Plant Physiol. (1990) 94, 320–327
0032-0889/90/94/0320/08/$01.00/0

Received for publication February 9, 1990
Accepted May 3, 1990

Degradation of Native Starch Granules by Barley α-Glucosidases[1]

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ABSTRACT

The initial hydrolysis of native (unboiled) starch granules in germinating cereal kernels is considered to be due to α-amylases. We report that barley (*Hordeum vulgare* L.) seed α-glucosidases (EC 3.2.1.20) can hydrolyze native starch granules isolated from barley kernels and can do so at rates comparable to those of the predominant α-amylase isozymes. Two α-glucosidase charge isoforms were used individually and in combination with purified barley α-amylases to study *in vitro* starch digestion. Dramatic synergism, as much as 10.7-fold, of native starch granule hydrolysis, as determined by reducing sugar production, occurred when high pI α-glucosidase was combined with either high or low pI α-amylase. Synergism was also found when low pI α-glucosidase was combined with α-amylases. Scanning electron micrographs revealed that starch granule degradation by α-amylases alone occurred specifically at the equatorial grooves of lenticular granules. Granules hydrolyzed by combinations of α-glucosidases and α-amylases exhibited larger and more numerous holes on granule surfaces than did those granules attacked by α-amylase alone. As the presence of α-glucosidases resulted in more areas being susceptible to hydrolysis, we propose that this synergism is due, in part, to the ability of the α-glucosidases to hydrolyze glucosidic bonds other than α-1,4- and α-1,6- that are present at the granule surface, thereby eliminating bonds which were barriers to hydrolysis by α-amylases. Since both α-glucosidase and α-amylase are synthesized in aleurone cells during germination and secreted to the endosperm, the synergism documented here may function *in vivo* as well as *in vitro*.

Although the first discovered enzyme is usually considered to be diastase (23), the malt enzyme that promotes starch hydrolysis, the enzymes responsible for initiation and high rates of starch granule degradation during cereal seed germination have never been conclusively established. Summarily, two main viewpoints on which enzymes are involved in this process have dominated over the last half century. The currently favored view is that α-amylases are the only enzymes that can attack native starch granules, and the function of other 'starch-degrading' enzymes (α-amylases, debranching enzyme, and α-glucosidase) is to hydrolyze the dextrins released by the α-amylases from the granules (3). The second viewpoint, more prevalent in earlier decades and now largely neglected, is that α-amylases alone have either no action or insignificant and very slow effects on native starch granules. Another factor distinct from α-amylase was thought to be needed for efficient digestion of native starch granules (26).

Evidence of a non-α-amylase starch hydrolyzing factor in malted wheat was reported by Blish *et al.* (4). They found a 'raw starch catalyst,' a factor separate and distinct from α-amylase, which greatly increased α-amylase hydrolysis of native starch granules but did not significantly alter the activity on soluble starch. Work with the wheat system was not pursued; hence, the activating factor has not been elucidated. However, a marked supplementing effect of *Aspergillus oryzae* amylase preparations on the hydrolysis of corn starch by pancreatic α-amylase has been reported (2). The supplementing factor in the mold was later traced to the presence of α-glucosidase (maltase) (21). Similar effects of α-glucosidase have also been termed 'amylase activation' and 'complementary action.' As no enzymes other than α-amylases have been demonstrated to be capable of attacking native starch granules from tissues of higher plants, these α-glucosidase effects have been attributed to its degradation of maltose, which is both an end product and an inhibitor of α-amylolysis.

We report here the identification of α-glucosidase (EC 3.2.1.20) as the enzyme responsible for the α-amylase 'activation' in germinating barley and that this supplementing effect is not just due to simple removal of maltose. We demonstrate (a) that α-glucosidase isolated from germinating barley seeds is capable of initiating attack on native barley starch granules in the absence of α-amylase, (b) that these α-glucosidases hydrolyze several different α-glucosidic bonds, and (c) that a dramatic synergism occurs between α-glucosidase and α-amylase activities in the hydrolysis of granular starch.

MATERIALS AND METHODS

Plant and Enzyme Sources

Barley (*Hordeum vulgare* cv Morex) and barley malt used in all experiments except aleurone secretion studies were obtained from the U.S.D.A. Cereal Crops Research Unit, Madison, WI. Aleurone secretion studies were done with cv Himalaya obtained from the Washington State University.

α-Glucosidase was obtained from barley seeds germinated in the dark at room temperature (about 20°C). After 4 d

[1] Supported by the U.S. Department of Agriculture, Agricultural Research Service, the American Malting Barley Association, The Graduate School of the University of Wisconsin, and USDA Competitive Research Grants Office grant No. 87-CRCR-1-2324. Funding for Z. S. was also provided by the Shenyang Agricultural University, People's Republic of China.

germination seedlings were lyophilized. Coleoptiles and husks were removed and remaining kernels were ground to a flour. Flour was extracted with 50 mM Na phosphate (pH 9.0), containing 1 M NaCl and 20 mM Chaps.[2]

Separation of α-glucosidases from each other was achieved by chromatography on DEAE-Sephacel[3] (equilibrated in 50 mM ethylenediamine, pH 8.0). The high pI isoform eluted in the equilibration buffer wash. The low pI isoform was eluted with a NaCl gradient. The high pI isoform was next chromatographed through a CM-cellulose column equilibrated with 50 mM Na succinate (pH 4.5). The separated isoforms were each chromatographed on columns of hydroxylapatite (equilibrated with 10 mM Na phosphate [pH 6.8]; eluted with a NaCl gradient), concanavalin A-Sepharose (equilibrated with 50 mM ethylenediamine [pH 7.0], containing 0.5 M NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 1 mM $MnCl_2$; eluted with methyl α-D-mannoside) and cycloheptaamylose-Sepharose affinity resin (equilibrated and eluted with 50 mM NaOAc [pH 5.5], containing 1 mM $CaCl_2$).

α-Amylase was obtained from barley malted as described previously (9). High and low pI α-amylase activities were extracted and separated as before (10). The high and low pI α-amylase isozymes were individually chromatographed over a cycloheptaamylose-Sepharose affinity column (equilibrated with 50 mM NaOAc [pH 5.5], containing 1 mM $CaCl_2$; elution was with 10% cycloheptaamylose in equilibration buffer). Cycloheptaamylose was removed by dialysis against buffer (10 mM NaOAc and 1 mM $CaCl_2$).

Enzyme purity was assessed by SDS-PAGE (13.5% acrylamide) using the buffer system of Laemmli (11).

Enzyme Assays

α-Glucosidase activity was measured with *p*-NPG or maltose as the substrate. Assay buffer for both substrates was 50 mM Na succinate (pH 4.7). When *p*-NPG was the substrate, 1 mL of 0.1% *p*-NPG was mixed with up to 0.1 mL of enzyme preparation in a total volume of 1.1 mL. After incubation (37°C; 30 min) reactions were terminated by addition of 0.1 mL of 1 N NaOH. Released *p*-nitrophenol was quantitated at 420 nm. When disaccharides were used as substrates, enzyme, up to 0.1 mL, was mixed with 0.1 mL of 0.1 M disaccharide in a total volume of 0.2 mL. The reaction was incubated at 37°C and terminated by boiling for 5 min. Disaccharide dependent glucose release was quantitated by NAD^+ reduction (8). One unit is defined as the amount of enzyme which liberates 1 μmol *p*-nitrophenol or glucose from substrate per minute at 37°C.

α-Amylase activity was measured with boiled, Lintner potato starch ('soluble starch') as the substrate (8). One unit is defined as the amount of enzyme resulting in the increase of reducing power equivalent to 1 μmol maltose/min at 37°C as determined with dinitrosalicylic acid (8). Starch azure was also used as a substrate for determining endoamylase activity (9).

Reducing sugar production from native starch granules was determined by the Somogyi-Nelson method (20, 25).

Starch Granule Isolation

Morex barley seeds were washed with distilled water and steeped for 48 h in NaOAc (20 mM [pH 6.5], containing 0.02% Na azide). The softened kernels were ground (VirTis homogenizer, model 23) for 6 min at 80% full speed. The grist was passed through two sieves (250 and 75 μm opening).

Crude starch that passed through both sieves was purified by centrifugation (60 *g*; 2.5 min) through a layer of 65% (w/v) sucrose. Pelleted starch granules were recentrifuged one or two times under the same conditions, then resuspended in NaOAc (20 mM) [pH 6.5], 0.02% Na azide).

Starch Granule Degradation

Incubation mixtures containing 2.05% (w/v) starch, 0.5% (w/v) BSA, 0.02% (w/v) azide, 2 mM $CaCl_2$, 0.032 units of α-glucosidase, or 1.088 units of α-amylase, or both, were incubated for up to 72 h. Incubations were at 37°C and starch granules were kept suspended by constant shaking. Aliquots, the equivalent of 1.1 mU α-glucosidase and/or 36.6 mU α-amylase, were periodically removed for determination of glucose and total reducing sugars released from starch granules.

Scanning Electron Microscopy

Starch samples were prepared by rinsing twice with 0.5 M NaCl, rinsing for 10 min with aqueous solutions of increasing acetone concentration (10, 40, and 80%), followed by two 10 min rinses with 100% acetone. Rinsed starch grains were dried under a vacuum (48 h; 60°C). Dried starch granules were attached to stubs with double-sided tape, grounded with silver paint, and coated with gold. Samples were examined in a Hitachi S-570 scanning electron microscope at an accelerating potential of 5 kV.

Aleurone Secretion Study

Aleurone layers were removed from seeds as described by Chrispeels and Varner (7). Isolated aleurone layers (0.43 g) were incubated in 2 mL media (7). Media aliquots were periodically removed for analysis of enzyme activities. α-Amylase activity, determined after heating enzyme preparations at 70°C for 15 min in the presence of 3 mM $CaCl_2$, was measured with boiled soluble starch as the substrate. α-Glucosidase was measured with *p*-NPG as the substrate.

RESULTS

Enzyme and Starch Characteristics

The purity of the final enzyme preparations was assessed by SDS-PAGE followed by silver staining. The high pI α-glucosidase preparation contained only two bands (Fig. 1A, lane 2), whereas the low pI α-glucosidase preparation con-

[2] Abbreviations: Chaps, 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate; *p*-NPG, *p*-nitrophenyl α-D-glucoside; mU, milliunits.

[3] Mention of a trademark or proprietary product does not constitute a guarantee or warranty by the U.S. Department of Agriculture and does not imply its approval over other products that might also be suitable.

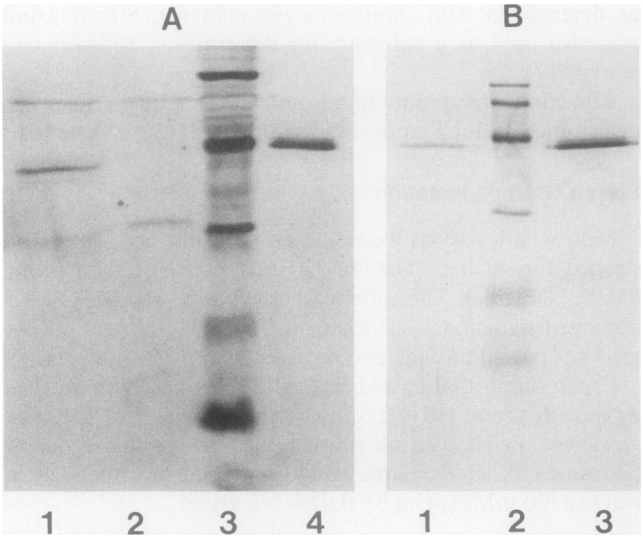

Figure 1. SDS-PAGE of A: 1, high pI α-glucosidase; 2, low pI α-glucosidase; 3, molecular mass standards (in order of decreasing molecular mass; phosphorylase B [92.5 kD], BSA [66.2 kD], ovalbumin [45 kD], carbonic anhydrase [31 kD], soybean trypsin inhibitor [21.5 kD]; 4, high pI α-amylase; and B: 1, low pI α-amylase; 2, mol wt standards; and 3, high pI α-amylase preparations used in starch granule hydrolysis studies. The same high pI α-amylase preparation is in panel A and B.

tained two major bands and several minor bands (Fig. 1A, lane 1). Both low and high pI α-amylases were purified to homogeneity (Fig. 1, A and B). These enzyme preparations contained very little or no cross-contamination between α-glucosidase and α-amylase activities (Table I). Although endolytic activity was present in the low pI α-glucosidase preparation (1.5% of the low pI α-amylase activity and 2.2% of the high pI α-amylase activity), evidence that this is not due to α-amylase contamination is: (a) the α-glucosidase preparations had been passed through cyclohexaamylose-Sepharose (an affinity ligand specific for α-amylases) columns to remove α-amylase; and (b) denaturing electrophoresis data do not indicate the presence of any α-amylase proteins in either α-glucosidase preparation. The low level of endolytic activity may be due to the low pI α-glucosidase itself or could be due to contaminating endolytic carbohydrases, such as α-amylase, which are not present in sufficient concentration to be detected in the silver stained gel. Neither high nor low pI α-glucosidase preparations were capable of hydrolyzing pullulan (data not shown), so the endolytic activity present in the low pI α-glucosidase preparation is not due to debranching enzyme. The high pI α-glucosidase preparation contained no endolytic activity. Both α-amylase preparations contained no maltase activity.

Both α-glucosidase charge isoforms readily hydrolyzed the α-1,4- and α-1,3-glucosidic bonds found in maltose and nigerose, respectively (Table II). Both isoforms were capable of hydrolyzing the α-1,2- and α-1,6-glucosidic bonds found in kojibiose and isomaltose, respectively. The low pI barley α-glucosidase hydrolyzed α-1,1-glucosidic bonds to an appreciable extent whereas the high pI α-glucosidase minimally hydrolyzed trehalose. The high pI α-glucosidase preparation, which had two protein bands on a denaturing polyacrylamide gel, exhibited catalytic activity with six of the seven carbohydrates tested (maltose, nigerose, kojibiose, isomaltose, trehalose, and granular starch). This enzyme preparation did not hydrolyze starch azure as previously discussed. As α-glucosidases from several other plant species have been proven to hydrolyze maltose and soluble starch (5, 27–29) and nigerose (6, 29), in addition to kojibiose, isomaltose, amylose, and amylopectin (27, 29), we conclude that the hydrolytic activities of the barley α-glucosidase preparations used in this study are indeed due to the α-glucosidases.

Barley endosperm starch consists of two distinct sizes of granules, *i.e.* large granules ≥15 μm in diameter and small granules of <5 μm in diameter (18). Only large starch granules were used in experiments reported here as they account for about 90%, by weight, of the total endosperm starch. Additionally, the large granules are more resistant to enzymolysis than are small starch granules (15).

Enzymatic Degradation of Starch Granules

All four enzyme preparations hydrolyzed granular starch (Table I), although they hydrolyzed either maltose or dyed-polysaccharide faster than granular starch. A time course of barley starch granule hydrolysis by α-glucosidases, α-amylases, and the combination of the two enzymes is shown in Figure 2. The amounts of α-amylase (36.3 mU with soluble starch as the substrate) and α-glucosidase (1.1 mU with maltose as the substrate) used in the starch granule degradation studies were selected based on differences in the maximal amounts of each activity that we have been able to extract from germinated barley seeds. We used maltose and soluble starch as the substrates in these assays as they are the commercially available substrates and therefore can easily serve as a standard reference. Although the amount of α-glucosidase activity was only one thirty-third of the α-amylase activity, as judged by hydrolysis of maltose and soluble starch, respectively, the degradation of starch granules by α-glucosidases was clearly detectable and the production of glucose and total reducing sugar was greater than or comparable to the production of sugars by the high pI α-amylase (Fig. 2, A and B).

The low pI α-amylase preparation was the most effective at granular starch hydrolysis when the four enzymes were tested individually (Fig. 2, A and B). The rate of granular starch

Table I. *Carbohydrase Activities of Barley Seed Enzyme Preparations*

	Substrate		
	Maltose	Starch azure	Granular starch[a]
	nmol product/min/mL enzyme		
Low pI α-Glucosidase	25	50	0.38
High pI α-Glucosidase	173	0	0.78
Low pI α-Amylase	0	3,320	21.2
High pI α-Amylase	0	2,220	1.42

[a] Data from granular starch hydrolysis were calculated with glucose as the standard.

Table II. *Disaccharidase Activities of Barley Seed α-Glucosidases*

Substrate	Low pI α-Glucosidase	High pI α-Glucosidase
	nmol glucose produced/min/mL	
Maltose (α-1,4)	25.0 (100)[a]	173.0 (100)
Nigerose (α-1,3)	23.6 (94)	152.2 (88)
Kojibiose (α-1,2)	10.0 (40)	65.7 (38)
Isomaltose (α-1,6)	5.5 (22)	38.1 (22)
Trehalose (α-1,1)	5.7 (23)	0.7 (<1)

[a] Values in parentheses are activities expressed as percent of that obtained with maltose as substrate.

digestion by low pI α-amylase was ≥10 times the digestion rate by high pI α-amylase, with the difference becoming greater as the digestion proceeded. Starch granule digestion by low pI α-amylase was greater than by either α-glucosidase isoform.

There was substantial synergism between α-glucosidases and α-amylases in degrading native starch granules (Fig. 2, C and D). When synergism is calculated as the ratio of digestion by the combination of two enzymes to the sum of digestions by the two enzymes acting alone, maximal synergism was achieved with high pI α-glucosidase combined with either high (ratio of combined activities to sum of separate activities = 10.7:1.0) or low (ratio of 8:1) pI α-amylase. Low pI α-glucosidase was also most synergistic (ratio of 7.6:1.0) when combined with high pI α-amylase. The absolute level of synergism varied somewhat depending upon hydrolysis time as well as upon the assay method and standard used to determine enzyme activity. If the endolytic activity present in the low α-glucosidase preparation is due to contamination by low pI α-amylase, it would not cause the observed synergism but would result in a simple additive effect.

Scanning Electron Microscopy of Starch Granules

The starch granules used here were large, lenticular in shape, uniform in size, and free of small granules (Fig. 3A). Scanning electron microscopy revealed distinct morphological differences resulting from the different enzymatic treatments. Degradation of starch granules by α-glucosidases alone was not detectable at ×800 and granules appeared to have smooth surfaces like those of the control treatment (Fig. 3A,B). At ×2500 magnification, extremely small bore holes were detected at the equatorial groove of starch granules treated with low pI α-glucosidase (Fig. 4B). Similar small holes were not detected in starch granules degraded with high pI α-glucosidase (Fig. 4C). There was no evidence of uniform degradation of the granule surface by either high or low pI α-glucosidase although such a pattern of hydrolysis may be difficult to visualize.

Hydrolysis by either α-amylase alone was readily visualized even at ×800 and was concentrated around the equatorial groove (Fig. 3, D and E). Upon examination at higher magnification, ×2500, it appears that once access through the first several lamellations of the granules had been gained, the digestion proceeded with greater ease throughout the starch granule interior (Fig. 4, D and E). A small number of shallow pits on granule surfaces other than the equatorial groove were

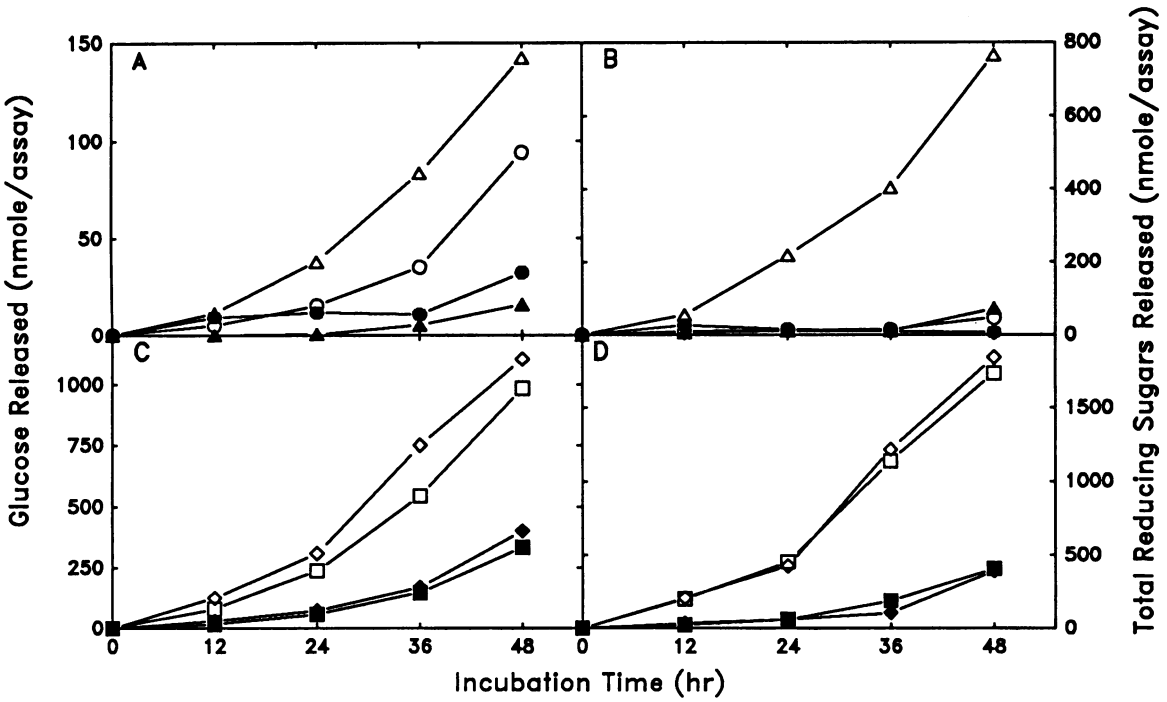

Figure 2. Time course of starch granule hydrolysis by α-glucosidases, α-amylases, and combinations of α-glucosidases and α-amylases. Panels A and C show production of glucose. Panels B and D show production of total reducing sugars. (○), Low pI α-glucosidase; (●), high pI α-glucosidase; (△), low pI α-amylase; (▲), high pI α-amylase; (□), low pI α-glucosidase plus low pI α-amylase; (■), low pI α-glucosidase plus high pI α-amylase; (◇), high pI α-glucosidase plus low pI α-amylase; (◆) high pI α-glucosidase plus high pI α-amylase.

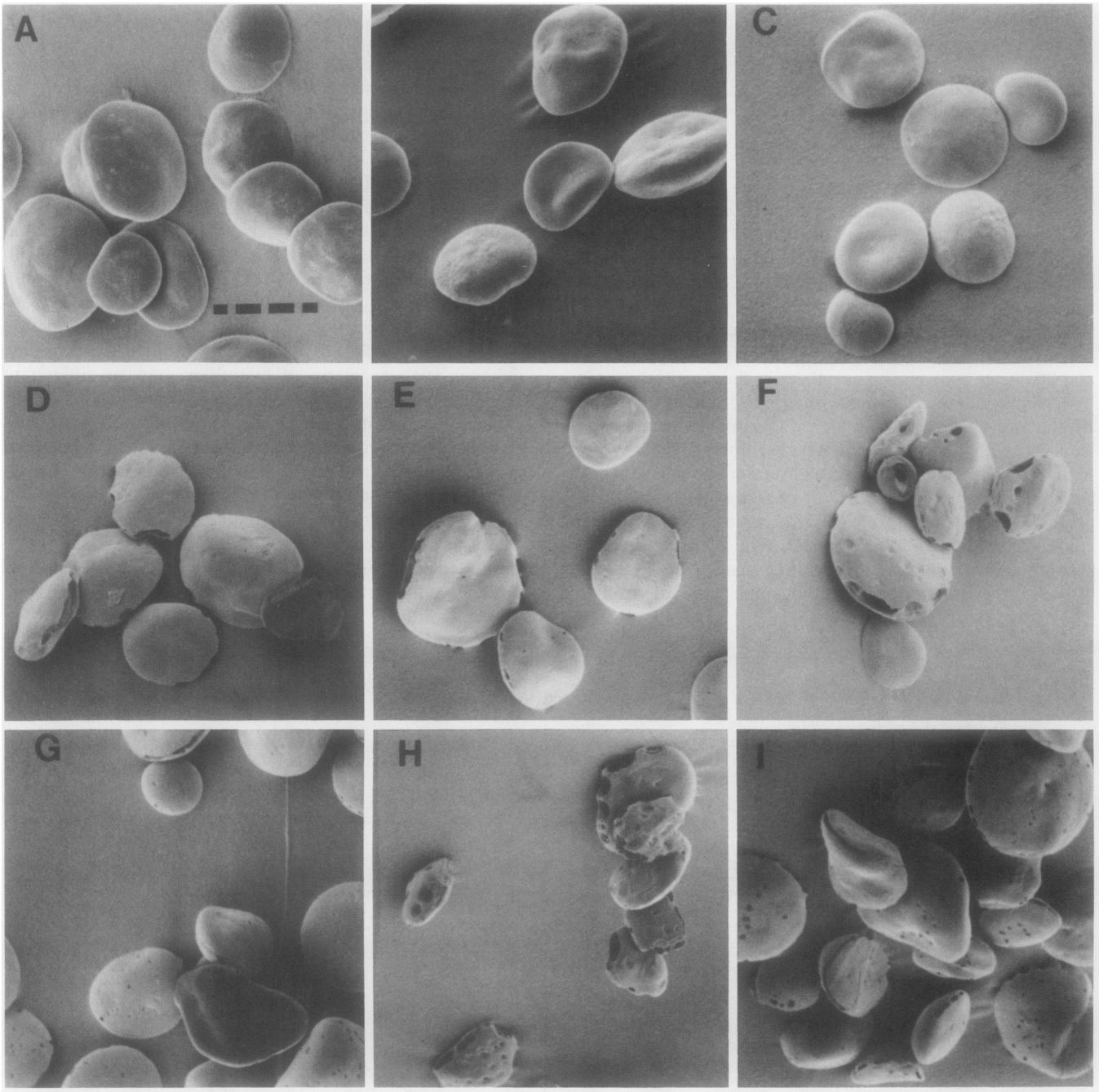

Figure 3. Scanning electron micrographs (×800) of starch granules after 72 h of hydrolysis treatments. Treatments were: A, no enzymes, control; B, low pI α-glucosidase; C, high pI α-glucosidase; D, low pI α-amylase; E, high pI α-amylase; F, low pI α-glucosidase plus low pI α-amylase; G, low pI α-glucosidase plus high pI α-amylase; H, high pI α-glucosidase plus low pI α-amylase; I, high pI α-glucosidase plus high pI α-amylase. Bar in (A) represents 20 μm in length and applies for all panels.

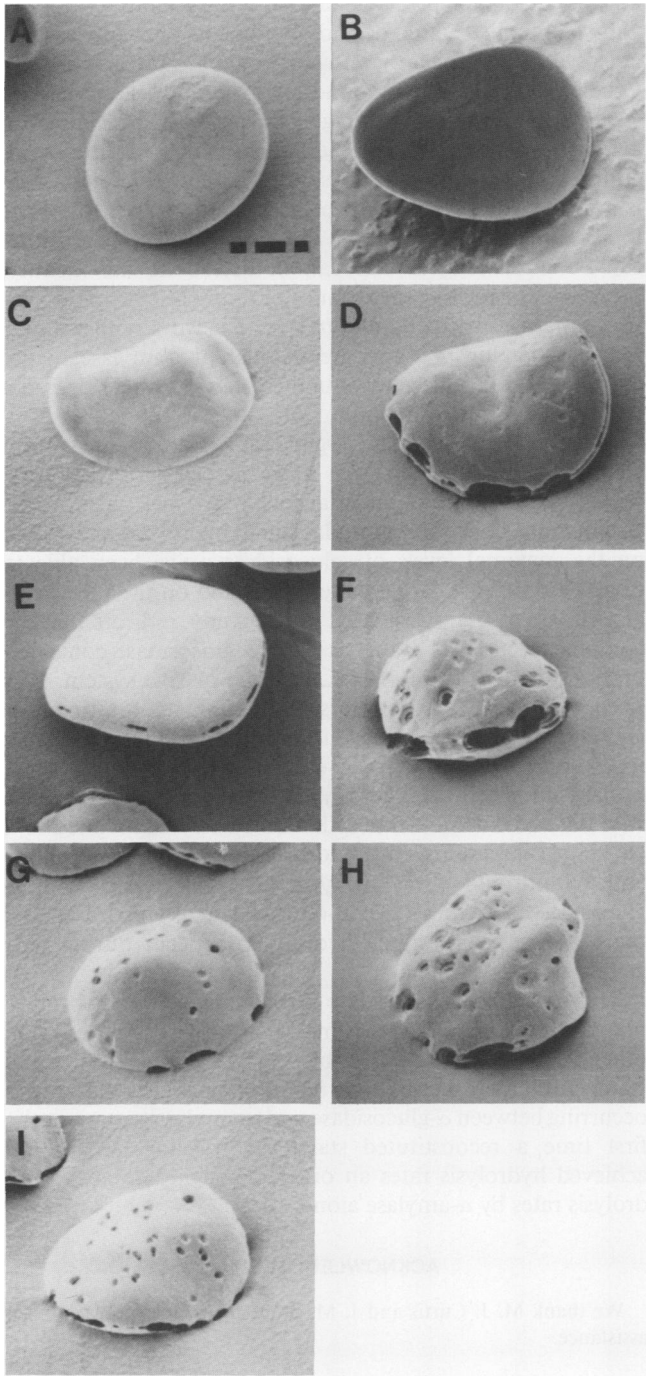

Figure 4. Scanning electron micrographs (×2500) of starch granules after 72 h of hydrolysis treatments. Treatments were the same as in Figure 3. Bar in (A) represents 8 μm in length and applies for all panels.

visible (Fig. 4D), although none were as extensive as holes at the equatorial groove.

Starch granules hydrolyzed by the combination of α-glucosidase and α-amylases (Figs. 3 and 4, F–I) exhibited far larger and more numerous craters on the surface than were found on granules attacked by α-amylases alone. Some of these craters appear to be deep enough to have extended well into the interior of the granules.

Enzyme Secretion from Aleurone Cells

The secretion of α-amylases from cereal seed aleurone cells is well documented. Isolated barley aleurone cells secreted both α-amylases and α-glucosidases, and secretion occurred approximately coincidentally (Fig. 5). Both charge isoforms of α-glucosidase were secreted (data not shown).

DISCUSSION

Currently, α-amylase is considered to be the only enzyme which can attack native, unboiled starch granules that have not been treated with organic solvents as is routinely done (15). The results presented here clearly demonstrate that barley α-glucosidases, in addition to α-amylases, are capable of hydrolyzing granular starch and should no longer be overlooked when native starch digesting enzymes are considered. Indeed, α-glucosidases alone could hydrolyze starch granules to an extent similar to that of high pI α-amylase. Hydrolysis of starch granules by the combined action of α-amylase (36.3 mU) and α-glucosidase (1.1 mU) was as much as 8 to 11 times that of the sum of the two enzymes alone. Synergism of this magnitude has never been reported for any other reconstituted starch hydrolyzing system. The synergistic effect of α-glucosidases on the digestion of starch granules by α-amylases can at least partially explain the inefficiency at which granular starches are degraded by α-amylases alone. Maeda *et al.* (12) reported that starch granule digestion by α-amylase alone was only slightly less than digestion by the combination of α- and β-amylases. Yet, dialyzed crude barley malt extract containing the same amount of α- and β-amylase activities

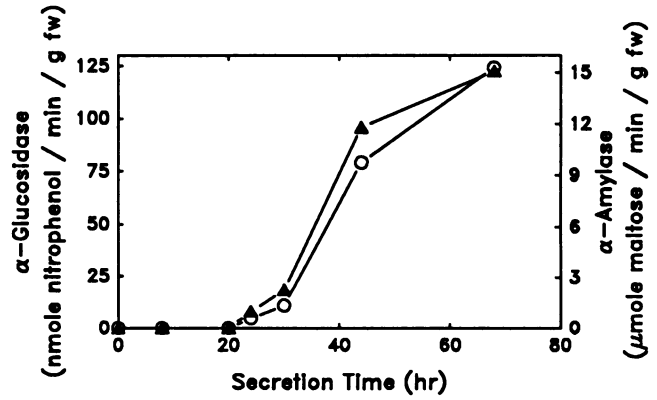

Figure 5. Time course of secretion of α-glucosidase (○) and α-amylase (▲) activities from isolated barley aleurone cells. Secretion was in the presence of 1 μM gibberellic acid and 20 mM $CaCl_2$.

resulted in twice the digestion rates of the α-amylase alone (12). These researchers considered debranching enzyme to be the missing factor, but found that the combined action of purified α-amylase and debranching enzyme gave the same extent of digestion as α- and β-amylase combined (13). Even when the three purified enzymes were combined, the degree of digestion only increased by about 25%, implying that other factors were still missing. Previous workers have attributed the resistance of native starch granules to digestion by amylases to be the result of a surface layer of amylopectin, protein, fat, hemicellulose, or phosphate. However, experiments designed to remove these putative protective layers have been either unsuccessful or inconclusive (2).

Regardless of whether or not α-glucosidase was the missing factor in studies done by Maeda *et al.*, what seems certain from the present study is that α-glucosidases are probably more important in native starch degradation than β-amylase or debranching enzymes although probably not more important than some α-amylase isozymes.

The mechanism by which α-glucosidases enhanced α-amylolysis of starch granules to such a great extent is not yet clear. Acceleration of starch granule degradation by combinations of α-glucosidases and α-amylases is probably partially due to the removal of inhibiting concentrations of maltose by α-glucosidase. Maltose has been suggested to interfere with a noncatalytic, raw starch binding site of α-amylase (22). α-Glucosidase removal of α-amylase-generated maltose should result in more active α-amylases. Additional enhancement of total reducing sugar production during granule hydrolysis may result from degradation of the maltodextrins released from starch granules by α-amylase. However, the synergism we report here cannot be entirely explained by maltose removal and degradation of released maltodextrins. We interpret the microscopy data presented here as an indication that previously unavailable attack sites become accessible to α-amylase by the action of α-glucosidase. This is unlikely to be due to the removal of maltose produced by α-amylase as initial starch granule attack, prior to accumulation of inhibitory levels of maltose, should have occurred uniformly across the various surfaces of the starch granule if the surfaces were indeed similarly susceptible to hydrolysis by α-amylase. Scanning electron micrographs (Figs. 3 and 4) and previous studies (17, 19) demonstrated that the equatorial groove of a starch granule was more susceptible to attack by α-amylases than were the broad, flat surfaces. Hydrolysis by α-amylases alone appears to be dependent upon penetration of the enzymes at the equatorial groove. Yet, when α-amylase is combined with α-glucosidase, hydrolysis occurs at many sites on the broad, flat surfaces in addition to the extensive hydrolysis at the equatorial groove.

We hypothesize that the marked synergistic effect of α-glucosidases on the initial stages of native starch granule degradation by α-amylases is due not just to maltose removal but also to the ability of α-glucosidase to remove non-α-1,4-glucosidic bonds from the granule surface which were acting as barriers to α-amylolysis. Both barley α-glucosidases used in this study can indeed hydrolyze, at variable rates, α-1,2-, α-1,3-, α-1,4-, and α-1,6-glucosidic linked disaccharides. Both α-1,2- and α-1,3-glucosidic bonds have been confirmed to exist in starch granules although their localization within the granule structure was not determined (1, 30). Work is currently underway to test this hypothesis.

We also report that low pI α-amylase, not high pI α-amylase, had an appreciable rate of starch granule hydrolysis. Native, unboiled starch degradation by low pI α-amylase was at least 10 times faster than that of high pI α-amylase (Fig. 2). Other workers have also found low pI α-amylase to degrade starch granules faster than high pI α-amylase (16). Based on activity toward boiled soluble starch, which is the case in almost all reported α-amylase studies, barley low pI α-amylase is a minor component that represents only 5 to 10% of the total α-amylase activity (14). The use of artificial substrates can be misleading as one cannot determine the identity, properties, or relative importance of α-amylase isozymes acting on native starch granules *in vivo*. The work reported here indicates that low pI α-amylase, not high pI α-amylase, makes the most significant contribution to starch granule degradation during seed germination.

As both α-amylases and α-glucosidases are secreted from aleurone cells into the starch filled endosperm cells during seed germination, the potential for *in vivo* synergism exists. Calculations of possible granular starch hydrolysis rates based on the maximal levels ofα-glucosidase we've been able to extract from germinating barley seeds and on the ratio of α-glucosidase to α-amylase used in this study, indicate that the combined action of α-amylase and α-glucosidase could hydrolyze granular starch faster than the *in vivo* system. It is likely that negative constraints (cellular pHs not optimal for activity, the presence of negative effectors, etc.) are probably imposed *in vivo*. Although no studies of minus-α-glucosidase mutants of cereal seeds have yet been reported, experiments with oat seeds demonstrated that dormancy was related to a lack of sugars resulting from decreased levels of α-glucosidase and that dormancy was highly negatively correlated with α-glucosidase activities (24). α-Glucosidase is apparently required for efficient starch hydrolysis to occur during seed germination.

This report of α-glucosidase alone being able to hydrolyze native starch granules is the first report of any plant enzyme other than α-amylase being able to initiate attack on native starch granules. Likewise, this report of synergistic hydrolysis occurring between α-glucosidase and α-amylase represents the first time a reconstituted starch hydrolyzing system has achieved hydrolysis rates an order of magnitude above hydrolysis rates by α-amylase alone.

ACKNOWLEDGMENTS

We thank M. J. Curtis and J. M. Stone for their expert technical assistance.

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