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SCANNING ELECTRON-MICROSCOPY OF STARCH GRANULES, WITH OR WITHOUT AMYLASE ATTACK*

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ABSTRACT

Scanning electron-microscopy (SEM) revealed that, for starch granules relatively susceptible to amylase, numerous pin holes could be observed on the surfaces of granules attacked by amylase. We also observed that the pores penetrated into the inner layers of granules during the enzyme action and some of the granules exhibited a terraced or step-shaped appearance in their inner portions. These internal characteristics are most probably indicative of layered internal structures of the granules. The other characteristic observations by SEM were striated structures on the surfaces of starch granules of banana, lily, and lotus attacked by pancreatin.

INTRODUCTION

Optical microscopy of an aqueous suspension of starch granules, for example those of potato, canna, lily, and lotus, shows the presence of layering (growth rings) within the granules. Starch granules show, in general, "Maltese cross" polarization patterns; the arms of the polarization cross also intersect the growth-rings perpendicularly. These observations mean that there is a high degree of molecular orientation within the granules, and it has long been supposed that the growth-rings are concentric shells of alternating refractive index[1,2].

Although the growth rings have been observed by transmission electron-microscopy (TEM) of thin sections of native[3,4] and acid-treated[5] maize-starch granules, TEM of the growth rings has been contradictory. Recently, scanning electron-microscopy (SEM) of enzymically[6–18], chemically[19], and physically[20] modified starch-granules revealed internal, terraced or step-shaped characteristics. These characteristics are the most likely indication of layered, internal structures of the granules.

This paper deals with observations by SEM of layered, internal structures of starch granules of maize, lily, and lotus attacked by pancreatin, potato-starch

*Dedicated to Professor Roy L. Whistler.

granules pretreated with acid and attacked by glucoamylase, and striated structures of the outer portion of starch granules of banana, lily, and lotus attacked by pancreatin.

MATERIALS AND METHODS

Starches. — Banana-starch granules were prepared by the method of Fujimoto *et al.*[21] from commercial green banana (*Musa cavendishii* L.), with or without ethylene gas treatment. Starch granules of potato, lily, and lotus were prepared from commercial tubers of potato (*Solanum tuberosum* L., cultivar Danshaku), bulbs of lily (*Lilium* spp.), and tubers of lotus (*Nelumbo nucifera* Gaertn), respectively, by a general procedure for tuber starches[22]. Corn-starch granules were prepared from commercial dent maize (*Zea mays* L.) by the method described previously[23].

Preparation of starch granules attacked by amylases, specimen mounting, and SEM. — Starch granules were attacked by amylases under the conditions reported previously[23]. Procedures for specimen mounting and SEM were followed as described earlier[15,17,18], except that starch granules were coated with 100–200 Å of a gold layer by ion sputtering with the use of an Ion Coater IB-3 from Eiko Engineering Co. Ltd., Japan.

RESULTS AND DISCUSSION

SEM of the inner, concentric shells of starch granules attacked by amylase. — Fig. 1 shows one of the typical views by SEM of maize-starch granules attacked by pancreatin. Attack usually started with small pits on the surfaces of the granules, the pits increased in number and in size, and pores penetrated into the inner portions of the granules toward the center. For some of the granules, it was possible to observe the inner portion, which appeared terraced or step-shaped. At the higher magnification (Fig. 1b), radial or onion-like layering in the granule is clearly visible. The

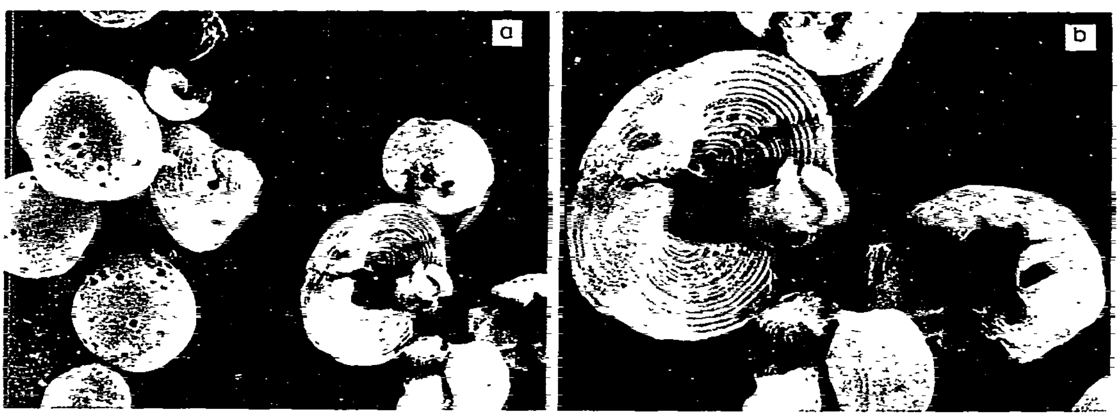

Fig. 1. Scanning electron-photomicrograms (SEM) of maize-starch granules attacked by pancreatin. a and b: degradation. 36%.

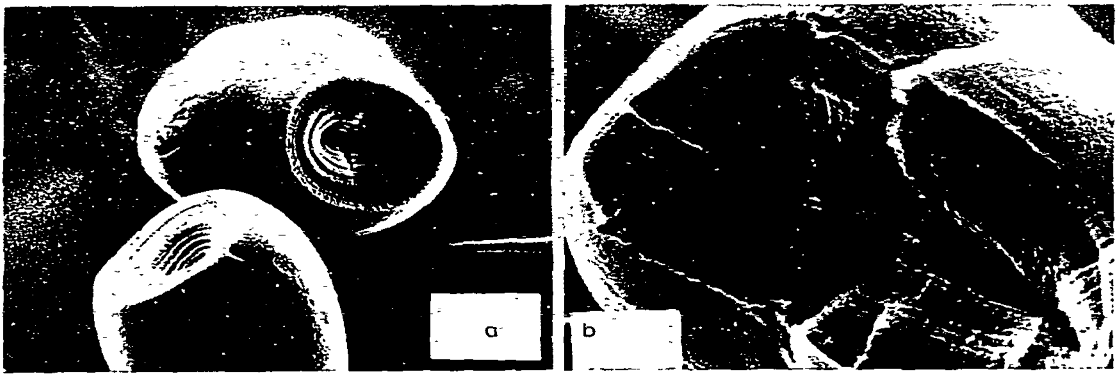

Fig. 2. SEM of potato-starch granules pretreated with hydrochloric acid and attacked by glucoamylase. a and b; degradation by glucoamylase, 16%.

Fig. 3. SEM of banana-starch granules and those attacked by pancreatin. a: green banana. b: green banana; degradation, 31%. c; yellow banana, d; yellow banana, degradation, 30%.

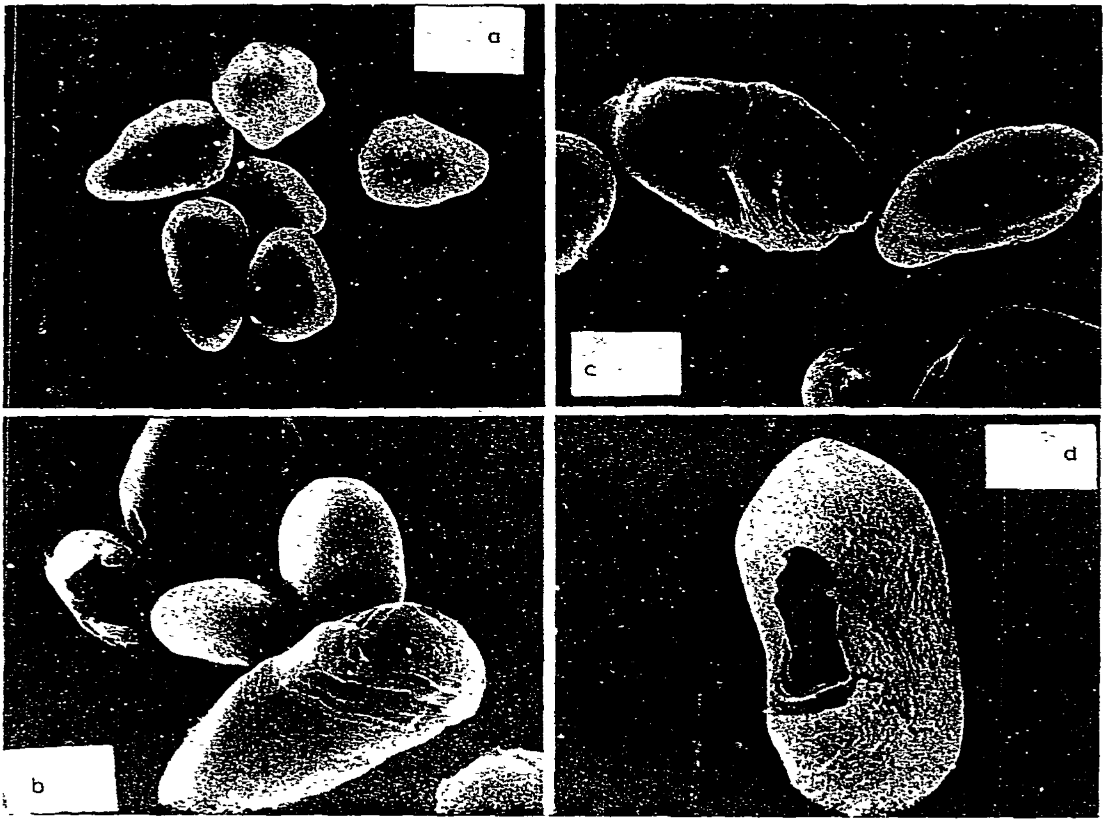

Fig. 4. SEM of lily-starch granules and those attacked by pancreatin. a: native. b and c: degradation, 20%. d: degradation, 42%.

SEM studies showed the layered, internal structures of starch granules of normal maize[11,17,18], several maize mutants[11,17,18], mung beans[16], sweet potato, lily (Fig. 4d), and lotus (Fig. 5d). Similar observations have been made by others[6~10, 12~14] on starch granules of normal maize, barley, rice, tapioca, wheat, and wrinkled pea attacked by amylase or physically modified. The layered, internal structures of starch granules were also visible in germinating seeds of barley[8,10], maize[14,15], pearl millet[14], rice[13], and wheat[7,14].

The concentric shells of potato-starch granules are obvious by light microscopy, however, SEM provides little or no evidence of the structures[20,24] except after pre-treatment of potato-starch granules with acid and/or amylase[25]. We showed such structures by SEM of potato-starch granules isolated from digestive tracts of rats fed a diet containing potato-starch granules as the sole source of carbohydrate[26], and also potato-starch granules pretreated with hydrochloric acid (M. 48 h, 37°) and attacked by glucoamylase (Figs. 2a and 2b).

SEM of the outer, striated structures of starch granules attacked by amylase. — Fig. 3a shows banana-starch granules isolated from the pulp of green fruits. The

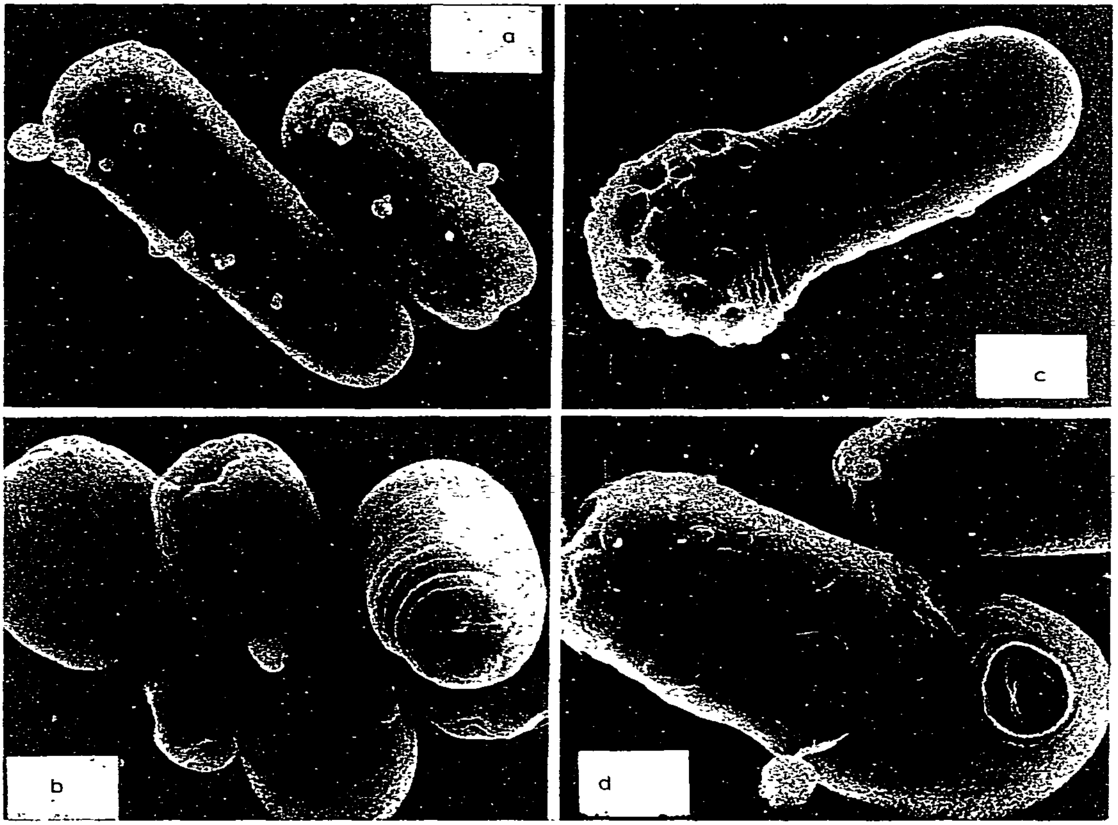

Fig. 5. SEM of lotus-starch granules and those attacked by pancreatin. a: native, b, c, and d: degradation, 51 %.

granules have smooth surfaces, are irregularly shaped, and are a mixture of flat and elongated ones[27]. Banana-starch granules isolated from the pulp of ripened fruits (green bananas were treated with ethylene gas for 24 h and kept for 8 days at 20–22°) showed characteristic striated structures on the surfaces (Fig. 3c), which were similar to those of green banana-starch granules attacked by pancreatin (Fig. 3b). These striated structures of ripened banana-starch granules were accented by treatment with pancreatin (Fig. 3d). Thus the crevices of the striated structures seem to be more susceptible to amylase attack. The striated structures were observed by SEM for some parts of the outer portion of starch granules of lily (Figs. 4b and 4c) and lotus (Figs. 5b and 5c) attacked by pancreatin.

Those starch granules that showed the striated characteristics on the outer portion upon attack by pancreatin had various irregular shapes. French[28] produced "optical maps" of various irregularly shaped starch-granules and stated that the molecular arrangement of chain is such that their long axes are perpendicular to the surface of the granule for these non-spherical granules. The appearance of the striated structures on the outer portion of such starch granules may be accounted for by removal of some of the surface layers of the original granule by enzyme attack, because we observed surface striations only on starch that had undergone a significant degree of enzymic erosion.

A large proportion of starch granules attacked by the enzyme did not show either the outer-striated or the inner-shell structure on the same granules, except those shown in Figs. 1a, 1b, and 5d. The present evidence is thus not yet conclusive as to whether these two kinds of observations are necessarily related or not. Further work is necessary to elucidate the relationship of these structures to the architecture of starch granules.

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