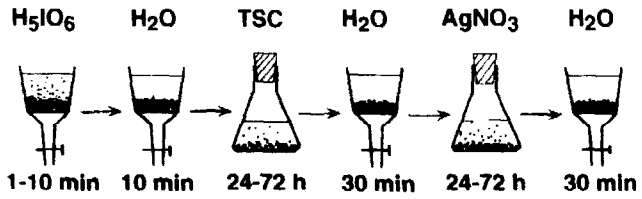

Fig. 4. The PATAg reaction (i.e. samples submitted to a mild periodic oxidation, followed by long thiosemicarbazide and silver nitrate permeations) on bulk samples (the starch granules) before resin embedding and sectioning. (From Gallant, 1974.)

is normally taken to be more electron dense due to increased crystallinity. Thus, following the PATAg procedure (either with or without enzymatic/acid hydrolysis), a double organization of the maize starch granule is revealed in granule cross-sections (Fig. 5a) as follows: (i) alternate tangentially dark and clear layers (soft and hard shells respectively); (ii) dark lines in the radial direction. The short dark lines in the radial direction indicate the presence of radial 'channels' or 'canals' of more amorphous material (i.e. less crystalline material which is able to be penetrated by the silver ions). Further evidence for the existence of pores and channels in starch granules is discussed below.

Figure 5b shows an oblique PATAg contrasted TEM section through the extreme edge (vertex) of the granule. Each of the lighter areas outlined by the crossing of the radial and tangential darker areas corresponds to one blocklet (thus, the blocklets are effectively viewed from above). It is apparent that the blocklets, as well as being stacked radially within the granule, are also arranged tangentially, in staggered rows (Fig. 5b). Their sizes (20–500 nm in diameter) are in the order of the estimated sizes for the macromolecules of amylopectin (Gallant, 1974), but it remains to be definitively proven if the blocklets are in fact composed of one discrete amylopectin molecule.

Figure 6a shows that the blocklets appear (more accurately) to be elongated and are not uniform with respect to shape and size. Figure 6b and c show higher resolution PATAg contrasted TEM cross-sections of a maize starch granule and reveal details of the amylopectin lamellae within the blocklets. The presence of over 10 thin crystalline lamellae is clearly evident (marked by arrows). These lamellae are also shown in Fig. 6c, which represents the highest magnification obtainable from the PATAg contrasted cross-section of a blocklet. From the thickness of the lamella (on average 9–10 nm) it is evident that the lamellae seen in these images are the proposed lamella structures of the amylopectin molecules (French, 1984; Oostergetel & van Bruggen, 1989; Jenkins *et al.*, 1993) thus confirming the average 9–10 nm dimensions of the amylopectin side chain clusters as proposed by French (1984), Manners (1989) and Jenkins *et al.* (1993). To date the lamellae have generally been accepted to be

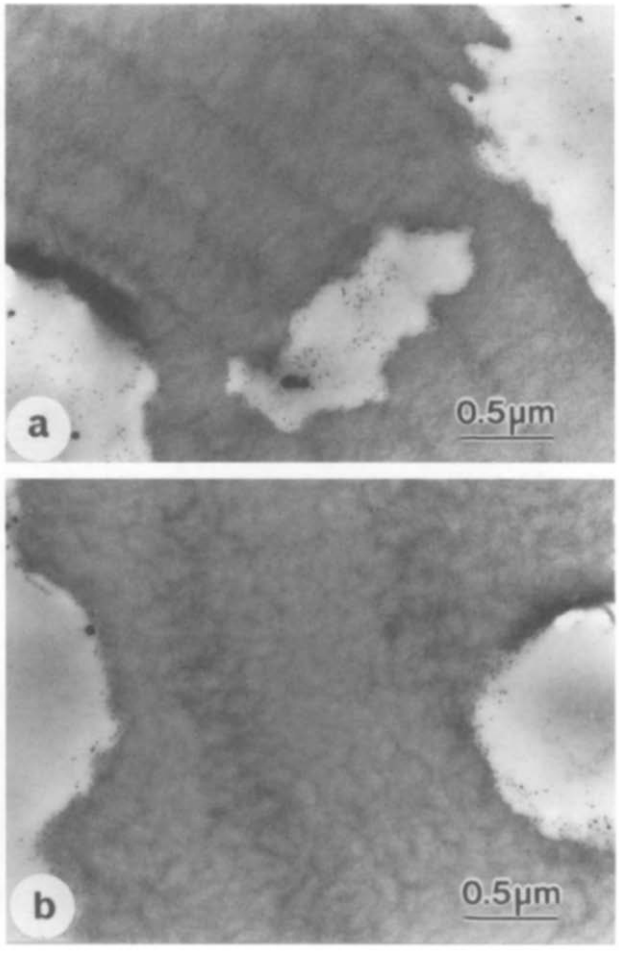

Fig. 5. Transmission electron micrographs of residual maize starch granules after partial hydrolysis by α-amylase of *Aspergillus fumigatus*, followed by PATAg treatment: (a) cross-section showing the double organization i.e. soft (dark) and hard (clear) shells alternating tangentially and dark lines in the radial direction through the layers; (b) tangential section showing blocklets arranged in staggered rows. Scale bar = 0.5 μm.

composed of continuous rows of the amylopectin side chain clusters interleaved with the more amorphous branching regions (Jenkins *et al.*, 1993). From Fig. 6b and c however, it is evident that the crystalline amylopectin side-chain clusters (the lighter areas) are not completely continuous in the direction of the lamellae, but are in fact separated and surrounded by amorphous material (which has been penetrated by the silver ions and therefore appears dark in the images). Furthermore, the crystalline amylopectin side chain clusters do not appear to be uniform with respect to their size (diameters *ca* 5–15 nm), shape or density. Consequently the lamellae do not appear to be uniformly straight or parallel. Whilst such observations fit with current thinking regarding the possible non-uniformity of the amylopectin side chain clusters and the associated crystalline lamellae (Oostergetel & van Bruggen, 1989; Jenkins & Donald, 1995), their implication on the validity of models in

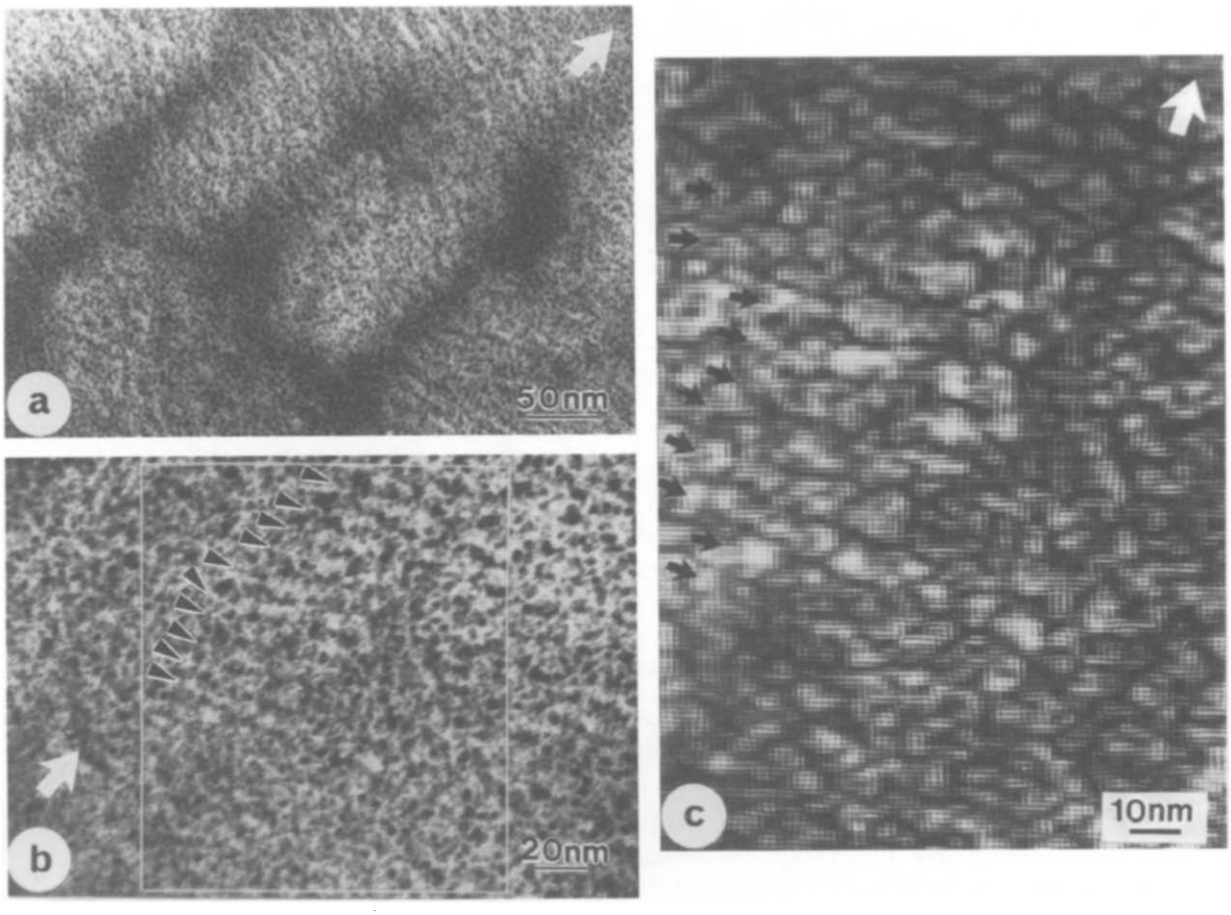

Fig. 6. PATAg contrasted TEM cross-sections through a maize starch granule. The large white arrows show the radial direction: (a) crystalline blocklets (light areas) surrounded by more amorphous regions (dark). Scale bar = 50 nm. (b) and (c) show regions of a PATAg contrasted TEM cross-section at higher resolution. Original contrast has been enhanced by image processing to reveal structural details; (b) stack of around 20 crystalline lamellae (light structures marked by black arrows) corresponding to the substructure of the blocklet. Each lamella and also each amylopectin side-chain cluster in the lamella, is separated by a black space (amorphous material). Scale bar = 20 nm; (c) ×2 magnification of (b). Crystalline lamellae (marked by black arrows) are not continuous and regular but are subdivided into discrete elements of about 10 nm, which are believed to correspond to the amylopectin side chain clusters. Scale bar = 10 nm.

which the lamellae are assumed to be continuous and infinite should be bourne in mind.

By taking the average diameter of an amylopectin side chain cluster to be 10 nm, it therefore follows that small blocklets (20–50 nm in diameter) are, on average, composed of between two and five amylopectin side chain clusters, whilst larger blockets (50–500 nm in diameter) contain between five and 50 amylopectin side chain clusters.

Furthermore, by considering the proposed dimensions of the A and B starch polymer crystalline unit cells (Imberty *et al.*, 1987; Imberty & Pérez, 1988) it is possible to estimate the number of double helical strands present in the average 10 nm diameter amylopectin side chain cluster from A and B type starch. Thus, for the A starch crystalline unit cell which has dimensions in the x,y plane of 2.12 and 1.17 nm, respectively, and contains two double helical strands, a 10 nm amylopectin side chain cluster would contain between nine and 17 double helical polymer strands. For the B starch crystalline unit cell which has dimensions in the x,y plane of 1.85 nm and contains two double helical strands, a 10 nm amylopectin side chain cluster would contain around 11 double helical polymer strands. These values represent estimates from the average size of the amylopectin side chain cluster, and as demonstrated above the size, and packing density of amylopectin side chain cluster appears to vary. The numbers of double helical amylopectin side chains in clusters from A and B starch agree, however, with the general concept that B starch is on average less densely packed than A starch.

FURTHER EVIDENCE OF A 'NEW' BLOCKLET LEVEL OF ORDER WITHIN STARCH GRANULES

Enzymatic degradation of large and small barley starch granules and of waxy maize starch granules (Bertoft, 1986) indicates that molecules of amylopectin are composed of 'super-clusters' (molecular weight *ca* 10^5)

arising from 'highly ordered regions of amylopectin'. Furthermore, specific (1–4)-α-D-glucosidic linkages between the 'super-clusters' appear to undergo preferential degradation during the initial stages of α-amylolysis thus producing these 'super-clusters'. Such observations fit with the idea that an extra level of amylopectin crystallization exists in starch granules and we hypothesize that the 'super-clusters' relate to the blocklet structure of starch. The preferentially degraded (1-4)-α-D-glucosidic linkages between the 'super-clusters' must, therefore, be located in the more-amorphous 'channel' regions between the blocklets, and are thus more readily accessible to degradation.

Another approach to structural determination of starch has been performed by Yamaguchi *et al.* (1979) and by Oostergetel and van Bruggen (1989, 1993). This approach used negative staining of lintnerized starch granules. Yamaguchi *et al.* (1979) described some 'worm-like ripple structures' in corn starch granules which they interpreted as 5 nm thick crystalline lamella created by the association of double helices perpendicular to the plane of the lamella. Oostergetel and van Bruggen with a more sophisticated procedure studied lintnerized wheat (1989) and potato starch (1993). Three-dimensional reconstructions of the residual crystallites in potato starch were carried out using negatives taken from a tilt series in the TEM, treated by a low-pass Fourier filter. A helical structure in 3D was observed in stereo mounts which revealed that the organization of the lamellae was much more complex than was previously thought to be the case (Fig. 7a). As a result they proposed the concept of 'super-helical' structure, as a level of structure between that of stacks of lamellae and the granule 'growth ring' (Fig. 7b).

De-branching studies of starch (Hizukuri, 1986) lend further support to the idea that a blocklet level of structure exists. Using HPLC, Hizukuri demonstrated that B chains of amylopectin can participate in more than one crystalline amylopectin side chain cluster. He therefore proposed a revised model of amylopectin structure and classified the B chains according to the number of side chain clusters in which they participate. Thus, B1 chains participate in one cluster, B2 and B3 chains extend into two or three clusters, respectively, while B4 chains link four or more clusters. It is, therefore, evident that B chains may link the amylopectin side chain clusters to form larger crystalline units. Such evidence corresponds well with the blocklet concept of starch structure. Furthermore, Hizukuri (1986) demonstrated that the connecting B chains were more abundant in potato starch and proposed that they are probably characteristic of starches with the B crystal pattern, since such starches have higher amounts of the B2–B4 chain fractions. This may therefore explain our observation that, in general, blocklet size is larger in starches with the B crystal pattern.

Further confirmation of the blocklet structure at the

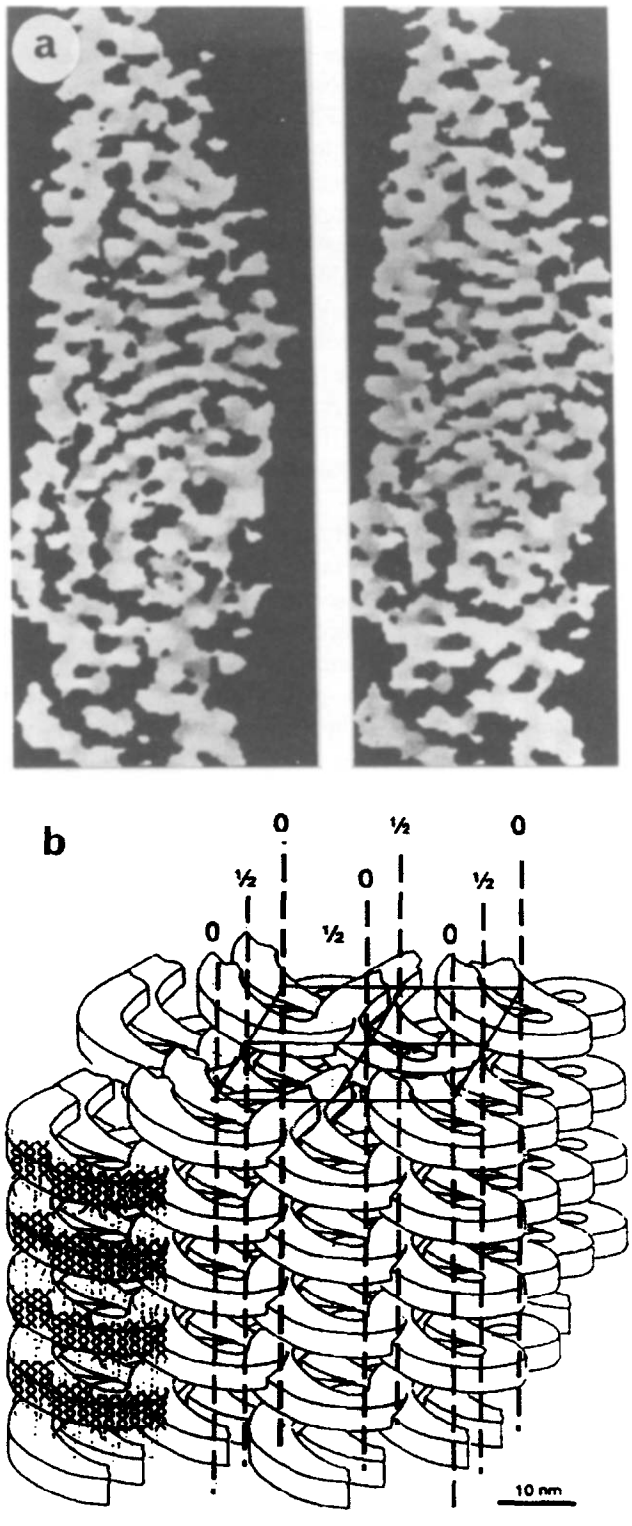

Fig. 7. (a) stereo mount of a 3D reconstruction of the semi-crystalline structure of potato starch amylopectin; (b) schematic model for the arrangement of amylopectin in potato starch showing the 'super-helical' structure and the amorphous areas inside the crystalline organization. (From Oostergetel and van Bruggen, 1993.)

granule surface can be drawn from the only other published AFM study of the starch granule surface (Thomson *et al.*, 1994). In this study it was reported that

the surface of wheat starch from the cultivar 'Timmo' was 'dotted with features between 50 and 450 nm in diameter'. Such features compare well with the surface protrusions seen in the AFM studies by Baldwin (1995) which correspond to the blocklet structures. Thomson *et al.*, however, wrongly believed them to be a surface contaminant, possibly protein. This deduction was reached because only one starch type (wheat starch) was observed in the study. Baldwin (1995), however, studied wheat and potato starch surfaces and it thus becomes clear that the surface protrusions are carbohydrate in nature. This is because the features are far more common on granule surfaces of potato starch than on wheat starch and because the surface protein content of potato starch is far lower (0.05%) than that of wheat starches (5.0%) (Baldwin, 1995).

More recently, Helbert and Chanzy (1996) reported the use of hydrophilic melamine resin for preparation of ultrathin starch granule sections for TEM observation. This preparation was recommended by both Gallant and by Oostergetel (Chanzy *et al.*, 1990), in order to reduce the potential of generating imaging artifacts compared with some of the older embedding techniques. In the resulting images, individual blocklets (with dimensions of a few hundred nanometers) were seen within the granule growth rings.

Considerable evidence therefore exists for a level of granule organization between that of the lamellae and the 'growth rings' and it seems possible that 'super-clusters' and/or 'super-helices' correpond to 'blocklets'. It is certain, however, that due to the sizes of the blocklets (from 20 to 500 nm) and the corresponding thickness of the semi-crystalline and crystalline shells of the granule (120–400 nm), the blocklets must play an important role in the structure and organization of the crystalline (and possibly semi-crystalline) granule shells. Furthermore, as we have already shown in the images obtained with the PATAg reaction (Fig. 6), and also as revealed by the Oostergetel and van Bruggen mounts (Fig. 7), the importance of the 'amorphous' fraction co-existing with the crystalline regions is confirmed.

A NEW MODEL OF AMYLOPECTIN SIDE CHAIN CLUSTERS AND LAMELLAE STRUCTURE

When the starch granule is treated with acid according to Lintner, each of the large semi-crystalline layers of the granule is preferentially hydrolyzed, thus leaving the more resistant crystalline regions of the granule. In the scheme drawn by Robin *et al.* (1974) in order to explain such biochemical results (Fig. 8) the A and B chains of amylopectin are grouped forming crystalline zones (clusters), each separated by a very narrow region designated as the branching area. This branching region is considered as 'amorphous', and therefore much more susceptible to acid hydrolysis (Fig. 8b). From Fig. 6b and c it is clear that the region between amylopectin side chains in the lamellae should also be considered as 'amorphous'. Robin *et al.*'s scheme should, therefore, be reconsidered including this new level of complexity. This is shown schematically in Fig. 9. In this diagram we have incorporated the following features:

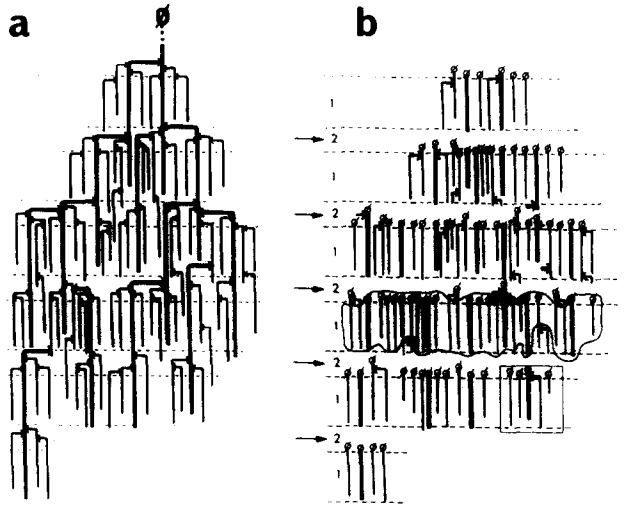

Fig. 8. Scheme of macromolecular organization of potato starch amylopectin: (a) in native starch, the cluster organization showing crystalline and amorphous lamellae, corresponding, respectively, to the areas without any α-(1–6) branchings and the areas where α-(1–6) branching occurs; (b) the same organization but after acid treatment according to Lintner. The branching zones are hydrolyzed, liberating the crystalline lamellae (From Robin *et al.*, 1974).

- the double helical structure of the amylopectin side-chains;
- amylopectin side-chain clusters are of varying sizes;
- each side-chain cluster can contain between nine and 17 double helical chains, each with about three turns of the double helice;
- individual amylopectin chains may participate in more than one cluster as demonstrated by Hizukuri (1986);
- amorphous zones are present between both the crystalline lamellae and the amylopectin side-chain clusters;
- lamellae are not straight, parallel or of uniform thickness;
- the starch polymers are not necessarily aligned exactly at right-angles to the direction of the lamellae.

For simplicity, amylose, protein and lipid have not been included in this diagram, since their exact location and interaction with amylopectin is still not certain. The most probable location of amylose is as randomly interspersed radial chains (Jane *et al.*, 1992; Kasemsuwan & Jane, 1994; Blanshard, 1987) with an increasing concentration of amylose (in non-mutant starches) towards the granule exterior (Geddes *et al.*, 1965; Morrison & Gadan, 1987). Furthermore, it is currently

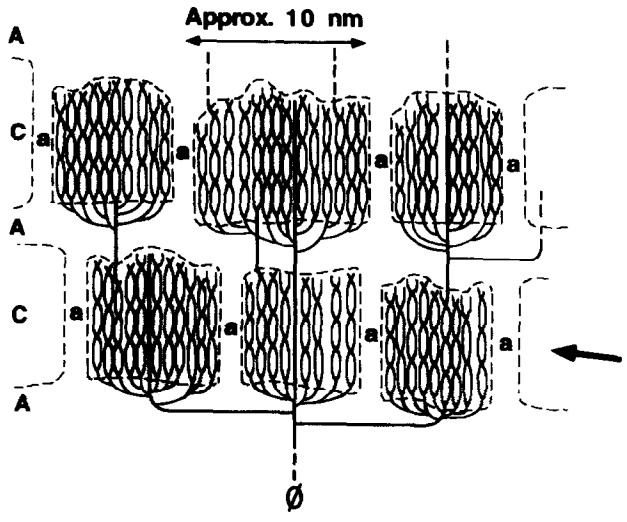

Fig. 9. Schematic diagram of amylopectin side chain clusters within lamellae. Each cluster contains between nine and 17 side-chains, and the double helical structure of the polymers is represented. Amorphous zones are shown both between the crystalline lamellae and between the individual side chain clusters. The lamellae are not completely straight, parallel or of uniform thickness and, consequently, the starch polymers are not always aligned at right angles to the direction of the lamellae. The general direction of the lamellae is shown by the large black arrow. C, Crystalline lamellae (amylopectin side chain clusters, on average 6 nm length); A, amorphous lamellae (branching zone) on average 4 nm length; a, amorphous regions between crystalline clusters.

hypothesized that amylose may be predominantly located in the amorphous zones of the granule and that increased interaction between amylose and amylopectin in these regions causes their decreased crystallinity (Zobel, 1988; Morrison *et al.*, 1994; Jenkins & Donald, 1995). If such a hypothesis is true it is possible that the presence of amylose may influence blocklet size since (as stated above) the crystalline blocklet size is generally found to be smaller in the amorphous shells of the granule (corresponding to the lower degree of crystallinity).

STARCH RESISTANCE TO ENZYMIC AND ACIDIC ATTACK

In gross terms, enzymatic and acidic attack of starch are similar since they both involve hydrolysis of the bonds in the starch polymers. Furthermore, in both processes the semi-crystalline (soft layers) of the granule are more easily and rapidly hydrolyzed than the crystalline (hard) layers (Buttrose, 1963). In more precise terms however, significant differences exist between the two types of attack with respect to bond specificity, mechanism of attack and extent of starch polymer degradation (Planchot, 1993; Zherebtsov *et al.*, 1995). The far smaller size of the acid molecule (e.g. hydrochloric acid) compared to the enzyme allows acidic attack to occur on a much finer scale than enzymic attack, although the extent of starch granule degradation by acid and the susceptibilities of the glycosidic bonds to acid are affected by a range of factors, including the acid concentration, temperature, and the presence of other compounds such as alcohols (Fox & Robyt, 1992). Furthermore, whilst different enzymes are specific for certain bonds (Macgregor, 1991), acid attack is non-specific with regards to the type of bonds hydrolyzed (i.e. both α-1,4 and α-1,6 bonds can be disrupted). Thus, after prolonged acid exposure, the acid is thought to penetrate even the crystalline regions of the granule and act on the α-1,6 bonds of the amylopectin molecules in the lamellae, as shown in Fig. 8 (Robin *et al.*, 1974). Acidic attack of starch granules therefore results in a higher proportion of shorter chain products than enzymatic attack, which are also less ramified (Planchot, 1993). Furthermore, increasing granule amylose content appears to decrease the extent of starch granule degradation by acid (Planchot, 1993). This relation appears to exist during enzymic attack of starches, however, it is not so clear cut, indicating that the amylose content alone cannot fully account for the granule resistance to enzymic attack (Planchot, 1993).

We therefore hypothesize that the hydrolysis rate of starch granules depends to a great extent on the distribution of the semi-crystalline and crystalline layers and on the size, identity and interaction of their constituents. Thus in potato starch and amylomaize (both with B cystalline pattern and considered resistant starches), it appears that the thick peripheral layer of large stacked blocklets explains the low rate of hydrolysis (Gallant *et al.*, 1992). Furthermore, less resistant starches such as wheat starch possess smaller blocklets than the resistant starches (Gallant *et al.*, 1992). However, the case of wrinkled pea is an exception. This starch has smaller blocklets than smooth pea (Gallant *et al.*, 1992) yet is more resistant to enzyme attack (Planchot, 1993; Planchot *et al.*, 1995). This exception therefore illustrates that other factors besides blocklet size influence granule crystallinity and enzyme resistance. As stated above, granule amylose content appears to be one of the factors involved in starch resistance (to both acid and enzymic attack). This is evident since starches containing high levels of amylose (e.g. amylomaize, wrinkled pea and the small B-granules of wheat) are more resistant to enzymatic attack than the corresponding starches of lower amylose content (i.e. normal and waxy maize, smooth pea and large A-wheat starch granules respectively). Non-mutant potato starch, however, has a normal amylose content [24% (Lineback, 1986)] and is highly enzyme resistant, thus indicating that granule amylose content alone cannot fully account for granule enzymic resistance. The enzyme resistance of potato starch may be linked to the large blocklet size, but may also indicate that the extent of amylose interaction with amylopectin influences crystallinity and resistance, as

supposed by Zobel (1988). Furthermore, the location of amylose within the granules may influence local crystallinity and resistance. It is now known that significant enrichment of amylose exists towards the granule surface in many starches including wheat and potato (Geddes *et al.*, 1965; Morrison & Gadan, 1987), which may be responsible for increased resistance towards the granule surface. It is, therefore, evident that the relationship between granule crystallinity and enzyme susceptibility is not yet fully understood, and requires further study.

EVIDENCE OF 'CHANNELS' WITHIN GRANULES: STRUCTURAL CHANGES DURING SWELLING

The above knowledge regarding starch granule structure may now be related to the structural changes which occur at the beginning of starch granule swelling. This is the phase when structural changes within the granule are just starting to occur, and relates to the region of the DSC curve when the endothermic peak is just beginning to form. This phase can, therefore, also be considered as the stage where the amorphous regions have been swollen due to water absorption and crystallite melting is just starting to occur (i.e. the beginning of the irreversible swelling process). At this point, SEM observation of cassava starch granules (Fig. 10a) shows the presence of groups of pores (0.05–0.1 μm diameter) which divide the surface of the granule into polygonal areas of about one micron diameter. At more advanced stages of swelling it has been reported that solubilization and leaching of amylose occurs and is partly responsible for the increase in viscosity of the starch dispersion (Miller *et al.*, 1973). This process is shown in Fig. 10b, where amylose can be seen to leach out of the starch granule, forming a network structure which fills the inter-granular space.

The amylose content of the starch granule (dark stained by reaction with PATAg) can be seen (Fig. 10c) both in the 0.05–0.1 μm diameter canals and discharged

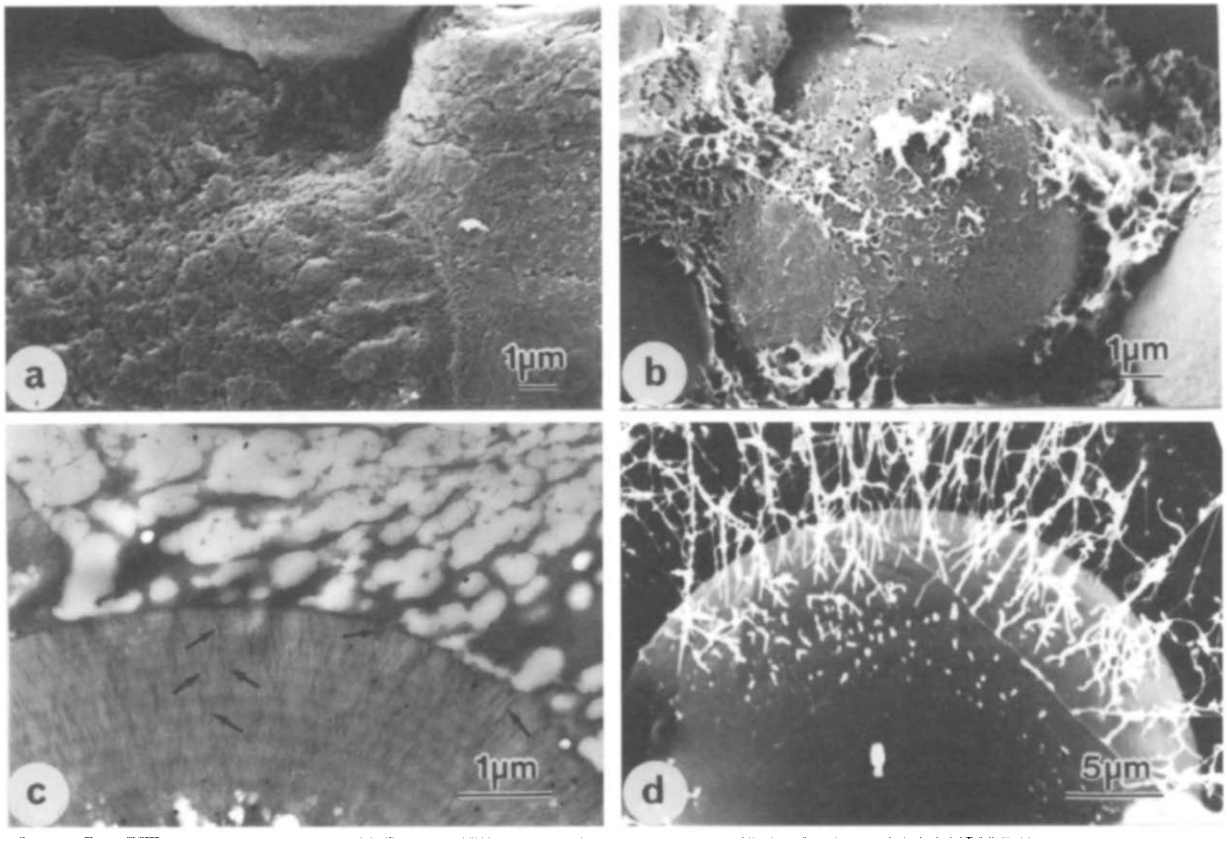

Fig. 10. Behaviour of starch at the beginning of swelling (a) scanning electron micrograph of cassava starch showing the appearance of small pores at the surface of the starch granules. Groups of pores (0.05–0.1 μm diameter) appear to divide the surface of the granule into polygonal areas. Scale bar = 1 μm; (b) scanning electron micrograph of the same sample at a more advanced stage of swelling showing amylose filaments leaching from the pores. Scale bar = 1 μm; (c) transmission electron micrograph of a PATAg stained cassava starch granule at the same stage of swelling as in (b) shows amorphous material (presumed to be amylose) within the radial channels (marked by arrows). Discharged (leached) amylose appears as a dark network external to the starch granule. Scale bar = 1 μm; (d) scanning electron micrograph of a potato starch granule at the beginning of swelling. Amylose filaments discharge from granule surface pores which appear during the gelatinization. The process here was stabilized by a special cryo-transfer stage inside the microscope. Scale bar = 5 μm. (a) and (b) are from Garcia (1996) and (d) is from Notté (1993).

exterior to the granule forming the dark network structure (top of image). This phenomenon of amylose leaching during gelatinization is observed with all other starch types including potato starch granules (Fig. 10d), although in potato and other starches of the type B, amorphous 'channels' do not appear to be present in the native state. This is collaborated by the fact that starch granules of the B crystalline type are progressively exo-eroded during α-amylolysis (or during germination of the tuber), without the formation of corrosion channels.

Collaborating evidence for the presence of channels within starch granules comes from the observations of Fannon *et al.* (1992a, b; 1993) who, using TEM and SEM, have observed surface pores and interior channels in corn, sorghum and millet starches and have observed surface pores along the equatorial groove of wheat, rye and barley starch granules. The surface pores and interior channels are believed to be naturally occuring features of the starch granule structure, with the pores being the external openings of the interior channels. The reported sizes of the channels (0.07–0.1 μm in diameter) corresponds well with the sizes of channels shown in Figs 5 and 10c (0.05–0.1 μm in diameter). Surface pores were reported to be larger (0.1–0.3 μm in diameter) than the channels, with neither feature being evident in potato starch. The interior channels in the starch granule were reported to be 'serpentine' i.e. to have a tortuous path, and to be roughly in the radial direction. Fannon *et al.* (1992a, b; 1993) further proposed that the channels facilitate enzyme attack of the granule during germination, but have not conclusively demonstrated whether the channels are void or filled with material.

In 1959, from observations made with the light microscope, Badenhuizen published a scheme closely related to the results shown in Fig. 10a–d, and which showed the concentric layers combined with very thin radial canals. Unfortunately, Badenhuizen thought that these two combined structures were the result of an optical illusion due to the superimposed structures seen in transmission through the light microscope, and the idea was not pursued. It is now evident, due to the use of far more powerful microscopic techniques that the two structures (alternating hard/soft layers and radial channels) may co-exist. From Figs 5a and 10c it appears that the radial channels are generally short, penetrating (at most) a few granule shells. This may be a false assumption, however, since the tortuous path of the channels may take them out of the plane of view (i.e. channels are 3D stuctures being viewed in an effectively 2D thin section). It seems, however, that the channels are comprised of amorphous starch polymers rather than being void (due to the penetration and binding of the silver ions in the PATAg contrasting procedure). We, therefore, postulate that the channels may form at the junction zones between the more crystalline blocklets. Furthermore, we are in agreement with Fannon *et al.* (1993) that the channels (and related surface pores) are naturally occuring features of the granule and are likely to pre-dispose the initial sites of enzymic attack during germination. Consequently, we hypothesize that the radial amorphous channels (in association with blocklet size) play a role in starch resistance to enzyme attack.

CONCLUSION

By combining old and new results provided over the years by a range of microscopic techniques, we have been able to gather together some of the pieces of the puzzle concerning starch granule internal structure and organization. This gathering of information is illustratively summarized in Fig. 11.

The crystalline fraction of starch granules has received the most intense study and is consequently the best understood. It is now widely accepted that the amylopectin polymer (which comprises around 75% of the granule composition in non-mutant starches) is predominantly responsible for granule crystallinity. Granule crystallinity is around 15–45% (Zobel, 1988) and can be characterized into three main X-ray diffraction patterns (type A, B or C) as a result of different packing of the amylopectin side-chain double helices. The crystalline regions are predominantly located in the hard (120–400 nm thick) layers of the granule and are composed of stacks of crystalline lamellae which form the backbone of the starch granule. The crystalline lamellae are around 9–10 nm thick on average, although it would appear that they are not always straight, parallel or of uniform thickness. The crystalline lamellae are believed to consist of the ordered double helical amylopectin side chain clusters and are inter-leaved (alternated) with more amorphous lamellae consisting of the amylopectin branching regions (according to the basic model of Robin *et al.* (1974)). The amylopectin side chain clusters within the crystalline lamellae have varying sizes but on average are around 10 nm wide by 9–10 nm long (the length represents the thickness of the lamellae). TEM images of the lamellae indicate that the region between the side chain clusters within the lamellae is also of an amorphous nature and that the crystalline lamellae are therefore not continuous.

Considerable evidence now exists from SEM, TEM, enzyme degradation studies and more recently from AFM, which indicates that the crystalline and amorphous lamellae of the amylopectin are organized into larger, more or less spherical structures, which have been termed 'blocklets'. Such an idea is not new, being first hinted at by Nägeli in 1858 and then by Badenhuizen in 1936, although at this time the resolution of the available microscopes was not sufficient to definitely prove this hypothesis. The blocklets range in diameter from around 20 to 500 nm depending on starch type (botanical source) and location in the granule. Enzy-

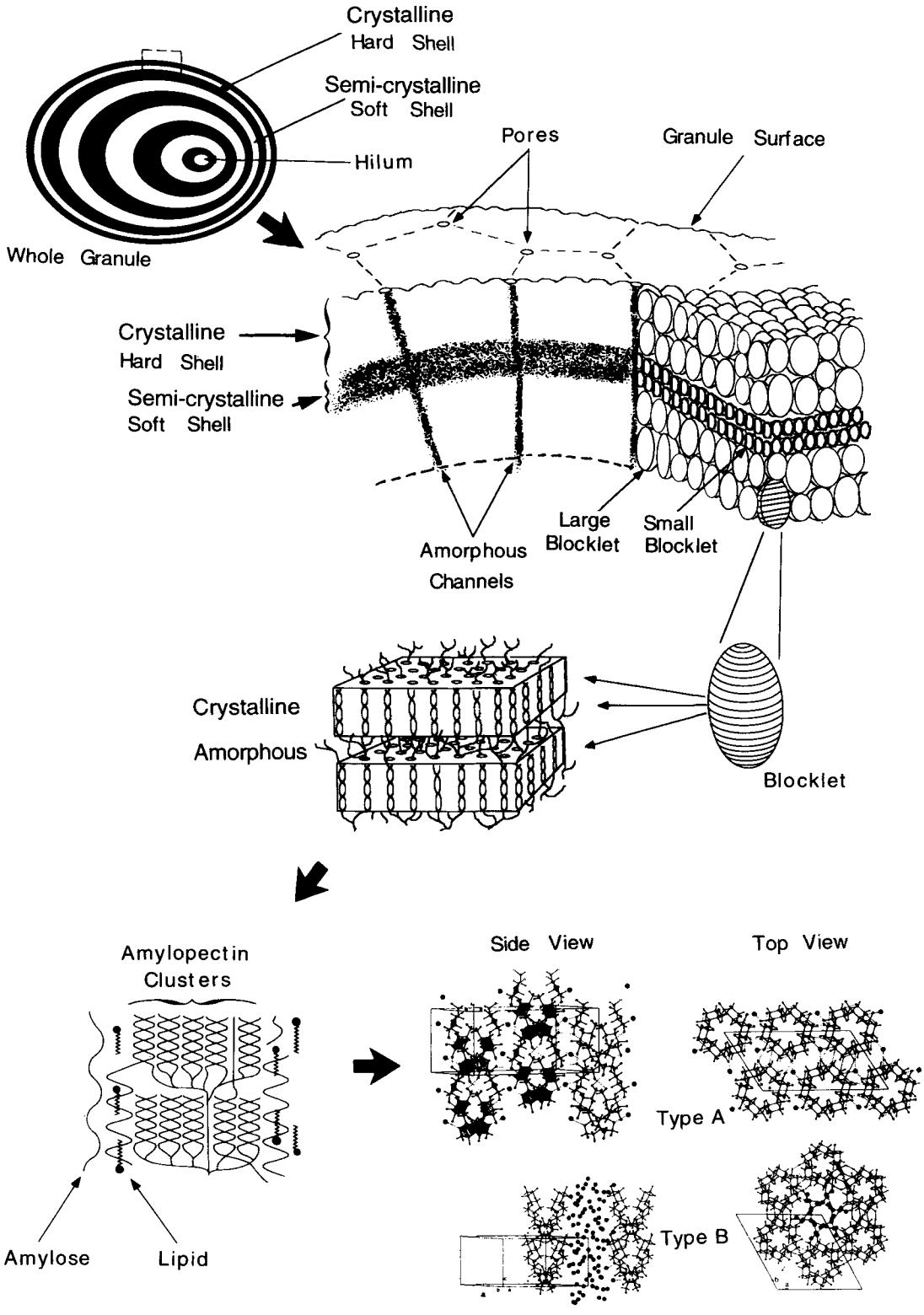

Fig. 11. Overview of starch granule structure. At the lowest level of granule organization (upper left), the alternating crystalline (hard) and semi-crystalline (soft) shells are shown (dark and light colours, respectively). The shells are thinner towards the granule exterior (due to increasing surface area to be added to by constant growth rate) and the hilum is shown off centre. At a higher level of structure the blocklet structure is shown, in association with amorphous radial channels. Blocklet size is smaller in the semi-crystalline shells than in the crystalline shells. At the next highest level of structure one blocklet is shown containing several amorphous crystalline lamellae. In the next diagram the starch amylopectin polymer in the lamellae is shown. The next image (from Blanshard, 1987) reminds us that amylose-lipid (and protein) feature in the organization of the amylopectin chains. At the highest level of order, the crystal structures of the starch polymers are shown (redrawn by Gallant *et al.*, 1992 from Imberty *et al.*, 1987 and Imberty & Pérez, 1988).

matic degradation studies in conjunction with SEM observation (Gallant *et al.*, 1992) indicate that resistant starches such as potato and amylomaize starch possess larger blocklets (50–500 nm) than less resistant starches. Blocklet size, therefore, appears to be an important factor in starch resistance, although other factors such as amylose content, location and interaction with amylopectin are also thought to be involved.

The bulk of the granule however, is not strictly crystalline material (more than 50% and up to 85% depending on starch type). The high amylopectin percentage content of non-mutant granules and the observation that the level of double helical order in the granule is significantly higher than the level of granule crystallinity (Gidley & Bociek, 1985) leads to the conclusion that amylopectin is located both in the crystalline and semi-crystalline shells. In the semi-crystalline shells amylopectin is therefore still predominantly in the double helical form, although its crystallinity is reduced (possibly due to increased interaction with amylose). These conclusions fit with observation that the semi-crystalline shells of the granule contain smaller blocklets (20–50 nm in diameter) which indicates a less crystalline organization.

The localization and functions of the amorphous fraction within the crystalline shells of the starch granule may be as follows: (i) between each lamella the 'more amorphous' branching zones may be responsible for the cohesiveness of the crystal stacks; (ii) between each amylopectin side chain cluster within the lamellae the 'more amorphous' region may allow some flexibility of the crystalline lamellae themselves; (iii) around each blocklet in the hard as well as in the soft shells the amorphous fraction will be responsible for the elasticity of the system. The amorphous fraction therefore controls the variation in granule volume due to its ability to absorb and release the free water of the raw starch granules. The less flexible crystalline shells of the granule will, however, at some point limit granule expansion (Morrison *et al.*, 1994); (iv) the radial 'channels' within starch granules are believed to be predominantly composed of semi-crystalline or amorphous material. The presence of these 'channels' (and the related surface pores) through which amylose can exit the granule during gelatinization, re-inforce the thinking regarding: (i) the real existence of granule radial structure; (ii) the pre-determination of clearly defined radial locals (possibly between the more crystalline blocklets) which are more easily degraded by enzymes, and (iii) their possible role in determining the resistance of starch (in association with other factors).

In conclusion, the continuing development of microscopic techniques with ever increasing resolving power and with different requirements for sample preparation, holds enormous potential for further microscopic investigation of the starch granule. This article demonstrates, however, the importance of periodically gathering together isolated published data to improve the overall state of current knowledge. Thus the considerable evidence regarding amylopectin lamellae structure, the blocklet organization of these lamellae in the starch and the presence of radially arranged amorphous channels in some starch types has been discussed.

REFERENCES

Badenhuizen, N. P. (1936) Abhanlung zur physikalischen Chemie der Stärke und der Brotbereitung. XXV. Weitere Beobachtungen über die Blöckchenstruktur der Stärkekörner. *Zeitschrift für Physik und Chemie* **A-175,** 383–395.

Badenhuizen, N. P. (1937) Die Strukur des Stärkekorns. *Protoplasma* **28,** 293–326.

Badenhuizen, N. P. (1959) Chemistry and biology of the starch granule, in *Protoplasmatologia. Handbuch der Protoplasmaforschung* II B 2b Delta, ed. L. V. Heilbrunn and F. Weber. Springer, Vien, pp. 1–74.

Baldwin, P. (1995) Studies on the surface chemistry, minor component composition and structure of granular starches. PhD. Thesis, The University of Nottingham, U.K.

Baldwin, P. M., Frazier, R. A., Adler, J., Glasbey, T. O., Keane, M. P., Roberts, C. J., Tendler, S. J. B., Davies, M. C. and Melia, C. D. (1996) Surface imaging of thermally-sensitive particulate and fibrous materials with the atomic force microscope: a novel sample preparation method. *Journal of Microscopy* **184,** 75–80.

Bertoft, E. (1986) Hydrolysis of amylopectin by the alpha-amylase of *B. subtilis*. *Carbohydrate Research* **149,** 387–397.

Blanshard, J. M. V. (1987) Starch granule structure and function: a physicochemical approach, in *Starch: Properties and Potentials*, Critical Reports on Applied Chemistry, Vol. 13, ed. T. Galliard. John Wiley and Sons, Chichester, U.K., pp. 16–54.

Buttrose, M. S. (1960) Submicroscopic development and structure of starch granules in cereal endosperms. *Journal of Ultrastructural Research* **4,** 231–257.

Buttrose, M. S. (1963) Electron microscopy of acid-degraded starch granules. *Starch/Stärke* **15,** 85–92.

Chanzy, H., Vuong, R. and Jésior, J.-C. (1990) An electron diffraction study on whole granules of lintnerized potato starch. *Starch/Stärke* **42,** 377–379.

Duprat, F., Gallant, D. J., Guilbot, A., Mercier, C. and Robin, J. P. (1980) L'amidon, in *Les Polymères Végétaux. Polymères pariétaux et alimentaires non azotés*, ed. B. Monties. Gauthier Villars, Paris, pp. 176–231.

Evers, A. D. and McDermott, E. E. (1970) Scanning electron of wheat starch. II. Structure of granules modified by alpha-amylolysis. Preliminary report. *Starch/Stärke* **22,** 23–26.

Fannon, J. E., Hauber, R. J. and BeMiller, J. N. (1992a) Surface pores of starch granules. *Cereal Chemistry* **69,** 284–288.

Fannon, J. E., Hauber, R. J. and BeMiller, J. N. (1992b) Use of low-temperature scanning electron microscopy to examine starch granule structure and behavior, in *Frontiers in Carbohydrate Research*, Vol. 2, ed. Chandrasekaran. Elsevier Applied Science, New York, pp. 1–23.

Fannon, J. E., Shull, J. M. and BeMiller, J. N. (1993) Interior channels of starch granules. *Cereal Chemistry* **70,** 611–613.

French, D. (1984) Organisation of the starch granules, in *Starch: Chemistry and Technology*, ed. R. L. Whistler, J. N. BeMiller and J. F. Paschall. Academic Press, Orlando, pp. 183–247.

Fox, J. D. and Robyt, J. F. (1992) Modification of starch granules by hydrolysis with hydrochloric acid in various alcohols, and the formation of new kinds of limit dextrins. *Carbohydrate Research* **227**, 163–170.

Fuwa, H., Glover, D. V., Sugimoto, Y. and Tanaka, M. (1978) Comparative susceptibility to amylases of starch granules of several single endosperm mutants representative of floury-opaque, starch-deficient, and modified starch types and their double mutant combinations with opaque-2 in four inbred lines of maize. *Journal of Nutritional Science and Vitaminology* **24**, 437–448.

Fuwa, H., Sugimoto, Y., Tanaka, T. and Nikuni, Z. (1979) Scanning electron microscopy of starch granules, with or without amylase attack. *Carbohydrate Research* **70**, 233–238.

Gallant, D. J. and Guilbot, A. (1969a) Etude de l'ultrastructure du grain d'amidon à l'aide de nouvelles méthodes de préparations en microscopie électronique. *Starch/Stärke* **21**, 156–163.

Gallant, D. J. and Guilbot, A. (1969b) Application de l'oxydation periodique à l'étude de l'ultrastructure de l'amidon de pomme de terre. *Journal de Microscopie* **8**, 549–568.

Gallant, D. J. and Guilbot, A. (1971) Artefacts au cours de la préparation de coupes de grains d'amidon. Etude par microscopie photonique et électronique. *Starch/Stärke* **23**, 244–250.

Gallant, D. J., Mercier, C. and Guilbot, A. (1972) Electron microscopy of starch granules modified by bacterial α-amylase. *Cereal Chemistry* **49**, 354–365.

Gallant, D. J., Derrien, A., Aumaitre, A. and Guilbot, A. (1973) Dégradation *in vitro* de l'amidon par le suc pancréatique. *Starch/Stärke* **25**, 56–64.

Gallant, D. J. and Guilbot, A. (1973) Développement des connaissances sur l'ultrastructure du grain d'amidon. I. L'amidon de blé. *Starch/Stärke* **25**, 335–342.

Gallant, D. J. (1974) Contribution à l'étude de la structure et de l'ultrastructure du grain d'amidon. PhD. Thesis, University of Paris VI, France. N° CNRS AO 10823.

Gallant, D. J. and Sterling, C. (1976) Electron microscopy of starch and starch products, in *Examination of Starch and Starch Products*, ed. J. A. Radley. Applied Science Publishers, London, pp. 33–59.

Gallant, D. J. and Bouchet, B. (1986) Ultrastructure of maize starch granules. A review. *Journal of Food Microstructure* **5**, 141–155.

Gallant, D. J., Bouchet, B., Buléon, A. and Pérez, S. (1992) Physical characteristics of starch granules and susceptibility to enzymatic degradation. *European Journal of Clinical Nutrition* **46**, S3–S16.

Garcia, V. (1996) Transitions thermiques de l'amidons de manioc en milieux peu hydratés. PhD. Thesis, Institut National Agronomique de Paris-Grignon, France.

Geddes, R., Greenwood, C. T. and Mackenzie, S. (1965) Studies on the biosynthesis of starch granules. Part III. The properties of the components of starches from the growing potato tuber. *Carbohydrate Research* **1**, 71–82.

Gidley, M. J. and Bociek, S. M. (1985) Molecular organisation in starches: A ^{13}C CP/MAS NMR study. *Journal of the American Chemical Society* **107**, 7040–7044.

Hanson, E. A. and Katz, J. R. (1934) Uber Versuche die gewachsene Struktur des Stärkekorns mikroskopisch sichtbar zu machen, besonders an lintnerisierter Stärke. *Zeitschrift fur Physik Chemie* **A168**, 339–352.

Helbert, W. and Chanzy, H. (1996) The ultrastructure of starch from ultrathin sectioning in melamine resin. *Starch/Stärke* **48**, 185–188.

Heyn, A. N. J. (1959) Electron microscope observation on helical structure in starch. *Textile Research Journal* **29**, 366–368.

Hizukuri, S. (1986) Polymodal distribution of the chain lengths of amylopectins, and its significance. *Carbohydrate Research* **147**, 342–347.

Imberty, A., Chanzy, H., Pérez, S., Buléon, A. and Tran, V. (1987) New three-dimensional structure for A-type starch. *Macromolecules* **20**, 2634–2636.

Imberty, A. and Pérez, S. (1988) A re-visit to the three-dimensional structure of B-type starch. *Biopolymers* **27**, 1205–1221.

Jane, J. L., Xu, A., Radosavljevic, M. and Seib, P. A. (1992) Location of amylose in normal starch granules. I. Susceptibility of amylose and amylopectin to cross-linking reagents. *Cereal Chemistry* **69**, 405–409.

Jenkins, P. J., Cameron, R. E. and Donald, A. M. (1993) A universal feature in the structure of starch granules from different botanical sources. *Starch/Stärke* **45**, 417–420.

Jenkins, P. J. and Donald, A. M. (1995) The influence of amylose on starch granule structure. *International Journal of the Biology of Macromolecules* **17**, 315–321.

Joseleau, J. P. and Ruel, K. (1985) A new cytochemical method for ultrastructural localization of polysaccharides. *Biol. Cell.* **53**, 61–66.

Kasemsuwan, T. and Jane, J. L. (1994) Location of amylose in normal starch granules. II. Locations of phosphodiester cross-linking revealed by phosphorus-31 nuclear magnetic resonance. *Cereal Chemistry* **71**, 282–287.

Kassenbeck, P. (1975) Eleckronenmikroskopisher Beitrag zur Kenntnis der Feinstruktur der Weizenstärke. *Starch/Stärke* **27**, 217–227.

Kassenbeck, P. (1978) Beitrag zur Kenntnis der Verteilung von Amylose und Amylopektin in Stärkekörnern. *Starch/Stärke* **30**, 40–46.

Lineback, D. R. (1986) Current concepts of starch structure and its impact on properties. *Journal of the Japanese Society of Starch Science* **33**, 80–88.

Manners, D. J. (1989) Recent developments in our understanding of amylopectin structure. *Carbohydrate Polymers* **11**, 87–112.

Macgregor, A. W. (1991) Starch degrading enzymes. *Fermentation* **4**, 178–182.

Miller, B. S., Derby, R. I. and Trimbo, H. B. (1973) A pictorial explanation for the increase in viscosity of a heated wheat starch-water suspension. *Cereal Chemistry* **50**, 271–280.

Morrison, W. R. and Gadan, H. J. (1987) The amylose content of starch granules in developing wheat endosperm. *Journal of Cereal Science* **5**, 263–275.

Morrison, W. R., Tester, R. F. and Gidley, M. J. (1994) Properties of damaged starch granules. II. Crystallinity, molecular order and gelatinisation of ball-milled starches. *Journal of Cereal Science* **19**, 209–217.

Mussulman, W. C. and Wagoner, J. A. (1968) Electron microscopy of unmodified and acid modified corn starches. *Cereal Chemistry* **45**, 162–171.

Nägeli, C. W., Die Stärkekörner. Pflanzenphysiologischer Untersuchungen. Heft 2. Verlag F Schülthess. Zürich, 1858.

Nikuni, Z. and Hizukuri, S. (1957) Structure of starch granules, in *Memoirs of the Institute of Scientific and Industrial Research*, Vol. 14. Osaka University, pp. 173–181.

Nikuni, Z. (1957) Proposed model of a starch granule or a starch molecule. *Chori Kagaku* (Japan) **2**.

Notté, C. (1993) Structure et fonctionnabilité des amidons modifiés chimiquement. DEA Cellular Biology. Faculty of Sciences and Technology, University of Nantes, France.

Oostergetel, G. T. and van Bruggen, E. F. J. (1989) On the origin of a low angle spacing in starch. *Starch/Stärke* **41,** 331–335.

Oostergetel, G. T. and van Bruggen, E. F. J. (1993) The crystalline domains in potato starch are arranged in a helical fashion. *Carbohydrate Polymers* **21**, 7–12.

Planchot, V. (1993) *alpha*-Amylases d'*Aspergillus fumigatus*. Mechanismes d'action en phase hétérogène. PhD. Thesis, University of Nantes, France.

Planchot, V., Colonna, P., Gallant, D. J. and Bouchet, B. (1995) Extensive degradation of native starch granules by *alpha*-amylase from *Aspergillus fumigatus*. *Journal of Cereal Science* **21,** 163–171.

Robin, J. P., Mercier, C., Charbonnière, R. and Guilbot, A. (1974) Lintnerized starches. Gel filtration and enzymatic studies of insoluble residues from prolonged acid treatment of potato starch. *Cereal Chemistry* **51**, 389–406.

Sterling, C. (1968) The structure of the starch grain, in *Starch and its Derivatives*, 4th edn, ed. J. A. Radley. Chapman & Hall, London, pp. 139–167.

Thomson, N. H., Miles, M. J., Ring, S. G., Shewry, P. R. and Tatham, A. S. (1994) Real-time imaging of enzymatic degradation of starch granules by atomic force microscopy. *Journal of Vacuum Science Technology* **B12,** 1565–1568.

Whistler, R. L. and Thornburg, W. L. (1957) Development of starch granules in corn endosperm. *Journal of Agricultural Food Chemistry* **5**, 203–207.

Whistler, R. L. and Turner, E. S. (1955) Fine structure of starch granule sections. *Journal of Polymer Science* **18**(87), 153–156.

Yamaguchi, M., Kainuma, K. and French, D. J. (1979) Electron microscopic observations of waxy maize starch. *Journal of Ultrastructural Research* **69**, 249–261.

Zherebstov, N. A., Ruadze, I. D. and Yakovlev, A. N. (1995) Mechanism of acid-catalyzed and enzymatic hydrolysis of starch. *Applied Biochemistry and Microbiology* **31**, 511–514.

Zobel, H. F. (1988) Molecules to granules: A comprehensive review. *Starch/Stärke* **40,** 1–7.