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A Difference in the Specificities of Human Liver *N*-Acetyl-β-hexosaminidases A and B Detected by their Activities towards Glycosaminoglycan Oligosaccharides

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N-Acetyl-β-hexosaminidases A and B differ in their activities towards oligosaccharides prepared from glycosaminoglycans. Trisaccharides from hyaluronic acid and desulphated chondroitin 4-sulphate were hydrolysed by *N*-acetyl-β-hexosaminidase A, but not by *N*-acetyl-β-hexosaminidase B.

Human *N*-acetyl-β-hexosaminidases A and B have very similar kinetic properties when assayed with synthetic substrates (Robinson & Stirling, 1968; Okada & O'Brien, 1969; Sandhoff & Wässle, 1971) and are indistinguishable by their activities towards a variety of oligosaccharides derived from glycoproteins (Bearpark *et al.*, 1977). *N*-Acetyl-β-hexosaminidase A (but not B) is thought to be responsible for hydrolysis of the ganglioside G_{M2} since the absence of this enzyme in patients with Tay-Sachs disease is associated with accumulation of the ganglioside in nervous tissues (Okada & O'Brien, 1969). Attempts to confirm this difference in the abilities of *N*-acetyl-β-hexosaminidases A and B to hydrolyse ganglioside G_{M2} *in vitro* have given inconsistent results (Wenger *et al.*, 1972; Srivastava *et al.*, 1974; Sandhoff, 1970; Li *et al.*, 1973; Tallman *et al.*, 1974), but Sandhoff *et al.* (1977) have now shown that in the presence of suitable detergents, *N*-acetyl-β-hexosaminidase A hydrolyses ganglioside G_{M2}, whereas *N*-acetyl-β-hexosaminidase B is inactive. Both enzymes hydrolyse asialo-ganglioside G_{M2}.

The degradation of glycosaminoglycans is thought to involve *N*-acetyl-β-hexosaminidase (Weissman *et al.*, 1964), but the role played by the two forms, A and B, is difficult to interpret. On one hand it is reported that glycosaminoglycans do not accumulate in the urine (Strecker & Montreuil, 1971) or tissues (Suzuki *et al.*, 1971; Applegarth & Bozoian, 1972) of patients with Tay-Sachs disease (hexosaminidase A missing) or Sandhoff's disease, in which hexosaminidases A and B are missing. On the other hand, crude extracts of Tay-Sachs fibroblasts did not hydrolyse a heptasaccharide prepared from chondroitin 4-sulphate, although normal fibroblasts had this activity (Thompson *et al.*, 1973). Cantz & Kresse (1974) made similar observations using [^{14}C]glucosamine oligosaccharides from hyaluronic acid and they were able to correct the storage of glycosaminoglycans by cultured Sandhoff fibroblasts with *N*-acetyl-β-hexosaminidase A, but not with B. Differences in the specificities of human *N*-acetyl-β-hexosaminidases A and B might be expected on the basis of these findings and the report by Werries *et al.* (1975) that the trisaccharide from hyaluronic acid (GlcNAc-GlcA-GlcNAc) was hydrolysed at a much higher rate by bovine *N*-acetyl-β-hexosaminidase A than by *N*-acetyl-β-hexosaminidase B, although one must be cautious about interspecific comparisons.

In the present paper we report on the activities of purified human liver *N*-acetyl-β-hexosaminidases A and B and chitobiase towards trisaccharides derived from hyaluronic acid and desulphated chondroitin 4-sulphate.

Materials and Methods

Materials

Human liver was obtained *post mortem* and stored at −20°C until required. Hyaluronic acid and chondroitin 4-sulphate were from BDH Chemicals, Poole, Dorset, U.K. *NN'*-Diacetylchitiobiose was from L'Industrie Biologique Française, 92115 Clichy, France. Sephadex G-100 was from Pharmacia (G.B.), London W.5, U.K. 4-Methylumbelliferyl 2-acetamido-2-deoxy-β-D-glucopyranoside and 2-acetamido-2-deoxygluconolactone were from Koch–Light Laboratories, Colnbrook, Bucks., U.K. Hyaluronidase, (bovine type IV) and bovine liver β-glucuronidase (B-10) were from Sigma (London) Chemical Co., Kingston-upon-Thames, Surrey, U.K.

Preparation of enzymes

Human liver *N*-acetyl-β-hexosaminidases A and B were purified as described by Bearpark *et al.* (1977). In this purification scheme chitobiase was separated from *N*-acetyl-β-hexosaminidase by chromatography on Sephadex G-75 and then further purified by chromatography on a column of CM-cellulose (10ml), equilibrated in 10mM-sodium citrate buffer, pH4.4; under these conditions chitobiase was

retained by the column and was eluted by application of a gradient of 0–0.5 M-NaCl in 400 ml of the column buffer.

N-Acetyl-β-hexosaminidase contamination of the chitobiase preparation was removed by passing the enzyme through an anti-hexosaminidase immunoadsorbent column as described by Braidman *et al.* (1974). The specific activity of the chitobiase preparation was 1.1 μmol/min per mg of protein.

Preparation of substrates

Chondroitin 4-sulphate was desulphated by the method of Kantor & Schubert (1957). The oligosaccharides, GalNAcβ1–4GlcAβ1–3GalNAc and GlcNAcβ1–4GlcAβ1–3GlcNAc were prepared from desulphated chondroitin 4-sulphate and from hyaluronic acid by digestion with hyaluronidase and β-glucuronidase as described by Suzuki & Strominger (1960) and Weissman *et al.* (1964). Digestion with bovine liver β-glucuronidase was performed in the presence of 1 mM-2-acetamido-2-deoxygluconolactone, which was added in order to inhibit completely the *N*-acetyl-β-hexosaminidase that contaminated this enzyme preparation. Oligosaccharides were isolated from the digestion mixtures as described by Flodin *et al.* (1964) and these had characteristic R_F values on paper chromatography (Suzuki & Strominger, 1960; Weissman *et al.*, 1964).

Enzyme assays

Fluorimetric assay of *N*-acetyl-β-hexosaminidase activity was by the method described by Braidman *et al.* (1974). Chitobiase was assayed by the method of Stirling (1974). Oligosaccharase activities of purified enzymes and the crude extract were assayed by incubating 50 μl of the enzyme with 50 μl of oligosaccharide (1 mM) and 50 μl of McIlvaine's sodium phosphate/citric acid buffer at an appropriate pH for 2 h at 37°C. The incubation mixture was passed through 1 ml columns of Dowex 1 (X8, formate form) and Dowex 50 (X1, H^+ form) to separate free *N*-acetylhexosamine from charged oligosaccharides. Columns were eluted with deionized water (10 ml) and the *N*-acetylhexosamine, which was unretained by the columns, was taken to dryness by evaporation at 50°C under reduced pressure on an Evapomix (Buchler Instruments, Fort Lee, NJ, U.S.A.) before assay by the method of Reissig *et al.* (1955). The recovery of test samples of *N*-acetylglucosamine passed through the columns was 95%.

Results

Purified *N*-acetyl-β-hexosaminidase A hydrolysed the trisaccharides from hyaluronic acid and desulphated chondroitin 4-sulphate, although there was a 7-fold difference in their rates of hydrolysis (Table 1). Under identical conditions *N*-acetyl-β-hexosaminidase B had no detectable activity towards the trisaccharides. Both preparations of *N*-acetyl-β-hexosaminidases A and B hydrolysed *NN'*-diacetylchitobiose at the same rate. Chitobiase, although having some activity towards the trisaccharide from hyaluronic acid, was unable to hydrolyse the corresponding trisaccharide from desulphated chondroitin 4-sulphate.

Assay of a crude extract of human liver showed that both hyaluronate oligosaccharase and chondroitin oligosaccharase activities were higher in relation to *N*-acetyl-β-hexosaminidase (4-MeUmb-GlcNAc) than they were in purified *N*-acetyl-β-hexosaminidase A. The expected oligosaccharase

Table 1. *Oligosaccharase activities of a crude extract and purified N-acetyl-β-hexosaminidases A and B and chitobiase from human liver*

Enzymes were assayed for oligosaccharase activity as described in the Materials and Methods section. Activities are the means of triplicate determinations.

		Enzyme activity (nmol/h per ml)				
		Hexosaminidase				
Substrate	pH	A	B	Chitobiase	Crude extracts	Calculated oligosacharide activity contributed by hexosaminidase A*
4-MeUmb–GlcNAc	4.5	106×10^3	98×10^3	—	11.6×10^3	8.1×10^3
GlcNAcβ1–4GlcNAc	3.5	420	420	1500	—	—
GlcNAcβ1–4GlcAβ1–3GlcNAc	2.5	—	—	12	342	
	3.5	222	0	8	246	16
	4.5	174	0	—	228	13
GalNAcβ1–4GlcAβ1–3GalNAc	2.5	—	—	0	144	—
	3.5	0	0	0	126	—
	4.5	24	0	—	162	2

* Activity contributed by *N*-acetyl-β-hexosaminidase A, which is 70% of total *N*-acetyl-β-hexosaminidase activity was calculated from the oligosaccharase activity of the purified enzyme.

activities based on the activity of *N*-acetyl-β-hexosaminidase A in the crude extract are given in Table 1, and are insufficient to explain the observed oligosaccharase activities.

Discussion

Although the precise role of *N*-acetyl-β-hexosaminidase A and B in the hydrolysis of glycosaminoglycans is not yet understood, the presence of *N*-acetyl-β-hexosaminidase A is known to be necessary for the hydrolysis of a chondroitin 4-sulphate heptasaccharide by fibroblast extracts (Thompson *et al.*, 1973), *N*-acetyl-β-hexosaminidase B apparently being inactive. We have shown that highly purified *N*-acetyl-β-hexosaminidases A and B from human liver also show a marked difference in their specificities when assayed with trisaccharides from hyaluronic acid and chondroitin 4-sulphate; *N*-acetyl-β-hexosaminidase A alone being active towards these substrates. This difference in specificity is in contrast with the very similar activities that *N*-acetyl-β-hexosaminidases A and B exhibit when assayed with a range of chitin oligosaccharides (T. M. Bearpark & J. L. Stirling, unpublished observations) and a variety of oligosaccharides derived from glycoproteins (Bearpark *et al.*, 1977).

There seems to be consistency in the differences of specificity between *N*-acetyl-β-hexosaminidases A and B observed so far. Substrates hydrolysed by *N*-acetyl-β-hexosaminidase A alone invariably have a subterminal negatively charged sugar. In the glycosaminoglycan oligosaccharides used here (GlcNAcβ1–4GlcAβ1–3GlcNAc and GalNAcβ1–4GlcAβ1–3GalNAc) the subterminal sugar is glucuronic acid, whereas in the ganglioside G_{M2} the subterminal galactose carries *N*-acetylneuraminic acid. The C-4 configuration of the *N*-acetylhexosamine in natural (or synthetic) substrates does not seem to be an important determinant of the difference in specificity between *N*-acetyl-β-hexosaminidases A and B.

There is general agreement that the structural differences between *N*-acetyl-β-hexosaminidases A and B reside in their subunit compositions (Srivastava & Beutler, 1974; Geiger & Arnon 1976; Beutler *et al.*, 1976). Form A is envisaged as $(\alpha\beta)_n$ and form B as $(\beta\beta)_n$. Since subunits occur in form A, but not in B, we suggest that their function is to facilitate the binding of acidic substrates by the enzyme. Sandhoff *et al.* (1977) suggested this role for the α-subunit in relation to ganglioside-G_{M2} hydrolysis, but from our results this would seem to be a more general function.

N-Acetyl-β-hexosaminidase activity alone is not sufficient to account for the rates of hydrolysis of glycosaminoglycan oligosaccharides measured in crude extracts of human liver. It is possible that the oligosaccharase activity of *N*-acetyl-β-hexosaminidase A is stimulated by an activator present in the crude extracts, but our finding (T. M. Bearpark & J. L. Stirling, unpublished observation) that chondroitin oligosaccharase can be partly separated from *N*-acetyl-β-hexosaminidase and chitobiase may indicate the presence of a distinct enzyme. From the results of Thompson *et al.* (1973) it would appear that fibroblasts do not have a chondroitin oligosaccharase, distinct from *N*-acetyl-β-hexosaminidase A.

The relative importance of these glycosidases in hydrolysing glycosaminoglycans remains to be established, but from our results it seems that *N*-acetyl-β-hexosaminidase A and chitobiase might hydrolyse hyaluronate oligosaccharides, whereas *N*-acetyl-β-hexosaminidase A and possible a chondroitin oligosaccharase might hydrolyse oligosaccharides derived from chondroitin 4-sulphate. The combined activities of these hepatic enzymes appear to be sufficient to prevent detectable accumulation of glycosaminoglycans in the tissues of patients with Tay-Sachs and Sandhoff's disease.

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References

Applegarth, D. A. & Bozoian, G. (1972) *Clin. Chim. Acta* **39**, 269–271
Bearpark, T. M., Bouquelet, S., Fournet, B., Montreuil, J., Spik, G., Stirling, J. L. & Strecker, G. (1977) *FEBS Lett.* **84**, 379–384
Beutler, E., Yoshida, A., Kuhl, W. & Lee, J. E. S. (1976) *Biochem. J.* **159**, 541–543
Braidman, I., Carroll, M., Dance, N. & Robinson, D. (1974) *Biochem. J.* **143**, 295–301
Cantz, M. & Kresse, H. (1974) *Eur. J. Biochem.* **47**, 581–590
Flodin, P., Gregory, J. D. & Rodin, L. (1964) *Anal. Biochem.* **8**, 424–433
Geiger, B. & Arnon, R. (1976) *Biochemistry* **15**, 3484–3494
Kantor, T. G. & Schubert, M. (1957) *J. Am. Chem. Soc.* **79**, 152–159
Li, Y. T., Mazzotta, H. Y., Wan, C. C., Orth, R. & Li, S. C. (1973) *J. Biol. Chem.* **248**, 7512–7515
Okada, S. & O'Brien, J. S. (1969) *Science* **165**, 698–700
Reissig, J. L., Strominger, J. L. & Leloir, L. F. (1955) *J. Biol. Chem.* **217**, 959–966
Robinson, D. & Stirling, J. L. (1968) *Biochem. J.* **107**, 321–327
Sandhoff, K. (1970) *FEBS Lett.* **11**, 342–344
Sandhoff, K. & Wässle, W. (1971) *Hoppe-Seyler's Z. Physiol. Chem.* **352**, 1119–1133
Sandhoff, K., Conzelmann, E. & Nehrkorn, H. (1977) *Hoppe-Seyler's Z. Physiol. Chem.* **358**, 779–787
Srivastava, S. K. & Beutler, E. (1974) *J. Biol. Chem.* **249**, 2054–2057
Srivastava, S. K., Awastai, V. C., Yoshida, A. & Beutler, E. (1974) *J. Biol. Chem.* **249**, 2043–2048
Stirling, J. L. (1974) *FEBS Lett.* **39**, 171–175

Strecker, G. & Montreuil, J. (1971) *Clin. Chim. Acta* **33**, 395
Suzuki, S. & Strominger, J. L. (1960) *J. Biol. Chem.* **235**, 267–273
Suzuki, Y., Jacob, J. C., Suzuki, K., Kutty, K. M. & Suzuki, K. (1971) *Neurology* **21**, 313–328
Tallman, J. F., Brady, R. O., Quirk, J. M., Villalba, M. & Gal, A. E. (1974) *J. Biol. Chem.* **249**, 3489–3499
Thompson, J. N., Stoolmiller, A. C., Matalon, R. & Dorfman, A. (1973) *Science* **181**, 866–867
Weissman, B., Hadjiioannou, S. & Tornheim, J. (1964) *J. Biol. Chem.* **239**, 59–63
Wenger, D. A., Okada, S. & O'Brien, J. (1972) *Arch. Biochem. Biophys.* **153**, 116–129
Werries, E., Neue, I. & Buddecke, E. (1975) *Hoppe-Seyler's Z. Physiol. Chem.* **356**, 953–960