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α-D-MANNOSIDASE. PREPARATION AND PROPERTIES OF FREE AND INSOLUBILIZED ENZYME

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Summary

α-D-Mannosidase (α-D-mannoside mannohydrolase, EC 3.2.1.24) has been purified to homogeneity as demonstrated by polyacrylamide gel electrophoresis and ultracentrifugation. The molecular weight of the enzyme is approx. 200 000; the protein appears to contain 4 subunits, with molecular weights of 66 000 and 44 000. The enzyme was immobilized on Sepharose and the properties of the coupled and free enzyme were compared. Both were stable up to 70°C with rapid loss of activity between 75—80°C; both retained 25—30% activity in 6 M urea and 65% of the original activity could be restored in the coupled preparation by removal of the urea. The pH maximum of each form was approximately the same, with the maximum of the immobilized enzyme shifted slightly to a lower pH.

The coupled α-D-mannosidase presented in this report offers the possibility of digesting high molecular weight substrates, such as glycoproteins, with the advantages of (1) recovering large quantities of digested substrate; (2) recovery of the active glycosidase; and (3) digestion at high temperatures and under conditions that denature many proteins.

Introduction

α-D-Mannopyranosyl residues are common components of glycoproteins, the carbohydrate compositions of which may be relatively simple, such as is found in ovalbumin [1] or complex as in α_1-acid glycoprotein [2] of human serum. Structural studies of glycoproteins and the modification of the carbo-

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hydrate groups have frequently involved specific hydrolysis by pure glycosidases, one of which is α-D-mannosidase (α-D-mannoside mannohydrolase, EC 3.2.1.24).

α-D-Mannosidase has been isolated and partially characterized from a variety of mammalian, bacterial, and plant sources [3]. Li [4,5] and Li and Li [6] described a purification of α-D-mannosidase from jack bean. It was free from other glycosidases and was homogeneous on gel electrophoresis. The enzyme, molecular weight 190 000 by gel filtration, showed maximal activity between pH 4.0 and 4.5 and lost about one-half of its activity after heating at 70°C for 5 min. Snaith and Levvy [7] and Snaith [8] modified the procedure, removing contaminating *N*-acetyl-β-D-glucosaminidase and β-D-galactosidase activities by treatment with 2 mM pyridine. It was also demonstrated that Zn^{2+} was essential for α-D-mannosidase stability. Their preparation of α-D-mannosidase was homogeneous using gel electrophoresis and ultracentrifugation, and a molecular weight of 230 000 was determined. A more recent description of a preparation of α-D-mannosidase from jack bean was based on a modification of the method of Li [5], reported by Chu and Turner in 1975 [9].

The present paper describes some properties of jack bean α-D-mannosidase in solution, and compares these properties with those of an insolubilized form of the enzyme.

Materials and Methods

Materials. Jack bean meal was obtained from Nutritional Biochemical Corporation. *p*-Nitrophenyl-α-D-mannopyranoside, -β-D-mannopyranoside, and -*N*-acetyl-β-D-glucosaminide, and -α-L-fucoside were purchased from Pierce Chemical Company; *p*-nitrophenyl-α-D-galactopyranoside from Sigma; and *p*-nitrophenyl-β-D-galactopyranoside from Calbiochem. *p*-Nitrophenyl-α-D-mannoside was also prepared according to the method of Conchie and Levvy [10]. Sepharose 4B was purchased from Pharmacia.

Ovalbumin was prepared according to the method of Kekwick and Cannan [11] and used as the desalted, lyophilized material. Asparaginyl-carbohydrate fractions were prepared from ovalbumin by the method of Huang et al. [12].

Enzyme assay. With *p*-nitrophenyl-α-D-mannoside as substrate, one unit of activity was defined as that amount of enzyme releasing 1 μmol of *p*-nitrophenol per min at 25°C. Enzyme assays were carried out in 0.05 M sodium acetate buffer, pH 4.5, containing 0.01 M NaCl and 0.1 mM $ZnSO_4$ (assay buffer).

1. Colorimetric assay. An aliquot of enzyme solution (10—100 μl containing approximately one unit) was added to 1.0 ml of 2 mM substrate in assay buffer. After 2 min, the reaction was stopped by the addition of 2 ml of 0.2 M sodium carbonate. The liberated *p*-nitrophenol was measured quantitatively at its absorbance maximum, 400 nm (ϵ_{400} for *p*-nitrophenol = $17.8 \cdot 10^3\ M^{-1} \cdot cm^{-1}$).

2. Continuous spectrophotometric assay. For testing possible inhibitors of α-D-mannosidase, a continuous assay was used as described previously [13]. The assay recorded the disappearance of *p*-nitrophenyl-α-D-mannoside at its absorbance maximum, 305 nm (pH 4.5). A Beckman DU spectrophotometer with a Gilford photometer was used to record the data. Assay mixtures contained 200 μl of 2.5 mM substrate in assay buffer, to which was added 1.0 ml of buffer for

control runs or 1.0 ml of a 0.01 M inhibitor solution. Enzyme (10 μl containing approx. 0.1 unit) was added and the reaction followed. The compounds tested for possible inhibition of α-D-mannosidase activity were 1,5-anhydro-D-glucitol and -D-mannitol; 1,4-anhydro-D-mannitol; 1,6-dichloromannitol; 2-deoxysorbitol, mesoerythritol; lyxosamine; D-mannitol; myoinositol; mucoinositol; L-chiroinositol; and D-glucuronic acid.

Protein determination. Protein was determined by measuring the absorbance of a solution in water at 230 nm, using bovine serum albumin as a standard. Enzyme solution (10—50 μl) was diluted with 2.5—10.0 ml of water to give an absorbance of less than 1.2 units. The absorbance at 230 nm for bovine serum albumin was found to be linear over a concentration range of 0—250 μg/ml. The method of Lowry et al. [14] was also used with bovine serum albumin as standard and gave similar values.

Purification of α-D-mannosidase. The purification of the α-D-mannosidase was a modification of the procedure for the preparation of *N*-acetyl-β-D-glucosaminidase reported by Li and Li [15], chromatography on hydroxyapatite being used to purify further the α-D-mannosidase fraction (fraction I) from DEAE-Sephadex A-50 chromatography (Figs. 1 and 2). The enzyme fraction from hydroxyapatite chromatography was heated finally at 65°C for 30 min in a constant-temperature water bath. The resulting turbid solution was centrifuged and the precipitate discarded. The enzyme was stored as a suspension in saturated ammonium sulfate. For use in further experiments, an aliquot of the homogeneous suspension was centrifuged, the precipitate dissolved in assay buffer, and dialyzed against assay buffer to remove any ammonium sulfate.

Gel electrophoresis

1. System I. This method was based on the procedure of Davis [16], using a pH 8.6 Tris/glycine buffer system and 7.5% acrylamide in the gels. The gels were stained for both protein, using Amido Schwartz, and enzyme activity. The zone of α-D-mannosidase activity was visualized by immersing the whole gel after electrophoresis in a dilute *p*-nitrophenyl-α-D-mannoside solution (approx. 0.05%) at pH 4.5 [13]. After 15—30 min, a yellow band appeared on the gel where the enzyme activity had migrated.

2. System II. This method was a modification of the procedure described by Chalkley and Panyim [17], and was used for most routine electrophoresis of the α-D-mannosidase preparation. Enzyme solutions, made 6 M with solid urea, were subjected to electrophoresis on polyacrylamide gels containing 2.5 M urea and 10% acrylamide for approx. 2 h. The gels were stained for protein using Amido Schwartz, and for carbohydrate using the method of Zacharius et al [18].

Molecular weight determinations

1. Native enzyme. (a) The molecular weight of the native enzyme was determined using Sephadex G-200 chromatography in 1 mM Tris · HCl, pH 7.4 with 0.1 M KCl; standard protein markers were aldolase (160 000), bovine serum albumin (68 000), ovalbumin (45 000) and cytochrome *c* (12 400).

(b) Molecular weight of the native enzyme was also determined by sedimentation in a sucrose density gradient, using bovine catalase (Sigma) as a marker

protein (mol. wt. 232 000). A sucrose gradient of 5—20% sucrose in 0.05 M Tris · HCl buffer, pH 7.5 was prepared in a total volume of 4.3 ml. α-D-Mannosidase (50 μl) containing approx. 0.5 units was mixed with 50 μl of a catalase solution and the solution layered on the top of the gradient. The tube was centrifuged at 39 000 rev./min for 12 h using an SW 50.2 rotor in a Beckman L3-40 centrifuge. The gradient was eluted from the tube. The activity of α-D-mannosidase was measured using *p*-nitrophenyl-α-D-mannoside. Molecular weight of α-D-mannosidase was calculated from the following formula [19]:

$$\frac{X_1}{X_2} = \left(\frac{M_1}{M_2}\right)^{2/3}$$

where X_1 and X_2 are elution volumes of catalase and α-D-mannosidase respectively, of molecular weights M_1 and M_2.

2. Subunits of α-D-mannosidase. Gel electrophoresis in sodium dodecyl sulfate (SDS)-mercaptoethanol according to Weber and Osborn [20] was used to determine subunit molecular weights. Enzyme solutions (20—60 μg/100 μl) were heated at 37°C (2 h) or in a boiling water bath (5 min) in the presence of 1% SDS and 1% mercaptoethanol. Gels were stained for protein using Amido Schwartz.

Ultracentrifugation. Sedimentation velocity experiments were performed in a Spinco Model E analytical ultracentrifuge at 56 000 rev./min and 20°C. Sedimentation coefficients were measured in the following buffer systems: assay buffer (pH 4.5); 6 M guanidine hydrochloride with 1% mercaptoethanol; 6 M urea, pH 2.6; and 6 M urea, pH 4.5. The enzyme concentration in each solvent was approx. 5 mg/ml. Synthetic boundary cells were used for velocity runs in denaturing solvents.

Temperature stability. The temperature stability of the pure enzyme was determined by incubating the enzyme in assay buffer at temperatures from 0—80°C for 5 min. An aliquot (100 μl) of the solution was then added to substrate at room temperature and the activity measured.

Thermal transition of α-D-mannosidase. The melting temperature of pure α-D-mannosidase was measured according to the method of Bull and Breese [21], using a protein concentration of 1.5 mg/ml in 0.5 M NaCl. A constant temperature rise of 0.63°C per min was used.

Coupling of α-D-mannosidase to Sepharose

α-D-Mannosidase, free of any contaminating glycosidase activities and protease activity (determined by the method of Kunitz [22]) was coupled to Sepharose according to the method of Porath et al. [23].

1. Activation of Sepharose with cyanogen bromide. Sepharose (35 g) was added to 35 ml of cold 5 M potassium triphosphate solution, brought to pH 11 with sodium hydroxide. Cyanogen bromide (3.5 g in 35 ml of water) at 5°C was added to the Sepharose slurry and the cold mixture stirred at room temperature for exactly 7 min. The activated gel was immediately washed on a Buchner funnel with cold distilled water (1.5 l), followed by cold 0.1 M $NaHCO_3$ (1.5 l).

2. Coupling of α-D-mannosidase to activated Sepharose. The enzyme (350 mg protein; 120 units) was dissolved in 15 ml of 0.05 M sodium acetate buffer,

pH 4.5 with 0.01 M sodium chloride, 0.1 mM Zn^{2+}, and 1 mM methyl-α-D-mannoside. An equal volume of cold 0.1 M $NaHCO_3$ was added to this solution and the mixture was brought to pH 8.0 with NaOH. This solution was then poured into the activated Sepharose slurry at 5°C, stirred for 5—10 min, and left overnight (16—18 h) at 5°C. The enzyme-Sepharose complex was then washed on a Buchner funnel alternately with 1 l of 0.1 M Tris · HCl (pH 8.0) and 1 l of 0.05 M sodium acetate buffer, pH 4.5, with 0.01 M NaCl and 0.1 mM $ZnSO_4$. The coupled enzyme was stored at 5°C in the sodium acetate buffer with 0.001% Thimerasol added to prevent bacterial growth. The activity of the coupled enzyme was measured by taking a 100 μl aliquot of the gel slurry, adding this to the *p*-nitrophenyl-α-mannoside, and the assay carried out as usual. The reaction mixture was filtered and the absorbance of the filtrate read at 400 nm. The recovered activity was equivalent to about 75 units of active coupled enzyme.

pH optimum. The effect of pH on the activities of the solubilized and immobilized α-D-mannosidase was studied using *p*-nitrophenyl-α-D-mannoside (2 mM) in 0.05 M sodium citrate or 0.05 M sodium phosphate buffers ranging from pH 3.0 to 8.0.

α-D-Mannosidase activity in 6 M urea, pH 4.5. Activity of the pure soluble enzyme in 6 M urea (pH 4.5) was compared to the activity of the α-D-mannosidase-Sepharose under the same conditions. Solid urea and assay buffer were added to concentrated enzyme and enzyme-Sepharose solutions to yield a final urea concentration of 6 M in a volume of 1 ml. Aliquots (100 μl) were removed and added to 0.9 ml of a 5 mM *p*-nitrophenyl-α-D-mannoside solution, and the reaction allowed to proceed.

Effect of temperature on the catalytic activity of soluble and immobilized α-D-mannosidase. The effect of temperature on the hydrolysis of *p*-nitrophenyl-α-D-mannoside was studied using both free and coupled enzyme in the range of 25—80°C. Substrate solutions (1 ml) ranging from 0.5 to 10 mM were incubated for 5 min at the desired temperature. The enzyme (100 μl) was then added and the reaction allowed to proceed at the same temperature.

Digestion of ovalbumin and asparaginyl-carbohydrate by α-D-mannosidase-Sepharose. Native ovalbumin (2 mg) or asparaginyl-carbohydrate (0.5 mg) was dissolved in 1.0 ml of assay buffer containing 0.001% Thimerasol. α-D-Mannosidase-Sepharose (1 ml containing approx. 2 units) was added and the mixture incubated at 37°C. The reaction was terminated by filtering off the enzyme-Sepharose beads. The absorbance at 280 nm of the filtrate was measured on the ovalbumin solution for quantitation of the protein ($\epsilon_{280}^{1\%}$ 7.5) and total mannose in the ovalbumin and asparaginyl-carbohydrate solutions was analyzed by the method of Dubois et al. [24]. An aliquot of the solution (1 ml) was removed, evaporated and analyzed for free mannose using anion-exchange chromatography in borate buffer as described by Lee et al. [25].

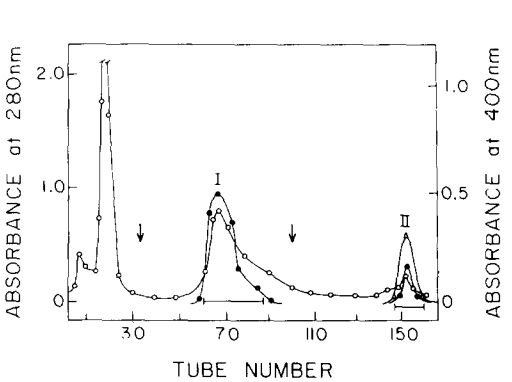
Results and Discussion

Studies of the structure or the roles of the carbohydrate groups in glycoproteins often include the modification of the native protein by the hydrolysis of specific carbohydrate residues. Thus, the terminal locations of sialyl and L-fucosyl residues in glycoproteins has been demonstrated many times [26] and

the metabolic rate of some of the serum proteins has been studied following the enzymatic removal of the sialic acid [27]. Problems arise however when the rate of the hydrolysis is slow, leading one to use larger amounts of enzyme, or the isolation of the undenatured modified glycoprotein is required. These obstacles were encountered in the modification of native ovalbumin with α-D-mannosidase and led to a study of the properties of the enzyme in free solution and after coupling to an insoluble matrix of Sepharose.

In an attempt to prepare a specific affinity column for purification of α-D-mannosidase, many compounds were tested as possible competitive inhibitors. Levvy and Snaith [28] found that D-mannono-(1→5)-lactone was a strong specific inhibitor of jack bean α-D-mannosidase, but no other specific inhibitors had been reported. Of the compounds tested in this study, only D-glucuronic acid showed slight inhibition at 10 mM concentration. Since no specific inhibitor was found suitable for attachment to a solid support, purification of α-D-mannosidase by affinity chromatography was not pursued. Following the completion of this work, Robinson et al. [29] described the affinity chromatography of human α-D-mannosidase using a column of immobilized D-mannosylamine.

α-D-Mannosidase used in this study was prepared from jack bean by a modification of the method of Li and Li [15] for purification of *N*-acetyl-β-D-glucosaminidase, since both *N*-acetyl-β-D-glucosaminidase and α-D-mannosidase could be isolated pure. The two glycosidases were separated on DEAE-Sephadex (Fig. 1), the α-D-mannosidase fraction being purified on hydroxyapatite chromatography followed by heating at 65°C for 30 min, yielding a preparation with a specific activity of 20 units/mg. Without this final heating step, it was noted that the gel electrophoretic patterns became complicated by additional



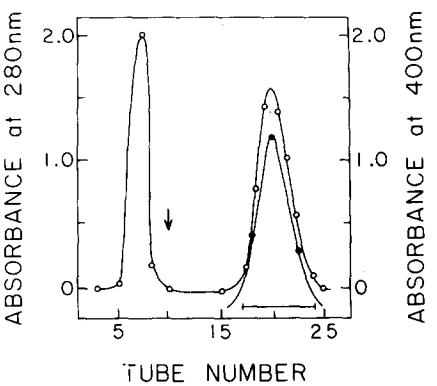


Fig. 1. Chromatography on DEAE-Sephadex A-50. The enzyme fraction from Sephadex G-100 chromatography containing both α-D-mannosidase and *N*-acetyl-β-D-hexosaminidase in 0.05 M sodium phosphate buffer, pH 7.0, was applied to a DEAE-Sephadex A-50 column (5.0 × 30 cm) equilibrated with the same buffer. The arrows indicate positions of the buffer changes as described in the text. Fractions of 10 ml were collected. ○———○, absorbance at 280 nm; ●———●, α-D-mannosidase activity; △———△, *N*-acetyl-β-D-hexosaminidase activity. Pooled fractions are indicated by the black bars.

Fig. 2. Hydroxyapatite chromatography. Fraction I from DEAE-Sephadex, containing α-D-mannosidase, was applied to a column (2.5 × 30 cm) of hydroxyapatite at room temperature. The column was equilibrated with 0.1 M sodium phosphate buffer, pH 7.0. Fractions of 10 ml were collected. The arrow indicates the position of the buffer change. ○———○, α-D-mannosidase activity; ●———●, absorbance at 280 nm. Pooled fractions are indicated by the black bar.

minor protein bands, although no proteolytic activity was detected by the Kunitz method [22]. *N*-Acetyl-β-D-glucosaminidase was further purified by chromatography on CM-Sephadex as described by Li and Li [15].

The pure α-D-mannosidase was free from other glycosidase activity as tested using the *p*-nitrophenyl glycopyranosides of β-D-mannose, *N*-acetyl-β-D-glucosamine, α- and β-D-galactose, and α-L-fucose. Gel electrophoresis in a Tris/glycine buffer (pH 8.6) showed the enzyme to move as a single protein band, containing all of the enzyme activity. Electrophoresis in 6 M urea at pH 2.6 gave two protein bands, containing approximately equal amounts of protein. Both bands stained for carbohydrate, similar to that reported for the enzyme from hog kidney [30].

The molecular weight of the native enzyme determined by chromatography on Sephadex G-200 was 200 000. The same value was obtained by sucrose density centrifugation and agrees closely with molecular weights of 190 000 [6] and 230 000 [8] found previously for the jack bean enzyme. α-D-Mannosidases from other sources have all shown molecular weights of at least 100 000: 190 000 for the enzyme from *Phaseolus vulgaris* [31]; 170 000—180 000 for the soy bean α-D-mannosidase [32]; 125 000—150 000 for the enzyme from *Vicia sativa* [33]; 100 000 for the hog kidney enzyme [30]; and 250 000 for the enzyme from hen oviduct [34].

Heating the enzyme in SDS and mercaptoethanol at 100°C produced subunits with molecular weights of 66 000 and 44 000 as determined by the method of Weber and Osborne [20]. The enzyme appears to be a tetramer containing four subunits, two each of 66 000 and 44 000 molecular weight. It was of interest to find that treating the native enzyme with SDS and mercaptoethanol at 37°C formed a component with an apparent molecular weight of 110 000 on SDS disc electrophoresis. Recognizing that this intermediate form is probably not completely denatured and may not have an electrophoretic mobility that can be compared to standard denatured proteins, it is perhaps fortuitous to find the molecular size equal to the sum of the two dissimilar subunits. A dimeric structure has been proposed for the hog kidney enzyme [30].

Sedimentation velocity studies were carried out to investigate the homogeneity of the enzyme in various solvents. A single protein peak was observed in assay buffer (pH 4.5), 6 M guanidine hydrochloride and 1% mercaptoethanol, and 6 M urea, pH 2.6, with $S_{20°,w}$ values of 9.65, 2.95 and 2.15, respectively. The enzyme in 6 M urea at pH 4.5 showed a broad peak that moved off the meniscus early and subsequently separated into two broad peaks for which $S_{20°,w}$ values could only be estimated as 3 S for the slower peak and 11 S for the faster peak. Combining this result with the fact that the enzyme retains some activity in 6 M urea at pH 4.5 (see Table II), one might conclude that the enzyme is only partially denatured, leaving a significant portion in the native form. A summary of molecular weight and sedimentation velocity data is given in Table I.

Several reports have been published concerning the heat stability of α-D-mannosidase. The enzymes from hen oviduct [34], hog kidney [35], *Turbo cortunis* [36], and beef liver [37] were all stable from 60—70°C. In the isolation of α-D-mannosidase from *T. cortunis* and hen oviduct, heating was used during the purification of the enzyme, resulting in both cases in a significant

TABLE I

A SUMMARY OF MOLECULAR WEIGHT AND SEDIMENTATION VELOCITY DATA

Preparation	Mol. wt.	$S_{20°,w}$
Native enzyme	200 000 [a]	9.6
Enzyme heated at 37°C for 30 min in 1% SDS/1% mercaptoethanol	110 000 [b]	
Enzyme heated at 100°C for 5 min in 1% SDS/1% mercaptoethanol	66 000 [b] 44 000 [b]	
6 M urea pH 2.6		2.9
6 M urea pH 4.5		2.7, 10.9
6 M GuHCl [c] + 1% mercaptoethanol		2.1

[a] Molecular weight obtained by gel filtration on Sephadex G-200 and by sucrose density centrifugation.
[b] Molecular weights obtained on SDS-gel electrophoresis.
[c] GuHCl = guanidine hydrochloride.

loss of contaminating proteins and an increase in specific activity. Li reported that the jack bean enzyme lost about 50% of the original activity after heating at 70°C for 5 min [5]. In the present study, the α-D-mannosidase remains fully active at 70°C after 5-min incubation, but is almost completely inactivated after heating at 80°C for 5 min (Fig. 3). These results are consistent with the study of the "melting" temperature of the enzyme as measured by the method of Bull and Breese [21]. A major conformational transition occurs at 76°C, which correlates well with the loss in catalytic activity.

α-D-Mannosidase was coupled in the presence of 1 mM methyl α-D-mannopyranoside to Sepharose 4B, activated by cyanogen bromide. The activity present in the bound form represented approximately 60% of that initially added, but in the absence of a measure of the bound protein it was impossible to arrive at a value for the specific activity. The reduction in activity that followed the coupling reaction may have been the result of incomplete reaction of the enzyme protein, some inactivation of the enzyme in the coupling system, or a decrease in specific activity as a result of coupling. However, the similarity in the properties of the bound and free α-D-mannosidase would indicate little alteration in the protein structure.

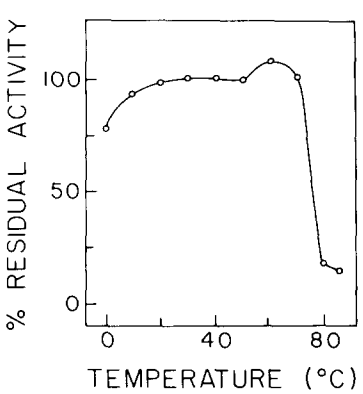


Fig. 3. Effect of heating on α-D-mannosidase. The enzyme was incubated at various temperatures for 5 min, an aliquot was removed, and the assay carried out at 25°C. See text for further experimental details.

Both the soluble and insoluble forms of the enzyme are stable at high temperature, and show a continuous increase in catalytic activity up to 75°C, a property which offers the advantage of carrying out hydrolyses for shorter times and under conditions which minimize bacterial growth. It is clear that the conformational changes in both forms of the enzyme resulting from increases in temperature are reversible at temperatures below 75°C and do not change the catalytic property of the protein. The double reciprocal plots of 1/velocity versus 1/[S] are linear over the range of 25—70°C as shown in Fig. 4. Furthermore, the activation energies for the free and bound enzymes are approx. 13.7 and 9.6 kcal per mol, respectively, suggesting that the coupling of the enzyme to the Sepharose does not significantly change the conformation of the enzyme at the active site.

The insolubilized enzyme was similar to the free enzyme in solution with respect to several other properties. Using *p*-nitrophenyl-α-D-mannoside as the substrate it is seen (Fig. 5) that the pH optimum for the free α-D-mannosidase is around 4.5 compared to 4.0 for the coupled enzyme.

It had been noted earlier that free α-D-mannosidase rapidly loses activity in 1% SDS containing 1% mercaptoethanol and at pH 2.6, but retains some activity in 6 M and 10 M urea at pH 4.5 and 2 M guanidine hydrochloride [13]. The enzyme coupled to Sepharose also retains about 25—30% of the original activity in 6 M urea but unlike the free enzyme recovers approx. 66% of the original activity after the urea is removed. Additional stabilization of structure appears to occur when the enzyme is coupled to Sepharose, the active site in both forms retaining some catalytic activity in 6 M urea such that hydrolyses of α-D-mannosides can be effected, a property that is applicable to studies of glycoprotein conformation. The free enzyme, however, does not regain any lost activity when the urea is dialyzed away. This added catalytic stability of the coupled enzyme is further exemplified by the fact that preparations can be repeatedly used. The preparation described in this paper has been used for hydrolyses of ovalbumin and ovalbumin glycopeptides many times over a period of several months, the insolubilized enzyme being recovered after each reaction by filtration. The enzyme-Sepharose beads are washed with assay buffer and stored at 5°C in suspension with 0.001% Thimerasol. A comparison of the

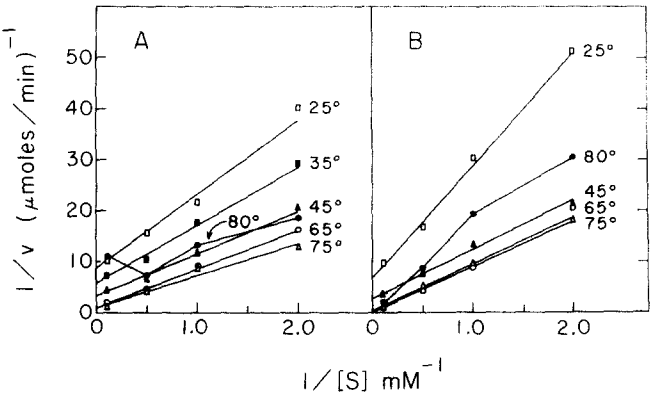
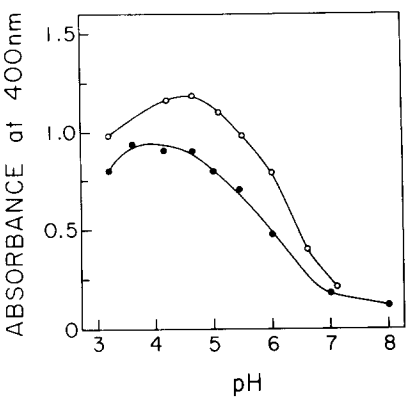


Fig. 4. Double reciprocal plot of 1/velocity vs. 1/[S]. Frame A: α-D-mannosidase; frame B: α-D-mannosidase-Sepharose.



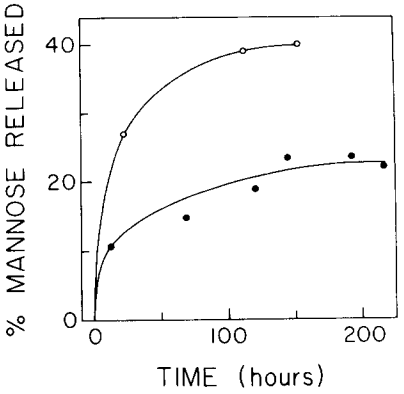


Fig. 5. pH optimum of α-D-mannosidase (○———○) and α-D-mannosidase-Sepharose (●———●).

Fig. 6. Mannose released from ovalbumin (○———○) and its asparaginyl-carbohydrate (●———●) by α-D-mannosidase-Sepharose.

TABLE II

A COMPARISON OF THE PROPERTIES OF SOLUBLE AND IMMOBILIZED α-D-MANNOSIDASE

	α-D-Mannosidase	α-D-Mannosidase-Sepharose
pH optimum	4.5—5.0	4.0—4.5
Activity retained in 6 M urea	35%	25%
% original activity recovered after urea	35%	65%
Maximum temp. stability	75	75

properties of the two forms of the enzyme is shown in Table II.

The free and coupled enzymes act in a similar way upon ovalbumin and ovalbumin glycopeptides. Using two units of the α-D-mannosidase coupled to Sepharose, approx. 22% of the total mannose in native ovalbumin is hydrolyzed at 37°C. Similar results with the free enzyme have been reported by Li [5]. It is also found that the hydrolysis of α-D-mannosyl residues from the asparaginyl carbohydrate fractions from ovalbumin by both forms of the enzyme are similar [12]. Results are shown in Fig. 6 as a release of mannose with time for ovalbumin and unfractionated asparaginyl carbohydrate. These results will be reported in greater detail in a forthcoming publication.

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