Purification to Homogeneity and Properties of Glucosidase II from Mung Bean Seedlings and Suspension-cultured Soybean Cells*

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Gur P. Kaushal, Irena Pastuszak, Ken-ichi Hatanaka†, and Alan D. Elbein
From the Department of Biochemistry, University of Texas Health Science Center, San Antonio, Texas 78284

Glucosidase II was purified approximately 1700-fold to homogeneity from Triton X-100 extracts of mung bean microsomes. A single band with a molecular mass of 110 kDa was seen on sodium dodecyl sulfate gels. This band was susceptible to digestion by endogluco-
saminidase H or peptide glycosidase F, and the change in mobility of the treated protein indicated the loss of one or two oligosaccharide chains. By gel filtration, the native enzyme was estimated to have a molecular mass of about 220 kDa, suggesting it was composed of two identical subunits. Glucosidase II showed a broad pH optima between 6.8 and 7.5 with reasonable activity even at 8.5, but there was almost no activity below pH 6.0. The purified enzyme could use p-nitrophenyl-
α-D-glucopyranoside as a substrate but was also active with a number of glucose-containing high-mannose oligosaccharides. Glc2ManαGlcNAc was the best sub-
strate while activity was significantly reduced when several mannose residues were removed, i.e. Glc3Manα-GlcNAc. The rate of activity was lowest with Glc4ManαGlcNAc, demonstrating that the innermost glucose is released the slowest. Evidence that the en-
zeyme is specific for α1,3-glycosidic linkages is shown by the fact that its activity on Glc4ManαGlcNAc was inhibited by nigerose, an α1,3-linked glucose disaccha-
dride, but not by α1,2 (koldjiose)-, α1,4(maltose)-, or α1,6 (isomaltose)-linked glucose disaccharides. Glu-
cosidase II was strongly inhibited by the glucosidase processing inhibitors deoxynojirimycin and 2,6-di-
dexoxygenojirimycin and 2,6-dideoxy-2,6-imino-l-ribohexosyl-l-d-glyc-
ecero-1-gulose, but less strongly by castanosper-
mine and not at all by australine.

Polyclonal antibodies prepared against the mung bean glucosidase II reacted with a 95-kDa protein from suspension-cultured soybean cells that also showed glu-
cosidase II activity. Soybean cells were labeled with either [2-3H]mannose or [6-3H]galactose, and the glu-
cosidase II was isolated by immunoprecipitation. Es-
sentially all of the radioactive mannose was released from the protein by treatment with endogluco-
saminidase H. The labeled oligosaccharide(s) released by endogluco-
saminidase H was isolated and characterized by gel filtration and by treatment with various en-
zymes. The major oligosaccharide chain on the soybean glucosidase II appeared to be a Manα1,2(GlcNAc)2 with small amounts of Glc4Manα(GlcNAc)2.

In plants, as in animals, the biosynthesis of the various types of N-linked oligosaccharides involves two distinct series of reactions that occur in the various compartments of the cell (1-3). The first series of reactions occurs in the endo-
plasmic reticulum and produces the common intermediate, i.e. Glc4Manα(GlcNAc)2-PP-dolichol, that gives rise to all of the N-linked oligosaccharide structures (4-6). In these reac-
tions, the sugars, GlcNAc, mannose, and glucose, are added one at a time from their nucleoside diphosphate sugar donors or from their dolichyl monophosphate sugar derivatives to the dolichyl-P carrier to form the precursor lipid-linked oligosac-
charide, Glc4Manα(GlcNAc)2-PP-dolichol (7, 8). This lipid-
linked saccharide intermediate then serves as the donor of oligosaccharide to specific asparagine residues on the protein (9, 10). The transfer of oligosaccharide to protein is a cotrans-
lational event and occurs in the endoplasmic reticulum while the protein is still being synthesized on membrane-bound polysomes (11-13).

Following the transfer of oligosaccharide to protein, a sec-
ond series of reactions occurs that result in alterations in the structure of the initial oligosaccharide to give rise to the various types of high-mannose, hybrid, and complex N-linked oligosaccharides (14, 15). These reactions are referred to as processing or trimming reactions and have been well studied in animal cells, with a number of the enzymes having been isolated and highly purified (16). However, these reactions are not nearly as well understood in plants, and only a few of the enzymes have been partially purified (17). The initial reactions of this processing pathway begin in the endoplasmic reticulum, probably while the protein is still being synthet-
ized. Two membrane-bound glucosidases, called glucosidase I and glucosidase II, remove the 3 glucose residues to give high-mannose structures (18). Thus, glucosidase I removes the outermost α1,2-linked glucose (19, 20), whereas glucosi-
dase II removes the next 2 α1,3-linked glucose (21-23). Both of these enzymes have been purified to homogeneity from various animal tissues (24-29), but only glucosidase I has been partially purified from plants (30). Since these enzymes are apparently quite stable and can therefore be utilized to test for new inhibitors of glycoprotein processing (31), and since very little was actually known about processing in plants, we decided to purify the various processing enzymes from mung bean seedlings and to compare their properties with those of animal cells. In addition, since these enzymes remain in the endoplasmic reticulum or the Golgi apparatus, we were inter-
ested in determining the signals that retain the various en-
zymes in their particular compartment.

In the present paper, we describe the purification to ho-
mogeneity of glucosidase II and the properties of this enzyme. The oligosaccharide substrate specificity and the affect of various processing inhibitors were also examined with this purified enzyme preparation. Antibody against the enzyme purified from mung bean seedlings cross-reacted with the

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† Present address: Institute of Industrial Science, University of Tokyo, Tokyo 106, Japan.
enzyme from suspension-cultured soybean cells. This antibody was used to isolate \(^{[3]H}\)mannose-labeled glucosidase II from soybean cells and to determine the carbohydrate structure of this glucosidase II.

**EXPERIMENTAL PROCEDURES AND RESULTS**

**Purification and Properties of Glucosidase II**—As outlined in the Miniprint Section of this paper, glucosidase II from mung bean seedlings was purified more than 1700-fold to a homogeneous state (see Figs. 1–5). As shown in Fig. 10, the enzyme at the final purification stage gave a single band on SDS\(^{-12}\) gels that had a subunit molecular mass of about 110 kDa. Peak A and peak B, initially separated from each other by DEAE-cellulose chromatography (see Fig. 1), were each purified individually using the procedures outlined in the Miniprint Section. It can be seen from Fig. 10 that both the purified peak A, and the purified peak B gave the same molecular weight band on these gels, indicating that the difference between these two peaks of glucosidase II activity is likely to be due to surface charge. In addition, the data in Fig. 10 demonstrate that both of these peaks were susceptible to endoglucomannanase H, and this treatment gave a faster moving band suggesting the loss of one or two high-mannose types of oligosaccharides. The structure of the oligosaccharide chain of glucosidase II isolated from suspension-cultured soybean cells is described below.

As indicated in Figs. 1–4 of the Miniprint Section, glucosidase II is able to remove the \(\alpha_1,3\)-linked glucose from the Glc\(_3\)Man\(_3\)GlcNAc and is also active with the \(p\)-nitrophenyl-\(\alpha\)-glucopyranoside. Fig. 11 compares the substrate specificity of the purified enzyme with a number of different glucose-containing high-mannose oligosaccharides. Each of these substrates was prepared from \(^{[3]H}\)glucose-labeled Glc\(_3\)Man\(_3\)GlcNAc (GlcNAc)\(_2\)-peptide as follows. Glc\(_3\)Man\(_3\)(GlcNAc)\(_2\)-peptide was treated with jack bean \(\alpha\)-mannosidase, and the resulting Glc\(_3\)Man\(_3\)(GlcNAc)\(_2\)-peptide was isolated by chromatography on a long (1 \(\times\) 150 cm) calibrated column of Bio-Gel P-4. This glycopeptide was treated with Endo H to give Glc\(_3\)Man\(_3\)GlcNAc, which was further purified on the Bio-Gel P-4 column. The Glc\(_3\)Man\(_3\)GlcNAc was treated for short times with partially purified glucosidase I to give Glc\(_3\)Man\(_3\)GlcNAc which was separated from the Glc\(_3\)Man\(_3\)GlcNAc by several chromatographies on the Bio-Gel P-4 column.

It can be seen that the enzyme exhibited the greatest rate of activity on the Glc\(_3\)Man\(_3\)GlcNAc oligosaccharide and appeared to be slightly less active towards this oligosaccharide when it was attached to asparagine (i.e. Glc\(_3\)Man\(_3\)(GlcNAc)\(_2\)-Asn). On the other hand, when several mannose residues were removed by the action of jack bean \(\alpha\)-mannosidase, there was a significant decrease in the rate of release of the glucose from Glc\(_3\)Man\(_3\)GlcNAc by the purified glucosidase II. The enzyme also exhibited a slower rate of hydrolysis of the innermost glucose as indicated by the rate of activity with Glc\(_3\)Man\(_3\)GlcNAc. In this experiment, this substrate was present at a 2-fold higher concentration (in terms of mass) in the incubation mixture than the other substrates, since the same amount of radioactivity was used with each substrate. Nevertheless, the data indicate that it was much less effective as a substrate for glucosidase II. This latter finding fits earlier studies on in vivo processing that demonstrated that the 3rd glucose was removed at a much slower rate than the 2 outer glucose residues (19).

**Effect of Various Processing Inhibitors on the Activity of Glucosidase II**—A number of glucosidase inhibitors were tested on the purified glucosidase II to compare their effectiveness against this enzyme. The results of these studies are shown in Fig. 12. The inhibitors were compared using both the \(p\)-nitrophenyl-\(\alpha\)-glucopyranoside (Aryl) as substrate and the Glc\(_3\)Man\(_3\)GlcNAc oligosaccharide (Processing) as substrate. The data show that deoxynojirimycin (DNJ) and 2,6-dideoxy-2,6-imino-7-O-(\(\beta\)-d-glucopyranosyl)-d-glycero-L-gulohexitol (MDL) were fairly potent inhibitors of this enzyme with 50% inhibition with the aryl substrate occurring at inhibitor concentrations of about 2–5 \(\mu M\), whereas with the oligosaccharide substrate, 50% inhibition required about 5–10 \(\mu M\). 7-O-(\(\beta\)-D-Glucopyranosyl)-d-glycero-L-gulohexitol has been shown to be a good inhibitor of glucosidase II but to have very weak activity towards glucosidase I (32). Fig. 12 also shows that castanospermine (CAST) also inhibited glucosidase II, but was much less effective than either of the
with the oligosaccharide substrate, the concentration of cas-

tospermine to give 50% inhibition was about 50-75 μM. Cas-

tospermine has been shown previously to be a potent

inhibitor of glucosidase I (33). When the oligosaccharide

products were examined at several levels of castanospermine

inhibition (50 and 100 μM), they were found to be a mixture

of mostly Glc-Man-GlcNAc and small amounts of Man-GlcNAc. Australine (AUST), on the other hand, had no

activity toward the purified glucosidase II, although this al-

kaloid is a fairly good inhibitor of glucosidase I (34).

**Immunoprecipitation of Mung Bean and Soybean Glucosi-

dase II**—Polyclonal antibody was prepared against the puri-

fied mung bean glucosidase II as indicated under “Experi-

mental Procedures.” This antibody was tested for its ability to

react with the glucosidase II from suspension cultured soy-

bean cells as shown in Fig. 13. In this experiment, membrane

preparations of mung bean seedlings or soybean cells were

solubilized with Triton X-100, and the clarified extracts were

incubated with various amounts of antiserum. Following an

incubation of 1 h, a suspension of Staphylococcus aureus was

added to precipitate the antibody-antigen complex, and the

mixture was centrifuged. The supernatant liquid was assayed

for glucosidase II activity. It can be seen from the figure that

the antiserum removed the glucosidase II activity from the

mung bean extracts and also from extracts of soybean cells,

and the antibody appeared to be almost equally effective

against either protein. No effect on enzyme activity was

observed when preimmune serum was used instead of anti-

body (Fig. 13).

**Characterization of Oligosaccharide Structure of Soybean

Glucosidase II**—Since it was not feasible to obtain sufficient

amounts of glucosidase II from mung bean seedlings to deter-

mine the structure of its carbohydrate chain(s), and since it

was not possible to label these glycoproteins in this system,

we used suspension-cultured soybean cells to produce enzyme

labeled in the carbohydrate portion of the molecule. Soybean

cells were grown in culture and labeled with either [2-3H]
mannose or [6-3H]galactose. Glucosidase II was initially ex-

tracted from the membrane fraction with detergent and par-

tially purified on DEAE-cellulose columns. The glucosidase

II was then immunoprecipitated with the polyclonal antibody

as described in Fig. 13. An aliquot of the precipitate was run

on SDS gels and the gels exposed to film to determine the

purity of the radioactive proteins. As seen in Fig. 14, a single

radioactive band, that had a subunit molecular weight of about

95 kDa, was seen on these films, indicating that the antibody

recognized only a single protein in these extracts and that

this protein was slightly smaller in size than the corresponding

protein of mung bean seedlings. However, this soybean glu-

cosidase showed the same susceptibility to Endo H as did the

mung bean enzyme. The remainder of the radiolabeled im-

munoprecipitate was suspended in 1% SDS containing 5 mM

β-mercaptoethanol and heated to dissociate the complex and
denature the protein. The mixture was then diluted to 0.1% SDS with citrate buffer, and Endo H was added to release the oligosaccharides. The protein was then precipitated with trichloroacetic acid, and the oligosaccharide chain(s) was identified by gel filtration on a column of Bio-Gel P-4. Endo H released essentially all of the mannose label, since no radioactivity remained associated with the trichloroacetic acid-precipitated protein after Endo H treatment.

The mannose-labeled and glucose-labeled oligosaccharides, released from the soybean glucosidase II with Endo H, were chromatographed on calibrated columns of Bio-Gel P-4 as seen in Fig. 15. The upper profile shows that the mannose-labeled oligosaccharide emerged in a fairly sharp symmetrical peak in an area just ahead of and with the Man\textsubscript{9}GlcNAc (peak B of the standards), suggesting that it was a mixture of Hexose\textsubscript{9}GlcNAc and Hexose\textsubscript{10}GlcNAc, but probably mostly Hexose, G1cNAc. As seen in the middle profile, the pooled oligosaccharide peak was mostly susceptible to digestion by jack bean \( \alpha \)-mannosidase, and the two main peaks resulting from this treatment were free mannose and a smaller peak migrating near the Man\textsubscript{9}GlcNAc area. This latter peak is probably Glc\textsubscript{1}Man\textsubscript{2}GlcNAc. Treatment of the original oligosaccharide peak with both glucosidase II and \( \alpha \)-mannosidase gave almost the same results as the treatment with \( \alpha \)-mannosidase alone (lowest profile), although this did give a slight increase in the amount of mannose released. As indicated above, glucosidase II acts very slowly on the Glc\textsubscript{1}Man\textsubscript{9}GlcNAc peak with both glucosidase II, and \( \alpha \)-mannosidase substrates and even more slowly on oligosaccharides containing fewer mannose residues. Since the mannosidase is quite as active compared with the glucosidase II, it is likely that a number of mannoses are removed before glucosidase II has a chance to work. Since it was difficult on this basis to determine whether the oligosaccharide did also contain small amounts of Glc\textsubscript{1}Man\textsubscript{2}GlcNAc, we took another approach to determine the presence of glucose in the oligosaccharides of glucosidase II.

The oligosaccharide was also synthesized with label in the glucose portion by growing the soybean cells in [6-\( ^{3} \)H]galactose and isolating the Endo H-sensitive oligosaccharide from the immunoprecipitate. In this case, the amount of radioactivity in the oligosaccharide was fairly low, but the intact oligosaccharide migrated at almost the same position as the peak tube of the mannose-labeled oligosaccharide seen in Fig. 15. In order to determine whether this oligosaccharide peak contained 1 or 2 glucose residues, it was subjected to periodate oxidation and after reduction with NaBH\textsubscript{4} and acid hydrolysis, the products were identified by paper chromatography. The glucose-labeled Glc\textsubscript{1}Man\textsubscript{2}G1cNAc standard prepared in influenza virus-infected Madin-Darby canine kidney cells gave two radioactive peaks corresponding to glycerol and glucose, and the ratio of radioactivity in these peaks was about 2:1 (glucolylglucose). On the other hand, the standard Glc\textsubscript{1}Man\textsubscript{2}GlcNAc also gave radioactive peaks corresponding to glucose and glycerol but in this case the ratio of radioactivity was 1:1. The unknown oligosaccharide from soybean cells also gave two radioactive peaks, one of which was clearly identifiable as glycerol and the other which ran faster than glucose and is likely to be mannose. The ratio of radioactivity in these two peaks was about 1:1. Although the presence of labeled mannose is unexpected, this sugar could arise from labeled galactose by known metabolic pathways. However, the absence of radioactive glucose after periodate oxidation but the presence of labeled glycerol, coupled with the slightly increased migration rate of the oligosaccharide on the Bio-Gel P-4 columns, indicates the presence of some Glc\textsubscript{1}Man\textsubscript{2}GlcNAc in the initial peak.

**DISCUSSION**

Glucosidase II is a key enzyme in the processing and maturation of N-linked glycoproteins, since it removes the two innermost \( \alpha \)-1,3-linked glucose residues to give rise to the high-mannose types of oligosaccharides (i.e. Man\textsubscript{9}(GlcNAc)\textsubscript{2}). These high-mannose chains are then the precursors to all of the hybrid and complex N-linked oligosaccharides. The removal of these last two glucose units may be a critical step in the transfer of glycoproteins from the endoplasmic reticulum to the Golgi apparatus. Thus, studies with cultured hepatocytes showed that when the removal of the glucose residues was inhibited with the glucosidase I inhibitor, deoxynojirimycin, the rate of secretion of some of the serum proteins (i.e. \( \alpha \)-1,3-hydroxycholesterolemia, the C3 component of complement) were only marginally affected (35). Similar results were observed with regard to biosynthesis and targeting of the low density lipoprotein receptor in human fibroblasts and in certain smooth muscle cells (36). In this case, when these cells were incubated in the presence of the glucosidase I inhibitor, castanospermine, the N-linked oligosaccharides on the low density lipoprotein receptor had Glc\textsubscript{1}Man\textsubscript{2}(GlcNAc)\textsubscript{2} structures, and these inhibited cells had only 40% as many low density lipoprotein receptors at their cell surface as did control cells. However, the total number of low density lipoprotein receptors in the inhibited cells was about the same as that in the control cells. Preliminary evidence suggests that inhibited cells have an accumulation of low density lipoprotein receptors in an intracellular compartment (probably the endoplasmic reticulum) (36). These studies and others cited in a review (37) suggest that removal of glucose residues may be critical for proper transport of some N-linked glycoproteins from the ER to the Golgi apparatus.

Glucosidase II removes the two \( \alpha \)-1,3-linked glucose residues from the Glc\textsubscript{1}Man\textsubscript{2}(GlcNAc)\textsubscript{2} chain of the N-linked glycopro-
teins. In vivo studies had indicated that the first α1,3-linked glucose (actually the middle glucose of the Glc2 structure) is released fairly rapidly, but the last glucose, i.e. the second α1,3-linked glucose, is removed much more slowly (19). In vitro studies with the purified glucosidase II as reported in this paper show that glucose release from Glc4Man4GlcNAc is significantly faster than glucose release from Glc4Man4GlcNAc. It is not known why there is this difference in rate between these two glucose units, but it should be noted that the innermost α1,3-linked glucose (that is, the one released most slowly) is attached to mannose, whereas the next α1,3-linked glucose is attached to another glucose. It is also interesting to note that glucosidase II does not release glucose more rapidly from the glycotope (i.e. Glc4Man4GlcNAc2-Asn) that it does from the oligosaccharide (Glc4Man4GlcNAc). It will be interesting to determine whether glucose is released more rapidly from the intact protein or from a denatured protein. Such studies would help to answer the question as to whether the protein portion of these glycoproteins has any effect, or plays any role, in glycoprotein processing.

Glucosidase II appears to be relatively specific for α1,3-glucosidic linkages. This was shown by comparing the inhibition capacity of a number of α-linked glucose disaccharides on the release of glucose from [3H]Glc4Man4GlcNAc by the purified glucosidase II. The α1,3-linked glucose disaccharide nigerose was a fairly good inhibitor of this enzyme showing 50% inhibition at about 1 mM concentrations. However, kojibiose (α1,2-linked), maltose (α1,4-linked), isomaltose (α1,6-linked), and trehalose (α1,α1-linked) were all poor inhibitors or were somewhat stimulatory. The reason for the stimulation observed by isomaltose and trehalose is not known. It is also not clear why nigerose only inhibits the reaction up to 75%, and no further inhibition is seen even at much higher concentrations. Although glucosidase II is fairly specific for α1,3-linked glucose, it also catalyzes the release of glucose from α-ribofuranosyl-α-glucose. Thus, it is not surprising that this substrate also inhibits the release of glucose from Glc4Man4GlcNAc.

The mung bean glucosidase II appears to have a molecular mass of about 290 kDa and is apparently composed of two identical subunits of about 110 kDa. However, the soybean glucosidase II is probably a Manα4GlcNAcP, with a molecular weight of about 290 kDa with two subunits of about 130 kDa. These two proteins apparently have different subunit molecular weights than those mentioned above. Thus, Burns and Toaster (24) reported a subunit molecular weight for rat liver glucosidase II of about 65,000, whereas the native enzyme on polyacrylamide gels appeared to be a 262,000-dalton tetramer (24). The mammary gland glucosidase II was also reported to have a molecular mass of about 290 kDa with two subunits of 62 and 64 kDa (26). Except for the two plant systems described in this report, glucosidase II has not been purified from any other plant tissue. Future studies will determine the cross-reactivity of our antibody with other plant species and the structures of the glucosidase II subunits.

REFERENCES


Additional references are found on p. 16277.

Continued on next page
Plant Processing Glucosidase II

SOMATICAL MATERIAL TO:

Development of Immunochemicals: Enzyme Inhibition of Glucosidase II from Morel Green Muskingum and Subterranean Cucumber Seedling Cells.

By Qur S. Kamal, Irene Faktas, Yaa Nakaza and Alan D. Elbeen

EXPERIMENTAL PROCEDURE

Materials:
- [E]-N-Methylacetyl (20 Ci/mmol) and [2-14C]-glucosamine (15 Ci/mmol) were purchased from New England Nuclear. Sodium dodecyl mercaptan was purchased from Fisher Scientific. The reagents were used as received.

Buffers:
- Buffer A: 100 mM sodium phosphate, pH 7.0, 0.1% Triton X-100, 100 mg/l glycine, 0.1% sodium deoxycholate, 0.1% sodium dodecyl sulfate, and 0.1% sodium azide. Buffer B contained 7 M urea and 0.1% sodium deoxycholate.

Purification of Glucosidase from Seed Extracts:
- The enzyme was purified by affinity chromatography on a Sepharose 4B column, followed by gel filtration on a Sephacryl S-200 column. The purified enzyme was stored at -70°C.

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Plant Processing Glucosidase II

A. First DAE-cellulose Chromatography. A 4-5 cm column of DAE-

cellulose column was applied to a 2.0-2.5 cm column of hydroxyap.

phosphate buffer and eluted with 1 liter of a linear gradient of 0 to 0.4 M NaCl in buffer A. As described in the methods, this was the only gradient used and no additional buffer was added. The major peak was eluted between 200-270 mM NaCl, while the minor peak eluted at 300 mM NaCl. The two peaks were pooled separately and dialyzed against buffer A. Each of the peaks shown in this figure (lanes 2 and 3) represents a peak at these peaks were eluted at 200-270 mM NaCl. The dialyzed fractions containing glucosidase II were pooled and 10-20 mg of enzyme were used in each of the subsequent experiments.

B. Second DAE-cellulose Chromatography. The dialyzed enzyme solution from peak A (Fig. 3) was applied to a 2.0-2.5 cm column of DAE-cellulose column. The column was washed with buffer A and the enzyme was eluted with 200 mM NaCl in buffer A. The fractions containing glucosidase II were pooled and 10-20 mg of enzyme were used in each of the subsequent experiments.

C. Chromatography on Carbohydrate-Sepharose. The purified enzyme was applied to a column of 1.5 ml of Sepharose CL-6B and eluted with 0.1 M NaCl in buffer A. The fractions containing glucosidase II were pooled and 10-20 mg of enzyme were used in each of the subsequent experiments.

D. Sepharose 2-100 Column Chromatography. The enzyme from Sepharose 2-100 column was applied to a column of Sepharose 2-100 and eluted with 0.1 M NaCl in buffer A. The fractions containing glucosidase II were pooled and 10-20 mg of enzyme were used in each of the subsequent experiments.

References for Supplemental Material


Figure 4: Purification of Glucosidase II on First DAE-cellulose. The purified enzyme was applied to a 2.0-2.5 cm column of DAE-cellulose column. The column was washed with 200 mM NaCl in buffer A. The fractions containing glucosidase II were pooled and 10-20 mg of enzyme were used in each of the subsequent experiments.

Effect of pH and Metal Ions: The effect of pH on the activity of glucosidase II was determined as described in the methods. The enzyme was stable at pH 6.0-8.0. The effect of metal ions on the activity of glucosidase II was determined as described in the methods.

E. Effect of Metal Ions: The effect of metal ions on the activity of glucosidase II was determined as described in the methods. The enzyme was stable in the presence of 1.0 M NaCl. The enzyme was stable at pH 6.0-8.0. The effect of metal ions on the activity of glucosidase II was determined as described in the methods.

The biosynthesis of glucosidase II activity was regulated by the addition of divalent metal ions such as Mg2+, Mn2+, or Ca++. However, a number of metal ions such as Co++, Cu++, and Zn++ had little or no effect on the enzyme. The enzyme was, however, rapidly inactivated in the presence of FUM.
Figure 3: Purification of Glucosidase II on a Column of Hydroxypropyl A. The enzyme was eluted with a 10 to 30% phosphate gradient and every other fraction was assayed for enzyme activity as described in the text.

Figure 4: Purification of Glucosidase II on Second DEAE-Cellulase Column. The column was prepared and equilibrated as described in the text. Enzyme activity was assayed as described in the text. The DEAE-cellulose chromatography except that both processing and purification were performed on a second DEAE-cellulose column.

Figure 5: Determination of Molecular Weight of Native Glucosidase II by Sephacryl 300 Chromatography. The activity of purified glucosidase II was measured by the method of Tamm and Stipanovic. The elution volumes of the standard markers were recorded, and the molecular weight of glucosidase II was determined by comparison with the elution volumes of the standard proteins.

Figure 6: SDS-Gel Electrophoresis of Glucosidase II Fractions. The fractions were separated by SDS-gel electrophoresis and stained with Coomassie blue. The migration distance of the protein bands was measured and the molecular weight was determined by comparison with the standard proteins.

Figure 7: Purification of 1.6 A R E N Z Y M E. The enzyme was purified by column chromatography and gel electrophoresis. The resulting enzyme was assayed for activity as described in the text.

Figure 8: Purification of Glucosidase II on a Sephacryl S-300 Column. A 1.5 x 1.0 m gel filtration column was prepared and equilibrated in buffer A. The enzyme from Concanavalin A-Sepharose (1 ml) was applied and eluted in the same buffer. Glucosidase II activity was assayed using both Cy3-Phenylmethyl and p-nitrophenyl-α-D-glucoside as substrates.
Figure 8: Effect of pH on the Activity of Glucosidase II. The pH of the reaction mixture was varied using MES buffer from 4.5 to 6.5, phosphate buffer from 6.5 to 8.2, and glycine buffer from 8.5 to 9.5. The enzyme activity was assayed using both p-nitrophenyl-β-D-glucoside as substrates.

Figure 9: Inhibition of Glucosidase II Activity by Glucose Disaccharides. Various amounts of glucose disaccharides, nigerose (mM), kojibiose (mM), trehalose (mM), maltose (mM), and iminose (mM) were added to the incubation mixture and after addition of the enzyme the incubation was done for 1h. The release of p-glucose from p-glucuronidase substrate was determined as described.

Table I

<table>
<thead>
<tr>
<th>Loop</th>
<th>Value</th>
<th>Units</th>
<th>Activity</th>
<th>Units</th>
<th>Yield %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Solubilized-Enzyme</td>
<td>840</td>
<td>6597</td>
<td>38930</td>
<td>6</td>
<td>100</td>
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<tr>
<td>2) 1st Phos-Cellulase</td>
<td>340</td>
<td>4530</td>
<td>39880</td>
<td>45</td>
<td>98</td>
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<td>3) Hydroxyapatite</td>
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<td>17876</td>
<td>241</td>
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<tr>
<td>4) 2nd Phos-Cellulase</td>
<td>86</td>
<td>21.5</td>
<td>9228</td>
<td>4294</td>
<td>24</td>
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<tr>
<td>5) Con A-Sepharose</td>
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<td>1.6</td>
<td>6656</td>
<td>4160</td>
<td>17.5</td>
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<tr>
<td>6) Sepacryl S-300</td>
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<td>0.46</td>
<td>4566</td>
<td>9927</td>
<td>12</td>
</tr>
</tbody>
</table>

* The unit of Glucosidase II is defined as the amount of enzyme that catalyzes the release of 1 nmoL of glucose from p-glucuronidase substrate in 60 min.
Purification to homogeneity and properties of glucosidase II from mung bean seedlings and suspension-cultured soybean cells.
G P Kaushal, I Pastuszak, K Hatanaka and A D Elbein