Isolation of the Liver *N*-Aspartyl- β -glucosaminidase in Aspartylglucosaminuria

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(Received 30 December 1975)

The isolation of liver *N*-aspartyl- β -glucosaminidase in human aspartylglucosaminuria, where this enzyme activity is diminished, yields an enzyme molecule with the same molecular weight and pH optimum as the normal enzyme. Its activity is 10% of that of the control preparation. Combination of both enzymes results in the summation of both activities, and the pathological enzyme does not inhibit the control preparation. It is concluded that no change into a totally different isoenzyme has occurred in aspartylglucosaminuria.

The activity of *N*-aspartyl- β -glucosaminidase (aspartylglucosylaminase, EC 3.5.1.26) is diminished in aspartylglucosaminuria (Somer *et al.*, 1975). Aspartylglucosaminuria is an inborn error of metabolism characterized by mental retardation and connective-tissue lesions (Pollitt *et al.*, 1968). As a consequence of the enzyme defect the patients secrete variable amounts of glycoasparagines in their urine (Palo *et al.*, 1973).

The exact nature of the enzyme defect is not known, although no change into a totally different isoenzyme has occurred in aspartylglucosaminuria (Somer *et al.*, 1975). The present paper is aimed at the isolation of the defective enzyme molecule for its comparison with the normal enzyme.

Materials and Methods

The pathological *N*-aspartyl- β -glucosaminidase was isolated as described by Conchie & Strachan (1969) from 230 g of liver of an aspartylglucosaminuric patient who was autopsied 48 h after her death. The normal enzyme was isolated similarly from 200 g of liver from a patient who died without any liver disease. The final enzyme preparation from the above-mentioned procedure was purified further by polyacrylamide-gel electrophoresis (Ornstein & Davis, 1964).

To locate the proteins the gels were stained with 1% (w/v) Amido Black in 7% (v/v) acetic acid. The electrophoretograms were scanned and the approximate molecular weight of the enzyme was calculated (Gonenne & Lebowitz, 1975). The enzyme band was recovered from the unstained gels by mincing the appropriate area of the gel in 5 ml of 20 mM- Na_2HPO_4 /KH₂PO₄ buffer, pH 7. The suspension was stored

overnight at 4°C, and the polyacrylamide fragments were removed by centrifugation. The supernatants were collected for enzyme activity analyses.

The enzyme activity was assayed at 37°C with 2-acetamido-1- β -L-aspartamido)-1,2-dideoxy- β -D-glucose as substrate. The liberated hexosamine was determined by using the Morgan–Elson reaction (Reissig *et al.*, 1955), with glucosamine as standard. The general assay buffer was 20 mM- Na_2HPO_4 /KH₂PO₄ buffer, pH 7, except for pH-optimum experiments, when 50 mM- Na_2HPO_4 /KH₂PO₄ buffer of appropriate pH was used. The final substrate concentration in the reaction mixture was 2.8 mM. Total protein was measured by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

For combination experiments both enzyme preparations before the electrophoretic separation were mixed in equal amounts of protein and the resulting enzyme activity was measured as described above. Further, the pathological *N*-aspartyl- β -glucosaminidase was also combined with the electrophoretically purified normal enzyme and the resulting activity was similarly assayed.

Results and Discussion

The *N*-aspartyl- β -glucosaminidase of the control liver homogenate liberates 0.9 nmol of glucosamine/min per mg of protein, whereas the activity in the diseased liver is 0.13 nmol/min per mg of protein. The control enzyme activity before the final electrophoretic separation is 192 nmol of glucosamine liberated/min per mg of protein, and the pathological enzyme at this step liberates 20 nmol/min per mg of protein.

The activity of the normal enzyme as recovered from the gel is 205.2 nmol of glucosamine liberated/min per mg of protein, and that of the pathological

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enzyme is 20nmol/min per mg of protein. This indicates 228-fold and 155-fold purification for the control and the pathological enzyme preparations respectively.

The electrophoresis reveals minor protein contaminants in the control preparation (Plate 1a). The contaminant proteins display no 2-acetamido-1-(β -L-aspartamido)-1,2-dideoxy- β -D-glucose-cleaving activity after their extraction from the gel. They amounted to 20% of the total protein in the control preparation, but in the aspartylglucosaminuria sample their amount is negligible (Plate 1b). In the pathological enzyme preparation the sole 2-acetamido-1-(β -L-aspartamido)-1,2-dideoxy- β -D-glucose-cleaving activity is associated with the major protein band in the electrophoretogram. The molecular weight of both the control and the pathological N-aspartyl- β -glucosaminidase is 150000.

The pH optimum of the control human liver N-aspartyl- β -glucosaminidase is similar to that found for the purified rat liver enzyme (Conchie & Strachan, 1969). The activity of the enzyme at pH7 is 205.2nmol of glucosamine liberated/min per mg of protein, whereas at pH6 it is 92% (188.8nmol/min per mg of protein) and at pH8 90% (186nmol/min per mg of protein) of the highest activity; at pH9 the activity is even less.

The pathological enzyme has a similar pH optimum, although differences are more conspicuous. At pH7 the activity is 20nmol/min per mg of protein, whereas at pH6 it is 16 and at pH8 14.5nmol/min per mg of protein.

Combination of the pathological enzyme with the whole normal enzyme preparation and with the electrophoretically separated enzyme results in the summation of both activities. When the assay is

carried out at pH7 the sum activity is 106nmol/min per mg of protein for the pathological enzyme plus the preparation before electrophoresis, and it is 112nmol/min per mg of protein for the pathological enzyme plus the electrophoretically separated normal enzyme. Thus the normal enzyme cannot restore the pathological enzyme activity to normal. On the other hand, the pathological enzyme does not inhibit the normal enzyme. The minor contaminating proteins are not essential to the normal enzyme function, nor can they restore the pathological activity to normal, as both combination experiments give similar results.

The electrophoresis of the normal enzyme obviously causes some loss in its activity, as the observed activity of the separated enzyme is less than would be expected if the contaminating proteins are assumed to be removed by other means.

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EXPLANATION OF PLATE I

(a) *Polyacrylamide disc gel electrophoretograms of normal and aspartylglucosaminuria N-aspartyl- β -glucosaminidase preparations at pH 8.3 and (b) scanning curves of these electrophoretograms*

(a) A 100 μ g sample of control and 200 μ g of pathological enzyme preparation were applied on to the gel. Cathode is at the top. One major molecule is seen in the aspartylglucosaminuria preparation, whereas the control displays a few contaminant proteins. (b) Cathode is on the right. The contaminant proteins constitute 20% of the total protein in the control preparation. The contamination in the aspartylglucosaminuria sample is negligible.