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Partial Characterization and Subcellular Localization of Three α-Glucosidase Isoforms in Pea (*Pisum sativum* L.) Seedlings[1]

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ABSTRACT

Three isoforms of α-glucosidase (EC 3.2.1.20) have been extracted from pea (*Pisum sativum* L.) seedlings and separated by DEAE-cellulose and CM-Sepharose chromatography. Two α-glucosidase isoforms (αG1 and αG2) were most active under acid conditions, and appeared to be apoplastic. A neutral form (αG3) was most active near pH 7, and was identified as a chloroplastic enzyme. Together, the activity of αG1 and αG2 in apoplastic preparations accounted for 21% of the total acid α-glucosidase activity recovered from pea stems. The vast majority (86%) of the apoplastic acid α-glucosidase activity was due to αG1. The apparent K_m values for maltose of αG1 and αG2 were 0.3 and 1.3 millimolar, respectively. The apparent K_m for maltose of αG3 was 33 millimolar. The respective native molecular weights of αG1, αG2, and αG3 were 125,000, 150,000, and 110,000.

α-Glucosidase (EC 3.2.1.20) has been extracted from a variety of plant sources including: the seeds of sugar beet (20), buckwheat (19), and barley (18); and cultured cells of sugar beet (23), carrot (16), *Convolvulus arvensis* (12), and soybean (21). α-Glucosidases are *exo*-type carbohydrases catalyzing the hydrolysis or transfer of the terminal α-D-glucosyl residues of α-D-glucosidically linked derivatives. Examples of substrates for hydrolysis include maltose, maltotriose, isomaltose, panose, kojibiose, soluble starch (18, 20), and native starch grains (18). Products of transglycosylation reactions catalyzed by plant α-glucosidases include: maltotriose from maltose by soybean callus α-glucosidase (21); isomaltose and panose from maltose by sugar beet α-glucosidase (20); and kojibiose, nigerose and maltose from soluble starch by buckwheat α-glucosidase (5).

The ability of some sugar beet α-glucosidases to hydrolyze soluble starch led Yamasaki and Konno (23) to suggest that the *in vivo* substrates for α-glucosidases may include not only maltose, released by the combined action of α-amylase and β-amylase on starch, but starch as well. Recently, Sun and Henson (18) have shown that barley α-glucosidase does hydrolyze native starch granules. They observed a high level of synergism when both α-amylase and α-glucosidase were incubated with starch granules, and proposed a role for α-glucosidase in increasing the susceptibility of starch grains to attack by α-amylase.

To date, no chloroplastic starch-degrading enzyme has been shown to be capable of hydrolyzing native starch granules isolated from leaves. It is possible that α-glucosidase plays a role in the degradation of transitory leaf starch similar to that proposed by Sun and Henson (18) for barley seed α-glucosidase. To do so, α-glucosidase must be present within the chloroplast, the site of starch accumulation and degradation. While several investigators have reported the presence of acid α-glucosidase in the cell wall (12, 16, 22), the existence of chloroplastic α-glucosidase is less well documented. Pongratz and Beck (17) found significant levels of α-glucosidase in spinach chloroplasts; while in other cases, chloroplasts from both spinach and pea were found to contain little or no α-glucosidase activity (11, 13–15). In light of the significance of the results obtained by Sun and Henson (18) using α-glucosidase from barley seeds, it seemed appropriate to reexamine chlorophyllous tissue for α-glucosidase activity and determine the subcellular localization of that activity.

We present here the first subcellular localization and partial characterization of the three isoforms of pea α-glucosidase. Two forms are most active under acid conditions and are probably apoplastic enzymes. The third form is a chloroplastic neutral α-glucosidase.

MATERIALS AND METHODS

Plant Tissue

Pea (*Pisum sativum* [L.] cv Laxton's Progress No. 9) seeds (J. W. Jung Seed Co., Randolph, WI) were surface-sterilized and sown as described by Beers and Duke (3). Plants were grown in a glasshouse under natural light supplemented with fluorescent lamps placed 70 cm above the surface of the vermiculite. Plants were watered with tap H_2O as needed and with nutrient solution (6) every 7 d.

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Chl and Protein Measurements

Chl was measured according to Arnon (1). Protein was measured using bicinchoninic acid (Sigma) according to the Sigma procedure.

Chloroplast Isolation

Chloroplasts were isolated from fully expanded leaves of 3-week-old plants by the method of Kakefuda *et al.* (11). The buffer used throughout the procedure consisted of 50 mM Hepes (pH 7.5), 330 mM sorbitol, 5 mM isoascorbate, 1 mM $MnCl_2$, 1 mM $MgCl_2$, 2 mM EDTA, and 20 μM leupeptin. Following the final wash, the buffer was removed and chloroplasts were stored as pellets at −20°C. Pelleted chloroplasts were ruptured by resuspension in 20 mL of grinding buffer (40 mM Hepes-NaOH [pH 7.2], 1 M NaCl, 3 mM DTT, 1 mM PMSF, and 2 mM EDTA).

DEAE-Cellulose Chromatography

Shoots (all above ground tissue) (125 g), stems (16 g) and chloroplasts (32 mg chl) were from 3-week-old seedlings. Shoots were homogenized with a Polytron PT 10-35 homogenizer (Kinematica, GmgH, Lucerne, Switzerland) equipped with a PTA 35 generator in cold (0–4°C) grinding buffer. Stems were homogenized in a chilled mortar with grinding buffer. Buffer was used at a 2:1, buffer:tissue ratio and leupeptin (20 μM final concentration) was added just prior to homogenization. Homogenized tissue was filtered through four layers of cheesecloth and centrifuged at 20,000*g* for 20 min at 4°C. The supernatant was decanted and either stored at −20°C or dialyzed overnight against DEAE-cellulose column buffer consisting of 10 mM Hepes-NaOH (pH 7.0) and 1 mM MTG[3]. Following dialysis, the samples were centrifuged at 20,000*g* and the supernatant was loaded on a DEAE-cellulose (Sigma) column (2.5 × 9 cm). The column was washed with two or three column volumes of column buffer before the bound protein was eluted with 200 mL of a 0 to 0.5 M NaCl gradient, in column buffer. DEAE-cellulose chromatography was conducted at 4°C. Fractions (4 mL) with α-glucosidase activity were pooled and concentrated on an Amicon YM30 membrane and stored at −20°C.

CM-Sepharose Chromatography

Acid α-glucosidase activity which did not bind to DEAE-cellulose at pH 7.0 (αG1) was dialyzed against 10 mM Na-acetate (pH 5.0), 1 mM MTG (CM-Sepharose column buffer). Following dialysis, the sample was centrifuged at 20,000*g* and the supernatant was loaded on a CM-Sepharose column (2.5 × 9 cm) equilibrated with column buffer. The column was washed with two volumes of column buffer before the bound protein was eluted with 200 mL of a 0 to 0.5 M NaCl gradient, in column buffer.

[3] Abbreviations: MTG, monothioglycerol; PNPG, *p*-nitrophenyl α-D-glucopyranoside; IE, infiltration-extraction; αG1, αG2, αG3, isoforms of α-glucosidase; NAD-MDH, NAD-malate dehydrogenase; NAD-GAPDH, NAD-glyceraldehyde-3-phosphate dehydrogenase.

Gel Filtration

Sephacryl S-300 gel filtration medium (Sigma) was used to estimate the native mol wt of pea α-glucosidases. The column buffer consisted of 20 mM Hepes-NaOH (pH 7.0), 150 mM NaCl, 1 mM MTG, and 0.02% Na-azide. Standards (Sigma) used were Cyt *c* (12.4 kD), carbonic anhydrase (29 kD), BSA (66 kD), alcohol dehydrogenase (150 kD), and apoferritin (443 kD). Gel filtration was conducted at 4°C.

Enzyme Assays

Marker enzyme assays were described previously (3). Glucosidase activity was detected using 30 mM PNPG in either 50 mM Na-acetate (pH 4.5) or 50 mM Mes (pH 6.5). Various concentrations of maltose were used for the detection and characterization of maltase activity; they are indicated in the text and legends. Maltase assays were terminated by boiling a 0.2 mL aliquot (from a 1 mL assay) for 10 min. Invertase assays were conducted using 292 mM sucrose, also at pH 4.5 and pH 6.5. The release of glucose from PNPG was determined from the change in A_{420} (4). Assays were conducted for

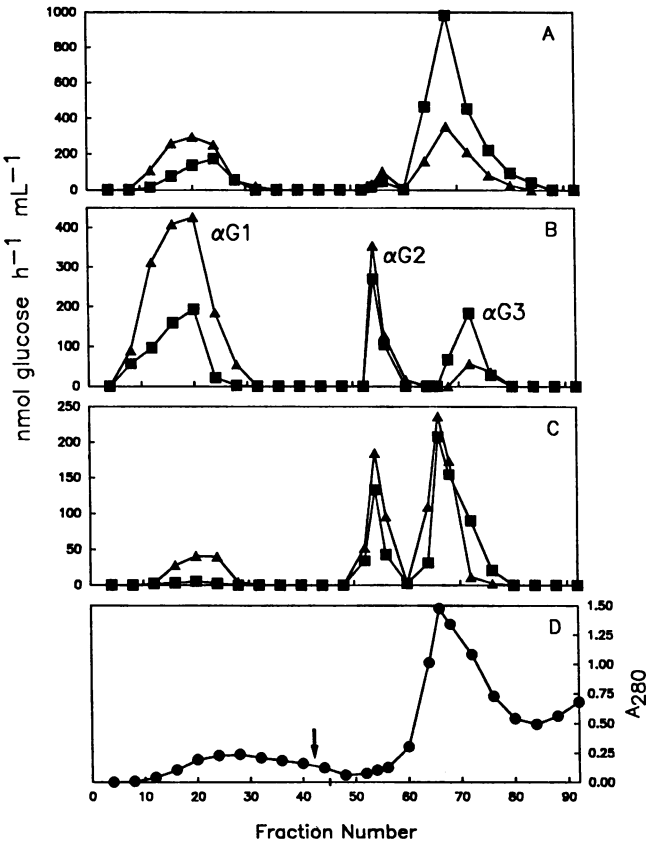

Figure 1. DEAE-cellulose chromatography of a pea shoot extract (32 mg chl) showing invertase activity with (A) 292 mM sucrose, (B) α-glucosidase (maltase) activity with 25 mM maltose, (C) glucosidase activity with 30 mM PNPG, and (D) A_{280}. Aliquots (0.2 mL) were incubated with each substrate in either 50 mM Na-acetate (pH 4.5) (▲) or 50 mM Mes (pH 6.5) (■) for 1 h at 30°C. α-Glucosidase isoforms αG1, αG2, and αG3 are identified in B. *Arrow* indicates the start of a 0 to 0.5 M NaCl gradient.

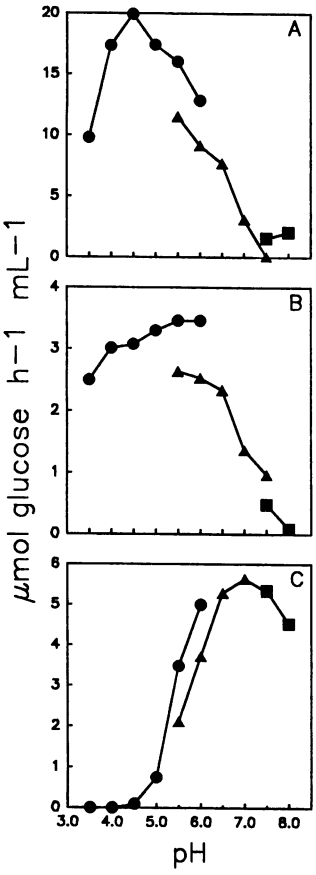

Figure 2. Partially purified αG1 (A), αG2 (B), and αG3 (C) pH profiles of activity with maltose. Buffers were 40 mM Na-acetate (●), 40 mM Mes (▲), and 40 mM Tricine (■). α-Glucosidase activity was extracted from 100 g of pea shoots. Isoforms were separated by DEAE-cellulose chromatography. Aliquots from a YM30 concentrate of each isoform were used for each profile. Assays were terminated after 1 h at 30°C. All assays were linear for 1 h. Maltose concentration was 25 mM for αG1 and αG2 and 50 mM for αG3.

1 h at 30°C. Activity of αG1, αG2, and αG3 was linear for at least 1 h. The release of glucose from maltose and sucrose was determined from the reduction of NAD^+ (change in A_{340}) coupled to glucose production via hexokinase and glucose-6-phosphate dehydrogenase, as described elsewhere (4). Values reported for glucose released with maltose as substrate are one-half of actual values, to indicate the number of hydrolytic cleavages. Production of reducing sugars from Lintner soluble starch (Sigma) was measured using 3,5-dinitrosalicylic acid reagent (4).

Stem IE

On two separate dates, four 2 g replicates of stems were subjected to six cycles of vacuum IE as described by Beers and Duke (3). Four additional 2 g replicates which were not subjected to the IE procedure served as controls. The IE buffer consisted of 40 mM Hepes-NaOH (pH 7.2), 200 mM NaCl, 3 mM $CaCl_2$, and 3 mM DTT. Stems were homogenized as described above under DEAE-cellulose chromatography. Total soluble acid α-glucosidase activity recovered from pea stems which have been subjected to 6 cycles of vacuum IE is the sum of the following two components: the activity remaining in stems after 6 cycles of IE, and subsequently extracted by homogenization of those stem segments (IE-stem), and the activity extracted from the apoplast by six cycles of vacuum IE.

RESULTS

pH Optimum, K_m, V_{max}, Molecular Weight, and Substrate Specificity of Partially Purified α-Glucosidases

Four glucosidases capable of hydrolyzing PNPG were detected during anion exchange chromatography of pea shoot extracts (Fig. 1C); however, maltase activity coeluted with only three of the PNPG-hydrolyzing enzymes (Fig. 1B). Those three α-glucosidases (maltases) are the subjects of this report. Two α-glucosidases (αG1 and αG2) were most active in the acid pH range (Fig. 2, A and B). Acid α-glucosidase activity recovered from the void volume during DEAE-cellulose chromatography of pea shoot extract (αG1) (Fig. 1B), eluted as a single peak of maltase (Fig. 3) or PNPG-hydrolyzing (data not shown) activity from CM-Sepharose when a 0 to 0.5 M NaCl gradient was applied, indicating that a single enzyme was responsible for αG1 activity. The application of a 0.5 to 1 M NaCl gradient did not release additional α-glucosidase activity (data not shown). The second peak of acid maltase activity coeluted with PNPG-hydrolyzing activity and eluted very soon after the start of the salt gradient (Fig. 1B). The third form (αG3) was most active near neutral pH (Fig. 2C) and eluted at approximately 0.25 M NaCl (Fig. 1B). At pH 6.5 only, αG3 activity was detected as a slight shoulder on the third peak of PNPG-hydrolyzing activity (Fig. 1C), thus representing the fourth PNPG-hydrolyzing enzyme and the third maltase detected following anion exchange chromatography. Again, no additional forms of α-glucosidase eluted during a 0.5 to 1 M NaCl gradient (data not shown).

When assayed at pH 4.5, partially purified αG1 from 100 g of pea shoots (see "Materials and Methods") had an apparent K_m for maltose of 0.3 mM and a V_{max} of 19.6 μmol glucose released h^{-1} mL^{-1} (Table I). Under the same conditions, αG2

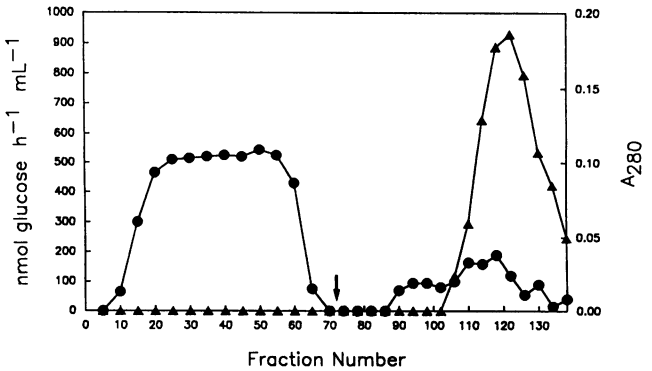

Figure 3. CM-Sepharose chromatography of partially purified (from DEAE-cellulose void) αG1 showing α-glucosidase activity with 25 mM maltose in 50 mM Na-acetate (pH 4.5) (▲) and A_{280} (●). Assays were terminated after 1 h at 30°C. *Arrow* indicates the start of a 0 to 0.5 M NaCl gradient.

Table I. *K_m for Maltose and V_{max} for Hydrolysis of Maltose by Partially Purified α-Glucosidases*

α-Glucosidase activity was extracted from 100 g of pea shoots and separated into αG1, αG2, and αG3 by DEAE-cellulose chromatography. Within each isoform, fractions containing α-glucosidase activity were pooled and concentrated on a YM30 membrane. The K_m for maltose was determined by incubating the concentrated enzyme preparations with maltose in either 50 mM Na-acetate, pH 4.5 (αG1 and αG2), or 50 mM Mes, pH 6.5 (αG3), for 1 h at 30°C. All assays were linear for 1 h. The concentration of maltose ranged from 69 μM to 55 mM. V_{max} was calculated from the *Y* intercept of Hanes-Woolf plots.

Enzyme Preparation	K_m	V_{max}
	mM maltose	*μmol glucose h^{-1} mL^{-1}*
αG1	0.3	19.6
αG2	1.3	5.3
αG3	33.0	8.7

had an apparent K_m for maltose of 1.3 mM and a V_{max} of 5.6 μmol glucose released h^{-1} mL^{-1}. At pH 6.5, the apparent K_m for maltose of αG3 was 33 mM. The V_{max} for maltose hydrolysis by αG3 was 8.3 μmol h^{-1} mL^{-1}. Gel filtration (Sephacryl-300) revealed the following apparent mol wt for pea α-glucosidases: 125,000 (αG1), 150,000 (αG2), and 110,000 (αG3) (Fig. 4).

Acid and neutral invertase activity did not coelute with αG1 during cation exchange chromatography (data not shown) or with αG2 or αG3 during anion exchange chromatography (Fig. 1A). Fractions containing the neutral α-glucosidase were capable of hydrolyzing soluble starch (Fig. 5A). In contrast, following anion exchange chromatography of apoplastic proteins, starch-degrading activity was not detected in fractions containing either form of acid α-glucosidase (Fig 6A).

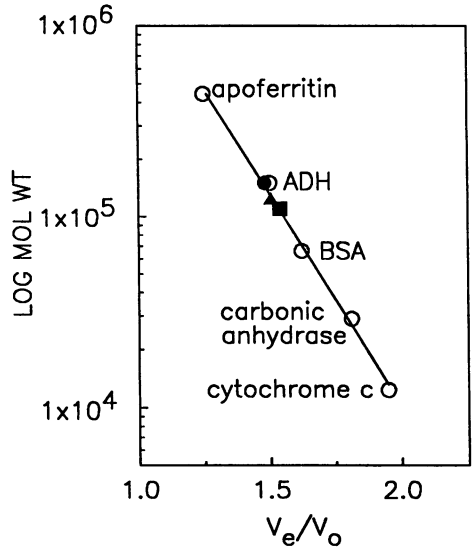

Figure 4. Determination of M_r of pea αG1 (125,000) (▲), αG2 (150,000) (●), and αG3 (110,000) (■) by Sephacryl S-300 gel filtration. V_e/V_0 is the volume to elute proteins divided by the void volume. α-Glucosidase activity was detected using 25 mM maltose in 50 mM Na-acetate (pH 4.5) for αG1 and αG2, or 50 mM maltose in 50 mM Mes (pH 6.5) for αG3. ADH = alcohol dehydrogenase.

Purity of Subcellular Fractions

To determine the subcellular location of pea α-glucosidases, chloroplastic and apoplastic extracts were prepared. The cytosolic marker enzyme PEP carboxylase was not detected in extracts of chloroplasts. After six cycles of IE, only 0.8% of the cytosolic marker enzyme NAD-MDH was detected in the apoplastic solution from pea stems. Another cytosolic marker enzyme NAD-GAPDH was not detected in the apoplastic extract. These results indicate that the chloroplastic and apoplastic preparations used in this study contained negligible levels of cytosolic contamination (*cf.* refs. 3 and 4).

Subcellular Localization of Acid and Neutral α-Glucosidases

When pea chloroplast extract was subjected to DEAE-cellulose chromatography, no activity (at pH 4.5) corresponding to the acid forms of α-glucosidase (αG1 and αG2, Fig. 1B) was detected (data not shown). Only a single peak corresponding to the neutral α-glucosidase (αG3) extracted from pea shoots (Fig. 1B) was observed (Fig. 5). This peak was

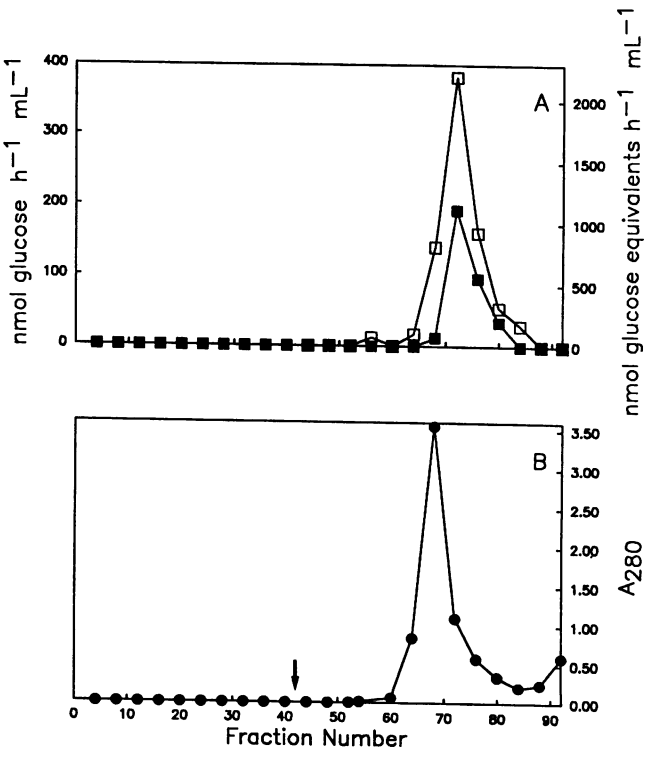

Figure 5. DEAE-cellulose chromatography of pea chloroplast extact (32 mg Chl) showing α-glucosidase activity (nmol glucose h^{-1} mL^{-1}) with 25 mM maltose (■), starch-degrading activity (nmol glucose equivalents h^{-1} mL^{-1}) with 10 mg mL^{-1} soluble starch (□) (A) and A_{280} (B). Aliquots (0.2 mL) were incubated with each substrate in 50 mM Mes (pH 6.5) for 1 h at 30°C. *Arrow* indicates the start of a 0 to 0.5 M NaCl gradient.

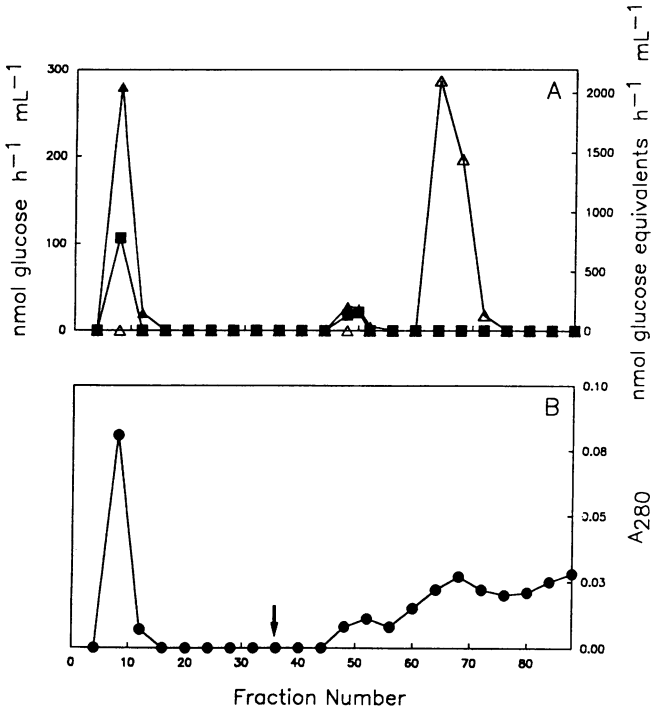

Figure 6. DEAE-cellulose chromatography of apoplastic proteins extracted from 16 g of pea stem segments by six cycles of vacuum IE, showing α-glucosidase activity with maltose (nmol glucose h^{-1} mL^{-1}) or soluble starch (nmol glucose equivalents h^{-1} mL^{-1}) (A), and A_{280} (B). Aliquots were incubated with 25 mM maltose in either 50 mM Na-acetate (pH 4.5) (▲) or 50 mM Mes (pH 6.5) (■) or with 10 mg mL^{-1} soluble starch in 50 mM Na-acetate (pH 4.5) (△). *Arrow* indicates the start of a 0 to 0.5 M NaCl gradient.

nearly identical both qualitatively and quantitatively to that detected in an equivalent (total Chl basis) pea shoot extract (compare Figs. 1B and 5). Thus, chloroplasts appear to contain only αG3.

Of the total acid α-glucosidase activity recovered from pea stems following six cycles of vacuum infiltration-extraction (IE-stem plus apoplastic activity, see "Materials and Methods") 21% was extracted from the apoplast (Table II), indicating that at least one acid α-glucosidase was apoplastic. Total acid α-glucosidase activity recovered from stems subjected to IE was 22% greater than that extracted from control stems (*i.e.* stems not subjected to IE) (Table II). As expected, αG3 activity was detected in the extract of control stems (Fig. 7), but not in the apoplastic preparation (Fig 6A).

Relative Contribution of αG1 and αG2 to Total Apoplastic α-Glucosidase Activity

To determine whether one or both acid α-glucosidases were being extracted by IE, proteins extracted by homogenization of stems subjected to IE and apoplastic proteins extracted by IE were separated by anion exchange chromatography. Both αG1 and αG2 were detected in the apoplastic extract (Fig. 6A). Fractions containing acid α-glucosidase activity were pooled and maltase activity at pH 4.5 was determined. Most (86%) of the acid α-glucosidase activity recovered from the apoplast was due to αG1 (Table III). The activity of the partially purified αG1 recovered from pea stem apoplast was equal to 22% of the combined activity (IE-stem plus apoplastic activity) of partially purified αG1 (Table III). The activity of the partially purified αG2 recovered from the apoplast was equal to 15% of the combined activity of partially purified αG2 (Table III). Since both αG1 and αG2 were present in the apoplastic extract at levels much higher than that determined for contamination by cytosolic marker enzymes (0.8%), both are probably apoplastic enzymes.

DISCUSSION

Pea shoots appear to contain three isoforms of α-glucosidase; two apoplastic forms that are most active under acid conditions, and one chloroplastic form that is most active near neutral pH. Native mol wts were estimated to be 125,000, 150,000, and 110,000 for αG1, αG2, and αG3, respectively. The three forms differ in their affinity for maltose, with the most striking difference being that between either acid form and αG3. The apparent K_m values for maltose of αG1 and αG2 were 0.3 and 1.3 mM, respectively. The apparent K_m for maltose of αG3 was 33 mM.

Following dialysis, DEAE-cellulose chromatography and YM30 concentration, the combined activities of αG1 and αG2 extracted by IE were responsible for 21% of the total acid α-glucosidase activity recovered by IE and homogenization of stems following IE (Table III). This is identical to the percentage of acid α-glucosidase activity observed in dialyzed apoplastic preparations, *i.e.* prior to separation via anion exchange chromatography (Table II). When compared, these results indicate that the apparent contribution of apoplastic αG1 and αG2 (22 and 15%, respectively) to total activity recovered from IE-stems plus apoplastic extract (Table III) is probably not an artifact of the purification and concentration steps. We do not know why 22% more acid α-glucosidase activity was recovered in the IE-stem plus apoplastic fractions than in the control stem extract (Table II). The repeated IE of stem segments may have stimulated the synthesis of α-glucosidase. Alternatively, α-glucosidase activity extracted with the apoplastic solution may have been protected from proteolytic degradation occurring in the extracts from both control and IE-stem preparations.

α-Glucosidases have been identified as apoplastic proteins

Table II. *Acid α-Glucosidase Activity in Control and IE Stem Fractions*

Values in parentheses represent recovery as a percentage of the sum of the activity in the soluble fraction of IE stems plus activity recovered from the apoplast by six cycles of IE. Control stems were not subjected to IE prior to homogenization. All samples were dialyzed prior to α-glucosidase assays. Samples were incubated for 1 h at 30°C in 25 mM maltose, 50 mM Na-acetate (pH 4.5).

Enzyme Preparation	Enzyme Activity
	nmol glucose h^{-1} g^{-1} fresh wt
Control stems (no IE)	712
IE stems (6 IEs)	690 (79)
Apoplast (6 IEs)	178 (21)

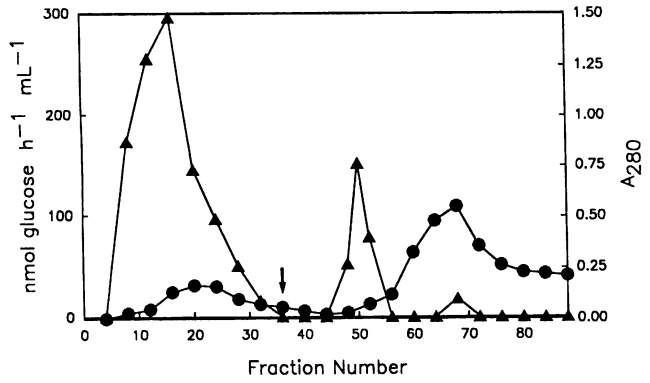

Figure 7. DEAE-cellulose chromatography of proteins extracted from 16 g of control pea stems, *i.e.* stems which were not subjected to vacuum IE, showing acid α-glucosidase activity with 25 mM maltose in 50 mM Na-acetate (pH 4.5) (▲) and A_{280} (●). Assays were terminated after 1 h at 30°C. *Arrow* indicates the start of a 0 to 0.5 M NaCl gradient.

by other investigators (12, 16, 22). In these cases, however, the enzyme was recovered from cell cultures using techniques other than IE, making comparisons with stem or leaf extractions difficult. Previous use of the IE technique in our laboratory resulted in the recovery of a much greater proportion of another carbohydrase from cell walls of pea (*i.e.* 87% of total α-amylase activity) (3). The percentage of acid α-glucosidase recovered from pea (21%, Tables II and III) agrees more closely with the 32% of linamarase extracted from the apoplast of *Phaseolus lunatus* L. leaves by Frehner and Conn (7).

The pH optima of pea α-glucosidases are consistent with their compartmentation. Plants appear to be able to regulate the pH of their cell walls in the range of 4 to 6 (8). Although the existence of apoplastic α-glucosidases has been known for some time, only the forms secreted by the barley aleurone have an apparent function (18), *i.e.* the degradation of starch granules during germination. Neither αG1 nor αG2 extracted from pea stem cell walls had any detectable starch-degrading activity (Fig. 6A). Barley α-glucosidases, however, are 10- to 20-fold more active with maltose as substrate than with soluble starch. Hence, the use of more active preparations of αG1 and αG2 might have indicated that starch is a substrate for pea acid α-glucosidases; albeit with very low specific activity. However, since our enzyme preparations were only partially purified (*i.e.* they may contain contaminating carbohydrases) conclusive determinations concerning starch-degrading activity were not possible. *In vivo* substrates for apoplastic α-glucosidases in chlorophyllous tissue have not been identified.

Hydrolysis of maltose by αG2 appeared to be inhibited by concentrations of maltose greater than 2.5 mM (50% inhibition at 50 mM maltose) (data not shown). This inhibition was not evident when increasing concentrations of PNPG were used as the substrate, indicating that transglycosylation may have depleted glucose released during the hydrolysis of maltose. It is possible, therefore, that αG2 can catalyze a condensation reaction between glucose and maltose.

Maltose is produced in the dark in pea chloroplasts (13) and it is the major product of starch degradation by pea

Table III. *Acid α-Glucosidase Recovery following DEAE-Cellulose Chromatography of IE Stem and Apoplast Preparations*

Within each isoform, fractions containing acid α-glucosidase activity (αG1 and αG2) were pooled and concentrated on a YM30 membrane. The activities of αG1 and αG2 preparations from both IE stems and apoplastic extracts were determined by incubating the partially purified, concentrated samples as described for Table I. Values in parentheses represent the activity of αG1 and αG2 combined as a percentage of the sum of acid α-glucosidase activity recovered from infiltration-extracted stems plus acid α-glucosidase activity recovered from the apoplast.

Enzyme Preparation	Activity Recovered	Contribution within enzyme Preparation	Contribution within Isoform
	nmol glucose h^{-1} g^{-1} fresh wt	*% total*	*% total*
Isoform			
IE stems (6 IEs)			
αG1	435	79	78
αG2	116	21	85
	(79)		
Apoplast (6 IEs)			
αG1	123	86	22
αG2	20	14	15
	(21)		

chloroplast extracts (11) as well as intact chloroplasts (13). Although the actual concentration of maltose in pea chloroplasts is not known, concentrations in the mM range are not unlikely (2). The activity of αG3 would be favored by the stromal environment at night, when the pH is close to neutral (9). When extracts of purified chloroplasts were subjected to DEAE-cellulose chromatography, starch-degrading activity coeluted with αG3 maltase activity (Fig. 5). This may represent the hydrolysis of soluble starch by extrachloroplastic amylases (which sometimes contaminate pea chloroplasts) (4), chloroplastic amylases, αG3 or any combination of these three. As with αG1 and αG2, conclusive assessment of the ability of αG3 to hydrolyze soluble starch would only be possible with a preparation of αG3 shown to be free of other carbohydrases. Hence, while pea chloroplast α-glucosidase probably has some role in the hydrolysis of chloroplastic maltose, its role, if any, as a starch-degrading enzyme is not known. Pea chloroplastic α-glucosidase may also be involved in tranglycosylation reactions as is pea chloroplast D-enzyme (10).

Previous investigators have reported finding little or no maltase activity in pea chloroplasts (11, 13, 14). While their assays were conducted at pH values near the pH optimum of αG3, it is possible that the extraction procedures used did not favor the preservation of α-glucosidase activity. It is also likely that the duration of the assays (10–30 min) (11, 13, 14) and low substrate concentrations (relative to the K_m for maltose of αG3) (11, 14) did not permit detection of α-glucosidase activity.

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LITERATURE CITED

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