NOTE

Interior Channels of Starch Granules[1]

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ABSTRACT

Microscopic evidence is presented that the pores previously observed on the external surface of sorghum starch granules are openings to serpentine channels that penetrate into the granule interior.

Recently, our laboratory reported the presence of pores on the external surfaces of starch granules from the subfamily Panicoideae (corn, sorghum, millet) (Fannon et al 1992a,b). Evidence was presented that these pores are normal, real, anatomical features of the starch granules, as opposed to being artifacts produced during the isolation, sample preparation, or observation processes for electron microscopy. After reviewing previous reports of starch granule pores, fissures, and cavities and their possible relationship to enzyme-catalyzed digestion and chemical reactivity, Fannon et al (1992a) concluded that there has been no direct evidence that surface pores are openings to channels penetrating into the granule interior, although certain behaviors, such as the pattern of enzyme-catalyzed digestion (Smith and Lineback 1976, Lineback and Ponpipom 1977, Fannon et al 1992a), suggest that this is the case.

We offer evidence here that the pores observed on the surfaces of corn and sorghum starch granules are openings to serpentine channels that penetrate in a roughly radial direction.

MATERIALS AND METHODS

Transmission Electron Microscopy

Sorghum seeds of a pure line (cultivar P721N) grown at the Purdue University Agronomy Research Center near West Lafayette, IN, during the 1988 crop season were hand-harvested at 20 days after half-bloom, sliced transversely into 1-2-mm pieces with a razor blade, and fixed at 4°C in 1% glutaraldehyde (v/v) and 4% paraformaldehyde (v/v) in 0.1*M* phosphate buffer, pH 6.8. Fixed tissues were rinsed in 0.1*M* phosphate buffer, pH 6.8. Tissue was postfixed with 2% osmium tetraoxide in 0.05*M* phosphate buffer (pH 6.8) for 4 hr, then rinsed in deionized water and dehydrated in a graded ethanol series (10 min each in 10, 30, 50, 70, 90, 95, 100, 100, and 100% ethanol). Dehydrated, fixed tissue was infiltrated for 2 hr each in 20, 40, 60, and 80% L. R. White resin monomer (Polysciences, Inc., Warrington, PA) in ethanol. Samples were then transferred to 100% L. R. White resin monomer and left overnight at room temperature. Monomer was replaced with fresh monomer the following morning, and infiltration was continued for two days. Samples were then placed in aluminum embedding molds, and the monomer was polymerized at 55°C in a sealed oven purged with nitrogen gas. After 48 hr, blocks were removed from the molds, mounted on dowels, and trimmed until the tissue was exposed. Because of the hardness of the tissue, the samples were reembedded. Exposed, embedded tissue was again placed into 100% L. R. White resin monomer and reinfiltrated for two days. Samples were then placed in polyurethane embedding capsules, and the monomer was polymerized as previously described.

Copper grids were prepared with Formvar membranes (Polysciences) and carbon-coated. Ultrathin sections from the double-embedded material were cut with a diamond knife on a Reichert Om U3 ultramicrotome (Reichert-Jung, Cambridge Instruments Inc., Buffalo, NY). Sections were placed on prepared grids and poststained with 2.5% aqueous uranyl acetate for 5 min and with 0.1% lead citrate for 2 min. Sections were viewed in a Philips EM-200 microscope (Philips Electronic Instruments Co., Mahwah, NJ) at 60 kV.

Scanning Electron Microscopy

Common corn starch granules were prepared and examined as previously described (Fannon et al 1992a) after incubation with pronase (Calbiochem, San Diego, CA).

To prepare granule ghosts, a 0.2% slurry of starch was heated to boiling (100°C) on a hot plate for 10-15 min with moderate stirring. Ghosts were isolated by centrifugation in an Eppendorf centrifuge (Eppendorf Inc., Fremont, CA) for 1 min. The pellet was resuspended in hot water (100°C) and centrifuged again. The washing process was repeated twice more. The hot paste of isolated ghosts was removed from the microcentrifuge tube and placed in a wet filter-paper sandwich, which was then passed through a graded ethanol series (25, 50, 75, 95, 100, 100, and 100%, with 30-min intervals) at room temperature. The sample was then subjected to critical-point drying in carbon dioxide, mounted on two-sided cellophane tape stuck to aluminum stubs, sputter-coated with approximately 300 Å of gold-palladium, and examined in a JEOL SEM-840 scanning electron microscope (JEOL USA Inc., Peabody, MA) at 10kV.

RESULTS AND DISCUSSION

Short segments of what appear to be channels oriented in a radial direction were found in some thin sections of unisolated sorghum starch granules (Fig. 1). From these observations, it may be deduced that the channels follow a serpentine route as they penetrate granules. In some cases, tubelike structures found at the periphery of granules extended to the external surface. Two such observations are shown in Figure 1. One of these is quite prominent and offers the best evidence found for existence of a tubelike channel extending from an external pore into the interior of a granule. From this evidence and that already published (Fannon et al 1992a), we determined that the external openings are of the order of magnitude of 0.1-0.3 μm (1,000-3,000 Å) in diameter, and that the internal channels are approximately 0.07-0.1 μm (700-1,000 Å) in diameter.

Scanning electron microscopic examination of isolated ghosts of cross-linked corn starch revealed pores on the inner surface (Fig. 2), indicating that corn starch granules also have openings that penetrate at least to that depth. In yet another study (Hauber et al 1992), common corn starch was treated in several different ways to remove protein to determine the effect of protein removal on reactivity and patterns of enzyme digestion. The treated starch was then examined by scanning electron microscopy as previously

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described (Fannon et al 1992a). Some granules treated with pronase alone fractured during preparation. The two fractured granules presented in Figure 3 provide evidence of radially oriented channels along the fracture planes.

It is possible that all starch granules contain pores and channels that are unobserved either because they are covered over with metal during sputter coating or because they are too small to be resolved by the electron microscope, yet large enough for passage of water, reagents, and even enzymes. The fact that the same pattern of pores was seen by environmental scanning electron microscopy of common corn starch granules that were not sputter-coated (Fannon et al 1992a) tends to rule out being covered over with metal during sputter-coating. The fact that patterns of enzyme digestion correlate with pore patterns (Fannon et al 1992a) rules out their being too small to be resolved by the electron microscope. Also, there is the evidence that the total surface of corn and sorghum starch granules available for gas absorption is greater than the external surface (Hellman and Melvin 1950, Hauber et al 1992), indicating a macroporous structure.

These observations suggest that the pores on the surface of sorghum, corn, and millet granules, and probably those along the equatorial groove of the large granules of wheat, rye, and barley starches (Fannon et al 1992a,b), are openings to twisted, tubelike channels. Based on the observation that enzymic digestion of corn starch begins at the hilum (Leach and Schoch 1961), it is likely that at least some channels penetrate to the hilum. Their presence suggests that consideration of the surface area accessible to enzymes (Lynn and Stark 1992), the efficiency of absorption of enzymes onto surfaces (Bertoft and Manelius 1992), and the surfaces available for chemical reaction (Hauber et al 1992) must now take into account hidden surfaces of channels for those starches with pores on the external surface. In addition, future discussions of the ultrastructures of corn, sorghum, and millet starch granules must include these anatomical features. This has already been done to some extent by Gallant and Bouchet (1986), particularly with regard to amylase attack.

Channels opening to the granule exterior and penetrating into the granule interior were found in unisolated granules, that is, granules still imbedded naturally in endosperm tissue. This is further indication that the channels are normal structures and not artifacts. Their biological origin remains unknown. One

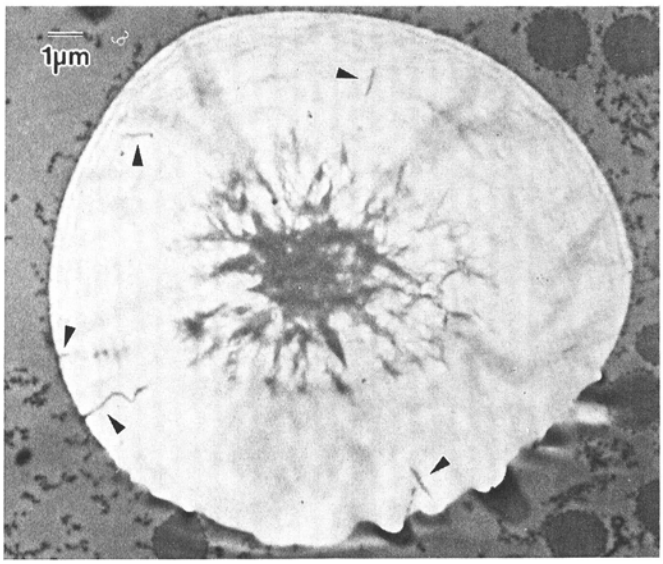


Fig. 1. Thin section of a sorghum starch granule (×4,300) contained in normal endosperm tissue from a seed collected 20 days after half-bloom. Arrows point to channels. The center of the granule appears as it does because of imcomplete penetration of the resin monomer and subsequent swelling when the thin section was floated on water, an unsolved problem in electron microscopy of starch granules (Gallant and Guilbot 1971).

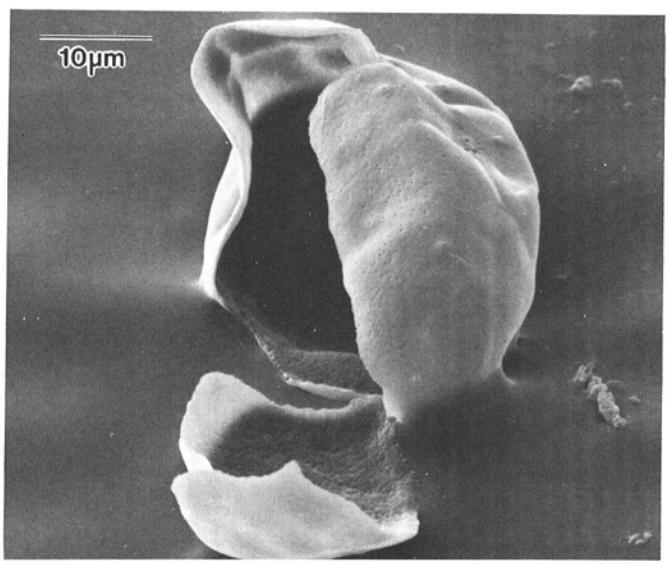
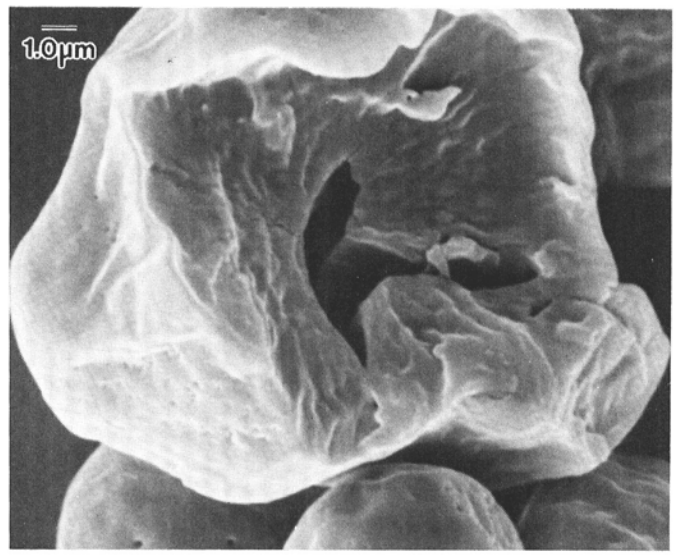


Fig. 2. Shell of a granule ghost isolated from a cross-linked common corn starch cooked and prepared for scanning electron microscopy by solvent-exchange dehydration and critical-point drying (×1,500).



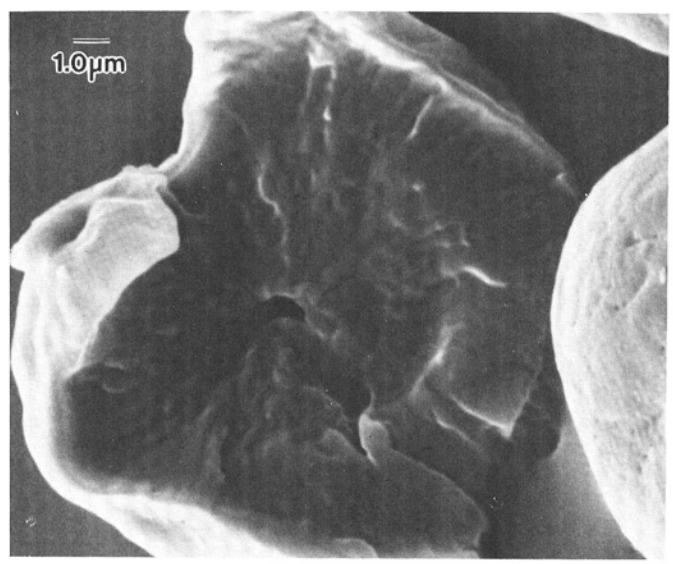


Fig. 3. Fractured common corn starch granules as visualized by low-temperature scanning electron microscopy following cryopreparation (×7,500).

possible function that has been proposed is the regulation of the rate of starch granule conversion into D-glucose during seed germination (Fannon et al 1992a).

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LITERATURE CITED

BERTOFT, E., and MANELIUS, R. 1992. A method for the study of the enzymic hydrolysis of starch granules. Carbohydr. Res. 227:269-283.

FANNON, J. E., HAUBER, R. J., and BeMILLER, J. N. 1992a. Surface pores of starch granules. Cereal Chem. 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. Pages 1-23 in: Frontiers in Carbohydrate Research, Vol. 2. R. Chandrasekaran, ed. Elsevier Applied Science: New York.

GALLANT, D. J., and BOUCHET, B. 1986. Ultrastructure of maize starch granules. A review. Food Microstruct. 5:141-155.

GALLANT, D. J., and GUILBOT, A. 1971. Artefacts au cours de la préparation de coupes de grains d'amidon. Staerke 23:244-250.

HAUBER, R. J., BeMILLER, J. N. and FANNON, J. E. 1992. Swelling and reactivity of maize starch granules. Starch/Staerke 44:323-327.

HELLMAN, N. N., and MELVIN, E.H. 1950. Surface area of starch and its role in water sorption. J. Am. Chem. Soc. 72:5186-5188.

LEACH, H. W., and SCHOCH, T. J. 1961. Structure of the starch granule. II. Action of various amylases on granular starches. Cereal Chem. 38:34-46.

LINEBACK, D. R., and PONPIPOM, S. 1977. Effects of germination of wheat, oats, and pearl millet on alpha-amylase activity and starch degradation. Staerke 29:52-60.

LYNN, A., and STARK, J. R. 1992. The action of isoamylase on the surface of starch granules. Carbohydr. Res. 227:379-383.

SMITH, J. S., and LINEBACK, D. R. 1976. Hydrolysis of native wheat and corn starch granules by glucoamylases from *Aspergillus niger* and *Rhizopus niveus*. Staerke 28:243-249.

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