THE HYDROLYSIS OF TAY-SACHS GANGLIOSIDE (TSG) BY HUMAN *N*-ACETYL-β-D-HEXOSAMINIDASE A

K.SANDHOFF

Max-Planck-Institut für Psychiatrie, Neurochemische Abteilung, München, Germany

Received 2 November 1970

In human tissues two main *N*-acetyl-β-D-hexosaminidases, A and B, have been found [1, 2]. A previous investigation [3] has shown that the normal pattern of these enzymes is altered in tissues of patients afflicted with Tay-Sachs disease. Three different types of enzyme pattern have been observed, corresponding to three variants of Tay-Sachs disease. All three variants are characterized by a strong accumulation of TSG (GalNAc*-β-1,4-[NeuNAc-2,3]-Gal-1,4-Glc-1,1-[2-*N*-acyl] sphingosine) and a minor one of trihexosylceramide (GalNAc-β-1,4-Gal-1,4-Glc-1,1-[2-*N*-acyl] sphingosine). In two of these variants a defect of *N*-acetyl-β-D-hexosaminidase A-activity was stated [4–6]. Up to now the enzymatic degradation of TSG had been established only with extracts from calf brain [7] and from human muscle [8], the latter, containing both *N*-acetyl-β-D-hexosaminidases A and B. In this paper it will be shown that the human *N*-acetyl-β-D-hexosaminidase A is responsible for the *in vitro* degradation of TSG. This was done with a purified enzyme preparation.

Fig. 1 shows the scheme for the purification of *N*-acetylhexosaminidases A and B from postmortem human liver. The A and B enzyme forms were enriched 3000-fold and 1000-fold respectively; the details of the purification procedure will be presented elsewhere. One mg of the purified enzyme A could hydrolyze 138.5 μmoles of *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide and 1.4 μmoles of trihexosylceramide per minute. One mg of the purified enzyme B splits 51.7 μmoles and 0.65 μmole per min of the same respective substrates. Both enzymes are also capable of acting upon kidney globoside (GalNAc-β-1,3-Gal-1,4-Gal-1,4-Glc-1,1[2-*N*-acyl] sphingosine). This lipid is additionally accumulated in the visceral organs of the variant of Tay-Sachs disease with both enzyme forms A and B missing [9].

The substrate, TSG, was radioactively labelled by hydrogenation of the sphingosine moiety with tritium gas and purified by column chromatography with silicic acid and florisil [10]. The specific activity of the labelled substrate was determined by dilution with non-radioactive TSG according to Snyder and Stephens [11] in a liquid scintillation counter and found to be 5500 cpm/nmole.

This radioactive TSG was then used to study the action of the purified enzyme preparation. 2 units** of *N*-acetyl-β-D-hexosaminidase A were incubated 24 hr at 37° with 40 μg TSG, 2.5 mg of a crude sodium taurocholate (Schuchardt, Munich), 50 μl 1 M citrate buffer in a final volume of 0.5 ml at pH 4.7. Subsequently, 40 μl aliquots of the incubation mixtures were analysed by thin-layer chromatography. The thin-layer plates were prepared from a suspension of 60 g Kieselgel G (Merck, Darmstadt) in 128 ml 0.06 M disodium tetraborate solution, air-dried and activated before use by treatment at 120° for 3 hr.

* *Abbreviations*:
Gal : D-galactose;
GalNAc : *N*-acetyl-D-galactosamine;
Glc : D-glucose;
NeuNAc: *N*-acetylneuraminic acid.

** One unit of *N*-acetyl-β-D-hexosaminidase hydrolyzes 1 μmole *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide in 0.1 M citrate buffer, pH 4.4 containing 0.01% albumin, in 1 min at 37°.

North-Holland Publishing Company - Amsterdam

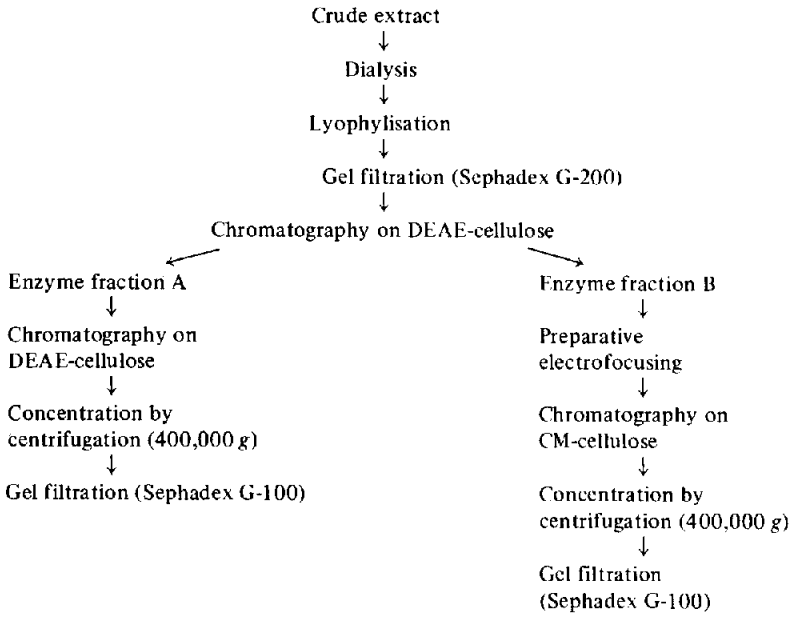

Fig. 1. Scheme for the purification of *N*-acetyl-β-D-hexosaminidases A and B from postmortem human liver.

The solvent system for the separation of the reaction products consisted of butanol–ethanol–0.01 M disodium tetraborate (20:10:7). The distribution of the radioactivity was determined by a scanning apparatus (Berthold, Wildbad). Under these conditions two radioactivity peaks were found, one (fig. 2, I) with the same mobility as TSG, and another peak (II) with a higher mobility. The unknown, labelled substance corresponding to peak II was eluated from an appropriately excised portion of the thin-layer material with chloroform–methanol (2:1) and subjected to further thin-layer chromatography. This substance migrated in three different solvent systems† with the same mobility as the ganglioside G_{M3}††(NeuNAc-2,3-Gal-1,4-Glc-1,1-(2-*N*-acyl) sphingosine) which was a generous gift from Dr. Wiegandt.

It seems likely then that by the action of *N*-acetyl-β-D-hexosaminidase A, labelled TSG (gangliodise G_{M2}) was transformed into labelled ganglioside G_{M3}. Under the conditions used 0.1 nmole TSG/min/mg protein are hydrolyzed. A significant hydrolysis was found between pH 4.2 and 5.0, the optimum was about 4.8. The reaction could not be observed in a control experiment in which the enzyme was first heat denatured (100°/10 min) and then incubated with the substrate (fig. 2).

It is possible that a slow conversion of the enzyme A to an enzyme form with the electrophoretic behaviour of the enzyme B as described by Robinson and Stirling [1] and as confirmed by us for the purified enzyme is also an active process during the 24 hr test incubation at 37°. It remains, therefore, questionable whether the enzyme A in its original or in its changed, enzyme B like form is responsible for the degradation of TSG. With the enzyme B itself as prepared according to fig. 1 we observed no significant degradation of TSG. It seems likely then that the enzyme A, unchanged or changed, must be responsible for the degradation of TSG.

From these *in vitro* experiments it seems reasonable that *N*-acetyl-β-D-hexosaminidase A could also catabolize TSG *in vivo*. The defect of this enzyme could then be the main cause for the accumulation of TSG in the neural tissue of the Tay-Sachs patients. The additional defect of the enzyme B in another

† Besides the system mentioned, the following systems were used: (1) Kieselgel G with propanol–water (7:3); (2) Kieselgel G with chloroform–methanol–water (120:80:13).

†† The ganglioside nomenclature of Svennerholm [12] is used.

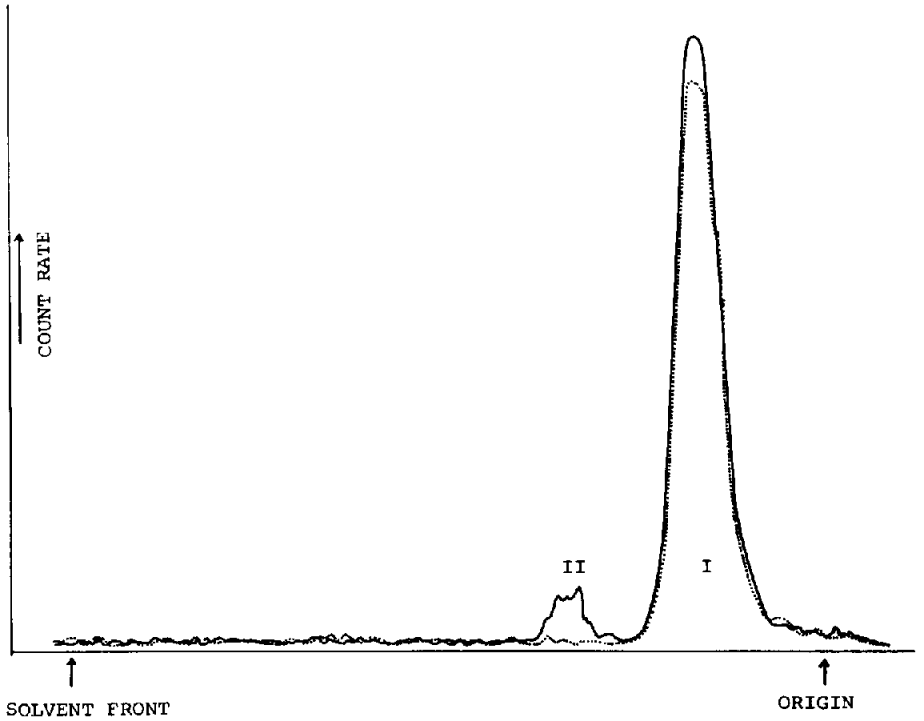

Fig. 2. Scan of a thin-layer radiochromatogram showing the result of the action of *N*-acetyl-β-D-hexosaminidase A on tritium-labelled Tay-Sachs ganglioside (= ganglioside G_{M2}). Experimental conditions see text. ——, active enzyme preparation; ....., heat denatured enzyme preparation. Peak I = Tay-Sachs ganglioside (ganglioside G_{M2}); peak II = degradation product with the mobility of ganglioside G_{M3}.

variant of Tay-Sachs disease with visceral involvement [3] could explain the concomitant accumulation of trihexosylceramide and of kidney globoside which both are substrates of the enzymes A and B.

Acknowledgements

I wish to thank Miss H.Nehrkorn and Mrs. C.Vogel, for skilled technical assistance.

References

[1] D.Robinson and J.L.Stirling, Biochem. J. 107 (1968) 321.
[2] K.Sandhoff, Z. Physiol. Chem. 349 (1968) 1095.
[3] K.Sandhoff, FEBS Letters 4 (1969) 351.
[4] S.Okada and J.S.O'Brien, Science 165 (1969) 698.
[5] B.Hultberg, Lancet II (1969) 1195.
[6] E.P.Young, R.B.Ellis, B.D.Lake and A.D.Patrick, FEBS Letters 9 (1970) 1.
[7] Y.Z.Frohwein and S.Gatt, Biochemistry 6 (1967) 2783.
[8] E.H.Kolodny, R.O.Brady and B.W.Volk, Biochem. Biophys. Res. Commun. 37 (1969) 526.
[9] K.Sandhoff, U.Andreae and H.Jatzkewitz, Pathol. European 3 (1968) 278.
[10] K.Sandhoff and W.Wässle, in preparation.
[11] F.Snyder and N.Stephens, Anal. Biochem. 4 (1962) 128.
[12] L.Svennerholm, J. Neurochem. 10 (1963) 613.