HUMAN MANNOSIDOSIS -- THE ENZYMIC DEFECT

M. Carroll, N. Dance, P. K. Masson, D. Robinson and B. G. Winchester

Department of Biochemistry
Queen Elizabeth College
Campden Hill
London W8 7AH

Received August 31,1972

Summary

Normal human liver α-mannosidase exists in at least 3 forms, separable by DEAE cellulose chromatography. The A and B forms are most active at pH 4.4 while activity of form C is maximal at pH 6.0. In two cases of mannosidosis, examined by ion exchange chromatography and isoelectric focusing, both A and B forms were absent and the residual α-mannosidase activity was due to the presence of the C form in normal amounts.

The presence of α-mannosidase has been demonstrated in many mammalian tissues including human (1,2). Genetic disorders involving this enzyme are rare but a case has been described in which accumulation of mannose-rich oligosaccharides in the tissues was accompanied by a decreased α-mannosidase activity (3). Hultberg (4) examined the residual activity in this patient and found it to have an apparently lower molecular weight and more neutral pH optimum than the normal enzyme. The possibility of the existence of more than one form of α-mannosidase in humans was raised but they could not be clearly separated by isoelectric focusing. With the use of ion exchange chromatography human liver α-mannosidase has now been separated into at least 3 forms, two of which are absent in liver samples from two patients with mannosidosis. Form C remained at approximately normal levels in the affected individuals.

Materials and Methods

Post-mortem samples of normal liver were stored at -12°. We are grateful to Dr. P. A. Öckerman for the frozen liver samples from mannosidosis patients. Tissue homogenates (10% w/v) were prepared in 0.01 M sodium phosphate buffer, pH 6.0 at 4° with a ground glass homogeniser. After centrifugation for 5 min at 36,000 g supernatants were applied to ion exchange columns of DEAE cellulose (Whatman DE23, W. R. Balston, Maidstone, Kent, U.K.) packed in 2 ml disposable syringes and pre-equilibrated with 0.01 M sodium phosphate buffer, pH 6.0.

Samples were first eluted with the same buffer and then with a linear sodium chloride gradient (0-0.2 M) in that buffer. Fractions (1.5 ml) were collected at a flow rate of 60 ml/h and assayed with 4-methylumbelliferyl-α-D-mannopyranoside substrate (Koch Light Laboratories, Colnbrook, Bucks., U.K.). The final assay (1 ml) contained 0.2 ml enzyme sample and 1.0 mM substrate in either 0.05 M sodium citrate buffer, pH 4.4 or 0.05 M sodium phosphate buffer, pH 6.0. After incubation for 2-4 h at 30° the reaction was stopped and fluorescence measured as described for other glycosidase assays (5).

Isoelectric focusing of distilled water homogenates, centrifuged as above, was carried out in ampholines producing a pH 3-8 gradient in an LKB column of approximately 110 ml capacity (6). After 40 h the column was drained off into 2.2 ml fractions and assayed as above except that McIlvaine phosphate-citrate buffers at pH 4.0 and 6.0 were used.

Results

The large decrease in α-mannosidase activity and shift of pH optimum towards a more neutral value in mannosidosis as compared to normal liver (4) has been confirmed. At pH 4.4 activities of normal liver varied from 70 to 80 nmoles/min/g tissue, within the accepted normal range (7), while the mannosidosis liver samples had activities of 1.2 and 3.3 nmoles/min/g tissue. The loss of activity measured at pH 6.0 was much less marked, falling from 10-73 in the normal liver to 8.1 and 31.4 in mannosidosis.

On DEAE cellulose chromatography the α-mannosidase of normal liver was resolved into three components (Fig. 1). The unabsorbed activity was called α-mannosidase A and the two peaks of activity eluted by the chloride concentration gradient were named B and C. Forms A and B had very similar pH-activity curves with maxima at pH 4.4 and were about three times more active at pH 4.4 than at pH 6.0. Form C was about twice as active at its optimum pH of 6.0 as at pH 4.4.

In contrast almost all the activity present in mannosidosis liver was eluted as form C, the amount of this form being closely comparable to the

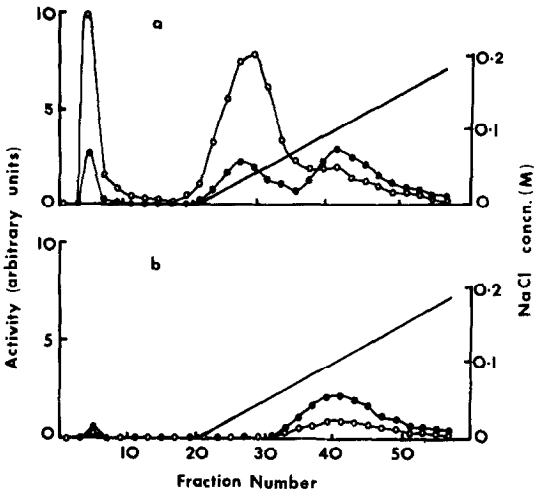

Fig. 1. DEAE-cellulose column chromatography of supernatant fractions (2 ml) from homogenates of (a) control human liver, (b) mannosidosis liver. ●, α-mannosidase activity at pH 6.0; o, α-mannosidase activity at pH 4.4; ——, NaCl gradient.

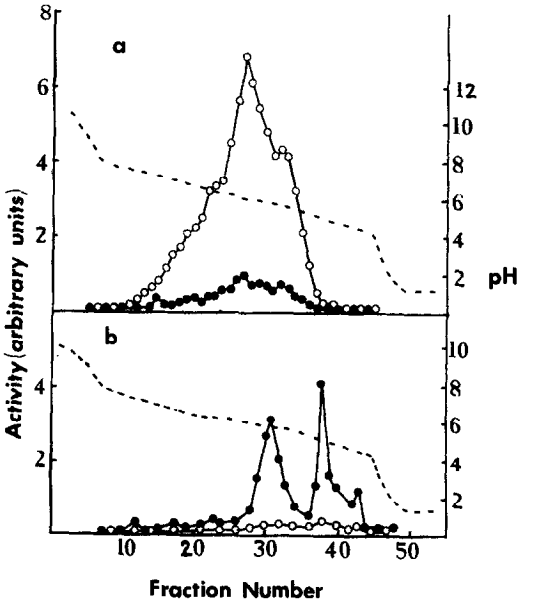

Fig. 2. Isoelectric focusing over the range pH 3-8 of supernatant fractions (2.4 ml) from homogenates of (a) control human liver, (b) mannosidosis liver. ●, α-mannosidase activity at pH 6.0; o, α-mannosidase activity at pH 4.0; ---, pH gradient.

amount of α-mannosidase C in normal samples.

In order to differentiate as far as possible between acid and neutral forms of the enzyme, the α-mannosidase activity of the eluates from the isoelectric focusing columns was assayed at pH 4.0 and 6.0 (Fig. 2). Measured at pH 4.0 the main component from normal liver had a pI of 5.9-6.0 with possible subsidiary peaks at about 5.7 and 6.3. None of the peaks showed higher activities at pH 6.0 although the ratio of 'neutral' to 'acid' activities was highest at pH 5.6, suggesting that form C was isoelectric at this point. Isoelectric focusing of mannosidosis liver gave clear peaks only when assayed at pH 6.0, two sharp peaks appearing with pI values of 5.6 and 4.9. However the latter peak was associated with a white precipitate that formed during the focusing procedure.

Discussion

From these experiments it appears that the enzymic defect in mannosidosis is a simultaneous loss of the 'acidic' A and B activities, the 'neutral' α-mannosidase C remaining normal. Similar 'acid' and 'neutral' α-mannosidase activities have been reported in rat and rabbit tissues (8) and Marsh and Gourlay (9) suggested that the 'acid' activity appeared in the lysosomal and the 'neutral' activity in the soluble fraction of rat liver homogenates. It is tempting to suppose that human α-mannosidases A and B are closely related lysosomal enzymes, under the same genetic control, while the C form, with more neutral pH optimum, is differently located in the cell. In a deficiency disease of Angus cattle that closely resembles human mannosidosis the residual activity of the affected cattle had a pH optimum of about 5.5-6.0 in contrast to the pH 4.0-4.5 optimum found normally (10), although separable forms comparable to the human enzymes have still to be reported.

The possibility that the residual α-mannosidase activity in human mannosidosis is still heterogeneous cannot be overlooked in view of the isoelectric focusing profile. It is possible however that the peak with an apparent pI of 4.9 arises from the association of α-mannosidase C of

pI 5.6 with the aggregated material that separated in this region.

This work was supported by grants from the Medical Research Council and Mental Health Research Fund.

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