# CHARACTERISATION OF HUMAN *N*-ACETYL-β-HEXOSAMINIDASE C

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## 1. Introduction

Besides the well-known *N*-acetyl-β-hexosaminidase A and B forms that occur widely in human tissues [1] there have from time to time been reports of further forms of this enzyme in particular physiological states, e.g. the presence of hexosaminidase P in pregnancy serum [2].

Particular attention has been paid to the nature of hexosaminidases in brain tissue in view of effects of the genetic hexosaminidase deficiencies in Tay Sachs and Sandhoff's diseases. Two recent reports [3, 4] have shown that a further form, hexosaminidase C can be detected in foetal and adult brain and in some other tissues by electrophoresis on cellulose acetate supports. This form of the enzyme has a more anodic electrophoretic mobility than hexosaminidase A and is most apparent during early life, being often difficult to detect in adult tissues.

The present report is a collaborative investigation by workers from three groups interested in this family of enzymes in an attempt to establish some characteristics of this new iso-enzymic form.

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## 2. Materials and methods

### 2.1. *Materials*

Brain and other tissues from normal individuals and from cases of Tay Sachs disease were obtained at post mortem and stored at −20°C until required. Homogenates (50% w/v) were made in the appropriate buffers for the subsequent treatment and centrifuged in an M.S.E. 18 refrigerated centrifuge for 30 min at 15 000 rpm and 4°C, the supernatant being used as the enzyme preparation.

### 2.2. *Enzyme assay*

Samples (20 μl) were incubated with a buffered solution of 4-methylumbelliferyl-β-D-*N*-acetylglucosaminide at a final concentration of 1 mM in a total volume of 120 μl. After 30 min at 37°C the reaction was stopped by the addition of 2 ml of 0.05 M glycine previously adjusted to pH 10.4 by the addition of 0.05 M NaOH. The fluorescence of the liberated aglycone was measured on a Locarte S.F. 5 fluorimeter.

### 2.3. *Electrophoresis*

Starch-gel electrophoresis at pH 7.0 and subsequent detection of enzymic activity was carried out as described by Robinson et al. [5]. Cellogel acetate electrophoresis was carried out at pH 6.6 in Cellogel strips (Chemetron, Milan, Italy) by the method of Poenaru and Dreyfus [4] and at pH 5.0 in Sepharose III strips (Gelman Instruments Co., USA) by the method of Hooghwinkel et al. [3].

### 2.4. *Serological methods*

Antisera to human liver hexosaminidase A and to a mixture of human liver hexosaminidases A and B were prepared by Carroll and Robinson [6]. Antisera raised to human placental hexosaminidase A were prepared by Poenaru, Weber and Dreyfus [7]. Any rabbit hexosaminidase occurring in the antisera was inactivated by incubation at 56°C for 1 hr before any serological tests were carried out. The testing of such antisera for precipitation of hexosaminidase has been previously described by Carroll and Robinson [6].

### 2.5. *Ion exchange chromatography*

Columns of Whatman DFAE 22 ion-exchange cellulose (W. R. Balston Ltd.) were equilibrated in 0.01 M sodium phosphate buffer pH 7.0 before chromatography of tissue supernatants in the same buffer. Fractions were eluted in a salt gradient rising to 0.5 M NaCl in the same buffer. Hexosaminidase B was eluted in 10 ml from a 10 cm × 1 cm column in this way and hexosaminidase A eluted at 44 ml, 34 ml from the start of the emergence of the salt gradient. Details of the method have been published previously [8].

## 3. Results and discussion

The most anodic band of hexosaminidase C could be demonstrated by all three methods of electrophoresis. The 'Cellogel' method gave the clearest separations and was the easiest to perform consistently. In addition to the A and B forms, the C form was seen to be present in normal adult brain as well as in foetal brain and neonatal spleen. A considerable amount of the C form was present in foetal Tay Sachs brain suggesting that the enzyme is unrelated to hexosaminidase A which is absent in this disease. Hexosaminidase C is also present, possibly in increased amounts, in the tissues of patients with complete deficiency of hexosaminidases A and B (Sandhoff's disease). Fig. 1 shows the results of a cellulose acetate electrophoresis of leukocytes extract from a child with Sandhoff's disease, his mother and a normal control. In this disease, hexosaminidase C is the only visible isozyme. The independent nature of C confirmed by the action of antisera raised to other hexosaminidase iso-enzymes. When these antisera were added to samples containing hexosaminidase C and the other forms only the A and B forms were precipitated. The supernatants from the immunoprecipitation were shown to contain the hexosaminidase C originally present. The same results were obtained whether normal brain containing forms A, B and C or Tay Sachs brain with form B and C were used.

Attempts to purify hexosaminidase C by ion exchange chromatography on DEAE cellulose were entirely unsuccessful even though the method gave good recovery of the A and B forms from normal brain and the B form from Tay Sachs brain. Form C appears to be either denatured or irreversibly bound to the ion-exchanger under these conditions. The former explanation is most likely since no enzymic activity could be detected when the column support was incubated with substrate after the above separations had been carried out.

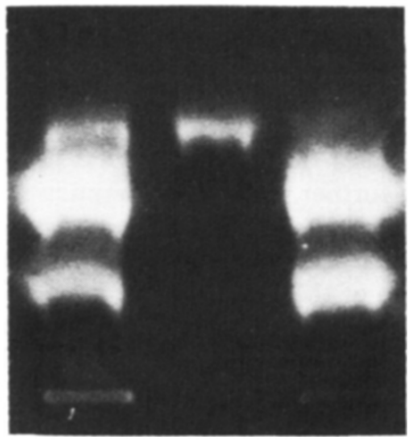


Fig. 1. Cellogel electrophoresis of leukocyte hexosaminidase. (1) Sandhoff heterozygote mother, (2) Sandhoff affected offspring of (1), (3) Normal control. The separation method is that of Poenaru and Dreyfus [4].

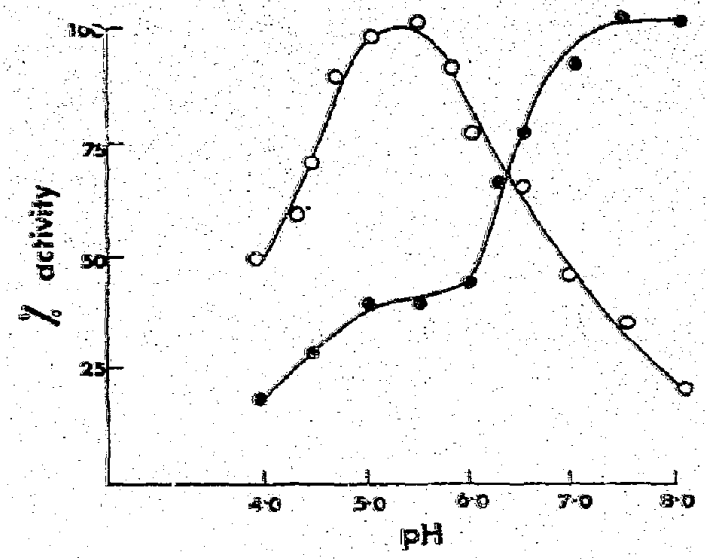


Fig. 2. pH-activity profile for human hexosaminidase. The effect of variation of pH on liver hexosaminidase B (o) and brain hexosaminidase C (•) prepared by immunoprecipitation of the other isoenzymes, was studied as described in the text.

A preparation of hexosaminidase C free of the A and B forms and suitable for preliminary characterisation purposes could be obtained however by use of the above antisera. Thus when a 50% w/v homogenate of normal brain tissue was incubated at 4° C for 16 hr with a suitable amount of anti-hexosaminidase A and B serum those two enzymic forms were almost completely removed from solution. After centrifugation the supernatant appeared to contain only hexosaminidase C on electrophoresis and maintained its activity and physical characteristics for at least 2 weeks at 4°C.

One reason why the presence of this activity may be overlooked is that it has a different pH optimum. Other hexosaminidases have been found to have typical optima of 4–5 as expected for lysosomal enzymes. Hexosaminidase C prepared as above has optimal activity at pH 6–7 (fig. 2) in agreement with the earlier results of Overdijk [9]. Its contribution to the total activity measured at the more usual acid pH's may be relatively small though significant under natural conditions. Thus when 225 units of activity from normal human brain were treated with the above antisera only 15.6 units as measured at pH 4.5 remained in solution. When the activity of the starting material was assayed at pH 7.0 it contained 95 units of which 70 units remained after immunoprecipitation. One may calculate that when each enzyme is measured at its pH optimum there is approximately three times as much hexosaminidase A and B together as there is hexosaminidase C and the latter enzyme no longer is insignificant.

The origin and function of hexosaminidase C requires explanation. Its presence is most readily detected in foetal and neonatal tissue and particularly in brain. Since it is present in Tay Sachs brain it may be suggested that it is ineffective in the hydrolysis of ganglioside $GM_2$ which accumulates in that condition. This is not surprising if hexosaminidase C is specific for glucosaminide structures since the terminal sugar of $GM_2$ has a galactosaminide configuration. The enzyme may be related to the early reports of Frohwein and Gatt [10] of evidence for specific β-glucosaminidases and β-galactosaminidases in calf brain.

A current hypothesis for the interrelations of hexosaminidases A and B proposes two different subunits, one of them common to both enzymic forms and the other being particular to the A form [11]. While this hypothesis readily explains the enzyme defect in Tay Sachs disease (specific unit defective) and in Sandhoff's disease (common unit defective) it is difficult to incorporate hexosaminidase C. Its different pH optimum, substrate specificity and immunological response all point to an independent genetic origin.

## Acknowledgements

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