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Degradation of keratan sulphate by β-*N*-acetylhexosaminidases A and B

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Enzymic cleavage of β-*N*-acetylglucosamine residues of keratan sulphate was studied *in vitro* by using as substrate a [^{3}H]glucosamine-labelled desulphated keratan sulphate with *N*-acetylglucosamine residues at the non-reducing end. Both lysosomal β-*N*-acetylhexosaminidases A and B are proposed to participate in the degradation of keratan sulphate on the basis of the following observations. Homogenates of fibroblasts from patients with Sandhoff disease, but not those from patients with Tay–Sachs disease, were unable to release significant amounts of *N*-acetyl[^{3}H]glucosamine. On isoelectric focusing of β-*N*-acetylhexosaminidase from human liver the peaks of keratan sulphate-degrading activity coincided with the activity towards *p*-nitrophenyl β-*N*-acetylglucosaminide. A monospecific antibody against the human enzyme reacted with both enzyme forms and precipitated the keratan sulphate-degrading activity. Both isoenzymes had the same apparent K_m of 4 mM, but the B form was approximately twice as active as the A form when compared with the activity towards a chromogenic substrate. Differences were noted in the pH–activity profiles of both isoenzymes. Thermal inactivation of isoenzyme B was less pronounced towards the polymeric substrate than towards the *p*-nitrophenyl derivative.

Intralysosomal degradation of sulphated glycosaminoglycans is accomplished by the sequential removal of the sulphate groups and sugar residues from the non-reducing terminus of the polysaccharide chain (for reviews see McKusick *et al.*, 1978; Rodén, 1980). Whereas the catabolism of chondroitin sulphate, dermatan sulphate and heparan sulphate is fairly well understood, detailed knowledge about the enzymic degradation of keratan sulphate is remarkably scanty.

The repeating disaccharide unit of keratan sulphate consists of *N*-acetyl-lactosamine. Sulphate groups can be present on the C-6 position of both glucosamine and galactose. The enzyme that hydrolyses the β-galactosyl residues is the same as that needed for hydrolysis of G_{M1} ganglioside (Tsay & Dawson, 1973; Groebe *et al.*, 1980). Sulphate release from 6-sulphated galactosyl residues is considered to result from the action of *N*-acetylgalactosamine 6-sulphate sulphatase (DiFerrante *et al.*, 1978). This enzyme is rendered inactive in Morquio disease type A (Matalon *et al.*, 1974; Singh *et al.*, 1976; Horwitz & Dorfman, 1978), a disorder characterized by faulty degradation of chondroitin 6-sulphate as well as of keratan sulphate. Purified and crude *N*-acetylgalactosamine 6-sulphate sulphatase, however, did not cleave galactitol 6-sulphate (Glössl *et al.*, 1979), the substrate used by DiFerrante *et al.* (1978) to demonstrate the deficiency of galactose 6-sulphate sulphatase in Morquio disease type A. The question of the identity of *N*-acetylgalactosamine 6-sulphate sulphatase and of galactose 6-sulphate sulphatase therefore remains unanswered. By analogy, *N*-acetylglucosamine 6-sulphate sulphatase was proposed to be active towards heparan sulphate and towards keratan sulphate (DiFerrante *et al.*, 1978; Ginsberg *et al.*, 1978). The recent observation of two patients with isolated deficiency of a heparan sulphate-degrading *N*-acetylglucosamine 6-sulphate sulphatase (Kresse *et al.*, 1980), however, characterizes the sulphatase activities directed towards heparan sulphate and towards keratan sulphate as distinct enzymic properties.

The enzymic cleavage of the β-*N*-acetylglucosamine residues of keratan sulphate has not yet been reported in the literature. We describe in the present paper that β-*N*-acetylhexosaminidases A and B (EC 3.2.1.52) can participate *in vitro* in the degradation of keratan sulphate.

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Experimental

Materials

Post-mortem samples of human liver were provided by the Institute of Pathology of this University and stored at −20°C before use. The following materials were purchased from the suppliers indicated: D-[6-^{3}H]glucosamine hydrochloride (sp. radioactivity 38 Ci/mmol; Amersham–Buchler, Braunschweig, Germany), concanavalin A–Sepharose (Deutsche Pharmacia, Freiburg, Germany), *p*-nitrophenyl glycosides (Paesel, Frankfurt, Germany), β-galactosidase (EC 3.2.1.23) from *Escherichia coli* (Boehringer, Mannheim, Germany), *N*-acetylneuraminidase (EC 3.2.1.18) from *Vibrio comma* (Behringwerke, Marburg, Germany), chondroitin ABC lyase (EC 4.2.2.4) (Sigma, Munich, Germany) and Dowex 1 (X2; 200–400 mesh) (Serva, Heidelberg, Germany). CH-Sepharose 4-B coupled with 6-aminohexyl 2-acetamido-2-deoxy-1-thio-β-D-glucoside, CH-Sepharose 4-B coupled with 6-aminohexyl 1-thio-β-D-galactoside and antiserum against human β-*N*-acetylhexosaminidase were kindly provided by Dr. U. Klein, Dr. R. Niemann and Dr. A. Hasilik respectively of this Institute. ^{3}H-labelled molecular-weight standards (mol.wts. 19000, 12400 and 5600) prepared from chondroitin sulphate were generously given by Dr. Å. Wasteson, University of Uppsala, Uppsala, Sweden.

Preparation of [^{3}H]glucosamine-labelled keratan sulphate

Bovine corneas (10 g) obtained immediately after slaughter were incubated in 40 ml of complete tissue-culture medium (Cantz *et al.*, 1972) containing 2.5 mCi of [6-^{3}H]glucosamine for 11 h at 37°C. A glycosaminoglycan-rich fraction was obtained from the tissue by papain digestion and stepwise chromatography on Dowex 1 (Kindler *et al.*, 1977). Material desorbing from the resin at between 0.5 M- and 3.0 M-NaCl was dialysed against water, and was subjected to digestion with Pronase P before ethanol fractionation (McCarthy & Baker, 1979). The keratan sulphate-rich fraction that became insoluble after increasing the proportion of ethanol from 30% to 70% (v/v) was collected by centrifugation, washed with 80% ethanol and 100% ethanol, and dried with ether. It was subsequently digested with chondroitin ABC lyase by adding 5 munits of enzyme/mg of material (Saito *et al.*, 1968) at the beginning and again after 8 h of incubation. The reaction was stopped after a further 16 h by addition of trichloroacetic acid (final concn. 100 g/litre). After removal of protein by centrifugation, the supernatant was dialysed against water. Keratan sulphate was precipitated by addition of 3 vol. of ethanol containing 1.3% (w/v) potassium acetate and dried as described above. The material thus obtained (40 mg) was treated with 1 unit of *N*-acetylneuraminidase in 6 ml of 0.11 M-sodium acetate buffer, pH 5.5, containing 0.16 M-NaCl, 3 mM-$CaCl_2$ and 3 mM-NaN_3 for 24 h at 37°C. The polysaccharide was recovered as described in the preceding step. Desulphation was performed essentially as described by Kantor & Schubert (1957). Of the material recovered 64% remained water-soluble.

A portion of this material (9 mg) was finally digested with 1700 units of β-galactosidase (free of β-*N*-acetylhexosaminidase activity) in 8 ml of 80 mM-potassium phosphate buffer, pH 7.0, containing 8 mM-KCl, 0.8 mM-$MgSO_4$, 3 mM-NaN_3 and 0.02% bovine serum albumin for 24 h at 37°C. Units were calculated from the hydrolysis of *p*-nitrophenyl β-D-galactopyranoside incubated under the same conditions (for definition of units see below). Pilot studies with [^{3}H]galactose-labelled keratan sulphate (Groebe *et al.*, 1980) indicated that this concentration of bacterial β-galactosidase was sufficient to remove all galactosyl residues from the non-reducing end of the polymer. The reaction was terminated by boiling, protein was removed by ultracentrifugation, and after dialysis against water the material was freeze-dried.

The keratan sulphate thus obtained had the following composition: hexosamine, 1.27 μmol/mg; galactose, 1.34 μmol/mg; sulphate, 0.03 μmol/mg; hexuronic acid, undetectable. The specific radioactivity was 1.4 μCi/mg. On chromatography on a Sephadex G-100 column (1.2 cm × 109 cm) equilibrated and eluted with 4 M-guanidinium chloride in 50 mM-sodium acetate buffer, pH 6.0, it had a mean $K_{av.}$ value of 0.48. According to the elution position of the molecular-weight standards this corresponds to a mean molecular weight of 10000.

Assay of β-N-acetylhexosaminidase activity

β-*N*-Acetylhexosaminidase activity was determined by using either [^{3}H]glucosamine-labelled keratan sulphate or *p*-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside as substrate.

For keratan sulphate the incubation mixture contained 5 μg of desulphated and β-galactosidase-treated keratan sulphate (about 8000 c.p.m.) and enzyme in 33 mM-sodium formate buffer, pH 4.2, containing 0.12 M-NaCl and 3 mM-NaN_3, in a final volume of 24 μl. Whenever possible, the activity of added enzyme was about 2 munits as measured with the chromogenic substrate. After 4 h at 37°C the mixture was spotted on Whatman no. 3 paper, and descending chromatography was performed in butan-1-ol/1 M-NH_3/acetic acid (2:1:3, by vol.). The paper was cut into 1 cm segments, which were placed in scintillation vials and eluted with 2.0 ml of water before the addition of 4 ml of Instagel (Packard, Frankfurt, Germany). All the migrating radioactivity exhibited the same mobility as *N*-

acetylglucosamine. No radioactivity co-migrated with D-galactose, D-mannose, L-fucose or *N*-acetylneuraminic acid when tested in this system or in a system consisting of ethyl acetate/pyridine/water (20:7:5, by vol.). Blanks contained less than 10c.p.m. of material behaving as *N*-acetylglucosamine. Under the assay conditions the enzyme was not saturated with substrate. Release of *N*-acetylglucosamine was linear with time and proportional to the amount of enzyme provided that not more than 3% (240c.p.m.) of the total radioactivity was converted into monosaccharide.

For *p*-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside enzyme activity was determined as described previously (von Figura, 1977).

One unit of activity was defined as the amount of enzyme catalysing the hydrolysis of 1μmol of substrate/min at 37°C under the condition of saturation with substrate.

Purification of β-N-acetylhexosaminidase

Crude enzyme preparation. Minced human liver (650g) was suspended in 2 litres of 2mM-sodium phosphate buffer, pH7.0, containing 10mM-NaCl and 3mM-NaN_3 and homogenized with an Ultra-Turrax homogenizer (Janke und Kunkel, Freiburg, Germany). The supernatant obtained after centrifugation at 11300*g* for 30min was filtered through cheese-cloth and made 70% saturated with solid $(NH_4)_2SO_4$. The precipitate, which was obtained by centrifugation for 30min at 80000*g*, was dissolved in 10mM-Tris/HCl buffer, pH7.0, containing 10mM-NaCl and 3mM-NaN_3 (buffer A), the final volume being 1400ml, and dialysed for 72h against five changes of 5 litres each of the same buffer.

Chromatography on concanavalin A–Sepharose. The non-diffusible material from the preceding step was loaded at 4°C on a concanavalin A–Sepharose column (3cm × 12.5cm), equilibrated with buffer A, at a flow rate of 60ml/h. The column was washed at 22°C with 500ml of 10mM-Tris/HCl buffer, pH7.0, containing 0.5M-NaCl and 3mM-NaN_3. Adsorbed material was eluted at the same temperature with 1025ml of buffer A containing 0.5M-methyl α-mannoside. The proteins that were desorbed by methyl α-mannoside were dialysed for 3 days against three changes of 5 litres each of 10mM-sodium acetate buffer, pH5.0, containing 0.1M-NaCl and 3mM-NaN_3 (buffer B), and concentrated to 215ml on an Amicon concentrator equipped with a PM 10 filter (Amicon, Witten, Germany).

Chromatography on CH-Sepharose 4-B coupled with 6-aminohexyl 1-thio-β-D-galactoside. To remove β-galactosidase, the enzyme preparation of the preceding step (144ml) was applied at a flow rate of 8.5ml/h to a column (2.5cm × 14cm) of substituted CH-Sepharose 4-B pre-equilibrated with buffer B. The column was eluted with 100ml of the same buffer. β-Galactosidase was desorbed by applying 130ml of buffer B containing 0.5M-NaCl and 0.1M-galactonolactone.

β-N-Acetylhexosaminidase-containing fractions were pooled and dialysed for 2 days against three changes of 5 litres each of 50mM-sodium phosphate buffer, pH6.2, containing 0.15M-NaCl and 3mM-NaN_3 (buffer C).

Chromatography on CH-Sepharose 4-B coupled with 6-aminohexyl 2-acetamido-2-deoxy-1-thio-β-D-glucoside. *β-N*-Acetylhexosaminidase was applied to a column (4cm × 8cm) of substituted Sepharose 4-B, pre-equilibrated with buffer C, at a flow rate of 10ml/h. After the column had been washed with buffer C (480ml), desorption was performed by 10mM-NaCl and 3mM-NaN_3 (480ml). Enzyme-containing fractions were concentrated to 7ml as described above.

Isoelectric focusing of β-N-acetylhexosaminidase. The material from the preceding step was subjected to isoelectric focusing in a 110ml column (LKB 8101; LKB Instrument, Gräfelfing, Germany) with Ampholine, pH3.5–10, at a final concentration of 1.2% (v/v), in accordance with the manufacturer's manual. The fractions obtained at the end of the run were dialysed against 10mM-sodium phosphate buffer, pH6.0, containing 0.15M-NaCl and 3mM-NaN_3.

Other methods

Analyses of hexosamine (Boas, 1953), galactose (Trevelyan & Harrison, 1952), sulphate (Greiling *et al.*, 1964) and uronic acids (Bitter & Muir, 1962) were performed as described. Radioactivity was determined in a Beckman LS 9000 liquid-scintillation spectrometer.

Skin fibroblasts were maintained in culture as described in detail elsewhere (Cantz *et al.*, 1972). Cell homogenates were prepared by ultrasonication.

Results

Keratan sulphate degradation by β-N-acetylhexosaminidases A and B

Keratan sulphate-degrading *β-N*-acetylhexosaminidase activity was measured in fibroblast homogenates from healthy individuals and patients with various enzyme deficiencies (Table 1). All samples except that from a patient with Sandhoff disease hydrolysed significant amounts of substrate, suggesting that *β-N*-acetylhexosaminidases A and/or B are involved in the catabolism of keratan sulphate.

β-N-Acetylhexosaminidase was therefore partially purified from a human source, and the isoenzymes A and B were separated by isoelectric focusing (Fig. 1). A coincidence of the activities measured towards *p*-nitrophenyl *β-N*-acetylglucosaminide and [^{3}H]-

glucosamine-labelled keratan sulphate was observed. The isoenzyme with the more basic isoelectric point (*β-N*-acetylhexosaminidase B), however, was relatively more active towards keratan sulphate than was the more acidic form (*β-N*-acetylhexosaminidase A).

Final proof that *β-N*-acetylhexosaminidases A and B participate in the degradation of keratan sulphate *in vitro* was obtained by immunological techniques. Isoenzymes A (pI 5.2) and B (pI 7.55) could be completely precipitated by a monospecific antibody against human *β-N*-acetylhexosaminidase (Hasilik & Neufeld, 1980). Activities towards *p*-nitrophenyl *β-N*-acetylglucosaminide and keratan sulphate were simultaneously removed from solution (Table 2). In the resuspended precipitate 46% (isoenzyme A) and 33% (isoenzyme B) respectively of the added keratan sulphate-degrading activity were recovered. This low yield most probably resulted from insufficient dispersion of the precipitate.

Table 1. *Keratan sulphate-degrading β-N-acetylhexosaminidase activity in homogenates of cultured human skin fibroblasts*
For full details see the text.

Genotype	Enzyme activity (pmol of *N*-acetylglucosamine/h per mg of cell protein)
Normal ($n = 4$)	123–607
Tay–Sachs disease	178
Sandhoff disease	8
Morquio disease type A	310
Morquio disease type B	238

Properties

The following results refer to the peak fractions of *β-N*-acetylhexosaminidases A and B as obtained after the electrofocusing experiment (Fig. 1). Keratan sulphate degradation by *β-N*-acetylhexosaminidases A and B was optimal at pH 4.2. The isoenzymes, however, differed in their pH–activity profiles, the A isoenzyme being less active at more-acidic pH values (Fig. 2).

On incubation of the two enzyme forms in the presence of 0.01–1.7 mmol of keratan sulphate/litre, in each case the plot of v against $v/[S]$ (Hofstee, 1959) revealed a straight-line relationship (Fig. 3),

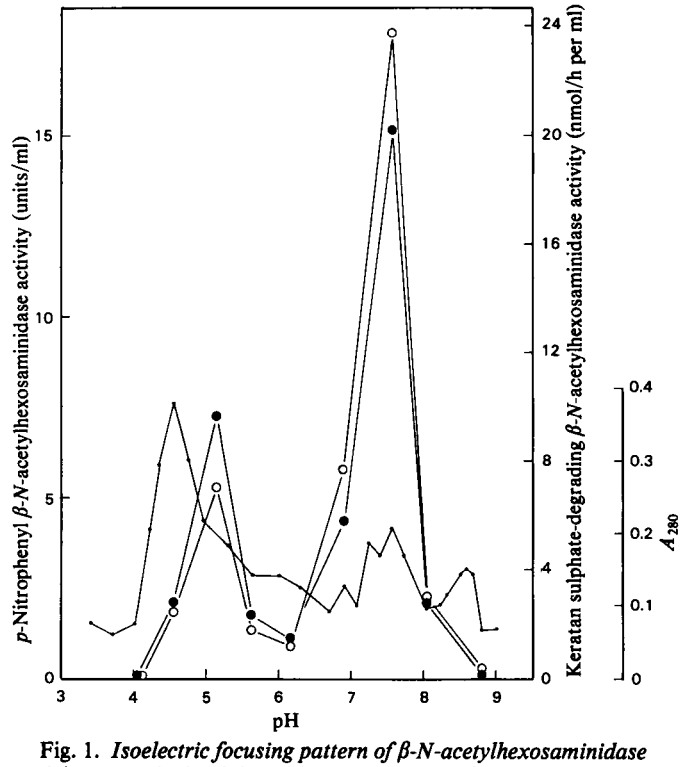

Fig. 1. *Isoelectric focusing pattern of β-N-acetylhexosaminidase*
•—•, A_{280}; O, enzyme activity towards desulphated keratan sulphate (nmol of *N*-acetylglucosamine released/h per ml); ●, enzyme activity towards *p*-nitrophenyl *β-N*-acetylglucosaminide (units/ml).

Table 2. *Immunoprecipitation of β-N-acetylhexosaminidases A and B*

Samples (50μl) of β-*N*-acetylhexosaminidases A (0.5 unit) and B (0.8 unit) were each mixed with 20μl of 0.4M-Tris/HCl buffer, pH 7.0, containing 1.6M-KCl and 4% (v/v) Triton X-100, and either 10μl of antiserum or 10μl of control serum. The mixtures were left for 30min at 20°C and then for 40h at 4°C before centrifugation at 10000*g* for 15min.

Enzyme form	Substrate	% of added activity in supernatant: With antiserum	With control serum
Isoenzyme A	Keratan sulphate	<0.2	107
	p-Nitrophenyl β-*N*-acetylglucosaminide	<0.1	107
Isoenzyme B	Keratan sulphate	<0.2	104
	p-Nitrophenyl β-*N*-acetylglucosaminide	<0.1	106

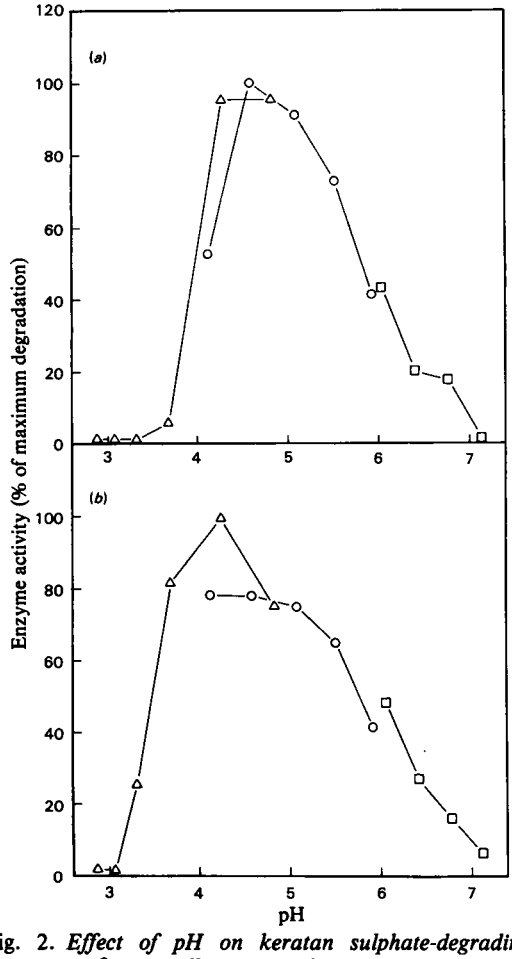

Fig. 2. *Effect of pH on keratan sulphate-degrading β-N-acetylhexosaminidase activity*

(*a*) β-*N*-Acetylhexosaminidase A (pI 5.2); (*b*) β-*N*-acetylhexosaminidase B (pI 7.55). The assays were performed in 33mM-sodium formate buffer (△), 33mM-sodium acetate buffer (○) and 33mM-sodium phosphate buffer (□), all the buffers containing 0.12M-NaCl. For each enzyme source the maximal activity was taken as 100%.

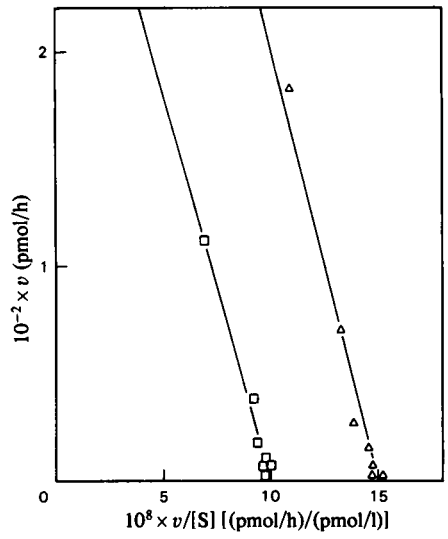

Fig. 3. *Relationship between β-N-acetylhexosaminidase activity and concentration of desulphated keratan sulphate*

The assay mixtures contained either 2.25munits of β-*N*-acetylhexosaminidase A (pI 5.2) (□) or 2.25munits of β-*N*-acetylhexosaminidase B (pI 7.55) (△) and 2.5–400μg of desulphated keratan sulphate. Results are plotted in accordance with the Hofstee (1959) procedure. Details are given in the Experimental section.

thus allowing the calculation of apparent Michaelis constants. The K_m of both isoenzymes was 4mM. These measurements were made with the use of identical activities towards the synthetic substrate. The maximal keratan sulphate-degrading activity of enzyme form B, however, was 1.7 times that of isoenzyme A.

β-*N*-Acetylhexosaminidase A has been shown to be more thermolabile than isoenzyme B (Kaback,

1972). This holds true in principle also for the keratan sulphate-degrading activity (Fig. 4), though after heat treatment isoenzyme B remained more active towards keratan sulphate than towards *p*-nitrophenyl β-*N*-acetylglucosaminide.

Keratan sulphate degradation by both isoenzymes was completely inhibited by 1 mM-Ag^+ and 1 mM-Hg^{2+}, as had been found previously for the hydrolysis of a chromogenic substrate (Marinkovic & Marinkovic, 1978).

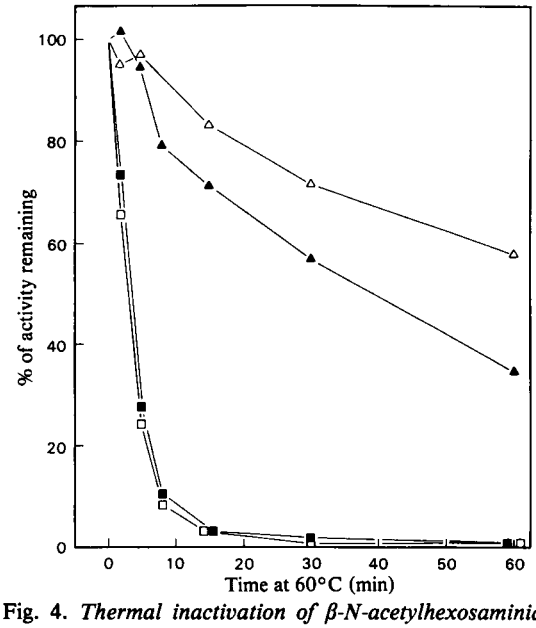

Fig. 4. *Thermal inactivation of β-N-acetylhexosaminidases A and B*

β-*N*-Acetylhexosaminidases A (□, ■; pI 5.2) and B (△, ▲; pI 7.55) were kept at 60°C for the times indicated and then incubated at 37°C with desulphated keratan sulphate (□, △) or with *p*-nitrophenyl β-*N*-acetylglucosaminide (■, ▲) as described in the Experimental section. Results are expressed as percentages of the activity of controls that had not been preincubated at 60°C.

Enzymic characterization of keratan sulphate

The demonstration that β-*N*-acetylhexosaminidases A and B can participate in the degradation of keratan sulphate allowed an enzymic characterization of the non-reducing end of keratan sulphate chains. The [^{3}H]glucosamine-labelled keratan sulphate used for this investigation was that material obtained after treatment with neuraminidase but before chemical desulphation. Enzyme sources were fibroblast homogenates from patients with deficiencies of β-*N*-acetylhexosaminidase (Sandhoff disease), of β-galactosidase (G_{M1} gangliosidosis) and of *N*-acetylgalactosamine 6-sulphate sulphatase (Morquio disease type A), and the corresponding purified normal human enzymes. The results described in Table 3 give evidence that sulphated or unsulphated *N*-acetylglucosamine residues are not present at the non-reducing terminus (assays I and V). They support the previous suggestion (DiFerrante *et al.*, 1978) that in fibroblasts from patients with Morquio disease type A a galactose 6-sulphate sulphatase is deficient (assays VII and VIII). As with *N*-acetylgalactosamine 6-sulphate sulphatase (Glössl *et al.*, 1979) the galactose 6-sulphate sulphatase should be inhibited by inorganic sulphate, whereas keratan sulphate-degrading *N*-acetylglucosamine 6-sulphate sulphatase should not (W. Fuchs & H. Kresse, unpublished work). One may then deduce that of the galactose residues present at the non-reducing end about 70% are sulphated (assays III, IV, VII and VIII). However, since the kinetics of these multiple reactions have not been studied in detail, a quantitative interpretation of the data should be approached with caution.

Table 3. *Degradation of N-acetylneuraminidase-treated keratan sulphate*

Incubation conditions were analogous to those described in the Experimental section except that 30 munits of purified β-*N*-acetylhexosaminidase B, 26 munits of purified β-galactosidase, an amount of purified *N*-acetylgalactosamine 6-sulphate sulphatase hydrolysing 3 nmol of trisaccharide/h (Glössl *et al.*, 1979) and Na_2SO_4 respectively were included as indicated in the assay mixture. Incubation was for 20 h at 37°C. Abbreviation: N.D., not detected.

Assay no.	Fibroblast homogenate	Purified enzyme	Sulphate (0.1 M)	*N*-Acetylglucosamine liberated (pmol)
I	None	β-*N*-Acetylhexosaminidase B	–	N.D.
II	Sandhoff disease	None	–	1.0
III	Sandhoff disease	β-*N*-Acetylhexosaminidase B	–	23.7
IV	Sandhoff disease	β-*N*-Acetylhexosaminidase B	+	6.7
V	G_{M1} gangliosidosis	None	–	N.D.
VI	G_{M1} gangliosidosis	β-Galactosidase	–	14.6
VII	Morquio disease type A	None	–	6.2
VIII	Morquio disease type A	*N*-Acetylgalactosamine 6-sulphate sulphatase	–	22.3

Discussion

The investigation of the enzymic removal of β-D-*N*-acetylglucosamine residues in keratan sulphate required the preparation of an appropriate substrate with exposed non-sulphated *N*-acetylglucosamine residues at the non-reducing end. This was achieved by sequential removal of *N*-acetylneuraminic acid, sulphate and terminal galactosyl residues from biosynthetically labelled corneal keratan sulphate. L-Fucose and D-mannose, which are also constituents of the polymer, did not have to be removed, since these sugars are constituents only of the polysaccharide–protein linkage region (Brekle & Mersmann, 1980). The use of a polymer as substrate, however, was inconvenient insofar as the proportion of radioactivity that could maximally be released by a β-galactosidase-free enzyme preparation was low (approx. 8%).

Several lines of evidence let us propose that both β-*N*-acetylhexosaminidases A and B participate in the enzymic degradation of keratan sulphate. (1) Fibroblasts deficient in β-*N*-acetylhexosaminidases A and B did not release measurable amounts of *N*-acetyl[6-^{3}H]glucosamine. (2) On isoelectric focusing the profiles of the activities directed towards *p*-nitrophenyl β-*N*-acetylglucosaminide and keratan sulphate were similar. (3) A monospecific antibody against β-*N*-acetylhexosaminidase reacted with both isoenzymes and precipitated the activity towards both substrates. β-*N*-Acetylhexosaminidases A and B, however, differed with respect to keratan sulphate degradation in their specific activities and in their pH–activity profiles.

It has been clearly established that β-*N*-acetylhexosaminidases A and B are involved in the breakdown of glycosphingolipids. An inactivity of the A form leads to Tay–Sachs disease, whereas the absence of both forms results in Sandhoff disease (O'Brien, 1978). Storage of oligosaccharides in the liver of patients with Sandhoff disease suggests a role of the enzymes in normal glycoprotein catabolism (Ng Ying Kin & Wolfe, 1974). With regard to glycosaminoglycans, at least hyaluronate and dermatan sulphate are susceptible to the action of both isoenzymes, though the A form seems to be the more active (Cantz & Kresse, 1974; Werries *et al.*, 1975; Bach & Geiger, 1978). On the other hand, a heptasaccharide from chondroitin 4-sulphate, containing a *N*-acetylgalactosamine residue at the non-reducing terminus, could be degraded only in the presence of isoenzyme A (Thompson *et al.*, 1973).

Though β-*N*-acetylhexosaminidase appears to be a key enzyme in the degradation of glycosaminoglycans, including keratan sulphate, it remains puzzling that patients with Sandhoff disease have no marked mucopolysaccharidurie (Strecker & Montreuil, 1971) and do not accumulate excessive amounts of glycosaminoglycans in their organs (Suzuki *et al.*, 1971; Applegarth & Bozoian, 1972). During the purification of β-*N*-acetylhexosaminidase we could not detect keratan sulphate-degrading activity in fractions devoid of activity towards the synthetic substrate. This finding does not support the possibility that in addition to the β-*N*-acetylhexosaminidases a specific keratan sulphate-degrading enzyme does exist. It seems possible, however, that the storage of keratan sulphate-derived oligosaccharides could have escaped detection, since no specific search for the presence of such material had been undertaken.

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