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THE RELATIONSHIP BETWEEN DIFFERENT FORMS OF HUMAN α-MANNOSIDASE

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Summary

The tissue distribution and some properties of human α-mannosidase (α-D-mannoside mannohydrolase EC 3.2.1.24) have been studied. The acidic forms of the enzyme were fairly stable, whereas the neutral forms easily lost enzymic activity. The acidic forms were sensitive to neuraminidase but the neutral forms were unaffected. The experiments indicate that the acidic components are closely related to each other, differing only in sialic acid content and possibly conformation. The neutral forms of the enzyme are probably quite different from the acidic forms both in structure and cellular function.

Introduction

The presence of α-D-mannosidase activity (α-D-mannoside mannohydrolase, EC 3.2.1.24) has been shown in several human tissues [1—7]. Carroll et al. [8] resolved the activity of α-mannosidase from normal human liver into three components, A, B, and C, by chromatography on DEAE-cellulose. Forms A and B had acidic pH optima (acid types) and form C a neutral pH optimum. Liver samples from patients suffering from mannosidosis, a lysosomal storage disease described by Öckerman [9], were also studied by Carroll et al. [8] who found that forms A and B were absent and form C was unaffected in this disease. Similar results have also been observed in a study of bovine mannosidosis [10]. Several other glycosidases show structural heterogeneity (for a review, see ref. 11). The aim of the present investigation was to study some properties of the α-mannosidase in normal human tissues.

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Experimental procedure

Preparation of tissue homogenates

Post-mortem samples of liver, kidney, spleen, pancreas, brain and lymph nodes, and a fresh placenta, were obtained from the University Hospital, Uppsala. All the samples were stored at −20 °C and unless otherwise stated, all experiments were carried out at 4 °C.

For all tissues, a 20% (w/v) homogenate was made in either redistilled water or in 10 mM sodium phosphate buffer, pH 6.0, using a Dounce tissue homogenizer. The sample was centrifuged at 32 000 × *g* for 20 min and the resulting supernatant was used in the following experiments.

Enzyme assay

Forms A and B were routinely assayed at pH 4.0 and form C at pH 6.0. The sample to be assayed (0.2 ml) was mixed with 0.1 ml of a 1.6 mM solution of 4-methylumbelliferyl-α-D-mannopyranoside (Koch-Light, Colnbrook, U.K.) in citrate-phosphate buffer (200 mM Na_2HPO_4 added to 100 mM citric acid). This was incubated at 37 °C for either 30 min or 1 h. Specific conditions are given in the individual experiments. The reaction was stopped by adding 1 ml of 250 mM glycine/NaOH buffer, pH 10.4, and the fluorescence measured using an Aminco-Bowman spectrofluorimeter (excitation wave-length 348 nm, emission wave-length 450 nm).

Ion exchange chromatography

The supernatant (1.8 ml in water), mixed with 0.2 ml of 100 mM sodium phosphate buffer, pH 6.0, was applied to a column (15 × 0.9 cm) containing Whatman DEAE-cellulose (type DE-52, W. and R. Balston Ltd., Maidstone, U.K.) pre-equilibrated with 10 mM sodium phosphate buffer, pH 6.0. The same buffer was used to elute 4 ml fractions at a flow rate of 60 ml/h for 40 min, after which a linear sodium chloride gradient in the eluting buffer was applied. The fractions containing high activity of the separated enzyme forms were pooled. The salt gradient was checked by measuring the concentration of chloride in the fractions using a Philips conductivity meter fitted with a 1.5 ml conductivity electrode.

An identical procedure was used for chromatography on Whatman CM-cellulose (CM-52, W. and R. Balston Ltd.) except that sample equilibration was performed against sodium phosphate buffer pH 5.0 and the column was eluted using the same buffer.

Effect of heat

A ten-fold dilution of liver, spleen, placenta and brain supernatant in buffer, and pooled fractions from liver, were heated at 50 °C. Aliquots (2 ml) were removed after timed intervals, cooled, and centrifuged at 1500 × *g* for 5 min. α-Mannosidase activity was measured as described in Fig. 2.

Treatment with neuraminidase

One ml of tissue supernatant prepared in 10 mM sodium phosphate buffer, pH 6.0, or purified enzyme fractions, were incubated at 37 °C for 3 h with

1 ml of citrate-phosphate buffer, pH 6.0, containing 0.5 mg of neuraminidase from *Clostridium perfringens* Type VI (Sigma Chemical Co., St. Louis, Mo., U.S.A.). This enzyme was found to be free of α-mannosidase activity, and under the conditions used is essentially free of proteinase activity. This purified preparation is supplied as a dialysed, lyophilized powder. No merthiolate was present [12]. After incubation, the reaction mixtures were cooled rapidly in ice and dialysed, and chromatographed as described.

Results

Effect of pH on α-mannosidase activity

The variations in α-mannosidase activity between pH 3.5 and 7.0 are shown in Fig. 1. The results obtained using liver tissue are shown in Fig. 1A, demonstrating the presence of a high neutral and lower acidic activity. A similar pattern was also obtained using spleen, placenta, and brain tissue. Different results were found using lymph nodes, kidney and pancreatic tissue (Fig. 1B).

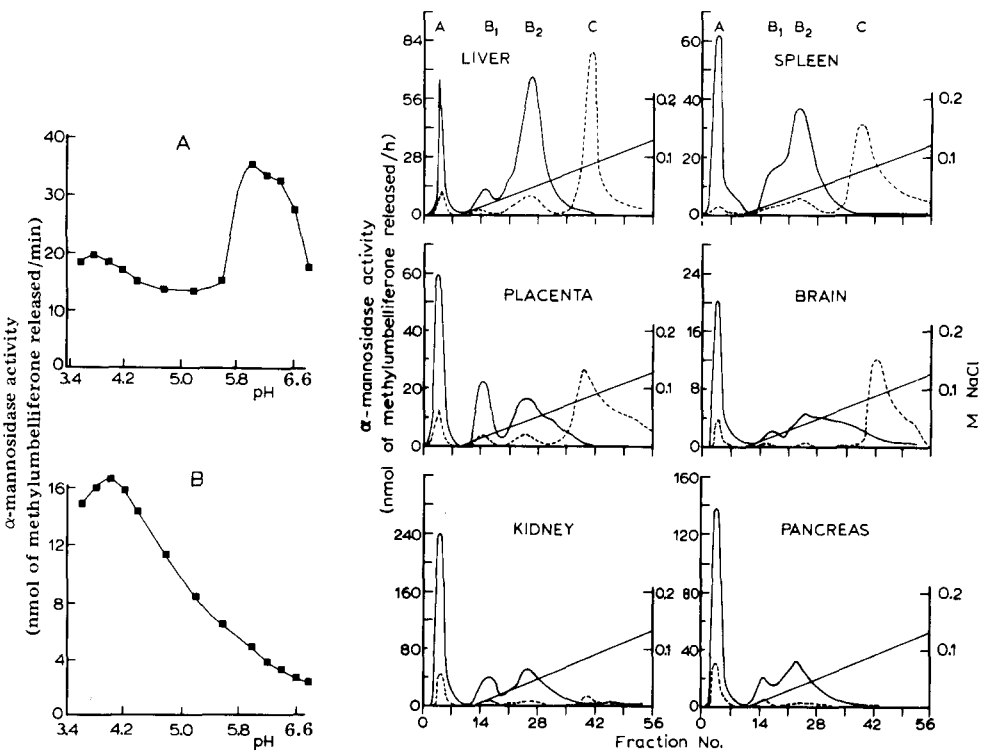

Fig. 1. Effect of pH on α-mannosidase activity. A, profile obtained using liver, spleen, placenta, and brain (Example shown is liver); B, profile obtained using kidney, pancreas and lymph nodes (Example shown is pancreas). Assay conditions: 0.2 ml of a 20% aqueous solution of supernatant, 0.2 ml citrate-phosphate buffer (pH 3.5—7.0) and 0.1 ml 1.6 mM 4-methylumbelliferyl-α-D-mannopyranoside in water was incubated for 30 min. Other procedures as given in Experimental Procedure.

Fig. 2. Separation of α-mannosidase components on DEAE-cellulose chromatography. ———, Activity of α-mannosidase at pH 4.0; – – –, activity of α-mannosidase at pH 6.0. The salt gradient is indicated by the straight line. Assay conditions: 0.2 ml column fraction and substrate in citrate-phosphate buffer, pH 4.0 or 6.0, was incubated for 1 h. Other conditions as in Experimental Procedure.

In these cases almost all the activity was acidic, only traces of neutral activity being apparent. The neutral activity, where present, showed different degrees of instability according to the tissue of origin. Keeping the samples at room temperature for 4 h resulted in a loss of about 70% of this activity in liver. The residual activity was quite stable and probably represents the major part of the neutral activity studied further in this work. In spleen the activity was almost halved in this period, whereas the activities in placenta and brain were unaffected. The activity in placenta was approximately halved after 24 h at room temperature, whereas there was no apparent loss of this activity in brain even after 48 h. The acidic activity in all tissues was unaffected by this treatment.

Chromatography on DEAE-cellulose

The fractionation of enzymic activities in all the tissues studied is shown in Fig. 2. The profile for lymph nodes was identical to that obtained for pancreas. A peak of acidic α-mannosidase activity (peak A) was eluted by the starting buffer, and after applying a linear salt gradient different patterns were obtained with each tissue. Two main peaks of acidic activity (B_1 and B_2) were eluted first, followed by a neutral component (peak C) which in some cases also showed heterogeneity. In each case 80—85% of the activity applied to the column was recovered, and the elution volume of each activity maximum was reproducible (±1 fraction).

Effect of heat and metal ions

When the supernatants of liver, spleen, placenta and brain, and column fractions A, B_2 and C from liver were heated at 50°C, the acidic α-mannosidase activities were stable and the neutral were labile. Marsh and Gourlay [13] reported that Co^{2+} and Mn^{2+} were most effective in stabilizing the neutral α-mannosidase activity from rat liver. To test whether Co^{2+} has a similar effect on the neutral activity from human liver, the supernatant was heated at 50°C, as described in Experimental Procedure, in the absence and presence of Co^{2+} (1 or 2 mM). In each case the percentage activity recovered was the same, indicating that the human enzyme, in contrast to the rat enzyme, was not stabilized by Co^{2+}.

Neuraminidase treatment of α-mannosidase

The tissue supernatants were chromatographed on DEAE-cellulose before and after neuraminidase treatment. The eluted fractions were assayed for α-mannosidase activity. Fig. 3 shows the results obtained using liver, which was representative of all the tissues studied. The acidic forms (B) were greatly diminished after this treatment, and a corresponding increase of the A type of enzyme was observed (Fig. 3A). On the other hand the elution profile of the neutral form of the enzyme (C) was not altered (Fig. 3B).

Fraction A obtained by DEAE-cellulose chromatography was re-chromatographed on CM-cellulose both before and after neuraminidase treatment (Fig. 4). The untreated fraction A under these conditions was resolved into a small non-adsorbed peak and a major component which was eluted by the salt gradient. However, neuraminidase-treated fraction A gave only one peak of activity which required a higher salt concentration for elution.

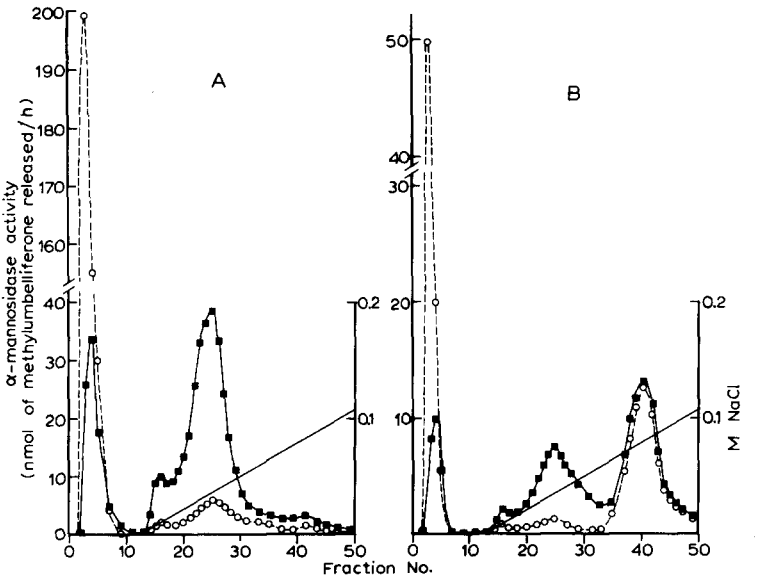

Fig. 3. DEAE-cellulose chromatography of liver supernatant before and after neuraminidase treatment. ■, Before neuraminidase treatment; ○, after neuraminidase treatment. (A) Enzyme activity measured at pH 4.0. (B) Enzyme activity measured at pH 6.0. ———, Salt gradient. Assay conditions as given in Fig. 2.

Fraction B_2 from the DEAE-cellulose chromatography of liver supernatant was re-chromatographed on DEAE-cellulose both before and after neuraminidase treatment. Eluted fractions were assayed for α-mannosidase activity (Fig. 5). The results show that after treatment with neuraminidase an almost

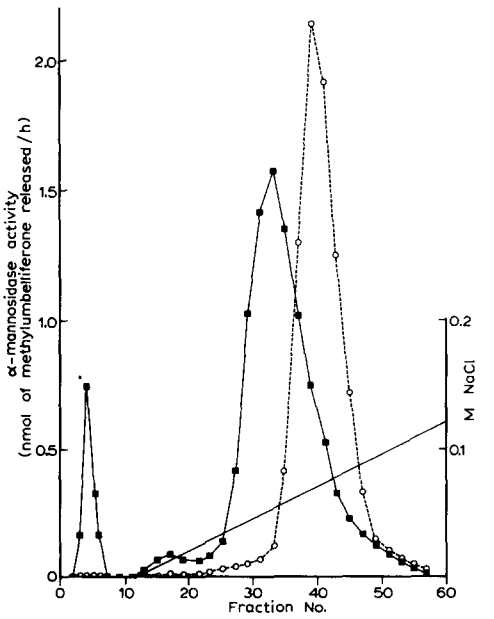

Fig. 4. CM-cellulose chromatography of α-mannosidase A from liver before and after neuraminidase treatment. Symbols and assay conditions as shown in Fig. 3. Enzyme activity measured at pH 4.0 only.

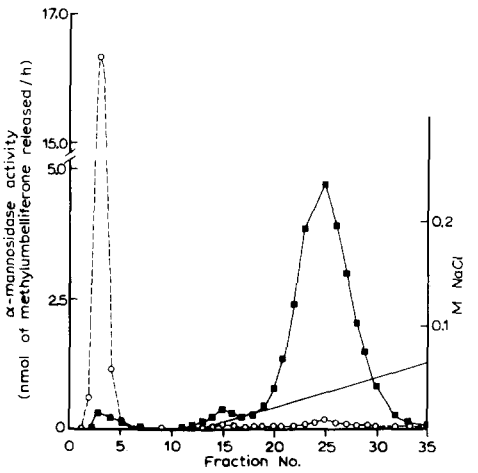

Fig. 5. DEAE-cellulose chromatography of α-mannosidase B_2 from liver before and after neuraminidase treatment. Symbols and assay conditions as shown in Fig. 3. Enzyme activity measured at pH 4.0 only.

complete conversion of the B form to an A type of enzyme had occurred. CM-cellulose chromatography of this A type of enzyme showed that it is probably identical with that obtained after neuraminidase treatment of form A. The same experiment was performed on the neutral enzyme (C), and in this case identical elution profiles were obtained both before and after neuraminidase treatment. Control experiments showed that these neuraminidase-induced conversions were not observed after the enzyme had been boiled.

Discussion

Several forms of α-mannosidase have been found in normal human tissues [7,8] but in mannosidosis the acidic forms of this enzyme were absent [8]. It was therefore considered important to investigate the distribution of the various forms in other human tissues and to obtain more information about the interrelationship between the different forms, which is necessary for a better understanding of the etiology of the disease.

The study of α-mannosidase activity at different pH's in a number of tissues gave two general patterns. In the one case (liver, spleen, placenta and brain) two maxima of activity were observed, at pH 4 and pH 6, the latter being more pronounced. In the other case (kidney, pancreas and lymph nodes) only one maximum was found (pH 4). The stability of the neutral activity varied considerably in different organs. The almost complete lack of neutral activity in kidney, pancreas and lymph nodes may indicate extremely unstable neutral forms and hence the observed absence in vitro does not necessarily prove an absence in vivo. Recently Phillips et al. [14] reported that the neutral enzymic activity of liver was unstable when kept at 45 °C. In similar experiments we were able to confirm this, and also show that this neutral activity was increasingly stable in liver, spleen, placenta and brain. We have also confirmed the results of Phillips et al. [14] on the effect of different divalent metal ions.

Carroll et al. [8] resolved the liver α-mannosidase into three components

using DEAE-cellulose chromatography. A study of several different organs using the same method but a lower salt gradient resulted in a separation of the B form of the liver enzyme into two forms (B_1 and B_2). Even greater heterogeneity was observed in the other organs studied.

A more detailed investigation on the interrelationship of the different forms of α-mannosidase was performed. Treatment of tissue supernatant with neuraminidase followed by DEAE-cellulose chromatography indicated that the B form lost sialic acid and was converted to an A type of enzyme. Component C was unaffected by this treatment.

The individual forms (A, B_2, and C) from liver, isolated after DEAE-cellulose chromatography, were treated separately with neuraminidase. Form A after rechromatography on CM-cellulose appeared as a single, less sialylated form, with no apparent loss of enzymic activity. Form B_2 after rechromatography on DEAE-cellulose was completely converted to an A type of enzyme and form C was totally unaffected. CM-cellulose chromatography of neuraminidase-treated form B_2 indicated that the product was the less sialylated form of A as mentioned above. These results seem to indicate that the forms A and B are structurally interrelated, differing mainly in sialic acid content, and possibly to a small extent in conformation.

After ion exchange chromatography, Phillips et al. [14] found that the A form from liver was partially converted to a B type of enzyme having a slightly higher molecular weight. In the present work a similar but less pronounced conversion of the A form to a B type under the same conditions was observed. It is possible that a slight change in conformation exposes more acidic groups and this conformational change results in an apparent increase in molecular weight. Another explanation of these findings is that deamidation of the enzyme occurs during chromatography [15].

The observation that the stable component in form C is unaffected by neuraminidase treatment and therefore probably contains no exposed sialic acid residues and yet has a much lower p*I* value than the A and B forms strongly suggests that this C form is structurally unrelated to the A and B forms of enzyme. The close relationship between the A and B forms may explain the fact that both are greatly reduced in mannosidosis and that at least the residual C type of enzyme is unaffected in the disease.

Acknowledgements

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