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N-Acetyl-β-D-hexosaminidase Component A

DIFFERENT FORMS IN HUMAN TISSUES AND FLUIDS

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1. Hexosaminidase A of human serum was resolved into two components, a minor form with properties identical with those of the single hexosaminidase A component of human liver, and a major form with significantly different properties. 2. The major serum hexosaminidase A form was eluted from a DEAE-cellulose column at a lower salt concentration than that required to elute the liver form. 3. A multiple-pass technique was used to elute the major serum enzyme A from a Sephadex G-150 column before that of liver enzyme A. 4. *Clostridium perfringens* neuraminidase converted the major component of serum hexosaminidase A into a form that was held less tightly by DEAE-cellulose, but the minor component of the A enzyme of serum, and the A enzyme of liver were not affected. 5. The hexosaminidase A from tears was similar to the A enzyme from serum, whereas those from several human tissues and from urine and lymph were similar to the liver form. 6. The A enzyme from serum may be derived from the A enzyme from liver by glycosylation before secretion.

Robinson & Stirling (1968) found that the *N*-acetyl-β-D-glucosaminidase activity (EC 3.2.1.30) of human tissue extracts could be attributed to two components, termed A and B, which could be resolved by starch-gel electrophoresis or anion-exchange chromatography, or distinguished by their different heat stabilities. They also found that the *N*-acetyl-β-galactosaminidase activity could not be distinguished from that of *N*-acetyl-β-glucosaminidase and therefore used the term 'hexosaminidase' to describe either activity. Interest in this enzyme has been greatly stimulated by the demonstration of a specific deficiency of component A in tissues from most cases of Tay–Sachs disease (Okada & O'Brien, 1969; Sandhoff, 1969; Hultberg, 1969) and a deficiency of both components in a variant form of the disease (Sandhoff, 1969). In addition to the major A and B forms, at least two minor components are present in tissue extracts and serum (Sandhoff, 1968; Young *et al.*, 1970; Price & Dance, 1972). The relationship between the various components is not clear, but studies on the action of neuraminidase indicate that hexosaminidase A contains sialic acid residues, and that their removal produces derivatives of similar electrophoretic mobility to the intermediates and to the B form (Robinson & Stirling, 1968; Goldstone *et al.*, 1971; Murphy & Craig, 1972). The immunological properties of hexoaminidases A and B are closely similar (Carroll & Robinson, 1973). It is implicit in previous reports that hexosaminidase A is the same in all human tissues and body fluids examined but evidence is now presented that at least two different forms occur. The separation and properties of the two forms of component A are described.

Experimental

The use of human tissues and fluids was approved by the Standing Committee on Ethical Practice of the Hospital for Sick Children, Great Ormond St., London.

Materials

Tissues, obtained within a few hours of death, were cut into slices not more than 1 cm thick, wrapped in aluminium foil, quickly frozen between blocks of solid CO_2, and stored at −20°C. For the preparation of serum, the blood of healthy adults was left to clot in glass tubes at room temperature for 30 min and then centrifuged at 3000*g* for 10min. The supernatant was removed, rapidly frozen in a methanol–solid CO_2 bath, and stored at −20°C. Leucocytes were prepared by the method of Kampine *et al.* (1967). For the collection of tears, four Shirmer Tear Test strips (Cooper Laboratories Inc., Wayne, N.J., U.S.A.) were kept in the eyes of a healthy adult until fully wet. Hexosaminidase activity was extracted by gentle shaking of the strips in 1.0ml of 10mM-phosphate buffer (NaH_2PO_4 adjusted to pH6.0 with NaOH) for 5min. Lymph was obtained from a child during surgery of a cystic hygroma.

Methods

Hexosaminidase assay. This was carried out as previously described (Young *et al.*, 1970).

DEAE-cellulose chromatography. Preparative and analytical runs were carried out by slight modifications to the method previously described (Young

et al., 1970). The flow rate could be greatly increased without significantly affecting the resolution of hexosaminidase components. For preparative runs, a column (30cm × 1.5cm internal diam.) of Whatman DEAE-cellulose (type DE-52, W. and R. Balston Ltd., Maidstone, Kent, U.K.) was equilibrated with 10mM-phosphate buffer (NaH_2PO_4 adjusted to pH6.0 with NaOH) at 2°C and liver supernatant [from 3ml of a 10% (w/v) homogenate in ice-cold water centrifuged at 2000*g* for 10min] or serum (10ml of non-diffusible material after dialysis at 2°C for 18h against 10mM-phosphate buffer, pH6.0) was applied. Phosphate buffer (10mM, pH6.0; 40ml) followed by a linear KCl gradient [prepared by connecting 10mM-phosphate buffer, pH6.0 (50ml), to an equal volume of 300mM-KCl in the same buffer in a gradient maker] were pumped through the column at 144ml/h. Fractions (4ml) were collected. Fractions from each peak of hexosaminidase activity were combined and concentrated sixfold with polyethylene glycol (PEG 6000, BDH Chemicals Ltd., Poole, Dorset, U.K.). The concentrates were frozen in a methanol–solid CO_2 bath and stored at −20°C. They were dialysed over-night before rechromatography. For analytical runs, body fluids and combined fractions from preparative columns were dialysed as described above for serum. Tissues were homogenized in ice-cold water and the supernatant used. Samples containing 1–10% of the hexosaminidase activities used for preparative work were applied to columns (15cm × 0.9cm internal diam.). Other conditions are given in the legend to Fig. 1.

Gel filtration. Recycling gel-filtration chromatography was carried out by the method of Porath & Bennich (1962) on a column (100cm × 2.5cm internal diam.) of Sephadex G-150 equilibrated at 2°C with 10mM-sodium phosphate buffer, pH7.6 (Gomori, 1955), made 400mM with respect to NaCl and 10mM to EDTA, at a flow rate of 15ml/h. The position of the peak of hexosaminidase activity was determined from a single-pass exploratory run, then, during the first pass, the leading and trailing edges were bled off at appropriate stages. The remaining activity (about 80% of the total applied) was cycled a further three times through the column before 3.2ml fractions were collected. Combined fractions were concentrated fivefold with PEG 6000, dialysed at 2°C for 18h against 10mM-phosphate buffer, pH6.0, and rechromatographed on analytical DEAE-cellulose columns.

Treatment with neuraminidase. Hexosaminidase component A of serum (10% of sample recovered from a preparative DEAE-cellulose column) or liver (2% of sample), or mixtures of the two, were incubated at 37°C with neuraminidase from *Clostridium perfringens* (0.25mg; Sigma fraction V; shown to be free of hexosaminidase activity; Sigma Chemical Co., Kingston-upon-Thames, Surrey, U.K.) in 1.0ml of McIlvaine's citric acid–sodium phosphate buffer, pH5.5 (Gomori, 1955), for 1.5 or 3h. The reaction mixtures were dialysed for 18h at 2°C against 10mM-sodium phosphate buffer, pH6.0, before chromatography on analytical DEAE-cellulose columns. Controls without added neuraminidase were treated in the same way.

Results

Ion-exchange chromatography

Two major peaks of hexosaminidase activity were seen after chromatography of normal serum or tissue extracts on anion-exchange cellulose columns (Fig. 1). The first component, which passed straight through the column, is equivalent to that defined as component B detected after starch-gel electrophoresis (Robinson & Stirling, 1968). The final component, eluted with the highest concentration of KCl on the linear gradient, is equivalent to component A. Two intermediate components of lower activity, I_1 and I_2, were eluted between the B and A peaks as previously described (Young *et al.*, 1970) and confirmed by Price & Dance (1972). These intermediates were most prominent in brain extracts (Fig. 1).

The positions of the peak maxima were highly reproducible (±one fraction) when repeated runs on the same type of sample were made. Thus the elution of serum hexosaminidase A (see line S in Fig. 1) six fractions earlier than component A of liver (see line T in Fig. 1) is significant and was confirmed by the chromatography of a mixture of hexosaminidase A components from serum and liver. No interaction appeared to have occurred; two partially separated peaks were seen and their maxima in fractions 34 and 40 corresponded to the positions of these components when chromatographed separately.

The peaks of hexosaminidase activity of tears were eluted from DEAE-cellulose at the same positions as those of serum, whereas human brain, spleen, kidney, leucocytes, urine, adrenal, pancreas, lymph node and lymph all gave peaks at the same positions as those of liver, though variations in the relative activities of the components were found in different tissues. Saliva gave the most complex hexosaminidase pattern of all materials examined, with major peaks corresponding to both serum and liver forms.

Though the hexosaminidase A peak of all tissues studied was symmetrical, that of serum trailed and in some runs a definite shoulder, suggestive of a minor component, was observed. When combined fractions from the front, middle or back regions of the peak of component A of liver from a preparative run were dialysed, concentrated and rechromatographed on analytical columns, a single peak with maximum activity in tube 40 (as for whole liver, see Fig. 1) was

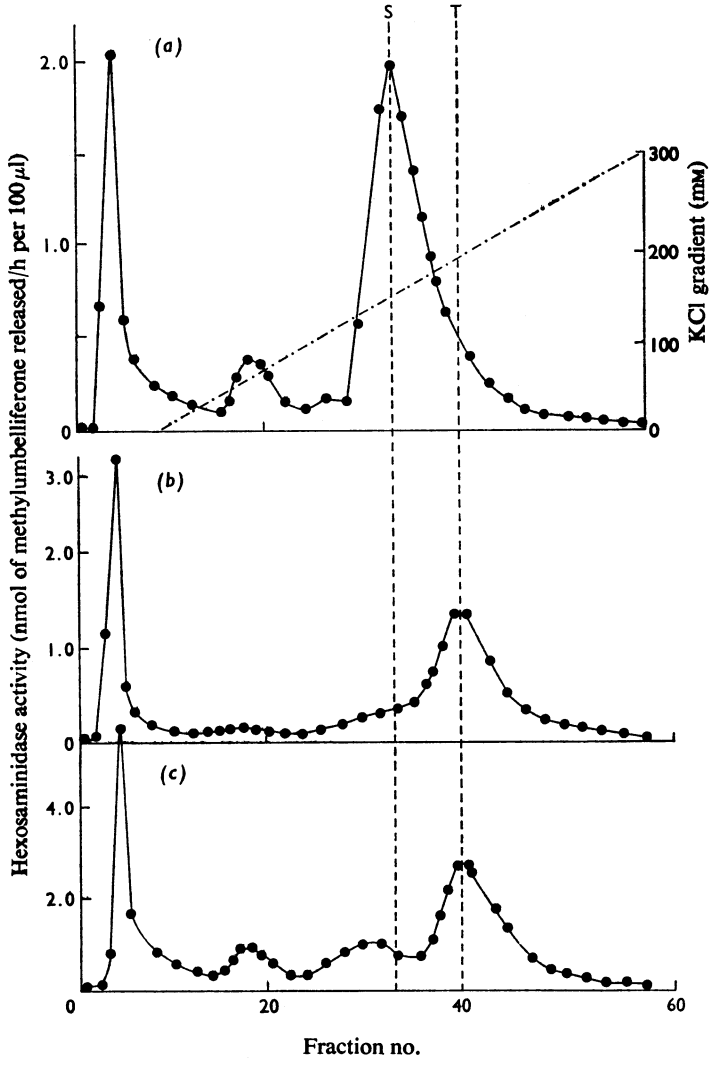

Fig. 1. *Resolution of hexosaminidase components by DEAE-cellulose chromatography*

Elution profiles are of (*a*) serum (1.0ml of non-diffusible material), (*b*) liver supernatant (1.0ml from a 1% homogenate) and (*c*) brain supernatant (1.0ml from a 5% homogenate). Other details are given under 'Methods'. Hexosaminidase activity was assayed as described by Young *et al.* (1970) and is expressed as nmol of methylumbelliferone released/h per 100µl of each fraction. All work was done in a cold-room at 2°C. The flow rate was 144ml/h and fraction size was 2.0ml. The KCl gradient (–·–·–) shown in (*a*) was identical for runs (*b*) and (*c*); 10mM-Na_2HPO_4, adjusted to pH6.0 with NaOH, was present throughout. The vertical line labelled 'S' is drawn through the peak of the A form of serum activity and that labelled 'T' passes through the peaks of the A form of tissue enzyme.

obtained in all cases. In contrast, fractions from the front and peak regions of serum component A rechromatographed as a single symmetrical peak with maximum activity in tube 34 (as for whole serum), but rechromatography of fractions from the tail region of serum component A gave a peak in tube 40 (at the position of the peak for tissue hexosaminidase A). Further work on the characterization of this minor component of serum hexosaminidase A is described below.

Though the major serum component A was eluted at a chloride ion concentration only slightly higher than that required for the elution of the intermediate component I_2 of liver, these were shown to be different by a study of cases of Tay–Sachs disease. Liver and serum hexosaminidase A were absent in cases of this disease, whereas component I_2, together with components B and I_1, were all present.

Gel filtration

Only one symmetrical peak of hexosaminidase activity was eluted from a Sephadex G-150 column after a single pass of a mixture of equal activities of hexoaminidase A prepared from serum and liver by ion-exchange chromatography. However, rechromatography on DEAE-cellulose of combined fractions from the leading and trailing edges of the Sephadex peak indicated that the A components of serum and liver were not evenly distributed within this peak; serum enzyme A was predominantly in the front and liver enzyme A in the rear portions.

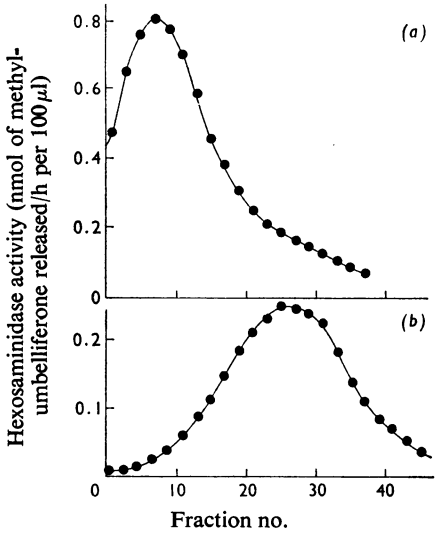

Fig. 2. *Gel-filtration behaviour of hexosaminidase A with the multiple-pass technique*

The purified forms used were prepared on DEAE-cellulose columns as described under 'Methods'. Elution profiles are of (*a*) the A enzyme from serum (50% of activity recovered from a preparative run) and (*b*) the A enzyme from liver (20% of activity from a preparative run) on Sephadex G-150 after four passes through the column. The flow rate was 15ml/h and all runs were done in a cold-room at 2°C. Fraction size was 3.2ml. Hexosaminidase activity is expressed as nmol of methylumbelliferone released/h per 100µl of each fraction. Conditions were as described in the text. The collection of fractions was started 46h after loading the sample.

The difference between the times required for the elution of hexosaminidase A components of serum and liver was greatly increased by the use of the recycling technique of Porath & Bennich (1962). When four passes through the Sephadex column were made, the peak activity of serum hexosaminidase A was eluted 15 fractions before that of the A enzyme of liver (Fig. 2). The difference in elution times between hexosaminidase A of serum and liver was confirmed by gel filtration of a mixture of the two by the recycling technique; though the separation was incomplete, two peaks were eluted at the same positions as those found for the components when run separately and their origin was confirmed by rechromatography on DEAE-cellulose.

The serum hexosaminidase A peak obtained after four passes through the Sephadex column had a tail extending through the region in which the liver component was eluted (Fig. 2). When combined fractions (nos. 25–35) from this tail were rechromatographed on DEAE-cellulose the peak activity was eluted in tube 40 at a position corresponding to that given by liver hexosaminidase A. However, combined fractions from either the front (fraction nos. 1–5) or central (fraction nos. 10–15) parts of the main serum peak from Sephadex gave maximal activity from DEAE-cellulose in tube 34, a position corresponding to that of the major serum hexosaminidase A.

When whole serum was subjected to recycling chromatography on Sephadex, no significant separation of the hexosaminidase B, I_1, I_2 and A components was found. A similar result was obtained with crude liver supernatant.

Treatment with neuraminidase

There was no loss of hexosaminidase activity when either serum or liver component A was incubated with neuraminidase from *C. perfringens* for periods of up to 3h. No change in hexosaminidase A of liver was detected by chromatography on DEAE-cellulose; it was eluted as a single peak with maximum activity in tube 40 (see line T in Fig. 3*c*), identical with that of the untreated control (Fig. 3*d*). In contrast, after incubation with neuraminidase for only 1.5h under the same conditions, about 90% of the activity of serum hexosaminidase A was eluted in a peak with maximum activity in tube 29 (line R in Fig. 3*a*), five fractions before the position of the peak in the untreated control (line S in Fig. 3*b*). No further change took place when the incubation period with neuraminidase was increased to 3h; the main peak was again centred around tube 29 and the other 10% of serum hexosaminidase activity remained as a well-

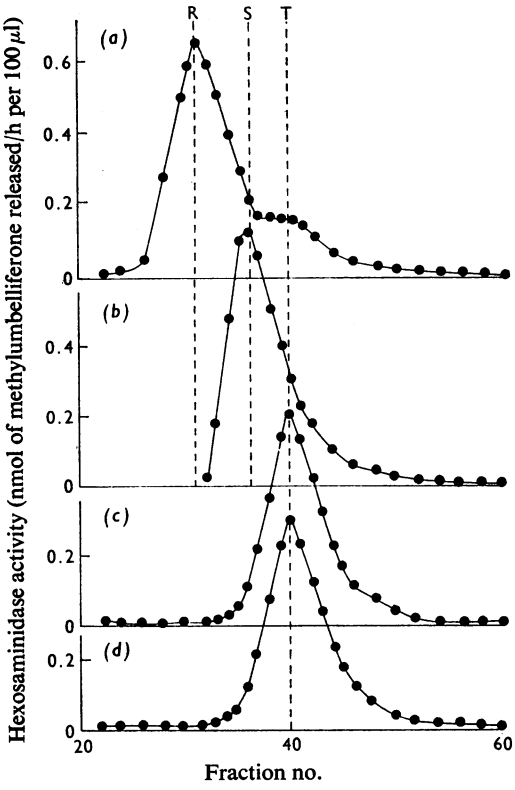

Fig. 3. *Action of neuraminidase of C. perfringens on hexosaminidase A*

Elution profiles on analytical DEAE-cellulose columns are of (*a*) the A enzyme from serum and (*c*) the A enzyme from liver after incubation with neuraminidase for 3h at 37°C. As controls, (*b*) the A enzyme from serum and (*d*) the A enzyme from liver were incubated under the same conditions, but without the addition of neuraminidase. Conditions are as described in the text and in Fig. 1. Fraction size was 2.0ml. No significant hexosaminidase activity was eluted in fractions 1–20. The vertical line 'R' is drawn through the new peak obtained after treatment of the A enzyme from serum with neuraminidase; lines 'S' and 'T' are as described in Fig. 1.

defined, though incompletely resolved, peak with maximum activity in tube 40 (line T in Fig. 3*a*).

When hexosaminidase A components of equal activity from serum and liver were mixed, treated with neuraminidase for 3h, and chromatographed on DEAE-cellulose, two peaks of similar size, with maxima in tubes 29 and 40, were obtained. This result is consistent with each component behaving exactly as it did when treated separately and suggests that the resistance of the liver component to neuraminidase was not due to an endogenous inhibitor.

Discussion

Evidence from three types of experiment is presented to support the conclusion that at least two forms of hexosaminidase component A can be isolated from human sources. Tissues such as liver, kidney, spleen and brain apparently contain only one form whereas serum contains a minor component similar to that of tissues and a major component of a significantly different type.

The difference between tissue and serum hexosaminidase A was first demonstrated by anion-exchange chromatography; the major serum form was eluted at a lower salt concentration than that needed to release the tissue form. This suggests that the tissue form has a relatively higher complement of negatively charged residues. The minor component of serum hexosaminidase A was eluted at the same salt concentration as that required for the tissue component. Similar separations were achieved by gel filtration after improvement of the resolution by a recycling technique; the main serum component was eluted before that of liver and the minor serum component corresponded to that of liver. Though this result could be ascribed to the major serum form having a slightly higher molecular weight than the liver form, the probable glycoprotein nature of these enzymes (Goldstone & Koenig, 1970) precludes any accurate estimate of the difference between them (Andrews, 1965).

Liver and serum hexosaminidase A were further distinguished by the difference in their susceptibility to the action of neuraminidase from *C. perfringens*; the liver form was not attacked whereas the major component of serum was modified to a form less tightly held by the anion-exchange cellulose. The possibility that the difference between these forms was due either to an endogenous activator in the serum preparation or to an inhibitor in the liver one was made unlikely by the finding that in experiments in which liver and serum A forms were mixed they behaved exactly as when they were treated separately. The observation that the minor component A of serum hexosaminidase was also neuraminidase resistant was in agreement with this and was further evidence for the identification of this component with the tissue form.

Plasma glycoproteins are synthesized mainly in the liver (Robinson *et al.*, 1964; Macbeth *et al.*, 1965) and their secretion is usually, though not invariably, preceded by glycosylation (Winterburn & Phelps, 1972). The differences between the hexosaminidase A forms of liver and serum described may reflect some modification of this kind. The finding that the hexosaminidase A of tears was similar to that of serum,

whereas that of a variety of tissues resembled the liver form, tends to support this view.

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