Purification and characterization of β -N-acetylhexosaminidase I₂ from human liver

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β -N-Acetylhexosaminidase I₂ was purified from human liver by a combination of concanavalin A chromatography, DEAE-cellulose chromatography, gel filtration and affinity chromatography on 2-acetamido-N-(6-aminohexanoyl)-2-deoxy- β -D-glucopyranosylamine coupled to CNBr-activated Sepharose 4B. Its specific activity was 130 μ mol/min per mg of protein compared with values of 150 and 320 μ mol/min/mg of protein for β -N-acetylhexosaminidases A and B purified from the same tissue. K_m values for I₂, A and B were 1.0 mM, 0.8 mM and 0.74 mM respectively. On gradient gel electrophoresis under non-denaturing conditions, hexosaminidase I₂ behaved similarly to A and appeared to have an M_r between 100 000 and 110 000. β -N-Acetylhexosaminidase I₂ was resolved into two major polypeptides, of M_r 56 000 and 29 000, on SDS/polyacrylamide-gel electrophoresis under denaturing conditions. Immunoblotting with anti-(hexosaminidase α -subunit) serum confirmed that the 56 000- M_r component was the α -subunit and anti-(hexosaminidase B) serum reacted with the 29 000 M_r component. β -N-Acetylhexosaminidase I₂ more closely resembles form A than B, but the features of its structure that allow it to be separated from A on the basis of net charge have not yet been found.

INTRODUCTION

In human tissues there are two major forms of β -N-acetylhexosaminidase, A and B, and a number of less abundant forms. Two of the minor forms, I₁ and I₂, have charge properties intermediate between those of A and B, judging by their behaviour on electrophoresis and on DEAE-cellulose, and represent a small proportion of the total activity in normal tissues. One of these (I₂) is increased very greatly in lymphocytes of patients with common acute lymphoblastic leukaemia (Ellis *et al.*, 1978), a characteristic that distinguishes this from other types of leukaemia. The intermediate forms of β -N-acetylhexosaminidase found in tissues have counterparts in plasma and in urine and they too may be increased in activity in some pathological conditions (Pott *et al.*, 1978; Tucker *et al.*, 1980; Kind, 1982) and also in maternal circulation during pregnancy when β -N-acetylhexosaminidase P becomes the major form (Stirling, 1972).

Until recently, almost nothing was known about the structures of the intermediate forms of β -N-acetylhexosaminidase extracted from tissues and their relationships with the A and B forms, but the intermediate forms from placenta have been purified and their polypeptide compositions determined by Lowden *et al.* (1984). β -N-Acetylhexosaminidase P has been purified and some of its characteristics described by Geiger *et al.* (1978). In the present paper we describe the purification of β -N-acetylhexosaminidase I₂ from human liver and report on some of its properties.

EXPERIMENTAL

Materials

Samples of human liver, brain and kidney were obtained *post mortem* and stored at -20°C . Placenta was obtained immediately after delivery and stored at -20°C . The affinity chromatography ligand 2-acetamido-N-(6-aminohexanoyl)-2-deoxy- β -D-glucopyranosylamine (ANAG) was prepared by the method of Koshy *et al.* (1975). 4-Methylumbelliferyl 2-acetamido-2-deoxy- β -D-glucopyranoside was purchased from Koch-Light, Colnbrook, Slough, Berks., U.K. Concanavalin A-Sepharose, Sephadex G-200 and Protein A-Sepharose were from Pharmacia, Hounslow, Middx., U.K. N-Acetylglucosamine, α -methyl glucoside, human serum albumin (fraction V) and Coomassie Brilliant Blue R-250 were from Sigma, Poole, Dorset, U.K. Acrylamide, NN' -methylenebisacrylamide, goat anti-(rabbit IgG)-horseradish-peroxidase conjugate and dithiothreitol were supplied by Bio-Rad Laboratories, Watford, Herts., U.K. DEAE-cellulose was from Whatman Biochemicals Ltd., Maidstone, Kent, U.K. 4-Chloro-1-naphthol was from Aldrich Chemical Co., Gillingham, Dorset, U.K. Nitrocellulose sheets BA 85 (0.45 μ m pore size) were obtained from Schleicher und Schüll, Dassel, Germany. All other reagents were purchased from BDH, Poole, Dorset, U.K.

Purification

Human liver (30 kg) was processed in two lots. Batches

Abbreviations used: ANAG, 2-acetamido-N-(6-aminohexanoyl)-2-deoxy- β -D-glucopyranosylamine; ASBI, 7-bromo-3-hydroxy-2-naphthol-anisole.

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of tissue were cut into small pieces and suspended in cold citrate/phosphate buffer (Gomori, 1955), pH 4.5, containing 200 mM-KCl in the proportions 1 kg of liver to 1 litre of buffer. The suspension was homogenized (Silverson homogenizer) and left overnight at 4 °C. Large particles were removed by filtration through two layers of cheesecloth and the filtrate centrifuged at 4 °C for 1.5 h at 3200 *g* in an MSE Coolspin centrifuge in batches of 4 litres. Supernatants were pooled and centrifuged under the same conditions for 30 min. Supernatants were again pooled and adjusted to 25% saturation with $(\text{NH}_4)_2\text{SO}_4$ by adding the solid salt. After stirring gently overnight at 4 °C the precipitate was removed by centrifugation at 3200 *g* for 30 min at 4 °C and discarded. Supernatants were adjusted to 65% saturation with $(\text{NH}_4)_2\text{SO}_4$ and stirred gently at 4 °C for 6 h. The precipitate was recovered by centrifugation as before, suspended in ice-cold water and dialysed against several changes of distilled water over 48 h. This 25–65% satd. $(\text{NH}_4)_2\text{SO}_4$ fraction was freeze-dried and stored at –70 °C. All subsequent steps were performed with small batches of the freeze-dried powder.

Lipids were extracted from the freeze-dried powder by adding butan-1-ol (dried over anhydrous Na_2SO_4) in the proportions 50 ml of solvent per g of powder, and mixing them to a fine paste in a glass homogenizer. The paste was stirred overnight at 4 °C and then centrifuged at 3200 *g* for 30 min at 0 °C. The butanol layer was removed and the pellet extracted once more, but this time the mixture was stirred in the cold for only 4 h. After centrifugation for 30 min at 3200 *g* at 0 °C the supernatant was discarded and the pellet kept at 0 °C. Acetone (30 ml), dried over anhydrous Na_2SO_4 and cooled to –20 °C, was added to the pellet, mixed quickly to a paste and centrifuged at –4 °C for 10 min at 3200 *g*. The acetone layer was removed and the pellet spread between two sheets of filter paper and allowed to dry. This powder was stored at –70 °C.

When required, the lipid-free powder (1 g) was dissolved in 10 mM-sodium phosphate buffer, pH 6.0 (50 ml), and centrifuged at 3200 *g* for 30 min at 0 °C. β -*N*-Acetylhexosaminidase in the supernatant represented 79% of the activity present in the freeze-dried powder, and automated DEAE-cellulose chromatography showed that the proportions of the isoenzymes was not altered by the solvent-extraction steps. For affinity chromatography on concanavalin A–Sephrose, the supernatant was loaded on to a 100 ml column of the gel and the unretained hexosaminidase washed through with buffer (50 mM-citric acid titrated to pH 4.5 with NaOH, containing 0.5 M-NaCl, 0.1 mM- MgCl_2 , 0.1 mM- MnCl_2 and 0.1 mM- CaCl_2). Enzyme retained by the column was eluted with 0.5 M- α -methyl glucoside in the column buffer and dialysed against 10 mM-sodium phosphate buffer, pH 6.0, for 18 h at 4 °C.

β -*N*-Acetylhexosaminidases A, B and I_2 were separated by applying the dialysed extract to a column of DEAE-cellulose (800 ml) equilibrated with 10 mM-sodium phosphate buffer, pH 6.0. Under these conditions the B-form was unretained and passed through the column; elution of hexosaminidases I_2 and A was achieved by the application of a linear KCl gradient that reached a concentration of 300 mM in 1.6 litre. The separated isoenzymes were stored at –70 °C. The whole of the hexosaminidase I_2 preparation was applied to a column (2.8 cm diam. $\times$ 86 cm) of Sephadex G-200 equilibrated in

10 mM-sodium phosphate buffer, pH 6.0, to remove contaminating proteins that were shown to persist through the affinity-chromatography step. All three forms of hexosaminidase were further purified by affinity chromatography on ANAG–Sephrose by the method of Coelho *et al.* (1981).

Automated DEAE-cellulose chromatography

β -*N*-Acetylhexosaminidase isoenzymes were separated on microcolumns of DEAE-cellulose DE52 and the eluate monitored continuously as described by Ellis *et al.* (1978).

Gradient gel electrophoresis

Electrophoresis of β -*N*-acetylhexosaminidases under non-denaturing conditions was performed in polyacrylamide gradient gels purchased from Uniscil Ltd., Southend, Essex, U.K. Immediately before use the gels were placed in the electrophoresis tank (Uniscil Ltd.) containing buffer (10.75 g of Tris, 5 g of boric acid, 930 mg of EDTA in 1 litre of water) and run at 125 V for 15 min. Samples (20 μ l) containing 10% (w/v) sucrose were applied and the gel run at 90 V until the samples had left the applicators and entered the gel. Electrophoresis was continued at 125 V for 48 h at 4 °C. Gels were removed from the glass cassettes, placed in 0.1 M-sodium citrate buffer, pH 4.5, for 30 min and stained for hexosaminidase by the method of Hayashi (1965), using naphthyl-ASBI β -*N*-acetylglucopyranoside as the substrate and Fast Garnet GBC as the coupling dye. Naphthol-ASBI 2-acetamido-2-deoxy- β -D-glucopyranoside (1.5 mg) was dissolved in 2-methoxyethanol ('ethylene glycol monomethyl ether') (0.5 ml) and mixed with a solution of Fast Garnet GBC in 0.1 M-citric acid adjusted to pH 4.3 with NaOH (1 mg/ml). The gel was incubated in this solution at 37 °C for 90 min or until a suitable staining was obtained at the site of the enzyme. The reaction was then stopped by immersing the gel in 7% (v/v) acetic acid. M_r values were determined by the method of Anderson *et al.* (1972).

SDS/polyacrylamide-gel electrophoresis

Polyacrylamide-gel electrophoresis in the presence of SDS was performed in 10%-(w/v)-polyacrylamide gels using the buffer system described by Laemmli (1970). Samples were prepared for electrophoresis by boiling them in sample buffer containing 50 mM-dithiothreitol for 5 min.

SDS/polyacrylamide gels were stained for protein with a 0.125% (w/v) solution of Coomassie Brilliant Blue R-250 in acetic acid/methanol/water (10:20:70, by vol.) and destained in the same solvent.

Western blotting and antibody staining

Transfer of β -*N*-acetylhexosaminidase I_2 from SDS/polyacrylamide gels to nitrocellulose sheets was by the method of Burnette (1981). Antibody detection of hexosaminidase I_2 subunits was performed by using rabbit anti-(human β -*N*-acetylhexosaminidase α -subunit) serum and rabbit anti-(β -*N*-acetylhexosaminidase B) serum by the method of Burnette (1981). Both antisera were kindly provided by Dr. E. Wilcox, University of California, San Diego, CA, U.S.A. Rabbit anti-hexosaminidase antibodies were detected by using goat anti-rabbit IgG coupled to horseradish peroxidase. For chromogenic detection of the antibody–peroxidase

Fig. 1. Automated DEAE-cellulose chromatography of hexosaminidase isoenzymes

Extracts of (a) kidney, (b) placenta, (c) liver and (d) brain were loaded on to microcolumns of DEAE-cellulose and eluted with a linear NaCl gradient. Column eluates were monitored by β -N-acetylhexosaminidase by an automated

conjugate, nitrocellulose sheets were immersed in a solution prepared by mixing 60 mg of 4-chloro-1-naphthol in ice-cold methanol with 100 ml of 50 mM-Tris/HCl (pH 7.5)/0.15 M-NaCl and 60 μ l of 30% (w/v) H₂O₂ added just before use.

Assay of β -N-acetylhexosaminidase, and determination of specific activities and K_m values

β -N-Acetylhexosaminidase in column eluates was assayed by incubating suitably diluted samples of the enzyme (100 μ l) with 100 μ l of 3 mM-4-methylumbelliferyl 2-acetamido-2-deoxy- β -D-glucopyranoside in citrate/phosphate buffer, pH 4.5 at 37 °C. The reaction was stopped, and fluorescence was developed by the addition of 0.25 M-glycine/NaOH buffer, pH 10.4 (2.3 ml). Fluorescence of the liberated 4-methylumbelliferone was measured by using a Perkin-Elmer fluorimeter with excitation between 340 and 360 nm and emission measured at 440 nm. The fluorimeter was calibrated with 0.4 μ M-4-methylumbelliferone in 0.25 M-glycine/NaOH buffer, pH 10.4. When specific activities and K_m values of the purified enzymes were determined, the assay mixture contained 0.5 mg of human serum albumin/ml (hexosaminidase-free) and 0.15 M-NaCl. Protein was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as a standard.

RESULTS

Choice of starting material for the purification of β -N-acetylhexosaminidase I₂

Samples of kidney, placenta, liver and brain were analysed by automated DEAE-cellulose chromatography for their constituent hexosaminidase isoenzymes. Placenta has been the usual choice as starting material for the purification of the A and B forms, but it has very small amounts of hexosaminidase I₂. Kidney and brain have appreciable hexosaminidase I₂ activities (Fig. 1), but liver was chosen as the starting material because it was the tissue with the greatest abundance of form I₂, where it accounts for about 10% of the total hexosaminidase activity. It is notable that whereas the retention times of the hexosaminidase A peaks in liver and kidney are the same, the placental enzyme is eluted earlier, indicating a less acidic net charge. Hexosaminidase A from brain is more acidic than the corresponding form from the other tissues analysed here. Hexosaminidase B always eluted as two peaks under the conditions used for DEAE-cellulose chromatography.

Purification of β -N-acetylhexosaminidases A, B and I₂

Although the purification scheme was primarily designed for the purification of hexosaminidase I₂, both hexosaminidase A and hexosaminidase B were also purified in parallel so that the properties of all three enzymes from one tissue could be compared. The results of the purification are given in Table 1. From its behaviour on concanavalin A-Sepharose, it would appear that hexosaminidase I₂ is glycosylated and in that

system of enzyme assay referred to in the Experimental section. The scale of β -N-acetylhexosaminidase activity is arbitrary and is different for each of the superimposed traces. Chromatographic runs were timed from the application of each sample. The profile of enzyme activity from each source was reproducible.

Table 1. Purification of β -N-acetylhexosaminidases from human liver

Step	Total vol. (litres)	$10^{-3} \times$ Total activity (units)	Total protein (g)	Specific activity (units/mg)	Purification factor	Yield (%)
Buffer extract	28.8	130	1667	0.078	1	100
25–65% -Satd. $(\text{NH}_4)_2\text{SO}_4$ fractionation		88.3	462	0.17	2	60
Butanol treatment		58	139	0.38	5	41
Concanavalin A–Sephrose	5.8	28	8.8	3.18	40	22
DEAE-cellulose*						
Hexosaminidase A	2.8	5.4	0.586	9.21	237	8.3
Hexosaminidase B	2.2	6.9	3.61	1.91	61	13.3
Hexosaminidase I_2	2.2	0.205	1.245	0.16	20	1.6
ANAG–Sephrose						
Hexosaminidase A	3.0	3.8	0.036	105	2699	5.85
Hexosaminidase B (+Protein A–Sephrose)	1.9	4.8	0.015	322	10320	9.2
Hexosaminidase I_2 (+Sephadex G-200)	0.06	0.102	0.001	102	13077	0.8

* Final yields and purification factors were calculated from proportions of activities of hexosaminidase A, B and I_2 in the crude homogenate of 5:4:1; the proportions were determined by calculating the area under the curves for hexosaminidases A, B and I_2 after separation on DEAE-cellulose.

respect resembles the A- and B-forms. Affinity chromatography on ANAG-Sephrose was a particularly effective purification step for all three forms of the enzyme, although a contaminant present in the hexosaminidase I_2 preparation was not removed by this method and had to be separated from hexosaminidase I_2 on Sephadex G-200 before affinity chromatography. Passage of the hexosaminidase B preparation through Protein A–Sephrose was used to remove traces of IgG that are the main contaminants of this form. There was complete recovery of hexosaminidase B from the Protein A–Sephrose column.

Specific activities and K_m values

Specific activities were determined for β -N-acetylhexosaminidases A, B and I_2 in the presence of human serum albumin (1 mg/ml) and 0.15 M-NaCl. At V_{max} , these values were 130, 370 and 150 $\mu\text{mol}/\text{min}$ per mg of protein for the A, B and I_2 forms respectively. K_m values of A, B and I_2 determined under the same conditions were 0.8 mM, 0.74 mM and 1.0 mM respectively.

Comparison of the M_r values of the native enzymes

Samples of β -N-acetylhexosaminidases A, B and I_2 were run on non-denaturing polyacrylamide gradient gels to establish approximate M_r values for the native enzymes. Hexosaminidase A formed a sharp band of activity with M_r of 100 000, but even after 48 h the B-form had not reached its exclusion limit in the gel and ran as a diffuse band of activity with incipient separation into two components (Fig. 2). This behaviour of hexosaminidase B is characteristic of the enzyme from both liver and placenta (L.C.B.B. Coelho & J. L. Stirling, unpublished work). Hexosaminidase I_2 behaved in a similar manner to hexosaminidase A and formed a sharp band of activity with an M_r between 100 000 and 110 000.

Subunit composition of β -N-acetylhexosaminidase I_2

Analysis of hexosaminidase A, B and I_2 by SDS/polyacrylamide-gel electrophoresis under reducing conditions showed that hexosaminidase A was resolved into two major components with M_r values of 56 000 and 29 000

Fig. 2. Comparison of the M_r values of the native enzymes

Samples of β -N-acetylhexosaminidases B, I_2 and A were run on polyacrylamide gradient gels for 48 h and then stained for enzyme activity as described in the Experimental section. The positions of M_r markers run on the same gel are shown; they were catalase (M_r 230 000) and bovine serum albumin (M_r 67 000).

(Fig. 3). In addition there were bands with M_r values in the range 26 000–22 000. Hexosaminidase B had a major component with an M_r of 29 000 and minor bands in the ranges 65 000–57 000 and 26 000–22 000. Hexosaminidase I_2 had bands with M_r values of 56 000 and 29 000 that were of approximately equal intensity and also minor bands in the ranges 65 000–57 000 and 26 000–22 000.

Immunological detection of subunits from β -N-acetylhexosaminidase I_2

Subunits of hexosaminidase I_2 separated by SDS/polyacrylamide-gel electrophoresis under denaturing conditions were transferred to nitrocellulose and treated with either rabbit anti-(human β -N-acetylhexosaminidase α -subunit) serum or with rabbit anti-(human hexosaminidase B) serum before chromogenic detection of the

Fig. 3. Analysis of hexosaminidase A, B and I₂ by SDS/polyacrylamide-gel electrophoresis under reducing conditions

β -N-Acetylhexosaminidases A, B and I₂ (3–5 μ g of protein) were reduced with dithiothreitol (50 mM) in sample buffer and boiled for 5 min before electrophoresis on a 10%-(w/v)-polyacrylamide gel in the buffer system described by Laemmli (1970). Polypeptides were detected by staining with Coomassie Brilliant Blue R-250. M_r markers (M) were: bovine serum albumin, M_r 67000; ovalbumin, M_r 45000; glyceraldehyde-3-phosphate dehydrogenase, M_r 36000; carbonic anhydrase, M_r 29000; trypsinogen, M_r approx. 24000; soya-bean trypsin inhibitor, M_r approx. 20000; α -lactalbumin, M_r 14200.

bound antibody with goat anti-rabbit IgG–peroxidase conjugate (Fig. 4). By using the anti-(α -subunit) serum, a component with the mobility of the α -subunit was detected in β -N-acetylhexosaminidase I₂ and there was also a prominent band in the 60000–65000- M_r range. A number of sharp minor bands of higher M_r were seen. With anti-(hexosaminidase B) serum the 29000- M_r component of hexosaminidase I₂ was detected and so too was a band in the M_r range 60000–65000. None of the higher- M_r bands was detected with this antiserum, but one of the components that ran ahead of the β -subunit (M_r 29000) could be seen.

DISCUSSION

β -N-Acetylhexosaminidase I₂ from human liver resembles the A-form from this tissue in a number of ways. Both I₂- and A-forms have similar specific activities and in that respect they differ from hexosaminidase B, which has more than double the specific activity. This difference in specific activity between the A- and B-forms of hexosaminidase has been observed with the enzymes from both liver (Sandhoff *et al.*, 1977) and placenta (Lee & Yoshida, 1976) and is to be expected if hydrolysis of synthetic substrates is catalysed predominantly by the β -subunits, since A is thought to be composed of α - and

Fig. 4. Immunological detection of subunits from β -N-acetylhexosaminidase I₂

Subunits of β -N-acetylhexosaminidase I₂ were separated by SDS/polyacrylamide-gel electrophoresis in 10% (w/v) gels under denaturing conditions, transferred to nitrocellulose paper and stained immunochemically as described in the Experimental section. Track (a) was stained with rabbit anti-(human β -N-acetylhexosaminidase B) serum and track (b) was stained with rabbit anti-(human β -N-acetylhexosaminidase α -subunit) serum.

β -subunits and B is composed of β -subunits alone (Srivastava & Beutler, 1974). β -N-Acetylhexosaminidases A, B and I₂ from human liver have similar K_m values with 4-methylumbelliferyl β -N-acetylglucosaminide as the substrate. From its behaviour on gradient gels, hexosaminidase I₂ appears to have an M_r of approx. 105000, compared with 100000 for the A-form, but the origin of its slightly larger size does not obviously reside in the nature of its constituent polypeptides, since I₂ and A both have 56000 and 29000- M_r polypeptides as major constituents. The feature of the polypeptide profiles that most clearly distinguishes hexosaminidases A and I₂ from hexosaminidase B is the presence of a 56000- M_r component. This is the α -subunit in A and its presence in I₂, confirmed by immunological reaction with anti-(α -subunit) antiserum, indicates that it too is composed of α - and β -gene products. It seems unlikely that I₂ is an unprocessed or partially processed form of hexosaminidase A. If a valid comparison can be made between the processing reactions that take place in liver and those that have been described for fibroblasts (Hasilik & Neufeld, 1980), the unprocessed precursors of α - and β -chains would be expected to have an M_r of 67000 and 63000 respectively. Although there is polypeptide material and also immunological staining with both anti-(α -subunit) and anti-B sera in this region of the gel, the amounts of protein, based on the intensity of Coomassie Blue staining, do not appear to be sufficiently large for these polypeptides to be in simple stoichiometric relationship with the α - and β -subunits.

One possibility to be considered is that the difference in net charge of the A and I₂ forms resides in differences in glycosylation, but we have no evidence for this. Both

I₂ and A forms are glycosylated, judging from their behaviour on concanavalin A-Sepharose, and they also behave similarly on columns of wheat-germ agglutinin immobilized on Sepharose (N. N. Dewji, unpublished work), but it is possible that there may be differences between them not revealed by binding to these lectins.

The polypeptide composition of hexosaminidases A and B from human liver which we report here are very similar to those found by Joziassse *et al.* (1981) (the differences in estimated M_r , probably being due to the use of different gel systems and M_r markers) and also to those from human placenta (Hasilik & Neufeld, 1980; Mahuran & Lowden, 1980). As with hexosaminidases A and I₂ from liver, the differences in the behaviour of the A-forms from liver and placenta on DEAE-cellulose do not appear to arise out of gross differences in polypeptide composition. However, there are subtleties in the structures of β -subunits isolated from β -N-acetylhexosaminidases A and B that are not revealed by their electrophoretic mobilities on SDS/polyacrylamide-gel electrophoresis or by their immunological cross-reactivity. Mahuran *et al.* (1982) have resolved the β -subunits of β -N-acetylhexosaminidases A and B from human placenta into two polypeptides with closely similar M_r but differing in charge; these are the B_a and B_b polypeptides. Moreover, the β -subunits from β -N-acetylhexosaminidases A and B are not identical, there being an additional peptide in the peptide map of subunits from A that is not present in B (Mahuran & Lowden, 1981). These differences in peptide composition were thought to be due to differences in proteolytic processing of β -N-acetylhexosaminidases A and B. In view of this heterogeneity of β -subunits and the lack of any obvious differences in the structure of hexosaminidases A and I₂, it is possible that the 'intermediate' behaviour of hexosaminidase I₂ may arise out of a stable association between an α -subunit and β -subunits of the B-type.

Hexosaminidase I₂ was the most abundant of the intermediate forms isolated from placenta by Lowden *et al.* (1984). Its native M_r was 140 000 and after reduction and alkylation there were three major polypeptides with M_r values of 61 000, 35 000 and 28 000. The presence of a 54 000- M_r component was considered to be due to contamination of I₂ with A. There are evidently considerable differences in the polypeptide compositions of the I₂ forms isolated from liver and placenta, only the 29 000- M_r polypeptide being common to both enzymes. Silver staining of SDS/polyacrylamide gels on which liver β -N-acetylhexosaminidase I₂ had been run under denaturing conditions (results not shown) revealed minor bands with M_r values in the range 60 000–65 000 and one of M_r 42 000. These bands were also present in small amounts in the hexosaminidase A and B preparations from liver. Lowden *et al.* (1984) noted that the 61 000–63 000- M_r polypeptide from placental I₂ reacted with both anti-(β -N-acetylhexosaminidase α) and anti-(β -N-acetylhexosaminidase β) sera, a property that it seems to share with a polypeptide of similar M_r in the

preparation of I₂ from liver, although in the latter case it is present only in small amounts, judging from the intensity of Coomassie Blue staining. It would appear that there are substantial differences between the structures of liver hexosaminidase I₂, placental I₂ and the pregnancy form, P, analysed by Geiger *et al.* (1978). The native M_r of hexosaminidase P was 150 000, compared with 100 000 for the A- and B-forms, and it was found to have identical antigenic specificity with form B. Lack of hexosaminidase A-specific determinants in hexosaminidase P (Geiger *et al.*, 1978) clearly distinguishes it from liver I₂, which has both α - and β -subunits, and placental I₂, which has polypeptides of M_r 61 000 and 35 000.

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