NOTE

Interior Channels of Starch Granules

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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% formaldehyde (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 tetroxide 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 reinfirtrated for two days. Samples were then placed in poly-

urethane 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 into 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 starchy 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 Bouchez (1986), particularly with regard to amylose 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
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


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