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Immunological Characterization of Human Liver α-D-Mannosidase

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Antiserum was raised against purified human liver α-D-mannosidase B. It precipitated α-mannosidases A and B from solution, demonstrating the close structural resemblance of these two forms of acidic α-mannosidase activity. A continuous enzymically active precipitin line with no spurs was obtained when α-mannosidase A and B were placed in adjacent wells on Ouchterlony double-diffusion plates. The antiserum precipitated acidic but not neutral α-mannosidase from an extract of human liver, confirming that the acidic and neutral activities are not closely related. Acidic activity was also precipitated from extracts of human brain, kidney and leucocytes by the antiserum. However, it did not cross-react with bovine acidic α-mannosidase activity or with the activity in human plasma that has an optimum pH of 5.5. The two acidic forms of human liver α-mannosidase, A and B, are immunologically identical but distinct from neutral α-mannosidase and that activity with an optimum pH of 5.5.

Two types of α-D-mannosidase (EC 3.2.1.24) have been described in normal human liver, one with acidic activity with a pH optimum of 4.0–4.5 (Öckerman, 1967) and one with neutral activity with a pH optimum of 6.2–6.5 (Carroll *et al.*, 1972). At least three peaks of activity can be separated from extracts of normal liver by ion-exchange chromatography on DEAE-cellulose (Carroll *et al.*, 1972). The activity in peaks A and B has an acidic pH optimum whereas the activity in peak C has a neutral pH optimum. In the lysosomal storage disease mannosidosis, there is a deficiency of acidic α-D-mannosidase and an accumulation of mannose-rich oligosaccharides in the tissues (Öckerman, 1967). Carroll *et al.* (1972) demonstrated that the acidic forms A and B were absent from the liver of a patient with mannosidosis and that a normal amount of the neutral activity accounted for the residual activity. A similar situation has been described in Angus cattle (Hocking *et al.*, 1972; Phillips *et al.*, 1974*a*). These observations suggested that the acidic and neutral activities have different metabolic functions and are not under the same genetic control. Characterization of the different forms of α-D-mannosidase in normal human liver(Phillips *et al.*, 1974*b*) has shown that the acidic and neutral activities also differ in their sensitivity to Zn^{2+}, Co^{2+} and EDTA, thermal stability, molecular weight and isoelectric points. The only detectable difference between the enzymes in the two acidic peaks A and B is in their charge, as evidenced by their separation by ion-exchange chromatography and isoelectric focusing. However, under certain conditions peak A is converted spontaneously into peak B, demonstrating that they are very closely related structurally (Phillips *et al.*, 1974*b*). A third type of α-D-mannosidase, which has a pH optimum of 5.5 and is particularly activated by Co^{2+}, occurs in human plasma. There is some evidence that it also occurs in tissues and may be related to the neutral activity (Phillips *et al.*, 1974*b*). The purpose of the present paper is to investigate some of the structural relationships between the various types of α-D-mannosidase activity and between the two acidic components A and B by using immunological techniques. A preliminary report of some of this work has been presented (Phillips *et al.*, 1975).

Experimental

Tissue

Post-mortem human liver, kidney and brain were stored at −20°C until required. Tissue homogenates (25%, w/v) were prepared in water in an MSE Ato-Mix homogenizer at 4°C. The supernatants obtained after centrifugation of the homogenates in the 8×50ml angle rotor of an MSE High-Speed 18 centrifuge for 15min at 4°C and 37000*g* ($r_{av.}$ 107mm) were used in the immunoprecipitation experiments. Heparinized plasma was prepared by centrifugation of fresh whole blood at 1500*g* for 10min. The buffy coat was also removed and resuspended in 10mM-sodium phosphate buffer, pH7.0, to lyse the erythrocytes. After 15min the leucocytes were collected as a pellet by centrifugation at 1500*g* for 10min. The pellet was redissolved in phosphate-buffered saline (0.15M-NaCl in 10mM-sodium phosphate buffer, pH7.0) and the suspension frozen and thawed three times to give the leucocyte extract used in immunoprecipitation.

Enzyme assays

α-D-Mannosidase was assayed by using 4-methylumbelliferyl α-D-mannopyranoside (Koch–Light Laboratories, Colnbrook, Bucks., U.K.) as described previously (Phillips *et al.*, 1974*b*). One unit of activity is that amount of enzyme which transforms 1 μmol of substrate/min under these conditions. The effect of Zn^{2+} and EDTA on the α-mannosidase activity was investigated by including 1mM-$ZnSO_4$ and 1mM-EDTA in the substrate solutions, giving a final concentration of 0.5mM in the incubation mixture. α-L-Fucosidase and *N*-acetyl-β-D-glucosaminidase were assayed by using the appropriate 4-methylumbelliferyl substrates (Koch–Light) by the procedures of Robinson & Thorpe (1974) and Leaback & Walker (1961) respectively. β-D-Mannosidase was assayed by using *p*-nitrophenyl β-D-mannopyranoside (Koch–Light). The reaction mixture (1ml) contained suitably diluted enzyme and 1mM substrate in 0.2M-sodium phosphate adjusted to pH4.25 with 0.1M-citric acid. The reaction was stopped by the addition of 2ml of 0.2M-glycine adjusted to pH10.7 with Na_2CO_3 and the absorbance of the liberated *p*-nitrophenol measured at 400nm.

Purification of α-mannosidase B

(1) *Initial fractionation.* Human liver (1kg) was homogenized in 4 litres of water and centrifuged batchwise in the 6×250ml angle rotor of an MSE High-Speed 18 centrifuge for 30min at 4°C and 23000*g* (r_{av}. 14.2cm). The material precipitated from the supernatant between 40 and 55% saturation with $(NH_4)_2SO_4$ was recovered by centrifugation and redissolved in 1 litre of 10mM-sodium phosphate buffer, pH6.0. The solution was incubated at 45°C for 1h and the material that precipitated was removed by further centrifugation at 23000*g* and discarded. The protein remaining in the supernatant was then precipitated by addition of solid $(NH_4)_2SO_4$ to give 60% saturation and subsequently redissolved in 10mM-sodium phosphate buffer, pH6.0. The enzyme was concentrated by this procedure in all subsequent steps unless otherwise stated.

(2) *Gel filtration on Sephadex G-150.* The material obtained by the initial fractionation was passed through a column (85cm×5cm diam.) of Sephadex G-150 [Pharmacia (G.B.) Ltd., London, W.5, U.K.], equilibrated in 10mM-sodium phosphate buffer, pH6.0. Fractions (9ml) were collected at 36ml/h and assayed for α-D-mannosidase. The material in the pooled fractions containing α-D-mannosidase was concentrated by precipitation by 60% saturation with $(NH_4)_2SO_4$ and redissolved in and dialysed against 10mM-sodium phosphate buffer, pH6.0.

(3) *Ion-exchange chromatography on DEAE-cellulose.* The α-mannosidase activity was separated into forms A and B by ion-exchange chromatography on a column (35cm×2.5cm diam.) of DEAE-cellulose (Whatman DE 23; Whatman Biochemicals, Maidstone, Kent, U.K.), equilibrated in 10mM-sodium phosphate buffer, pH6.0. Elution was started with the equilibration buffer and continued with a linear NaCl gradient (0–0.3M) in 600ml of the same buffer. Fractions (4.6ml) were collected at 36ml/h and assayed for enzymic activity. The salt gradient was followed by measuring the conductivity of the fractions with a conductivity-measuring bridge, type MC3 [Electronic Switchgear (London) Ltd., Hitchin, Herts., U.K.]. The unabsorbed activity, α-mannosidase A, and the absorbed activity, α-mannosidase B, were subsequently processed separately but in an identical manner, as described below.

(4) *Gel filtration on Sephadex G-200.* The two preparations (17ml) were passed through a column (96cm×2.5cm diam.) of Sephadex G-200 equilibrated in 10mM-sodium phosphate buffer, pH6.0. Fractions (3.6ml) were collected at 21ml/h.

(5) *Hydroxyapatite chromatography.* Hydroxyapatite was prepared by the method of Bernardi (1971), equilibrated in 5mM-sodium phosphate buffer, pH6.8, and packed into a column (10cm×2cm diam.). Elution was carried out by using a linear sodium phosphate buffer gradient (5–300mM, pH6.8) in a volume of 400ml. Fractions (6ml) were collected at 21ml/h and the phosphate gradient was monitored by measuring the conductivity of the eluate.

(6) *Rechromatography on Sephadex G-200.* The concentrated peaks (13ml) of α-mannosidase A and B from the hydroxyapatite chromatography were rechromatographed separately on the same Sephadex G-200 column under the conditions described above.

(7) *Rechromatography on DEAE-cellulose.* The conditions were the same as described previously, except that a smaller column (10cm×2cm diam.) was used and the linear salt gradient was 0–0.2M in 150ml of buffer. Fractions (3.6ml) were collected at 60ml/h.

(8) *Isoelectric focusing.* Material corresponding to peak A and peak B from the rechromatography on DEAE-cellulose was concentrated against polyethylene glycol 4000P (Shell Chemicals, London S.E.1, U.K.) and then dialysed against 1% (w/v) glycine, pH6.0 at 4°C. It was analysed by isoelectric focusing in an Ampholine type 8100 column (LKB Instruments, London S.E.20, U.K.) at 5°C as described by Vesterberg & Svensson (1966) and Phillips *et al.* (1974*b*).

Polyacrylamide-gel electrophoresis

Electrophoresis of the purified samples was carried out in 5% (w/v) polyacrylamide gels by using the discontinuous buffer system of Davis (1964). Separated protein bands were detected by staining the gels

with an 0.0025% solution of Coomassie Blue R250 (G. T. Gurr, London S.W.6, U.K.) in 10% (w/v) acetic acid. Duplicate gels were washed in 10ml of water. Enzymically active bands were then detected by immersion in 7.5ml of 0.2M-sodium phosphate adjusted to pH4.0 with 0.1M-citric acid, containing 1-naphthyl α-D-mannopyranoside (Koch–Light) (1mg/ml) and the diazonium salt of 4-chloro-*o*-anisidine (Koch–Light) (2mg/ml) at 37°C. Coloured bands locating enzyme activity generally appeared within 10min and were fully developed within 60min. The bands were stable when the gels were stored in 7% (v/v) acetic acid.

Preparation of antiserum

All rabbits were bled before immunization to provide control sera. Control sera and antisera were stored at −20°C in the presence of 0.2% (w/v) NaN_3 as preservative. The α-mannosidase B preparation containing 5mg of protein and having a specific activity of 0.24 unit/mg of protein was emulsified with an equal volume (5ml) of complete Freund's adjuvant (Difco Laboratories, Detroit, Mich., U.S.A.). A rabbit was given intramuscular injections of the antigen (2.0ml) at 10-day intervals over a period of 40 days, and was bled from the marginal ear vein 15 days after the last injection. The blood was allowed to clot at room temperature (18°C) for 2h before the serum was collected by centrifugation at 3000*g* for 10min.

Immunoprecipitation experiments

A constant amount (100μl) of human liver supernatant, peaks A, B or C separated on DEAE-cellulose as described previously (Phillips *et al.*, 1974*b*) or suitably diluted purified α-mannosidase A and B, was placed in a series of 15ml conical centrifuge tubes. Then 0–100μl of diluted (10 or 50-fold) antiserum was added to the tubes and the volume made up to 300μl with phosphate-buffered saline, pH7.0. After incubation at 4°C for 16h the tubes were centrifuged at 1500*g* for 15min. The supernatant was removed and 0.2ml assayed for α-D-mannosidase activity at pH4.0 or 6.5 by the normal procedure. The equivalence points were calculated by plotting the activity remaining in the supernatants against the volume of antiserum in the incubation mixture (Pollock, 1963).

Immunodiffusion

Double diffusion was carried out in 1mm-thick gels (3%, w/v) of a mixture of starch and agarose (3:1, w/w) in phosphate-buffered saline, pH7.0, at room temperature. After 24h the gels were washed in phosphate-buffered saline for 48h to remove the unprecipitated protein. Enzymically active precipitin lines were detected by the same procedure as for polyacrylamide-gel electrophoresis. Proteins were stained with Amido Black 10B [1% (w/v) in 12% (v/v) acetic acid] for 30min at room temperature.

Results

Purification of α-mannosidase B

α-Mannosidase B was purified by the procedure in Table 1. No neutral activity was detectable after the initial fractionation. Polyacrylamide-gel electrophoresis showed that the preparation consisted of four major protein bands, one of which possessed α-mannosidase activity (Fig. 1). The isoelectric point

Table 1. *Purification of human liver α-mannosidase B*

Details of the purification procedure are described in the Experimental section. The major enzymic contaminants of the α-mannosidase preparation were α-L-fucosidase (0.04 unit/mg of protein) and β-D-glucosaminidase (0.012 unit/mg of protein).

Purification step	Total activity (units)	Specific activity (units/mg of protein)	Yield (%)	Purification
Homogenate	231	0.0012	100	1
1. $(NH_4)_2SO_4$ precipitate, 40–55% saturation	135	0.0053	58	4.4
Heat treatment 50°C, 60min	124	0.021	54	17.5
2. Sephadex G-150	99	0.035	43	29
3. DEAE-cellulose chromatography (peak B)	31	0.055	13.4	45.8
4. Sephadex G-200	25	0.108	10.8	90
5. Hydroxyapatite	15	0.196	6.5	163
6. Sephadex G-200	15	0.287	6.5	239
7. DEAE-cellulose	4.5	0.245	2.0	204
8. Isoelectric focusing	2.4	0.244	1.0	203

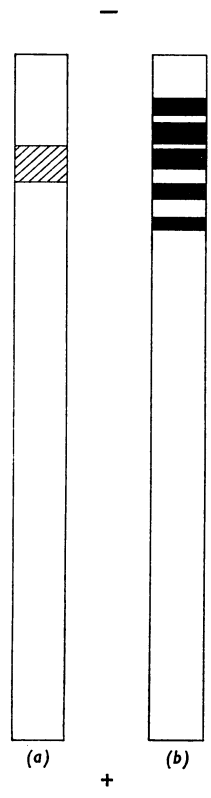


Fig. 1. *Polyacrylamide-gel electrophoresis of purified α-mannosidase B*

(*a*) α-Mannosidase activity; (*b*) protein components. Details of electrophoresis and staining methods are described in the Experimental section; 20 μl of purified α-mannosidase B was analysed.

of the α-mannosidase activity in this preparation was pH5.4, very close to the value found for α-mannosidase B derived from crude liver extracts (Phillips *et al.*, 1974*b*). There was no α-mannosidase isoelectric at pH5.9–6.1, the pI of α-mannosidase A. Therefore it was concluded that the preparation did not contain any α-mannosidase A. The activity in the preparation had a pH optimum of 4.25–4.5 and a K_m of 1.3mM. In contrast with unpurified α-mannosidase B it was not activated by Zn^{2+} or inhibited by EDTA. Acidic α-mannosidase activity which is unaffected by these reagents has been reported previously in crude liver extracts (Phillips *et al.*, 1974*b*). Antiserum was raised against this preparation.

Immunoprecipitation of α-mannosidase A and B

The antiserum raised against purified α-mannosidase B completely precipitated the antigen, α-

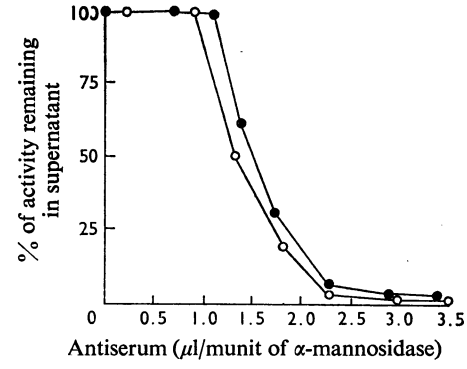


Fig. 2. *Immunoprecipitation of purified α-mannosidase A and B by anti-(α-mannosidase B) serum*

Details of immunization and assay procedures are described in the Experimental section. Various volumes of antiserum and a fixed volume of enzyme preparation were incubated together in phosphate-buffered saline, pH7.0. The supernatants (0.2ml) obtained on centrifugation of the incubation mixtures were assayed for α-mannosidase activity by the normal procedure in 0.2M-sodium phosphate adjusted to pH4.0 with 0.1M-citric acid. ●, α-Mannosidase A; ○, α-mannosidase B.

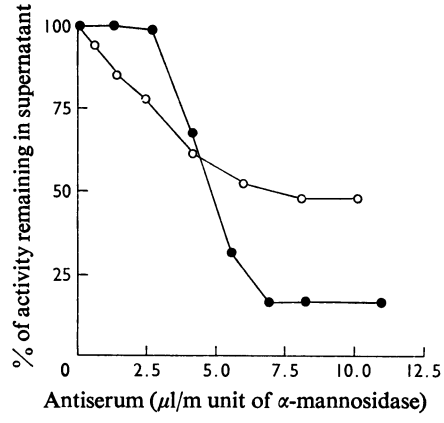


Fig. 3. *Immunoprecipitation of human liver supernatant α-mannosidase activity*

Details are as for Fig. 2 except that the supernatants were assayed at pH4.0 (●) and pH6.5 (○) by the normal procedure in 0.2M-sodium phosphate adjusted to pH4.0 or 6.5 with 0.1M-citric acid.

mannosidase B, from solution (Fig. 2). It was equally effective in precipitating α-mannosidase A that had been purified 1270-fold to a specific activity of 1.55 units/mg of protein by a procedure comparable with that for α-mannosidase B. The equivalence points for purified α-mannosidase A and B were 1.38 and

1.5ml of antiserum/unit of enzyme activity respectively.

To investigate whether the antiserum precipitated neutral α-mannosidase and unpurified acidic activity, human liver supernatant was used in an immunoprecipitation experiment as a source of α-mannosidase activity (Fig. 3). Over 80% of the acidic activity was precipitated, but the equivalence point of 6ml/unit of enzyme activity was considerably higher than for the purified acidic forms. Some of the neutral activity was also apparently precipitated, but the shape of the titration curve was not typical As the acidic forms of the enzyme have some activity at pH6.5, some of the loss of activity measured at pH6.5 was probably due to precipitation of the acidic activity.

The equivalence point for the acidic activity was independent of enzyme concentration. The antiserum did not precipitate any β-mannosidase activity but it did precipitate 25% of the α-L-fucosidase and 2% of the *N*-acetyl-β-D-glucosaminidase at a concentration at which all of the acidic α-mannosidase was precipitated. α-L-Fucosidase was the major enzymic contaminant in the antigen.

The antiserum was tested against α-mannosidase components A, B and C separated by ion-exchange chromatography on DEAE-cellulose to investigate specifically whether it precipitated any of the neutral activity. The equivalence points for the precipitation of the acidic activity in peak A and peak B were 6.2 and 6.3 ml of antiserum/unit of enzyme activity respectively, close to the value obtained for the precipitation of the acidic activity from human liver supernatant. Some apparent precipitation of the neutral activity in peak C was found, but it was very much less than the precipitation of the neutral activity from a liver extract. Most of this apparent loss of neutral activity could be attributed to the acidic activity known by isoelectric focusing to contaminate peak C (Phillips *et al.*, 1974*b*).

These results suggest that the antiserum raised against purified α-mannosidase B cross-reacts with the closely related α-mannosidase A but not with the neutral α-mannosidase C. Confirmation that the antiserum does not precipitate any neutral activity was obtained by incubating a sample of human liver supernatant with a twofold excess of antiserum calculated to precipitate all the acidic activity and analysing the supernatant by ion-exchange chromatography on DEAE-cellulose (Fig. 4). A quantitative comparison of this elution profile with that for a control incubation containing no antiserum showed that the antiserum had precipitated all of components A and B but none of the neutral component C. The elution profile for the supernatant treated with antiserum bore a striking resemblance to the elution profile for α-mannosidase activity from the liver of a patient with mannosidosis (Carroll *et al.*, 1972). These results support the hypothesis that the two

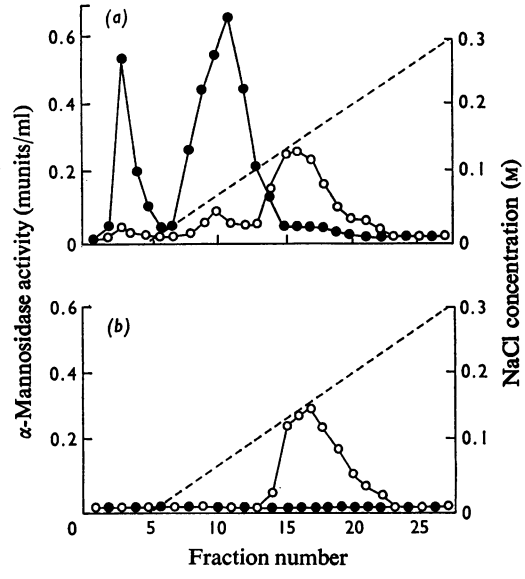


Fig. 4. *Ion-exchange chromatography on DEAE-cellulose of (a) control human liver supernatant and (b) residual activity in human liver supernatant treated with twofold excess of antiserum*

Human liver supernatant (2ml, 25%, w/v) was incubated with a twofold excess of antiserum contained in 0.5ml of phosphate-buffered saline for 24h at 4°C. In a control experiment (*a*) the supernatant was incubated with 0.5ml of phosphate-buffered saline only. Supernatants (2ml) obtained on centrifugation at 1500*g* for 15 min were applied separately to a column (2.5cm×0.75cm diam.) of DEAE-cellulose equilibrated in 10mM-sodium phosphate buffer, pH6.0. Elution was started with equilibration buffer and continued with a 0–0.3M-NaCl gradient in 50ml of the buffer. Fractions (2ml) were collected at 60ml/h and assayed for α-mannosidase activity at pH4.0 (●) and 6.5 (○). ----, NaCl gradient measured with a conductivity meter.

acidic forms A and B are very closely related structurally and genetically but that the neutral form C is structurally and genetically distinct.

The antiserum did not appear to contain antibodies specific for α-mannosidase A or B. When the supernatant obtained from the incubation of α-mannosidase B with its equivalent or less of the antiserum was tested against α-mannosidase A, no precipitation of this enzyme occurred. A similar result was found when the complementary experiment was performed, suggesting that α-mannosidase A and B are immunologically identical.

Ouchterlony double-diffusion experiments

Double diffusion of purified α-mannosidase A and B against the antiserum showed that both forms

produced multiple precipitin lines (Plate 1), several lines being common to both forms. Only one precipitin line possessed α-mannosidase activity and this was common to both α-mannosidase A and B. The line was continuous in adjacent diffusions of A and B and there was no evidence of spur formation, again suggesting that α-mannosidase A and B are immunologically identical. Double diffusion of human liver supernatant and the material in peaks A and B separated on DEAE-cellulose against the antiserum also produced multiple precipitin lines. When these were stained for α-mannosidase activity, faint and diffuse bands (not visible in Plate 1) were obtained because a longer incubation time had to be used on account of the lower specific α-mannosidase activity in these samples. However, all the enzymic-activity lines appeared to be continuous with those obtained for the purified α-mannosidase A and B. Thus purification of the enzyme does not appear to alter its immunogenic properties.

Nature of the α-mannosidase activity precipitated by antiserum

Unpurified α-mannosidase is activated by Zn^{2+} and inhibited by EDTA. As the α-mannosidase activity used to prepare the antiserum had lost this sensitivity during purification, we decided to see if the activity precipitated from human liver supernatant by the antiserum possessed this sensitivity. The precipitate was resuspended in phosphate-buffered saline, pH7.0, and assayed for α-mannosidase activity by the normal procedure over a range of pH values in the presence and absence of 0.5mM-$ZnSO_4$ or 0.5mM-EDTA. Zn^{2+} lowered the pH optimum to 3.75 and increased the activity and the EDTA decreased the activity as for the unpurified sample.

Immunoprecipitation of α-mannosidase from other human tissues

The antiserum raised against purified human liver α-mannosidase B was tested against the α-mannosidase activity in other human tissues (Table 2). In all the tissues examined precipitation of the acidic but not of the neutral activity occurred. The equivalence point for the acidic activity and the amount of soluble antigen–antibody complex varied widely.

Although there is little or no neutral activity in plasma, it contains in addition to acidic activity a relatively large amount of a third type of α-mannosidase activity, with an optimum pH of 5.5. The antiserum precipitated over 50% of the acidic α-mannosidase from plasma but none of the activity with an optimum pH of 5.5. A comparison of the pH curve of the untreated plasma with that of the activity remaining in the supernatant confirmed that only acidic activity had been removed by the antiserum.

Table 2. *Equivalence points for acidic α-mannosidase in various human tissues*

The equivalence point is the volume of antiserum calculated to neutralize 1 unit of α-mannosidase activity.

	Antiserum (ml)
Liver (i) whole supernatant	6.0
(ii) peak A (DEAE-cellulose)	6.2
(iii) peak B (DEAE-cellulose)	6.3
(iv) purified α-mannosidase A	1.4
(v) purified α-mannosidase B	1.5
Kidney	2.4
Brain	5.4
Leucocytes	9.3
Plasma	10.2

These results suggest that the activity with an optimum pH of 5.5 is also structurally different from the acidic activity.

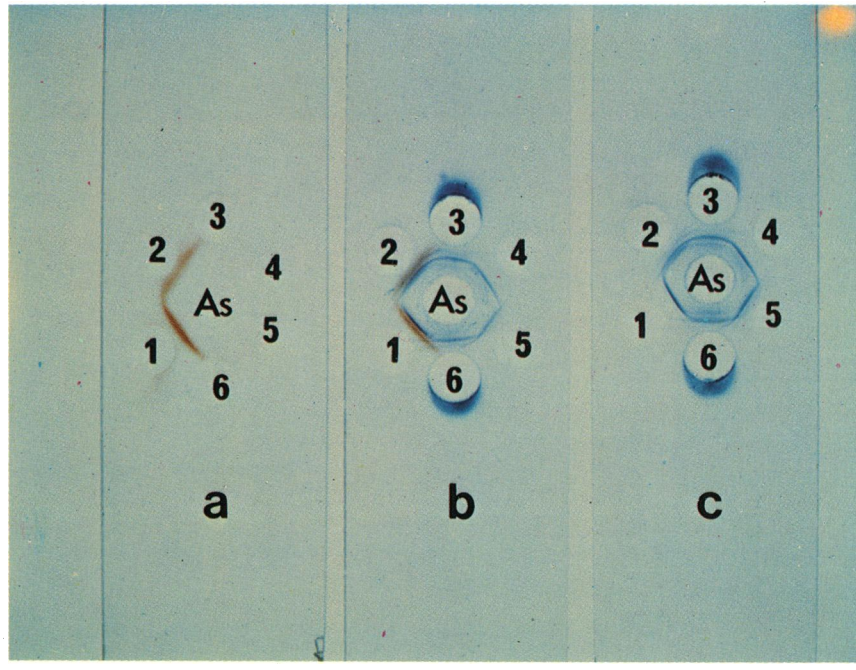
Lack of cross-reactivity between bovine α-mannosidase and anti-(human α-mannosidase) serum

The antiserum did not precipitate α-mannosidase activity from bovine liver and kidney. Conversely antiserum raised against purified bovine α-mannosidase did not cross-react with human α-mannosidase.

Discussion

Precipitation of purified α-mannosidase A by antiserum raised against purified α-mannosidase B showed that the two forms of acidic α-mannosidase were closely related. Further, the similarity of their equivalence points and the lack of spur formation in the continuous enzymically active precipitin line obtained when purified α-mannosidase A and B were placed in adjacent wells in an immunodiffusion experiment suggested that they were immunologically identical. The absence from the antiserum of antibodies specific for either α-mannosidase A or B supports this conclusion. α-Mannosidase A and B have very similar physical and chemical properties (Phillips *et al.*, 1974*b*) and are both absent in mannosidosis (Carroll *et al.*, 1972). The results in the present paper reinforce the view that there is a close structural, functional and genetic relationship between α-mannosidase A and B in human tissue.

Immunological identity of closely related forms of lysosomal enzymes has been reported previously for human liver hexosaminidases A and B (Carroll & Robinson, 1972; Srivastava & Beutler, 1972) and chicken liver cathepsin D isoenzymes (Dingle *et al.*, 1971). In contrast, α-galactosidases A and B, both of which have an acidic pH optimum and are lysosomal in origin, do not cross-react (Beutler & Kuhl, 1972), nor do sulphatases A and B (Neuwelt *et al.*, 1971).



EXPLANATION OF PLATE 1

Immunodiffusion between anti-(α-mannosidase B) serum and various preparations of human liver α-mannosidase

(*a*) Precipitin arcs stained for α-mannosidase with 1-naphthyl α-D-mannopyranoside; (*b*) precipitin arcs stained for α-mannosidase and post-stained for protein with Amido Black; (*c*) precipitin arcs stained for protein. As, anti-(α-mannosidase B) serum; (1), purified α-mannosidase A; (2), α-mannosidase B; (3) and (6), human liver supernatant; (4) and (5), peaks A and B respectively from DEAE-cellulose chromatography.

Presumably this lack of cross-reactivity is a reflexion of the different structure and functions of these isoenzymes.

The antiserum raised against acidic α-mannosidase did not precipitate neutral α-mannosidase from liver or other tissue extracts. The α-mannosidase profiles obtained by ion-exchange chromatography of a liver extract treated with the antiserum and of an extract of a liver from a patient with mannosidosis were very similar. These observations are consistent with acidic and neutral α- mannosidase activity having different physicochemical properties, functions and subcellular locations and being under different genetic control (Phillips *et al.*, 1974*b*). A similar situation has been found for other human glycosidases, where antiserum raised against the lysosomal isoenzyme(s) with an acidic pH optimum does not react with the form of the enzyme with a neutral pH optimum, e.g. hexosaminidase (Poenaru *et al.*, 1973; Braidman *et al.*, 1974), β-galactosidase (Norden *et al.*, 1974; Meisler & Rattazzi, 1974) and α-glucosidase (de Barsy *et al.*, 1972). For each of these enzymes there is a specific genetic deficiency of the acidic activity which does not affect the neutral activity. Therefore it can be concluded that in general the acidic and neutral forms of mammalian glycosidases are genetically and immunologically distinct.

The antiserum also failed to react with the activity in plasma that has an intermediate pH optimum of 5.5. The residual activity in the plasma after removal of the acidic activity by immunoprecipitation closely resembled the residual activity in the plasma of a patient with mannosidosis. Thus the activity with an optimum pH of 5.5 is also immunologically and genetically distinct from the acidic activity. It has been suggested previously that it is related to the neutral activity (Phillips *et al.*, 1974*b*). Although bovine and human acidic α-mannosidase have very similar properties the antiserum raised against the human enzyme did not cross-react with the bovine enzyme and vice versa. Similarly Dingle *et al.* (1971) found that serum raised against cathepsin D from one mammalian source did not react with cathepsin D from several other mammalian sources. However, antiserum to chicken cathepsin D reacted with cathepsin D from a variety of different species of bird.

The antiserum precipitated the acidic α-mannosidase activity from brain, kidney and leucocytes as well as liver. Therefore it seems unlikely that the enzyme exists in organ-specific forms that are immunologically distinct. However, the equivalence point did vary from organ to organ and it was much higher in crude tissue extracts or preparations than for the purified forms of the enzyme. Enzymically inactive cross-reacting material that is present in the crude preparations but not in the purified preparation probably accounts for these observed differences.

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