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Channels of maize and sorghum starch granules

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Abstract

The radial, tube-like channels of corn/maize and sorghum starch granules, which penetrate from the external surface inward toward a cavity at the hilum, were found to vary in depth of penetration from granule to granule. Most, but not all, channels spanned the entire granule matrix, from the outer surface to the central cavity. Under slight swelling conditions (water), cavities swelled somewhat closed, while channels appeared to remain open. Swelling also affected the permeability of the granule matrix to dye molecules. Penetration of an aqueous dye solution occurred primarily from the central cavity outward and laterally from channels. Even under the slight swelling conditions, colloidal gold particles filled channels and cavities, showing that they are voids.

Keywords: Channels; Cavities; Starch granules

1. Introduction

Most granular starch utilized in food and industrial applications is first modified chemically to improve its physicochemical properties in accordance with its intended function. Although industrial chemical modification processes have been studied extensively, the role of granule architecture in the reactivity of starch is not understood because of an incomplete understanding of granule structure. Ultimately, granule microstructure and ultrastructure must dictate accessibility of individual starch polymer molecules to chemical reagents and, thus, greatly influence the pattern of chemical reaction and location of individual reaction sites within granules.

Pores at the surfaces of starch granules, internal cavities at the granule hilum, and channels connecting the two are architectural features that could influence granule reactions. The pores observed on maize (corn), sorghum and millet starch granules were hypothesized to be openings to channels that provide access to the granule interior (Fannon, Schull & BeMiller, 1993). Later, the existence of channels and the relationship between pores, channels, and cavities were established unequivocally (Huber & BeMiller, 1997). The objective of this research was to utilize this improved knowledge of starch granule microstructure to gain insight into penetration of chemical reagents.

1.1. Surface pores

Pores were observed on the surfaces of some maize (corn), sorghum and millet starch granules, and along the equatorial groove of large wheat, barley and rye starch granules (Fannon, Hauber & BeMiller, 1992; Hall & Sayre, 1970a). Evidence was presented that the pores were not artifacts of processing or preparation of specimens for scanning electron microscopy (SEM). Their distribution appeared to be random, with varied numbers per granule (Fannon et al., 1992). Pores tended to occur in clusters and were reported to be more prevalent on spherical granules of the floury endosperm (Dombrink-Kurtzman & Knutson, 1997; Fannon et al., 1992).

1.2. Channels

Evidence that granule pores might be more than surface features, i.e. that pores might be openings to channels, was provided by Fannon et al. (1993). Short portions of what appeared to be channels within starch granules could be perceived in a transmission electron micrograph of sorghum endosperm. Channels appeared to be serpentine in nature and were estimated to range from 0.07 to 0.10 μm in diameter, which falls within the limits (0.005–0.400 μm) measured for waxy maize starch granules via mercury porosimetry by Karathanos and Saravacos (1993). Clear evidence that channels in corn and sorghum starch granules connect an internal cavity to the external environment was presented by Huber and BeMiller (1997).

Gallant, Bouchet and Baldwin (1997) argued that channels

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are not void spaces, but instead may contain amorphous material. Further, they suggested that channels are short, penetrating at most a few granule shells and that channels might represent junction zones between amylopectin blocklets, which they proposed as the building blocks of starch granules. Based on the model describing the structure of starch granule crystalline domains put forth by Oostergetel and van Bruggen (1993), Oates (1997) also attempted to explain the origin of pores and channels. In the model proposed by Oostergetel and van Bruggen (1993), a crystalline domain is comprised of adjacent chains of an amylopectin molecule, which together are oriented to form a supermolecular helix with a void large enough to accommodate an amylase molecule, but which may be plugged with an amylose–lipid complex, preventing access of enzymes (Oates, 1997). However, the Oostergetel and van Bruggen model was formulated for potato starch granules, which contain little amylose–lipid complex (perhaps none in ungelatinized granules) and which exhibit only exocorrosion when treated with hydrolytic enzymes (Fannon et al., 1992; Gallant, Derrien, Aumaitre & Guilbot, 1973), i.e. do not contain pores (Fannon et al., 1992). Channels formed by superhelices (~8 nm in diameter) are certainly not the much larger channels viewed by fluorescence microscopy (Huber & BeMiller, 1997) and seen externally as pores (Fannon et al., 1992).

1.3. Starch granule cavities

Central cavities, which are voids at the hilum of mature starch granules, were first reported by Reichert (1913), but since have been observed and described by others, beginning with Whistler and Turner (1955). By viewing aqueous suspensions of previously dried, native starches under the light microscope, Hall and Sayre (1973) detected internal cavities in common maize (corn), sorghum, and waxy maize, but not barley, oat, rice, or wheat starch granules. By crushing, they found internal cavities in potato and canna starch granules (Hall & Sayre, 1970b). Internal cavities were observed in commercial waxy maize starch granules using a freeze-etching technique coupled with transmission electron microscopy (TEM) (Chabot, Allen & Hood, 1978). Baldwin, Adler, Davies and Melia (1994) detected cavities in potato, rice and wheat (B only) starch granules using a combination of light microscopy, SEM, and confocal scanning laser microscopy (CSLM). Cavity size in potato starch was reported to vary widely among granules. No apparent relationship between granule size and cavity size was observed, and granules of all sizes possessed cavities. Huber and BeMiller (1997) presented additional evidence of the presence of cavities in maize and sorghum starch granules. Overall, the preponderance of evidence suggests that cavities observed at the hilum of some native starch granules are actual granule features and not artifacts of specimen preparation, although formation and development of cavities in native starch granules have been attributed to drying, which involves moisture loss, often at elevated temperatures (Baldwin et al., 1994; Whistler & Thornburg, 1957). Thus, the previous history of the starch may be related to the presence of cavities.

Amylolytic enzymes enlarge the cavities at the hilum of certain of starch granules (Giraud, Champailler & Raimbault, 1994; Revilla & Fernandez-Tarrago, 1986; Zhao, Madson & Whistler, 1996). It has been known for some time that maize starch granules are preferentially degraded from the inside out beginning at the hilum (Fuwa, Sugimoto, Tanaka & Glover, 1978; Helbert, Schuelein & Henrissat, 1996; Leach & Schoch, 1961; Nikuni, 1956; Nikuni & Whistler, 1957; Schwimmer, 1945), and it has been hypothesized that the presence of pores (Fannon et al., 1992) and possibly channels (Badenhuizen, 1959; Fannon et al., 1993) within maize and sorghum starch granules provides enzymes direct access to the granule interior. The central area of the granule around the hilum is believed to be the least organized region of the starch granule, since gelatinization, enzymic attack (maize), acid-catalyzed hydrolysis (maize) and cavitation all originate there (Chabot et al., 1978; Fuwa et al., 1978; Hoseney, Zeleznak & Yost, 1978; Leach & Schoch, 1961; Whistler & Thornburg, 1957).

Hence, as microstructural features, granules, pores, channels and cavities have the potential to influence starch granule reactions by connecting the cavity at the hilum to the granule exterior and increasing the surface area available for reagent infiltration into the granule matrix, especially into the less organized region surrounding the hilum. Thus, a greater understanding of these microstructural features was needed to shed light on their potential role in granule reactivity. The objectives of this study were to (1) provide further direct evidence for the existence of channels penetrating into the starch granule interior, (2) further examine structural relationships between pores, channels and cavities, (3) further investigate characteristics of pores, channels and cavities, and (4) investigate potential influences of pores, channels and cavities on starch granule reactions.

2. Materials and methods

2.1. Starch sources

Commercial common and waxy maize (corn) starches and intermediately processed commercial starches (wet milled, but not yet commercially-dried) of both aforementioned genotypes were donated by the A.E. Staley Manufacturing Company (Decatur, IL). Sorghum starch (isolated from genotype P721N), *hl* sorghum grain (genotype P-851171), and millet starch were provided by Dr Bruce R. Hamaker of the Whistler Center for Carbohydrate Research, Purdue University (West Lafayette, IN).

Steeping and wet milling of *hl* sorghum grain were done by a process similar to the method of Subrahmanyam and

Hoseney (1995). The ground slurry was filtered through a series of sieves (No.'s 50, 120, 200, 230), and the crude starch was purified by washing with a water–toluene mixture (10:1) and centrifuging. This process was repeated until the starch appeared white, after which the starch was recovered by filtration, washed briefly with ethanol and air-dried.

Fresh samples of dough-stage maize (Fielder's Choice 8205) were provided by Dr Robert Nielsen of the Department of Agronomy, Purdue University (West Lafayette, IN). Starch was isolated by lightly grinding wet kernels in a blender in the presence of NaCl solution (0.05 M). The resulting slurry was passed through a sieve (No. 325) to recover starch. Starch was washed with distilled water, followed by centrifugation and scraping of protein from the surface of the starch pellet. This process was repeated until the starch appeared white. Starch processed by this method was divided into two fractions. The first fraction was recovered by filtration and allowed to air-dry. The second fraction was not dried, but was stored in the hydrated state under aqueous NaN_3 (0.03% w/v) until further treatment.

2.2. Treatment with merbromin

Starch granule interior channels and cavities were flooded with a methanolic solution of merbromin as previously described (Huber & BeMiller, 1997). Treated starch was observed using light (Huber & BeMiller, 1997), fluorescence (Huber & BeMiller, 1997), confocal scanning laser, and scanning electron (Huber & BeMiller, 1997) microscopies.

A short-time merbromin treatment using an aqueous dye solution (1 g merbromin/12 ml water) was applied to commercial common maize starch granules. Starch granules were exposed to dye solution for 15, 30 or 60 s, after which they were recovered by filtration. Starch samples were washed on the filter with 100% ethanol to dehydrate the granules and halt progression of the dye. Specimens were analyzed using CSLM.

Commercial (isolated from dried maize kernels and redried), intermediately processed commercial (isolated from dried kernels but not redried), once-dried (isolated from dough-stage kernels and dried), and never-dried (isolated from dough-stage kernels but not dried) starch granules were suspended in a methanolic solution of merbromin for 90 min, recovered by vacuum filtration and air-dried (Huber & BeMiller, 1997). Treated granules were observed by fluorescence microscopy.

2.3. Treatment with colloidal gold

Sorghum starch granules were suspended in an aqueous suspension of colloidal gold particles (50 nm particle size; BBI International, Cardiff, UK). Following a 4 h treatment, starch was recovered by vacuum filtration, lightly washed on the filter with 100% ethanol, air-dried and viewed by SEM.

2.4. Preparation of 5-([4,6-dichlorotriazin-2-yl]amino)-fluorescein-derivatized waxy maize starch

Waxy maize starch was derivatized with 5-([4,6-dichlorotriazin-2-yl]amino)-fluorescein (DTAF) by a method similar to that described by Whistler, Madson, Zhao and Daniel (1998) for obtaining surface-reacted acylated and carboxymethylated corn starch granules. Starch (10.0 g db) was suspended in triethylamine (18.5 ml) with constant stirring for 30 min, after which DTAF (0.0025 g) in carbon tetrachloride (15.2 ml) was added. The reaction was allowed to proceed for 7.5 h in the dark. The mixture was then transferred to a screw-capped polypropylene tube, and the modified starch was recovered by centrifugation. The colorless supernatant, which indicated that the reagent remained on the starch granules, was discarded and replaced with anhydrous ethanol (35 ml) to wash unreacted dye from the starch. The tube containing the suspension of starch in ethanol was shaken briefly on a multi-wrist shaker (in the dark) and centrifuged to recover the derivatized starch. The supernatant, which was colored due to the presence of dye, was discarded and replaced with fresh EtOH. The tube was returned to the shaker and the washing process (addition of fresh EtOH, shaking, centrifugation, decanting of supernatant) was continued over the course of several days until all unreacted dye had been removed. The washed starch was recovered by vacuum filtration, allowed to air-dry, and stored in the dark at 4°C. A reaction control was generated by carrying native waxy maize starch through the same process, with the exception that no DTAF was added to the initial reaction mixture.

2.5. Fluorescent microscopy

Dry granules of DTAF-derivatized and merbromin-treated starch were mounted on slides in either water or immersion oil and overlaid with coverslips. Specimens were viewed with an Olympus Vanox photomicroscope equipped with fluorescence optics. The microscope head was equipped with a DM500 dichrome mirror, a 400–490 nm range exciter filter and a 0515 barrier filter to provide blue light illumination.

2.6. Confocal scanning laser microscopy

For merbromin-treated starch, dry granules were mounted onto slides that had been previously coated with a thin film of paraffin wax. To anchor granules, slides were heated just enough to melt the wax. Optical sections of granules were obtained using a BioRad MRC 1024 Confocal Scanning Laser Microscope (Hercules, CA) coupled to a Nikon Optiphot 300 (Melville, NY) inverted microscope. Excitation was achieved with an Argon laser (488 line) operating at

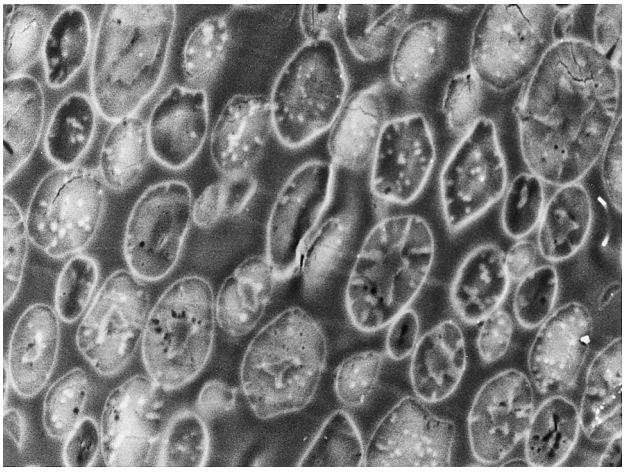

Fig. 1. Electron micrograph of granule sections from aqueous colloidal gold-treated *hl* sorghum starch visualized by SEM compositional BSE imaging.

10% power, while emission lines were selected with a F515 long pass filter.

2.7. Scanning electron microscopy

Colloidal gold-treated starches were embedded in Spurr's resin and sectioned as previously described (Huber & BeMiller, 1997). Dry sections were mounted directly onto double-sided carbon tape on aluminum stubs. Mounted specimens were carbon-coated and viewed in a JEOL SEM-840 scanning electron microscope (JEOL USA Inc., Peabody, MA) at 10 kV using compositional backscattered electron (BSE) imaging. All micrographs were obtained using Polaroid 55 sheet film (Cambridge, MA).

3. Results and discussion

3.1. Nature of channels and cavities and the effect of granule hydration on them

Results of previous work indicated that channels originated at the periphery of the granule and penetrated inward, with many extending to the cavity at the hilum (Huber & BeMiller, 1997). However, from viewing either whole granules or individual granule sections, it was difficult to determine whether all or just some of the channels penetrated all the way to the hilum. In this investigation, a three-dimensional image of a starch granule reconstructed from optical serial sections obtained by CSLM revealed that not all channels reached the hilum. Rather, channels penetrated the granule to varying depths, with most extending to the central cavity; so the hypothesis of Gallant et al. (1997) that channels penetrate only a few shells into the granule appears not to be true of the starches investigated in this work.

This pattern was not limited to starch granules of *hl* sorghum, which was used to improve the likelihood of success in viewing channels (Huber & BeMiller, 1997), but was also observed in normal sorghum, common maize, waxy maize and millet starch granules treated with methanolic merbromin and viewed by fluorescence microscopy. Channels occurring within granules of both maize starches appeared to be smaller in diameter and less numerous than those within sorghum starch granules. Also, channels appeared to be more abundant in granules of common maize starch as compared to those of waxy maize starch. Overall, it was clearly confirmed that the surface pores of maize, sorghum and millet starch granules reported by Fannon et al. (1992) are indeed openings to channels leading into the granule interior.

To determine if channels could have resulted from contact of granules with a dehydrating medium (methanol) during treatment with merbromin, *hl* sorghum starch granules were treated with an aqueous solution of merbromin and then examined for channels. Granules treated with aqueous merbromin stained very differently from granules treated with methanolic merbromin. SEM compositional BSE images of cross-sections of granules stained in water and viewed by SEM manifested the reverse or opposite staining pattern of those previously obtained from granules stained in methanol (Huber & BeMiller, 1997). While methanolic treatment confined the dye to granule surfaces, thus clearly outlining channels and cavities, aqueous treatment allowed the dye to permeate throughout the granule matrix, leaving channels and cavities as voids (Huber & BeMiller, 1997). Differences between the aqueous and methanolic merbromin treatments were corroborated by optical sections of maize starch granules obtained by CSLM which gave images identical to those obtained by physically sectioning granules and examining them by fluorescent microscopy and SEM compositional BSE imaging. Thus, the degree of granule swelling influenced permeability of the granule matrix to the dye.

Since the slight swelling produced by room temperature water alone was sufficient to promote penetration of granules by merbromin, a marker of greater size was needed to elucidate channels in an aqueous environment. By treating *hl* sorghum starch granules with an aqueous suspension of colloidal gold (50 nm in diameter), channels in granule cross-sections viewed by SEM compositional BSE imaging were nicely elucidated (Fig. 1). The fact that channels and cavities were penetrated by relatively large colloidal gold particles afforded some potentially useful information about their nature, viz., that channels are more likely voids rather than regions occupied by amorphous material as proposed by Gallant et al. (1997). This experiment also illustrated that channels could be detected, i.e. are present, in both aqueous and nonaqueous media. Thus, a dehydrating medium is not required for their presence and observation.

To further determine any effects of granule rehydration on channels and cavities, waxy maize starch was first surface reacted with a fluorescent dye (DTAF) and the granular pattern of reaction was observed by fluorescence

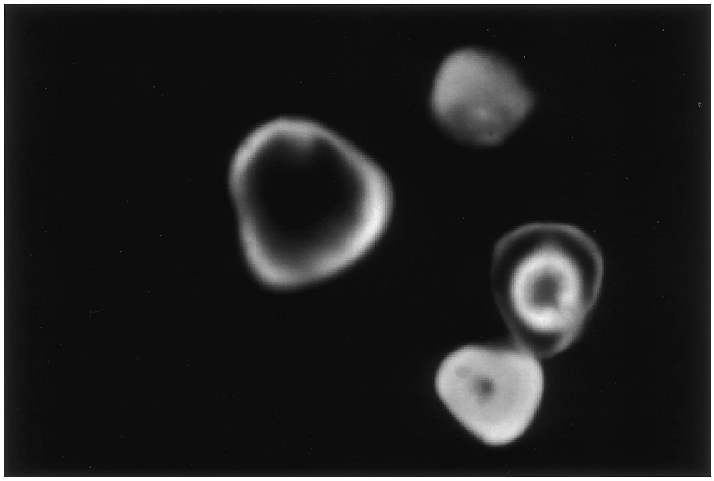

Fig. 2. Optical section of short-time, aqueous merbromin-treated commercial common maize starch granules viewed by CSLM that depicts variability of dye penetration among granules (partially from the inside out and from the outside in and completely).

microscopy. As anticipated, reacted dye was localized at granule surfaces, including those of both channels and cavities. While Whistler et al. (1998) had previously reported exclusive reaction of exterior granule and cavity surfaces of common maize starch granules using the same reaction conditions, they did not report visualization of granule channels. Although channel surfaces were likely derivatized in their study, channels probably were not resolved by the light microscopy procedure used by them. They did, however, credit channels for the delivery of reagent to cavities at the granule hilum.

Despite slight granule swelling in water, channels appeared to remain open. Further, in waxy maize starch granules that had been modified with DTAF only at surfaces (including those of channels and cavities), channels appeared to enlarge with slight swelling of granules. Using the fluorescence microscope, channels were more easily visualized in granules that were first hydrated on the slide, dried in air several minutes to remove visible water and mounted in immersion oil, as compared to those granules that were simply mounted in immersion oil without previous hydration. Thus, channels might have enlarged with granule hydration/swelling. For cavities, the opposite effect was observed. Cavities, which had been visible in dried DTAF-modified starch granules mounted in immersion oil and viewed by fluorescence microscopy, disappeared when granules were mounted in water. This result for corn starch granules parallels the report of Baldwin et al. (1994), who observed that cavities within potato starch granules disappeared upon rehydration. However, since cavities may not always be visible within hydrated granules due to complications of refractive index, Baldwin et al. (1994) could not conclusively associate cavity disappearance with cavity closure for potato starch granules in aqueous suspension. Nevertheless, for DTAF-modified waxy maize starch granules mounted in water, cavities appeared to swell somewhat shut, as the fluorescent dye, which outlined the boundaries of cavity surfaces within dried granules, now clearly highlighted the union of the converged cavity surfaces in rehydrated granules. However, it should be remembered that the well-documented attack of amylolytic enzymes in the area surrounding the hilum (already discussed) shows that the closure resulting from swelling is not so complete as to block entry of enzyme molecules, which is supported by the finding in this work that 50 nm colloidal gold particles filled cavities of *hl* sorghum starch granules in aqueous suspension.

3.2. Potential roles of channels and cavities in starch granule reactions

Channels, which could provide direct access of reagents to a loosely organized region at the hilum, have the potential to influence and possibly dictate patterns of reaction within starch granules. Since dehydration appeared to play a role in the development of both channels and cavities (at least development to a size observable microscopically), although their origin has not been determined, it was important to ascertain what effect, if any, granule rehydration would have on channel and cavity structure, as industrial starch reactions are conducted in aqueous media under slightly swelling conditions.

Since it was known that corn starch granules become permeated with merbromin upon suspension in aqueous dye solution, control of the degree of granule hydration, which affected permeability of the granule matrix to dye molecules, might be used to control the extent of reagent penetration into the granule during starch modification. Thus, determination of the mode of dye entry into the granule matrix might clarify the roles of channels and cavities in granular chemical reactions and simulate reagent flow into the granule. It was hypothesized that dye penetration would be most rapid through the region surrounding the central cavity, since the hilum region of the granule is believed to

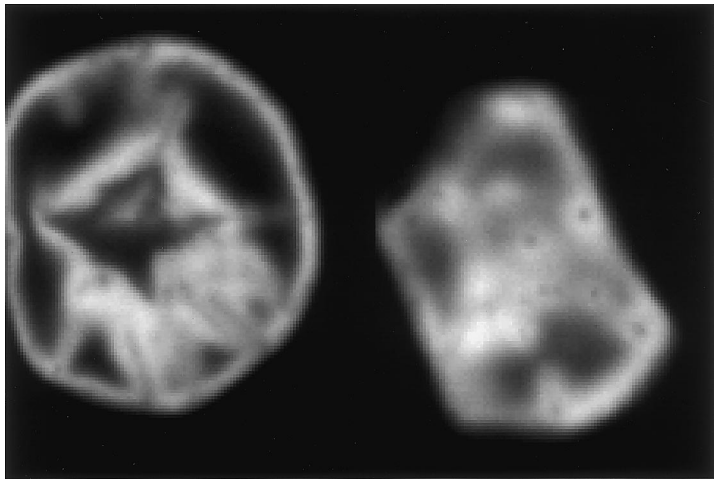

Fig. 3. Optical sections of short-time, aqueous merbromin-treated commercial common maize starch granules viewed by CSLM illustrating lateral penetration of dye from channels into the granule matrix. The granule on the left is sectioned along the length of the channels; the one on the right is sectioned across the channels.

be the least organized. To test this hypothesis, maize starch granules, which had been subjected to short-time treatment with aqueous merbromin solution (15, 30 or 60 s), were optically sectioned by CSLM to generate granule serial sections. The short-time treatment of maize starch granules, which was designed to produce intermediate rather than complete penetration, clearly revealed a flow of dye into the hydrated granule matrix through both channels (laterally) and cavities (from the center outward). Regardless of treatment length, the extent of dye penetration varied greatly among granules. This variation is depicted in Fig. 2, in which granules optically sectioned at approximately their geometric centers displayed both partial and complete penetration of dye.

While some variation might be attributable to the treatment process itself, it is evident that populations of starch granules are heterogeneous in terms of granular microstructure. This observed heterogeneity between granules might also be due in part to variations in porosity (variation in the number pores and channels per granule), and indicates the potential for non-uniform substitution in a population of granules during chemical modification of corn and sorghum starch. Since dye penetrated into the matrix laterally from channels, the channels no longer appeared as fine, distinct lines, (Huber & BeMiller, 1997) but became more indefinite as dye penetration progressed (Figs. 2–4). Channels further aided the permeation process by delivering dye into cavities (Fig. 4), which allowed dye entry into the granule matrix (from the hilum region outward). Even though evidence had indicated that cavities swell shut upon granule rehydration, the most rapid penetration of dye into the matrix still occurred from the inside out, rather than from the outside in. Thus, pores and channels of common maize starch increased the potential surface area available for reaction

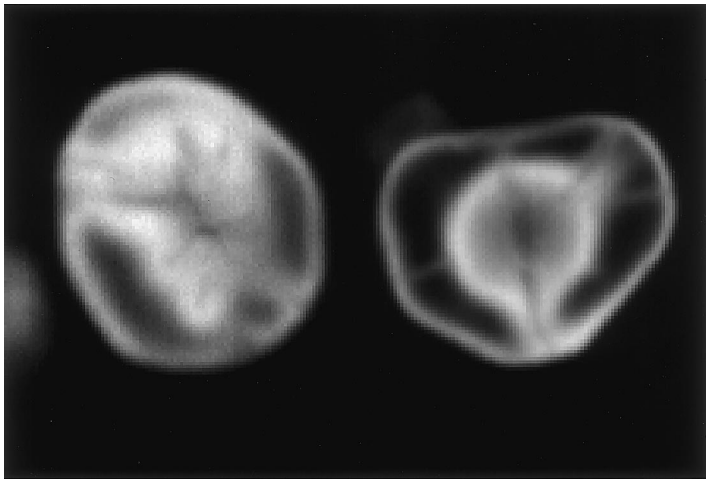

Fig. 4. Optical sections of two additional short-time, aqueous merbromin-treated commercial common maize starch granules viewed by CSLM depicting penetration of dye into the granule matrix both from the cavity and laterally from channels.

and fostered dye penetration into the granule matrix, despite apparent closure of the cavity upon granule swelling. The probable closure of the cavity did not appear to hinder dye penetration, just as it appears not to hinder enzymolysis (as described earlier), but instead could have aided it by increasing gaps between starch molecules, since the same amount of starch material would now fill a larger space. Thus, the hypothesis that the hilum is the least organized region of the granule is supported. However, the possibility that the dye could have penetrated from the outside in, in at least some granules (Fig. 2), could not be ruled out.

3.3. Origin of channels and cavities

Although this research was primarily designed to elucidate channels and cavities and their potential effects on granule chemical reactivity, it was hoped that insight into their origin might also be gained, as understanding the conditions surrounding the origin of channels and cavities might provide a means of controlling their frequency and occurrence, thereby controlling reactivity indirectly. Dough-stage (never-dried), intermediately processed (field-dried, wet-milled, but not commercially dried) and completely processed (field-dried, wet-milled and commercially dried) common maize starch granules were analyzed for the presence of channels and cavities by treating them with methanolic merbromin solution. Channels and cavities were observed by light and fluorescence microscopies in most granules of each of the three starch samples examined. Differences in cavity size were also perceived. Cavities observed in never-dried, dough-stage maize starch granules were substantially smaller (eye observation) than those in both intermediately and completely processed granules, which possessed cavities of comparable size. The observed difference in cavity size could be related to the smaller size of immature granules from dough-stage maize, although Baldwin et al. (1994) did not find any correlation between granule size and cavity size for potato starch. Definitive conclusions regarding channel and cavity existence, frequency, or size of the three starch samples are premature, since preparation of dough-stage and intermediately processed starch samples in methanolic merbromin undoubtedly resulted in granule dehydration, which has been shown to influence cavity frequency and size (Baldwin et al., 1994; Whistler, Spencer, Goatley & Nikuni, 1958).

Granule cavities can be seen clearly without the aid of dye by simply mounting dry or wet granules in a medium with a refractive index different from that of water and viewing them with a light microscope (Huber & BeMiller, 1997). Since it was not possible to disperse an aqueous slurry of starch in immersion oil for visualization of possible cavities, poly(ethylene glycol) (PEG, $M_n \cong 400$), which has a refractive index similar to immersion oil and is water miscible, was used as a mounting medium. Results for starches viewed with the light microscope in PEG paralleled those previously obtained for the methanolic merbromin treatment, except that cavity frequency appeared to increase with increased processing/drying (field and plant dried > field dried only > dough stage/never dried). These results also should be embraced with some caution, since some dehydration of granules in PEG cannot be ruled out. Further, since a commercial wet-milling company provided the intermediately and completely processed starches, their genotype was not known and likely differs from that of the dough-stage maize. (The commercial maize may also have been heat dried prior to storage.) However, use of an aqueous suspension of colloidal gold showed that a cavity area accessible to 50 nm particles is present in wet sorghum starch granules.

At this time, neither the time of nor the basis for formation of channels and cavities is known, although it would seem that their presence cannot be attributed solely to dehydration. Cavities, which seem to be independent of channels, as they occur in potato starch granules (Baldwin et al., 1994; Hall & Sayre, 1970b;), which have no pores, and presumably no channels, could be formed by crystallization of amylopectin molecules and concurrent shrinkage of the matrix as the granule grows and develops. Drying could then enlarge cavities.

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