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Isolation and Properties of α-D-Mannosidase from Human Kidney

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α-D-Mannosidase activity exists in three forms that can be separated by DEAE-cellulose chromatography. α-D-Mannosidase was isolated from human kidney in a homogeneous state, and was purified 2100-fold, with *p*-nitrophenyl α-D-mannoside as substrate. The purified α-D-mannosidase was practically free from all other glycosidases tested. The K_m of the synthetic substrate with the enzyme was 1×10^{-3}M and the pH optimum 4.5. It was inhibited by heavy metals, sodium dodecyl sulphate, urea and compounds that react with the thiol groups, and was activated by Zn^{2+}, Na^+, 2-mercaptoethanol, human albumin and γ-globulin. The mol.wt. of the enzyme was estimated to be 180000±4500. After pretreatment with 2-mercaptoethanol and sodium dodecyl sulphate, α-D-mannosidase dissociated into subunits of mol.wts. of 58000±600 and 30000±380 respectively. Subunits of the same molecular weights were also obtained after the enzyme was heated at 100°C.

The enzyme α-D-mannosidase (EC 3.2.1.24) is responsible for the hydrolysis of α-mannoside bonds in mucopolysaccharides, glycoproteins and glycopeptides. The purification and properties of the enzyme from jack-bean meal were described by Li (1966) and Snaith & Levvy (1968*a*,*b*). This enzyme has been shown to occur widely in living organisms. The presence of α-mannosidase has been detected in almost all the tissues investigated in the mouse and rat by Conchie *et al.* (1959). An enzyme deficiency, known as mannosidosis, has been described by Öckerman (1967, 1969), Hultberg (1970) and Van Hoof & Hers (1968). It has been shown that the normal liver contains A, B and C α-mannosidase components, separable by DEAE-cellulose chromatography (Carroll *et al.*, 1972). Components A and B are most active at pH4.4, whereas the maximum activity of the C component is at pH6.0. Components A and B were absent in two cases of mannosidosis, and the residual α-mannosidase activity was due to the presence of the C component in normal amount. Okumura & Yamashina (1970) have reported the presence of a neutral α-mannosidase in tissues from rabbit and rat which could be separated from the acid α-mannosidase by $(NH_4)_2SO_4$ fractionation.

In the present paper we report the isolation and characterization of α-D-mannosidase form A from human kidney as well as the first determination of some physical properties of this enzyme.

Experimental

Materials

Normal tissues were obtained from the Department of Forensic Pathology of the University of Texas Health Science Center. The tissues were washed with 0.9% NaCl at 4°C and were stored frozen at −80°C until used. Substrates used in the enzymic assays were obtained as follows: *p*-nitrophenyl α-D-mannoside, *p*-nitrophenyl β-D-mannoside, *p*-nitrophenyl α-L-fucoside and *p*-nitrophenyl β-D-fucoside, *p*-nitrophenyl α-D-glucoside, *p*-nitrophenyl β-D-glucuronide, *p*-nitrophenyl *N*-acetyl-β-D-glucosaminide, *o*-nitrophenyl β-galactoside from Sigma Chemical Co., St. Louis, MO, U.S.A. 4-Methylumbelliferyl α-mannopyranoside was purchased from the Koch-Light Laboratories Ltd., Colnbrook, Bucks., U.K. Standard proteins were obtained as follows: crystalline human serum albumin, human γ-globulin fraction II, ovalbumin, bovine haemoglobin, bovine fibrinogen from Sigma Chemical Co.; pepsin was from Worthington Biochemical Corp., Freehold, NJ, U.S.A.; pig angiotensin I-converting enzyme isolated from pig kidney by Oshima *et al.* (1974); β-galactosidase from *Escherichia coli* K12 3300 was purified by Marinkovic & Marinkovic (1976). Sephadex G-200 and Blue Dextran 2000 were purchased from Pharmacia Fine Chemicals, Uppsala, Sweden; DEAE-cellulose, type 40, was from Carl Scheicher and Schuell Co., Keene, NH, U.S.A.; CM-cellulose 32 was from Whatman, W. R. Balston Ltd., Maidstone, Kent, U.K.; hydroxyapatite was from Bio-Rad, Richmond, CA, U.S.A. The reagents for polyacrylamide-gel electrophoresis were purchased from Canalco, Rockville, MD, U.S.A. Other reagents were analytical grade. Human serum albumin and γ-globulin fraction II were free of α-D-mannosidase activity.

Tissue

The tissue was washed free of blood and homogenized in a Waring blender at 4°C for 4min. The

homogenates (20%, w/v) were prepared in 0.01M-potassium phosphate buffer, pH7.0, containing 0.1% Triton X-100 and 0.15M-KCl. The supernatants obtained after centrifugation of the homogenates, first for 30min at 14000*g* in a Sorvall RC-2 centrifuge, and then for 2h at 50000*g* in a Spinco L-2 ultracentrifuge at 4°C, were used in all the experiments.

Enzyme assay

Fresh homogenate was assayed for α-D-mannosidase, β-D-mannosidase, β-D-galactosidase, α-D-glucosidase, β-D-glucuronidase, *N*-acetyl-β-D-glucosaminidase, α-L-fucosidase and β-D-fucosidase activities. The following substrate solutions were used: 6mM-*p*-nitrophenyl α-D-mannoside in McIlvaine (1921) citrate/phosphate buffer, pH4.5; 6mM-*p*-nitrophenyl β-D-mannoside in citrate/phosphate buffer, pH4.5; 3mM-*p*-nitrophenyl α-L-fucoside in citrate/phosphate buffer, pH6.1; 3mM-*p*-nitrophenyl β-D-fucoside in citrate/phosphate buffer, pH6.1; 2mM-*p*-nitrophenyl α-D-glucose in citrate/phosphate buffer, pH5.0; 1.9mM-*p*-nitrophenyl β-D-glucuronide in citrate/phosphate buffer, pH5.0; 2mM-*p*-nitrophenyl *N*-acetyl-β-D-glucosaminide in citrate/phosphate buffer, pH4.5; 2mM-*o*-nitrophenyl β-galactosidase in citrate/phosphate buffer, pH3.0.

A portion (0.1ml) of the substrate solution was hydrolysed with 20 or 50μl of enzyme extract. The blank contained the same amount of substrate solution, but water instead of enzyme extract. After incubation at 37°C for 10min, the reaction was stopped by the addition of 0.1ml of 2M-NH_3, and diluted with water to a total volume of 1ml. The E_{400} of the liberated *p*-nitrophenol was read in a Perkin–Elmer spectrophotometer. The extinction coefficient for the *p*-nitrophenolate ion at 400nm was $18.1 \times 10^3 M^{-1}$ and was corrected with the blank as described by Armstrong *et al.* (1966). One unit of enzyme was defined as the amount of enzyme that hydrolyses 1μmol of substrate/min at 37°C. Specific activities were expressed as units/mg of protein.

Protein concentrations were determined by the procedure of Lowry *et al.* (1951) with crystalline plasma albumin as the standard.

Concentration of protein samples

Protein samples were concentrated at 4°C by using Amicon PM-30 Diaflo ultrafiltration apparatus.

Chromatographic procedures

DEAE-cellulose type 40, CM-cellulose 32 and Sephadex G-200 were dispersed in water and allowed to swell. After the fine particles had been decanted several times, the media were poured into chromatographic columns. The DEAE-cellulose and CM-cellulose were then each equilibrated in the first buffer to be used in the respective elution procedures. The E_{280} was measured with a Hitachi–Perkin model 120 spectrophotometer.

Electrophoretic analyses

Polyacrylamide-disc electrophoresis (Canalco) was performed as described by Davis (1964). Enzyme activity was detected by cutting the gel into 2mm slices, and incubating the homogenized slices with substrate and assaying as described above. Within 30min a yellow colour appeared in the region of the α-D-mannosidase.

Polyacrylamide-gel electrophoresis was also carried out at pH4.5 by the method of Reisfeld *et al.* (1962).

Molecular-weight determination

Gel filtration. For the determination of the molecular weight of α-mannosidase, a column (40cm × 2.2cm diam.) of Sephadex G-200 was prepared in 25mM-potassium phosphate buffer, pH6.0. The sample (10–15mg) was applied to the column and eluted in 3ml fractions with the equilibrating buffer at a flow rate of 12ml/h. The column was calibrated with the following standards (mol.wt. in parentheses): Blue Dextran (2000000); bovine fibrinogen (380000); pig angiotensin I-converting enzyme isolated by Oshima *et al.* (1974) (195000); human γ-globulin (157000); bovine serum albumin (69000); ovalbumin (45000). A plot of the elution volumes of the standards against the logarithms of their molecular weights was used to estimate the molecular weight of the α-mannosidase.

Subunit molecular weights. Molecular weights of subunits were determined by polyacrylamide-gel electrophoresis in sodium dodecyl sulphate by the method of Shapiro *et al.* (1967) with 5% gels. Samples (1mg) were suspended in 0.1ml of 0.05M-sodium phosphate buffer (pH7.1) containing 1% each of sodium dodecyl sulphate and 2-mercaptoethanol. After incubation at 37°C for 3h, a 10μl sample containing 0.1mg of protein was applied to the top of each gel with 10μl of 40% (w/v) sucrose. The buffer for electrophoresis was 0.1M-sodium phosphate (pH7.1) containing 0.1% sodium dodecyl sulphate and 0.1% 2-mercaptoethanol. The following standards were used for the calibration curve (mol.wts. in parentheses): subunits of β-galactosidase from *E. coli* (130000); bovine serum albumin (69000); ovalbumin (45000); pepsin (34000); bovine haemoglobin, α and β chains (16000). After electrophoresis for 3h at 6mA/tube, gels were fixed with 12% (v/v) trichloroacetic acid, stained with 0.05% Coomassie Brilliant Blue R-250 in water, and destained by diffusion in 7% (w/v) acetic acid.

K_m value. This was calculated by the double-reciprocal-plot method of Lineweaver & Burk (1934).

Purification of human α-D-mannosidase

α-D-Mannosidase was purified from human kidney as described below. All steps of the purification procedure were carried out at 4°C unless otherwise indicated. Approx. 1100g of kidney was minced and then homogenized in 5500ml of 0.01M-potassium phosphate buffer, pH7.0, containing 0.1% Triton X-100 and 0.15M-KCl. The homogenate was centrifuged as described under 'Tissue'.

Step 1: *treatment with acid.* The supernatant solution (5000ml) after a 50000*g* centrifugation, was adjusted to pH4.5 with solid citric acid, and warmed to 20°C over a period of 30min, during which time some inactive protein was precipitated. The suspension was left for 1h, centrifuged at 5000*g* for 20min and the pH of the clear supernatant was adjusted to pH7.0 with solid Tris.

Step 2: *concentration.* The supernatant was concentrated by ultrafiltration through Diaflo type PM-30 membrane to 800ml.

Step 3: *$(NH_4)_2SO_4$ precipitation.* $(NH_4)_2SO_4$ (344g) was added to the supernatant (800ml) with constant stirring to give 65%-satd. solution. The solution was stirred overnight, the precipitate recovered by centrifugation (20000*g* for 30min) and the supernatant discarded. The precipitate was dissolved in 0.01M-potassium phosphate buffer, pH6.0, to a final volume of 300ml, and dialysed for 2 days against four 1-litre changes of buffer.

Step 4: *DEAE-cellulose chromatography.* The material from step 3 (300ml) was added to a column (37cm×5cm diam.) of DEAE-cellulose prepared as described under 'Chromatographic procedures'. The column was eluted with the same buffer as in step 3 at a flow rate of 80ml/h. α-D-Mannosidase form A was not adsorbed and was eluted in 9.5ml fractions 31–150. α-D-Mannosidase forms B and C were then eluted with a linear KCl gradient (0–2%) in the same buffer.

Step 5: *CM-cellulose chromatography.* The fractions from step 4 containing enzyme-A activity were combined, and the volume was decreased to 190ml by ultrafiltration. The solution was dialysed against 0.01M-potassium phosphate buffer, pH6.85, and then applied to a column (85cm×2.5cm diam.) of CM-cellulose equilibrated with the same buffer. The α-D-mannosidase form A was not adsorbed and was eluted in 10ml fractions 17–40. These fractions were combined and concentrated to 30ml by ultrafiltration, and the concentrate was dialysed against three 3-litre changes of 1mM-potassium phosphate buffer, pH6.5.

Step 6: *CM-cellulose chromatography.* The dialysed solution with α-D-mannosidase A activity was applied to a column (80cm×2.5cm diam.) of CM-cellulose equilibrated with 1mM-potassium phosphate buffer, pH6.5. The α-D-mannosidase A was not adsorbed and was eluted in 10ml fractions 18–80, which were combined for a total volume of 700ml.

Step 7: *CM-cellulose chromatography.* The fractions from step 6 containing enzyme A activity were combined, and the volume was decreased to 100ml by ultrafiltration. The solution was dialysed against 0.01M-potassium phosphate buffer, pH6.0, and then applied to a column (33cm×2.2cm diam.) of CM-cellulose equilibrated with the same buffer. The α-D-mannosidase form A was adsorbed and was eluted in 10ml fractions 90–140, at 0.025M-KCl in the same buffer. These fractions were combined and concentrated to 260ml by ultrafiltration.

Step 8: *heat treatment.* To the enzyme solution (260ml) 0.13ml of 0.2mM-zinc acetate was added. The solution was placed in a water bath at 60°C until the temperature of the solution reached 60°C, and was then maintained at this temperature for 30min. The solution was cooled with ice and centrifuged. The precipitate was washed once with 0.01M-potassium phosphate buffer, pH6.5, and the clear supernatant fractions were combined. By this treatment, β-D-galactosidase, β-D-fucosidase, α-L-fucosidase, α-D-glucosidase, β-D-mannosidase and β-D-glucuronidase were completely deactivated, and 87% of the activity of *N*-acetyl-β-D-glucosaminidase was destroyed.

Step 9: *hydroxyapatite column chromatography.* The α-D-mannosidase fraction from step 8 was concentrated to 30ml and added to a column (11cm× 2.2cm diam.) of hydroxyapatite. The column was developed with a potassium phosphate gradient (0.05–0.50M) at pH6.5. α-D-Mannosidase A was eluted in 6ml fractions around tube 120 at 0.20M-potassium phosphate buffer. The active fractions were combined and the volume was concentrated to 2.0ml by ultrafiltration.

Step 10: *gel filtration on Sephadex G-200.* The kidney α-D-mannosidase form-A fraction from step 9 was subjected to gel filtration on a column of Sephadex G-200, equilibrated with 0.025M-potassium phosphate buffer, pH6.0. The α-D-mannosidase was eluted with the same buffer in 3ml fractions at about tube 29. The active fractions were combined and concentrated to 1ml.

Electrophoresis. Cellogel electrophoresis was carried out as described by Fluharty *et al.* (1971), with modification, for 2h at 4°C and 3mA/cm. The buffer was 0.04M-potassium phosphate, pH6.6. The staining substrate was 4-methylumbelliferyl-α-mannopyranoside as a 0.5mM solution in a 0.1M-citrate/phosphate buffer. Incubation was performed at pH4.5. Bands of Whatman 1 MM paper, saturated with the substrate, were pressed against the electrophoresis bands on a glass sheet. After 30min incubation at room temperature, the band was saturated with

Table 1. *Purification of human kidney α-mannosidase*

Purification steps/fraction	Total protein (mg)	Total activity (units)	Specific activity (units/mg of protein)	Recovery (%)
Crude extract	84700	403	0.0047	100
Supernatant (pH4.50)	44150	283	0.0064	70.2
Amicon PM-30	28600	280	0.0098	69.5
$(NH_4)_2SO_4$ (65% satn.)	19454	198	0.0101	49.1
DEAE-cellulose 40 (pH6.0)	6252	72	0.0115	17.8
CM-cellulose 32 (pH6.85)	3000	60	0.0200	14.8
CM-cellulose 32 (pH6.50)	1450	55	0.0379	13.6
CM-cellulose 32 (pH6.0)	455	38	0.0835	9.4
Heated (60°C for 30min)	142	35	0.246	8.7
Hydroxyapatite gel	18	30	1.666	7.4
Sephadex G-200	2	20	10.0	4.9

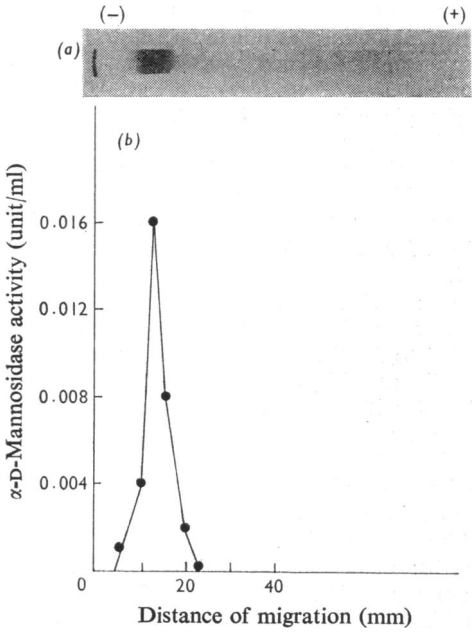


Fig. 1. *Disc-gel electrophoresis of human kidney α-D-mannosidase A at pH8.5*

(*a*) The gel was stacked at pH8.9 with sample containing 100μg of enzyme and run at 3mA/gel (Davis, 1964). The final α-D-mannosidase, after the last step of purification, was applied. The cathode is to the left. (*b*) The gel was cut into 2mm slices, which were homogenized with substrate and assayed as described in the Experimental section.

1M-NH_3. The fluorescent spots were detected with a u.v. lamp. At pH4.5 the α-D-mannosidase after the last step of purification showed that one sharp band migrated to the anode.

Results

Purification of α-D-mannosidase form A is summarized in Table 1.

Disc electrophoresis

The electrophoretic behaviour of the final product of the purified α-D-mannosidase form A from human kidney is shown in Fig. 1. A single band of protein and enzymic stain was detected. The α-D-mannosidase behaved as a basic protein. A single band was also obtained after disc-gel electrophoresis at pH4.4 (Plate 1, gel 1).

Molecular weights of the enzyme and enzyme subunits

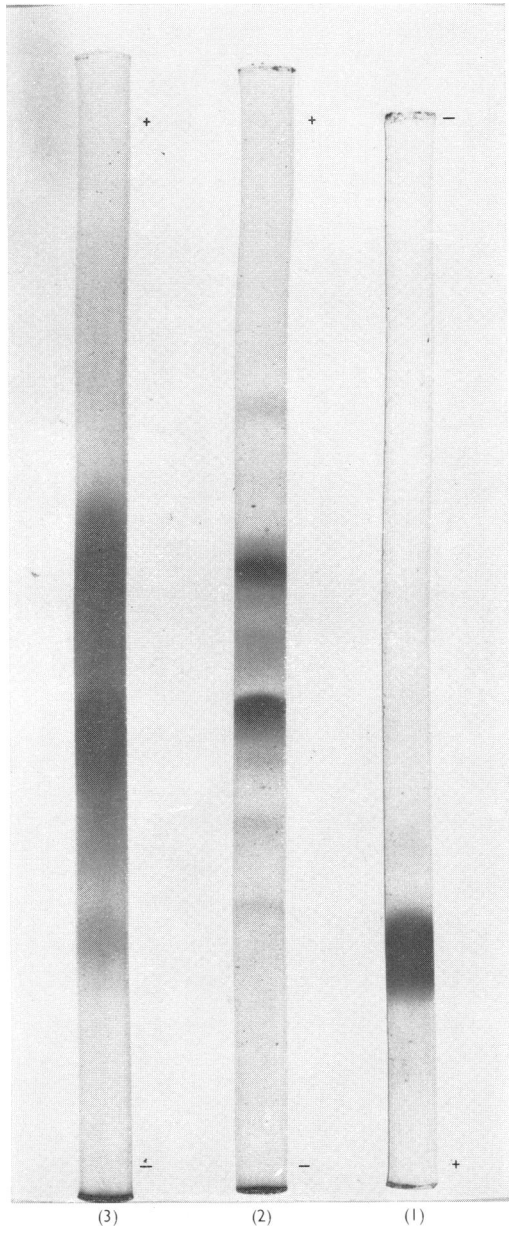
Gel filtration. For the determination of the molecular weight of α-D-mannosidase, gel filtration with marker proteins and α-D-mannosidase was done under identical conditions. Duplicate measurements of V_e/V_0 agreed within 0.7%, which would introduce an error of about 2%. The gel-filtration data for marker proteins were found to fit the following equation, obtained by the method of least squares:

$$\log M = 6.2465 - 0.664\, V_e/V_0 \qquad (1)$$

The value of the mol.wt. obtained by using eqn. (1) is 180000±4500.

Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of proteins was carried out in quadruplicate on 5% gels. Purified α-D-mannosidase was treated with sodium dodecyl sulphate and 2-mercaptoethanol and run on the above gels with marker proteins. The enzyme dissociated into two subunits. A least-squares analysis of the data fitted the equation:

$$\log M = 5.4859 - 1.4493\, R_m \qquad (2)$$



EXPLANATION OF PLATE 1

Disc-gel electrophoresis of human α-D-*mannosidase A at pH* 4.5 *and* 7.1

The gel samples are: (1) gel stacked at pH 4.5 with sample containing 100 μg of enzyme and run at 4 mA/gel (Reisfeld *et al.*, 1962); (2) sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of α-D-mannosidase A, after preincubation with sodium dodecyl sulphate and 2-mercaptoethanol for 3 h at 37°C; (3) the gel was the same as (2) but α-D-mannosidase was heated at 100°C for 5 min.

The plot of log M versus R_m is linear over the mol.wt. range 16000–130000 and is devoid of any inflexion for 5% gels. The reduced α-D-mannosidase in sodium dodecyl sulphate/polyacrylamide gel moved as two bands; their mol.wts. calculated from their relative mobilities by eqn. (2) are 58000±600 and 30000±380 respectively. After heating at 100°C for 5min in 1% sodium dodecyl sulphate, 1% 2-mercaptoethanol and 0.1M-sodium phosphate buffer, pH7.1, the α-D-mannosidase dissociated into subunits with mol.wt. of 58000±600 and 30000±380. The results are shown in Plate 1 (gel 3).

Enzymic properties of human kidney α-mannosidase

(1) *pH optimum.* The effects of pH on enzyme activity were studied by adjusting *p*-nitrophenyl α-D-mannoside to various pH values by using a wide-range buffer, which was 0.1M-citric acid/0.2M-sodium phosphate. The α-mannosidase was then assayed for its activity as described in the Experimental section. The pH optimum was near pH4.5 (Fig. 2).

(2) *Effects of inhibitors and activators on human kidney α-mannosidase.* Thiol inhibitors such as iodoacetic acid or *p*-chloromercuribenzoate produced considerable inhibition. The enzyme was also inhibited by heavy-metal ions, such as Hg^{2+} and Ag^{+}, at concentrations of 0.05–5mM, which suggests that a thiol group is part of the enzyme. α-Mannosidase was activated by metal ions such as Na^{+}, Mg^{2+} and Zn^{2+} as well as by mercaptoethanol, human albumin and γ-globulin. Table 2 lists various compounds affecting the activity of α-mannosidase of human kidney. The

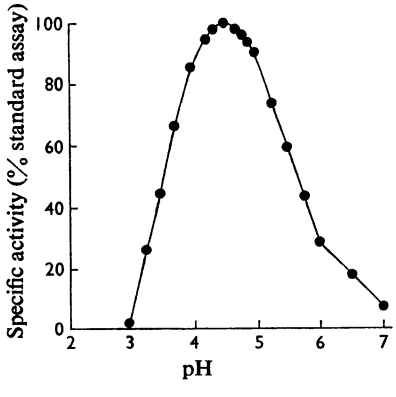


Fig. 2. *Effect of pH on activity of human kidney α-D-mannosidase A*

The activity is expressed as a percentage of that at pH4.5. Citrate/phosphate buffer (0.1M-citric acid plus 0.2M-Na_2HPO_4) was used to adjust the pH to the desired value.

K_m of the purified enzyme for *p*-nitrophenyl α-D-mannoside was determined to be 1.15×10^{-3}M.

(3) *Heat stability of human kidney α-mannosidase.* The enzyme was very stable at 50° and 60°C, but heating at 70° or 80°C led to a rapid loss of enzyme activity. Fig. 3 shows the effects of the temperature of incubation on stability of α-mannosidase enzyme activity. The zero-time controls were performed in the same way for samples (*a*) and (*b*) as for samples (*c*) and (*d*).

Discussion

In this paper we describe a procedure for the isolation of α-mannosidase from human kidney with a high degree of purity as indicated by electrophoretic and enzymic criteria. Human kidney contained three α-D-mannosidase activities. The isoenzymes can easily be separated and the purification method

Table 2. *Effects of various activators and inhibitors on human kidney α-mannosidase*

The enzyme was dialysed against water and assayed for enzymic activity as described in the Experimental section. The enzyme was incubated with the activator or inhibitor for 30min at 20°C before the assay. Results are expressed as percentage of the activity in the absence of added materials.

Activator or inhibitor	Concentration	Relative activity (%)
Mercaptoethanol	5mM	124
Iodoacetic acid	5mM	75
p-Chloromercuribenzoate	0.05mM	91
	5mM	50
EDTA	5mM	90
Sodium dodecyl sulphate	3mM	88
	6mM	84
	9mM	75
	15mM	58
	30mM	50
Na^{+}	50mM	117
	500mM	160
Ag^{+}	0.05mM	100
	5mM	7
Zn^{2+}	5mM	115
Co^{2+}	5mM	93
Mg^{2+}	5mM	105
Hg^{2+}	0.05mM	33
	5mM	5
Cd^{2+}	5mM	95
Urea	2M	91
	5M	50
	6M	39
	7M	25
	8M	11
Human albumin	0.025%	160
Human γ globulin	0.025%	160

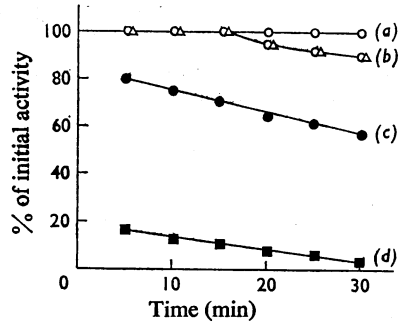


Fig. 3. *Effect of temperature on enzyme activity*

Tubes containing α-mannosidase in a total volume of 0.2ml were incubated at 50°C (*a*), 60°C (*b*), 70°C (*c*) or 80°C (*d*) for 5–30min in citrate/phosphate buffer, pH5.20. The extent of hydroylsis of substrate at 37°C was assigned a value of 100 and activities at other temperatures are expressed as relative values. α-D-Mannosidase activity was measured as described in the Experimental section.

described here gives a 5% yield of α-D-mannosidase form A. After 2100-fold purification, α-D-mannosidase appears homogeneous by polyacrylamide-disc-gel electrophoresis under two different conditions. We found that form A can be converted into form B, which confirms the results of Phillips *et al.* (1974*a*,*b*) and Chester *et al.* (1975). In the case of DEAE-cellulose column chromatography, in 0.01M-potassium phosphate buffer, pH6.0 (see Table 1, step 4) the proportion of activity in the peaks A, B and C was the same, 1:0.97:0.95. On the other hand, when fraction A was pooled and rechromatographed on CM-cellulose column in 0.01M-potassium phosphate buffer, pH6.0 (see Table 1, step 7), the ratio of activity A/B was found to be 1:0.645. We have shown by electrophoresis on cellulose acetate, followed by incubation with 4-methylumbelliferyl α-mannopyranoside at pH4.5, only one sharp band of α-D-mannosidase activity.

The purified α-D-mannosidase does not contain any measurable amount of other glycosidase activity, such as β-D-mannosidase, β-D-fucosidase, α-L-fucosidase, α-D-glucosidase, β-D-glucuronidase, *N*-acetyl-β-D-glucosaminidase or β-D-galactosidase activity. Our results agree with the data of Carroll *et al.* (1972), who observed three different forms of α-mannosidase from liver extracts separable on DEAE-cellulose, which they designated as A, B and C. The forms A and B are active at an acid pH, and C at neutral pH. We observed that our component-A α-mannosidase had a very similar pH–activity curve to human liver forms A and B with maximum activity at pH4.5. The human α-D-mannosidase is heat-stable and allowed us to obtain this enzyme practically free of other glycosidases. The heat stability was also described for the pig kidney α-D-mannosidase (Okumura & Yamashina, 1970).

The Golgi-membrane fraction of rat liver, which can be distinguished from the lysosomal and cytoplasmic fractions, has been shown by Dewald & Touster (1973) to contain α-mannosidase activity with a pH optimum of 5.3. We obtained a mol.wt. of 180000 by gel filtration for human kidney α-mannosidase. The mol.wt. of α-mannosidase from bovine kidney was between 275000 and 390000 (Phillips *et al.*, 1974*a*). Phillips *et al.* (1974*b*) found that mol.wts. of the acidic forms of the α-D-mannosidase from human liver were 250000–300000 and that of the neutral form 350000–400000. The enzyme from *Phaseolus vulgaris* was found to have a mol.wt. of 200000 by polyacrylamide-gel electrophoresis (Paus & Christensen, 1972), and that from soya bean a, mol.wt. of 180000 by gel chromatography on Sephadex G-200 (Saita *et al.*, 1971). Okumura & Yamashina (1973) reported that the mol.wt. of α-mannosidase from pig kidney as estimated from gel filtration was 100000, and as determined from gel electrophoresis in the presence of sodium dodecyl sulphate, 42000. Our α-mannosidase from human kidney dissociated into two subunits with mol.wt. of about 58000 and 30000 respectively when incubated at 37°C or heated at 100°C. It can be assumed that α-mannosidase may consist of two heavy and two light chains. The requirement for 2-mercaptoethanol for stability suggests that it is a thiol-dependent enzyme. We find that the enzyme activity is increased in the presence of 2-mercaptoethanol. The inhibition of enzyme activity after the addition of thiol inhibitors supports the result of other investigators (Snaith & Levvy, 1969; Dewald & Touster, 1973).

Our purification of the α-mannosidase form A from human kidney will allow it to be used for replacement therapy for patients with mannosidosis.

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