Ultrastructure of Maize Starch Granules. A Review

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Abstract

History of starch granule ultrastructure and the principal data obtained on maize starch granules are analyzed. New results are developed: i) growth and development of the maize starch granules during maturation depend on the maize varieties and the tissue site in the kernel, especially the horny and floury endosperms; ii) cytochemical studies of the starch granules differing from their amylose/amylopectin ratio show important differences in the distribution of their crystalline and amorphous zones that explain their behaviour under some hydrolytic treatments; iii) complexing between colloidal gold labelled Concanavalin-A and starch (amylopectin) permits new and greater specificity to ultrastructural study of the starch granule.

Key words: Starch granule ultrastructure, amylo-, maize, waxy and normal maizes, corn, scanning electron microscopy, transmission electron microscopy, histochemistry, lectins, Concanavalin-A.

History of the starch granule ultrastructure

Historically, the understanding of starch granule ultrastructure is deeply connected to the development of electron microscopy techniques and microscopes (Gallant, 1974). Studies and results which are summarized in some papers (Gallant, 1974; Gallant and Sterling, 1976; Nikuni, 1978; Hood and Liboff, 1982; French, 1984) can be divided into three principal periods:

1) The replica period: From 1950 to 1960 most of the experiments on the starch granule ultrastructure were limited to making surface replicas of commercially purified starch (Kore-Eda and Nikuni, 1955; Whistler et al., 1955) or internal starch granule replicas after sectioning (Whistler and Turner, 1955; Nikuni and Hizukuri, 1957; Whistler and Thornburg, 1957; Whistler et al., 1958). The starch granule surface basically appeared smooth with occasional indentations of small starch granules or protein bodies in the hard endosperm. Internally, very fine granular structures (microgranules of 20 to 30 nm diameter) with occasional central cracks were seen. Using similar techniques, Sterling and Spit (1957) provided evidence for the structural compositions of the internal fibrils and the surface papillae (about 20 nm diameter) which were believed to be the surface end of the internal microfibrils.

2) The sectioning and contrasting period: From 1960 to 1980 workers used the degradative treatments introduced by Buttrose (1960) in the study of the starch granule with mild acid or enzymatic (germination) treatments. These treatments were used to improve the in vivo or in vitro starch granule susceptibility to ultrathin sections of the degraded starch granules were studied. Neither Buttrose (1960), nor Mussulman and Wagoner (1968) used carbohydrate contrasting. Although the general results were of some interest, the resolution seen in the micrographs remained very poor. It was, in fact, impossible to discern the ultrafine structure of the starch granule except for what remained after hydrolysis.

Elsewhere, acid treatment was combined with the use of potassium permanganate (Innamorati, 1966), or chromic acid (Hölzl and Bancher, 1965). Potassium permanganate at low pH, or chronic acid reacted as oxidants of the primary alcohol of alpha-D-anhydroglucosone units and the precipi-
tation of Mn or Cr gave rise to a high contrast in the residual fraction of the hydrolyzed starch granules.

During this period, the chemical procedures for chelating oxidized cellulose were introduced by Edel (1962) in the textile field. Radioactive metals fixed by the alpha-glycols were chelated stoichiometrically and analyzed quantitatively, after specific oxidation of the C2-C3 secondary alcohols and thiosemicarbazide fixation. Such procedures were developed for electron microscopy of carbohydrates and the starch granules under the periodic acid-thiosemicarbazide-silver reaction (PATAg) which used silver nitrate as the contrasting reagent (Gallant, 1974; Gallant et al., 1972, 1973; Gallant and Guilbot, 1969a, b and 1973; Gallant and Sterling, 1976; Kassenbeck, 1975, 1978).

Also, chemical reactions were used to locate carbohydrates and starch granules on ultrathin sections and were developed by: i) the fixation of plumbite HPbO2, (Gallant, 1974) via hydrogen bonding according to Karnovskj (1961) in very alkaline medium; ii) the fixation using cesium butylate (Gallant et al., 1967; Gallant, 1974) according to Hagege (1967) via the free secondary alcohols R-OH of alpha-D-anhydroglucose units being substituted with cesium butylate (Cs2H5OCs). Such reaction with alkaline alcoholicates was confirmed by Mentre (1972) on starch granules and other polysaccharides using the thallium ethanolate Cs2H5OTl as a reactant; iii) the fixation of iodine as insoluble lead iodine (PbI2) as a reactant; iv) the fixation of iodine as insoluble lead iodine (PbI2) and modified by Yamanouchi et al. (1979) used uranyl acetate as a negative stain for wet mashed starch granules or amylopectin dispersions in order to outline ultrafine structure.

In summary for TEM: i) several ways can be used for nonspecific and specific detection of starch granules and other polysaccharides on ultrathin sections; ii) ultrastructural studies of starch granules require acid, enzymatic or/and oxidative treatments of the whole granules prior to staining. The starch granules appear to consist of alternate rings of crystalline and amor­phous materials; iii) the macromolecular structure is contro­versial with some workers convinced of the granular-like units (Gallant, 1974; Duprat et al., 1980) and others (Kassenbeck, 1978; Yamaguchi et al., 1979; French, 1984) suggesting that the fine structure is a periodic organization of superimposed clusters (amylopectin) radially oriented according to the model of amylopectin hypothesized by biochemists (French, 1975, 1984; Robin, 1976). 3) The scanning electron microscopy period: Since 1970 and the interesting paper series of Hall and Sayre (1969, 1970a, b, 1971a, b, 1973), Evers (1969, 1970, 1971), Evers and McClemont (1970), and Evers et al. (1971), numerous papers on the SEM of starch granules have been published on cereal (wheat, barley, maize, sorghum, and rice), tuber and legume starches, especially after enzymatic, physical, chemical and hydrothermic treatments. These studies have shown: i) a good correlation between SEM micrographs of the starch granule surfaces and micrographs of native granule replicas; ii) very useful three-dimensional views of the degradation by canals of corrosion; iii) very important structural differences between starch of different origins; and iv) very close correlation between SEM and TEM studies (Gallant, 1974; Gallant and Guilbot, 1973; Gallant et al., 1973).

Maize starch granule morphology and development

The structures of starch and the protein matrix were studied in the soft and hard endosperms of normal and opaque-2 maize kernels with SEM using a modified, natural drying process (Robutti et al., 1974). Starch from the floury and horny endosperms differed in shape; polygonal and tightly packed starch granules showed indentations made by zein bodies embedded in the protein matrix of the hard normal endosperm whereas soft endosperm contained intergranular air spaces, particularly in opaque-2 maize kernels and round starch granules.

These fundamental differences can be generally observed in all cereal seeds containing hard or soft endosperm, as shown in Figure 1 on waxy, normal maize and amylo maize. The differences are demonstrated by the heterogeneous shape and the smaller size of amylo maize starch granules and, in particular, by the weakness of the larger waxy maize starch granules in horny endosperm.

Physico-chemical characteristics of normal (24% amylose) and amylo maize (38 and 64% amylose) starch granules, A76, A62, and A36, respectively, have been followed during their formation and maturation (Mercier et al., 1970). Amylose content increased from the beginning of grain formation up to the 35th day after anthesis. Two weeks after anthesis, both A76 and A62 exhibited type A X-rays spectra, but A36 was typical of type B spectra. Amylo maize A62 shifted to type B spectra around the 22nd day after anthesis. Starch granule shape and size from floury endosperm was studied too. Normal maize starch granules increased in a regular manner in size and a new population of small granules appeared on the 72nd day after anthesis. Amylo maizes (A62 and A36) showed two populations of starch granules, spherical and filamentous.

We observed that in commercial amylo maize, the greater the amylose content in the starch, the greater the number of filamentous granules. Starch formation was studied during maturation (Mercier et al., 1970). The percentage of irregular starch granules in the 38% amylose increased between the 22nd and 29th day after anthesis and decreased by the 72nd day after anthesis. Inversely, the percentage of irregularly shaped starch granules in the 64% amylose, mostly filamentous in shape, increased continuously up to the 72nd day after anthesis (Fig. 2).

A comparison between the size and shape of starch granules in hard and soft endosperms was studied by Gallant (1974). As seen in Figure 3, 6
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Fig. 1. SEM of maize starch granules. a) waxy maize in floury and b) in horny endosperms; c) normal maize in floury and d) in horny endosperms; e) amylo maize in floury and f) in horny endosperms. cw: cell walls; fs: filamentous starch granules; i: zein bodies indentations; zb: zein bodies.
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<th>normal maize A76</th>
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<tr>
<td>72nd day</td>
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Fig. 2. Morphological evolution of maize starch granules in floury endosperms of normal maize (A76) and amylomaizes (A62 and A36) during maturation from 15th to 72nd day after anthesis as seen under polarized light. Note that filamentous starch granules of the amylomaizes show birefringency (Maltese cross) only from part to part in some nucleations. Percents are amylose contents during maturation.
Maize starch granule ultrastructure

Approximately ten years ago, SEM confirmed that in vitro alpha-amylolysis of commercial starch granules led to somewhat damaged granules and that the pattern of degradation was characteristic of each species (Gallant et al., 1973). SEM also was used for nutritional studies. It was used to study breakdown of maize starch granules by microflora in the digestive tract of chicken (Champ et al., 1981). In vitro evidence showed that Lactobacillus strain 207 hydrolyzed normal maize starch granules less than strain 220 did. A more extensive degradation (pin holes and internal cavities) was seen when starch was included in the feed indicating some mechanical damages due to processing (pelleting). No damage appeared in starch granules obtained from the crop of axenic (germ free) chicken. Maize starch granules drawn from the crop of holoxenic (conventional) and monoxenic (only one microorganism) chicken were scarcely damaged and erosion was always superficial.
When treated with porcine (hog) pancreatic juice (Gallant et al., 1973), normal and waxy maize starch granules showed numerous rapidly developing pin holes randomly distributed on the starch granule surface. Evidence of internal saw-tooth patterns inside the granules. Differences were observed mainly in the hydrolysis rate. Normal maize starch granules showed random endocorrosion, the radial rate of degradation being faster than the tangential one. Waxy maize starch granules showed random endocorrosion too; however, radially the degradation rate was slower than the tangential one. Starch granules stained after mild periodic oxidation were studied by TEM showing that in both cases a good correlation exists between zones which are most susceptible to amylolysis and those that are the less crystalline layers.

Amylomaize (amylose extender) starch granules treated with porcine pancreatic juice did not show such structure (Gallant et al., 1973). Using SEM, no evidence of exocorrosion occurred with granule surfaces remaining almost completely smooth except in some points where the enzyme had penetrated. Those points were seen to have narrow pin holes surrounded by circular protuberances, crater-like. Evidence of internal hydrolysis having taken place could be seen only by TEM. Such SEM observations were in agreement with Knutson et al. (1982) who noted that the mode of attack by hog pancreas alpha-amylase on amylomaizes V and VII was quite different from that on dent corn starch. For example, instead of typical corrosion type effects in dent corn, they only observed roughening of amylomaize granule surfaces. Beneath the surface protuberances, amyllose susceptible material was more or less digestible with the radial degradation being greater than the tangential degradation (Gallant et al., 1973). Nevertheless, this effect was not considered similar to normal and waxy maize starches with the shell organization being less pronounced or absent in case of amylomaize.

SEM of normal, waxy and high-amylose maize starch granules was studied by Takaya et al. (1978) using various strains of alpha-amylase (crude powder of Streptomyces hygroscopicus, Aspergillus oryzae Alpha-amylase, Rhizopus an-agarakkeniis glucoamylase and Streptomyces hygroscopi­cus alpha-amylase). Normal maize starch granules were also studied after treatment with Streptomyces precox alpha-amylase (Takaya et al., 1979). As previously reported (Evers and McDermott, 1970; Gallant et al., 1973; Gallant, 1974), amylase attack started with small pits on the granule surface. With time the pits increased in number and size, and pores penetrated toward the center of the granules. Streptomyces precox alpha-amylase seemed to hydrolyze starch granules better than other alpha-amylases (Takaya et al., 1979).

Using Rhizopus glucoamylase or crystalline bacterial alpha-amylase, Fuwa et al. (1978a, b) found a higher resistance in the granule of high-amylose maize starches showing shapes and surface similar to the native granules, but other maize starch granules presented the same pin holes already described.

On the other hand, Fuwa et al. (1978a) studied comparative susceptibility of several single endosperm mutants and their double-mutant combinations with opaque-2 in four inbred lines of maize, giving internal saw-tooth (ae), aeo2, waxy (wx), wxo2, sugary-1 (dull) and sul0 maize starch granules which were treated by Rhizopus glucoamylase and pancreatic. Relative susceptibility of single mutants and their normal counterparts were in increasing order, amylose extender, normal, opaque-2, waxy and sugary-1. Starch granules of double mutant combinations with the 02 gene were digested similar to their non-opaque single mutant counterparts.

Other studies (Gallant, 1974; Duprat et al., 1980) gave more details on granule structure. Starch granules appeared to be composed of successive layers or shells of more or less crystalline material (Fig. 4). Each layer in waxy maize starch granules was composed of one layer of 100 nm thickness being built with very compact crystalline blocklets or spherulites of 100 nm diameter. These were separated from one another by 200 nm of a less crystalline layer built with smaller blocklets (50 nm diameter). Normal starch granules were more complex in that the less crystalline layers were thinner (40 to 50 nm) while the more crystalline layer was thicker (200 to 300 nm).

Amylomaize starch granules were much different: i) the starch granule periphery was resistant to amylolysis; and ii) spherical and filamentous starch granules internally consisted of very uniform blocklets from 80 to 100 nm in diameter (Gallant, 1974; Duprat et al., 1980).

Such size ranges are not far from data obtained by Yang et al. (1985) using small angle X-ray scattering by hydrated wheat starch granules. These authors did not report the 10 nm defraction spacing reported by others; neither did we, except when the starch granule is limnirized (Gallant, 1974; Duprat et al., 1980).

The compact spherulitic structure of the starch granule has also been revealed by freeze-etching or freeze-fracturing of purified and modified starches (Allen and Hood, 1976; Allen et al., 1977; Chabot et al., 1978; Davis and Gordon, 1979; Höglund, 1981; Holzl, 1973; Hood, 1973; Mußlethalre, 1965) or starches in foods (Barlow et al., 1973; Bechtel, 1985; Cloke et al., 1982; Hsieh et al., 1981; Resmini and Pagani, 1983). Using these techniques, spherulites varied from 5 to 20 nm, which is a lower size range than we observed for the small blocklets of the less crystalline layer.

Working on Gallant's results (1974) and improving the techniques he used, Kassenbeck studied the fine structure of wheat (Kassenbeck, 1975) and maize (Kassenbeck, 1978) starches. Basic sample preparation was as follows: freeze-drying of 1-2 mm cross sectioned kernels, 48 h formaline fixation and 12 h washing, 40 to 50 μm thickness cryosectioning and PATAg reaction on the cryosections, embedding in methacrylate, ultrathin sectioning and post-staining. Kassenbeck explained that the aldehyde groups produced by periodic oxidation were involved in hemiacetal formation in the relatively less oxidized area. The hemiacetal linkages are
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Assuming that the stains proposed by Kassenbeck (1978) were not really specific for amyllose and amylopectin, preliminary evaluations by Miller et al. (1984) and Bouchet et al. (1984) suggest that using lectins as a tool will soon permit greater specificity to such studies.

Miller et al. (1984) treated glycol methacrylate sections of cereal grains with Lens culinaris agglutinin (LCA) labelled with FITC, and observed them under UV illumination (450-490 nm/520 nm). When applied to oat grain sections, LCA-FITC stained only the compound starch granules. When applied to wheat starch sections, the shell organization of lenticular starch granules was clearly seen and an intense fluorescence of the central core of the smaller starch granules was observed.

Bouchet et al. (1984) studied the specificity of Canavalia ensiformis agglutinin, namely Concanavalin-A (Con-A), towards maize starch granules (amylo maize, normal and waxy maize with an amylopectin content of 30, 76 and 99 %, respectively). Con-A interacts (Fig. 5) only with the non-reducing, end chain groups of alpha-D-glucosides, alpha-D-mannosides, alpha and beta-D-fructofuranosides, by coupling with the C3, C4 and C6 hydroxyl groups; and with the C2, C3, C5 hydroxyl groups of alpha and beta-D-arabinofuranosides (Goldstein, 1972, 1976). Reaction occurs only when Con-A is in its tetrameric conformation (pH = 7) in the presence of cations, Ca²⁺, Mg²⁺ and Mn²⁺ (Becker et al., 1976; Goldstein, 1976). Complexing was achieved for light microscopy with dichlorotriazinyl-amino-fluorescein, the starch suspension being observed under blue light illumination (450/530 nm). For TEM, colloidal gold labelled Con-A according to Horisberger and Rosset (1977) and Horisberger (1979) has been used. The following are new TEM results:
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Fig. 5. A1: diagram of the linear and helicoidal amylose macromolecule (after Nikuni, 1978); A2: the amylose macromolecule which contains about 2,000 anhydroglucose units shows one reducing end chain group (c, Fig. A1) and only one non-reducing end chain group (e, Fig. A1); B1: diagram of the grape-like clustering of branch points amylopectin macromolecule (after French, 1972); B2: the amylopectin macromolecule which contains 100,000 to 1 billion anhydroglucose units, shows only one reducing end chain group (c, Fig. B1) but 10,000 to several million non-reducing end chain groups (e, Fig. B1). Note that the molecular configuration with the 3 hydroxyl groups allowing Con-A coupling is only shown by the non-reducing end chain groups (amylose and/or amylopectin).

In the inset are the oligosaccharides (hexapyranosides and furanosides) reacting with Con-A. C1: alpha-D-glucose; C2: alpha-D-mannose; C3: alpha-D-arabinofuranose; C4: beta-D-arabinofuranose; C5: alpha-D-fructofuranose; C6: beta-D-fructofuranose. Specificity of Con-A is shown too for the non-reducing end chain groups of their polymers.

Inset symbols represent: □ (CH2), ○ (OH groups) not involved and ● (OH groups) involved in the coupling reaction with Con-A.

i) Colloidal gold labelled Con-A can be used specifically to reveal Con-A binding sites on the whole starch granule but after chemical or enzymatic treatments only (Bouchet et al., 1984).

ii) Contrary to LCA binding as shown by Miller et al. (1984), the reaction between Con-A and the whole starch granule was negative (Bouchet et al., 1984), possibly because the starch granule surface does not have free non-reducing end chains. It must be remembered that Miller and co-workers treated thin-sections with LCA and not the whole granules. That is confirmed by the strong surface reaction we obtained after mild periodic oxidation of the whole granule (Figs. 6a, 6b and 10b). Such release of the non-reducing end chains was also observed after alpha-amylolysis of the whole granules Figs. 7, 8a, 9a and 10a) but the intensity of coupling was always lower than that after periodic oxidation.

iii) Whole amylomaize starch granules always showed weak coupling. Their high amylose content confirmed interactions between Con-A tetramers and non-reducing end chains groups. Theoretically, as shown Figure 5, there was single coupling in the case of the amylose macromolecule (DP around 2,000 anhydroglucose units), but possibly ten thousand to several million couplings in case of the amylopectin macromolecule (DP reaching 100,000 to one billion anhydroglucose units). Such giant macromolecules which are of a crystalline, structured conformation, then must be fully closed; their external chains which may react with lectins are consequently those which can be released after mild treatments.

iv) Surface oxidized amylomaize starch granules reacted with Con-A, although the reaction was weak. The resistant surface membrane previously described (Gallant et al., 1973) contains sites of alpha-D-glucose which may be sites penetrable by alpha-amylase.

v) After mild periodic oxidation of whole normal and waxy maize starch granules, coupling did not occur inside the granule (Bouchet et al. 1984). Loss of the crystalline structure on macromolecules at the granule surface may allow better accessibility of the amylopectin non-reducing end chains to Con-A.

vi) Binding can be achieved with thin sections (Fig. 7, 6b, 9c and 10c) of starch granules which were treated with alpha-amylase. We observed gold labelling on the whole sections of waxy (Fig. 7 and 8b), normal (Fig. 9c) maize, and amylomaize (Fig. 10c) starch granules, and stronger reaction at levels of less crystalline layers (Fig. 7). It is interesting to note that waxy and normal varieties reacted as well as amylomaize. As for histochemical reactions, variations in binding seem more selective on the whole granule than on thin sections of the starch granules.
Fig. 7. ConA-Au coupling achieved with thin section of waxy maize starch granule which was treated with alpha-amylase.

Fig. 8. ConA-Au coupling of waxy maize A99 (99% amylopectin) starch granule. a) coupling of thin section of granule partly digested by alpha-amylase. Same abbreviations as Fig. 6.

Fig. 9. ConA-Au coupling of normal maize A76 (76% amylopectin) starch granule. a) coupling of the whole granule after partial alpha-amylolysis; b) after mild periodic oxidation of the whole granule; c) coupling of thin section after partial alpha-amylolysis of the whole granule. Same abbreviations as Fig. 6.
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Fig. 10. ConA-Au coupling of amylo maize A30 (30% amylopectin) starch granule. a) coupling of the whole granule after alpha-amylolysis. From stars to periphery there is the peripheral part resistant to amylolysis (after Gallant et al., 1973); b) after periodic oxidation of the whole granule; c) coupling of thin section after partial alpha-amylolysis of the whole granule. Same abbreviations as Fig. 6.

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Acknowledgments

Many thanks are due to Mrs Martine Chapeau for typing this paper.

Discussion with Reviewers

P. Resmini: Do the authors include in the term "replica" also the freeze-fracturing preparations?

Authors: In the term "replica" we only included replicas of whole granule surface as usually performed in the years before 1960 (see Gallant, 1974; Gallant and Sterling, 1976). A collodion or plastic replica was made first, and then was shadowed with heavy metal, either before or after removing the biological material, according to the different workers.

Freeze-fracturing methods were developed mainly after 1960 and used first by Mühlethaler (1965) for ultrastructural study of the starch granule.

P. Resmini: Does the amylo maize starch exhibit crystalline or amorphous properties?

Authors: As noted in this paper, and contrary to normal and waxy maize starch granules which show A-type spectra X-rays, mature amylo maize starch granules show B-type spectrum X-rays (Mercier et al., 1970).

Nevertheless, under polarized light, round amylo maize starch granules exhibited very clear Maltese crosses, whereas the filamentous ones never exhibited such birefringency (see Fig. 2).

P. Resmini: What were the conditions for alpha-amylolysis?

Authors: Ultrathin sections were incubated 24h (37°C) with a 0.01% solution bacterial alpha-amylase (from Bacillus subtilis, Sigma) in saline phosphate buffer (PBS) 0.01M (pH 7.2) containing 0.01% NaN3.

P. Resmini: In some Figures (8b, 9c and 10c), clumps of gold particles are visible. Do these areas correspond to particular aggregates of starch material (perhaps areas with high density of amylopectin molecules)?

Authors: We cannot say what the clumping of gold particles means as it was seen on previous photomicrographs. Since the review, we improved the coupling technique we used. Photomicrographs 7, 8a, 9c and 10c are new micrographs, showing better, more intense couplings than the ones which were originally submitted to the reviewers.

Clumping of gold particles may be areas showing higher density of amylopectin macromolecules; but may also be related to more numerous free non-reducing end chains. Now we are working on this reaction in order to understand better what happens at level of the starch granule section.

P. Resmini: Did the authors study the labelling with lectins on gelatinized starch granules and if so, is this possible without amylolysis or periodic oxidation?

Authors: As indicated in our paper (Bouchet et al., 1984), such a study was planned but has not yet been completed using colloidal gold labelled Concanavalin-A. We only tried to label swollen maize starch granules (normal, waxy maize and amylo maize) using the fluorescent (FITC) labelled Concanavalin-A. Reaction was negative with all these swollen starches.

S.H. Yiu: Con-A binds materials that contain free alpha-D-glycosyl and/or alpha-D-mannosyl side chains. In your opinion, can the Con-A-gold conjugate be used as marker for intact starch granules?

Do you think that more non-reducing side chains are exposed in starch granules obtained from thin-sectioning?

Authors: Theoretically, when intact starch granules contain free alpha-D-glycosyl side chains, coupling might occur. Unfortunately that has never been seen in our experiments.

We can consider the "grape-like clustering of branch points with longer straight chain portions" which has been proposed by biochemists (Rollings, 1985) as the most probable model for amylopectin. However, because granules did not react without additional treatment, such macromolecular conformations must be very close at the surface of native starch granules. Then, the blocklets, already shown by electron microscopists as real structural units could explain this lack of reactivity and must be taken into account in any serious hypothesis of the native starch granule ultrastructure.

Only some treatments are able to increase the porosity of the starch granule, thereby exposing the structural units, producing the release of non-reducing end chains and increasing number of end chain groups being able to react. Thus, in our opinion, Con-A-gold conjugate cannot be used as EM marker for intact starch granules.

Experiments have shown that coupling might occur in thin-sectioning starch granules because, when such closed structures are cut and then opened, more non-reducing side chains are released and more non reducing side chain groups are stoichiometrically free.
S.H. Yiu: Your findings show that oxidation or enzymatic hydrolysis is required for the binding between Con-A-gold and starch to take place. Have you observed binding of Con-A-gold, to mechanically damaged (due to processing) starch granules?

Authors: Although not yet investigated, it seems likely that damaged starch granules could react, at internal fissures due to processing, as well as with sectioned starch granules.

S.H. Yiu: How do you relate your findings to the ultrastructural organization of maize starch granules?

Authors: It is somewhat premature to discuss ultrastructural organization of maize starch granules in relation to the new findings using Con-A-gold. Multiple treatment combinations are now under investigation and results appear partly contradictory with what we knew before the experiments about starch organization.