An enzyme with properties similar to those of β -N-acetylhexosaminidase S is expressed in the promyelocytic cell line HL-60

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Extracts of the human promyelocytic cell line HL-60 contain a form of β -N-acetylhexosaminidase that is not retained on columns of benzenboronate-agarose ('phenylboronate-agarose') and has a pI value lower than that of β -N-acetylhexosaminidase A. It is clearly distinct from β -N-acetylhexosaminidase A in its behaviour on DEAE-cellulose columns, and it requires a higher concentration of salt for its elution. This 'extra' form has a higher ratio of activity towards 4-methylumbelliferyl β -N-acetylglucosaminide 6-sulphate and 4-methylumbelliferyl β -N-acetylglucosaminide than has β -N-acetylhexosaminidase A and is less stable when heated at 50 °C. It has a pH optimum of 4.5 and is therefore not β -N-acetylglucosaminidase C. Anti-(human β -N-acetylhexosaminidase α -subunit) serum precipitated both β -N-acetylhexosaminidase A and the 'extra' form, whereas an anti-(β -subunit) serum precipitated β -N-acetylhexosaminidase A but not the 'extra' form. Western blotting and immunodetection of polypeptides derived from the 'extra' form revealed a band corresponding in size to mature α -subunits. On the basis of this and of its behaviour on isoelectric focusing, chromatofocusing and its kinetic properties, we conclude that the 'extra' form is composed of α -subunits and resembles β -N-acetylhexosaminidase S, the residual form in Sandhoff's disease.

INTRODUCTION

There have been several accounts of alterations to the isoenzymes of β -N-acetylhexosaminidase in human leukaemic cells. In the first of these Ellis *et al.* (1978) described the increased abundance of the I₂ form in lymphocytes from patients with common acute lymphoblastic leukaemia (c-ALL), a characteristic that has been used as a marker in distinguishing common acute lymphoblastic leukaemia from other types of leukaemia (Greaves, 1981). β -N-Acetylhexosaminidase I₂ was initially demonstrated in leukaemic cells by DEAE-cellulose chromatography, but it has also been detected in pre B-ALL, AUL, Null-ALL and AML as well as in c-ALL cells by isoelectric focusing (Drexler *et al.*, 1984).

Chromatography of β -N-acetylhexosaminidase on columns of benzenboronate-agarose ('phenylboronate-agarose', PBA) revealed a distinct form of the enzyme that is present in leukaemic lymphocytes and myelocytes but not in lymphocytes or granulocytes (Orlacchio *et al.*, 1984). This 'extra' form is quite distinct from the intermediate form I₂.

Alterations to β -N-acetylhexosaminidase isoenzymes of an apparently different sort were described by Swallow *et al.* (1977) and Dreyfus *et al.* (1984), who found that certain lymphoblastoid cell lines, including RAJI, DAUDI, RAMOS and U698, had a decrease or even complete lack of hexosaminidases A and B. In these cell lines a form of the enzyme with an electrophoretic mobility faster than that of β -N-acetylhexosaminidase A was a major band, but in neither case was this characterized.

The promyelocytic cell line HL-60 (Collins *et al.*, 1977) is a continuous leukaemic cell line that is able to proliferate *in vitro* without changes in its state of differentiation. In HL-60 cells the

'extra' form of β -N-acetylhexosaminidase constitutes a high proportion of the total activity and is therefore a good source of the enzyme for further characterization. In its electrophoretic mobility it resembles the most anodic form described by Swallow *et al.* (1977) and Dreyfus *et al.* (1984). In the present paper we describe the nature of the 'extra' form of β -N-acetylhexosaminidase in HL-60 cells and report that it has several properties in common with β -N-acetylhexosaminidase S, the residual activity in patients with Sandhoff's disease.

EXPERIMENTAL

Materials

Roswell Park Memorial Institute (RPMI) 1640 medium and fetal-calf serum were obtained from GIBCO Europe (Breda, The Netherlands); culture flasks and Mono-Poly Resolving Medium were from Flow Laboratories (Milan, Italy); Miranol H2M was from Miranol Chemical Co. (Irvington, NJ, U.S.A.); 4-methylumbelliferyl 2-acetamido-2-deoxy- β -D-glucopyranoside (4-MUGlcNAc), 4-methylumbelliferone, N-acetylglucosamine, N-acetylgalactosamine and Naphthol-ASBI 2-acetamido-2-deoxy- β -D-glucopyranoside were from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Acrylamide, NN'-methylenebisacrylamide, Bio-Rad protein assay kit and bovine γ -globulin were from Bio-Rad Laboratories (Richmond, CA, U.S.A.); Matrex gel PBA 30 was from Amicon B.V. (Oosterhout, The Netherlands); concanavalin A-Sepharose 4B (10 mg of lectin/ml of gel), Polybuffer Exchanger PBE-94 and Polybuffer 74 for chromatofocusing were from Pharmacia Fine Chemicals (Uppsala, Sweden); DEAE-cellulose was from Whatman Biochemicals (Maidstone, Kent, U.K.); Ampholines were from

Abbreviations used: 4-MUGlcNAc, 4-methylumbelliferyl β -N-acetylglucosaminide; 4-MUGlcNAc-6-SO₄, 4-methylumbelliferyl β -N-acetylglucosaminide 6-sulphate; PBA, benzenboronate-agarose ('phenylboronate-agarose').

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LKB (Bromma, Sweden). 4-MUGlcNAc-6-SO₄ was purchased as an impure preparation from Koch-Light Laboratories (Haverhill, Suffolk, U.K.) and purified as described by Beccari *et al.* (1987). All other reagents were of analytical grade.

Cell culture

The HL-60 cell line was cultured in RPMI 1640 medium containing 10% (v/v) heat-inactivated fetal-calf serum in 75 cm² culture flasks in a humidified atmosphere containing 5% CO₂. Polymorphonuclear leucocytes were separated from peripheral venous blood by using Mono-Poly Resolving Medium, to give a preparation that was 87% pure.

Preparation of cell lysates and enzyme extraction

The cell samples (5×10^7), after three washings with 0.9% NaCl, were suspended in 1 ml of 10 mM-sodium phosphate buffer, pH 6.0, containing 0.1% (v/v) Miranol H2M, an ionically balanced detergent. They were then sonicated with a 100 WMSE ultrasonic disintegrator equipped with a micro-tip at 12 μ m wave amplitude (four sonications, 15 s each) followed by centrifugation at 36000 *g* (r_{av} 8.19 cm) for 20 min. All procedures were carried out at 4 °C. The supernatants were used as cell lysates.

Enzyme assays, kinetic properties and thermal stability of β -N-acetylhexosaminidase

Enzyme activity was measured as described by Beccari *et al.* (1987) with 3 mM-4-MUGlcNAc or 0.3 mM-4-MUGlcNAc-6-SO₄ in 0.1 M-citric acid/0.2 M-disodium phosphate buffer, pH 4.5. Fluorescence of the liberated 4-methylumbelliferone was measured on a Perkin-Elmer LS-3 fluorimeter (excitation, 360 nm; emission, 446 nm).

One unit is the amount of enzyme that hydrolyses 1 μ mol of substrate/min at 37 °C. Protein was measured by the method of Bradford (1976), with crystalline bovine γ -globulin as a standard. Specific activity is expressed as units/mg of protein.

K_m values were determined by Lineweaver & Burk (1934) plots. Samples of isoenzymes (50 μ l) were incubated with 4-MUGlcNAc in the range 0.27–2.27 mM or with 4-MUGlcNAc-6-SO₄ in the range 0.05–0.23 mM. In both cases the buffer was 0.1 M-citric acid/0.2 M-disodium phosphate buffer, pH 4.5, and incubations were at 37 °C. K_i values with 4-MUGlcNAc as the substrate were obtained by the method of Dixon & Webb (1958).

pH optima were determined in 0.1 M-citric acid/0.2 M-disodium phosphate buffers in the pH range 2.5–6.5 and in 0.1 M-sodium phosphate buffers in the pH range 7.0–8.0.

Thermal stability was determined by incubating samples of enzyme (50 μ l) in 0.1 M-citric acid/0.2 M-disodium phosphate buffer, pH 4.5, for various periods of time at 50 °C. Samples were cooled on ice for 2 h, then assayed for β -N-acetylhexosaminidase activity at 37 °C in the usual way. The results are expressed as percentage of a non-heated control.

Matrex gel PBA-30 chromatography

Cell lysates were dialysed overnight against 50 mM-Hepes/NaOH buffer, pH 8.5, containing 10 mM-MgCl₂ (starting buffer), and the precipitate that formed was sedimented by centrifugation in the SS-34 rotor of a Sorvall RC5B centrifuge at 13600 *g* for 15 min at 4 °C. There was no loss of β -N-acetylhexosaminidase activity in the precipitate. A sample of supernatant (0.5 ml) was applied to a Matrex gel PBA-30 column (0.5 cm diam. $\times$ 14 cm) equilibrated with the starting buffer. After the sample had been allowed to remain in contact with the gel for 30 min, the column was eluted with the same buffer at a flow rate of 24 ml/h. Fractions (2 ml) were collected until the A_{280} reached zero. Elution was continued with 50 mM-Hepes/NaOH buffer, pH 8.5, containing 15 mM-EDTA until the

A_{280} was zero, and then with 50 mM-Hepes/NaOH buffer, pH 8.5, containing 100 mM-sorbitol, again until the A_{280} was zero. Finally the column was washed with 100 mM-Tris/HCl buffer, pH 8.5. Chromatography was carried out at 4 °C.

DEAE-cellulose chromatography

DEAE-cellulose chromatography was performed as described by Robinson & Stirling (1968) with a 3 ml column equilibrated with 10 mM-sodium phosphate buffer, pH 6.0. Enzyme activity retained by the column was eluted in a linear gradient of NaCl that reached a concentration of 0.5 M in 120 ml of buffer.

Chromatofocusing

Automated chromatofocusing on Polybuffer Exchanger PBE-94 was performed by the method of Orlacchio *et al.* (1986a).

Chromatography on concanavalin A-Sepharose-4B

Lysates of HL-60 cells (1 ml) were dialysed overnight against 20 mM-Tris/HCl buffer, pH 7.4, containing 1 mM-MnCl₂, 1 mM-MgCl₂ and 1 mM-CaCl₂, and loaded on to a 5 ml concanavalin A-Sepharose column equilibrated with the same buffer. The column was eluted with this buffer until the A_{280} was zero. Glycoproteins retained by the column were eluted with a linear gradient of 0–0.5 M methyl α -mannoside in 50 mM-Tris/HCl buffer, pH 7.4, containing 1 M-NaCl (100 ml).

Determination of M_r values

β -N-Acetylhexosaminidase isoenzymes were dialysed against 10 mM-sodium phosphate buffer, pH 6.8, containing 0.1 M-NaCl, then analysed on a Spherogel TSK SW4000 column (0.75 cm $\times$ 30 cm), equilibrated with the above buffer, in a Beckman 332 gradient h.p.l.c. system. Samples (20 μ l) were loaded on to the column at a flow rate of 0.2 ml/min. Ovalbumin (M_r 45000), BSA (M_r 67000), fructose-bisphosphate aldolase (M_r 158000), catalase (M_r 232000) and ferritin (M_r 440000) at concentrations of 3–5 mg/ml were used to calibrate the column by monitoring A_{280} . Fractions collected at 1 min intervals were assayed for β -N-acetylhexosaminidase activity.

Isoelectric focusing

Samples of enzyme (in water) were applied to horizontal thin-layer gels containing 5% (w/v) polyacrylamide and 3% (v/v) Ampholine mixture (final pH range 3.5–9.5) in a Pharmacia FBE-3000 flat-bed apparatus. Runs were performed at 5 °C for 1.5 h at 25 W constant power, with 1 M-H₃PO₄ and 1 M-NaOH as electrode solutions. Isoenzymes were located on the gels by using the histochemical substrate Naphthol-ASBI 2-acetamido-2-deoxy- β -D-glucopyranoside by the method of Hayashi (1965).

Immunological methods

Antisera specific for the α - and β -subunits of β -N-acetylhexosaminidase were kindly provided by Dr. A. Hasilik. The anti-(placental β -N-acetylhexosaminidase A) serum was kindly provided by Dr. D. Mahuran. Immunoprecipitation was performed as described by Parkes *et al.* (1984), and the method for Western blotting and immunodetection was that described by Dewji *et al.* (1986).

RESULTS

Separation of β -N-acetylhexosaminidases from HL-60 cells by chromatography on PBA columns

When extracts of HL-60 cells were chromatographed on columns of Matrex gel PBA-30 there was a peak of non-retained β -N-acetylhexosaminidase that accounted for 30% of the applied activity (Fig. 1). This 'extra' peak was not present in extracts of

Fig. 1. Separation of β -N-acetylhexosaminidase from HL-60 cells by Matrex gel PBA-30 chromatography

Cell lysate, after dialysis against 50 mM-Hepes/NaOH buffer, pH 8.5, containing 10 mM-MgCl₂ was applied to a Matrex gel PBA-30 column equilibrated with the same buffer. After 30 min the column was eluted (24 ml/h) with the same buffer. Elution was continued with 50 mM-Hepes/NaOH buffer, pH 8.5, containing 15 mM-EDTA (arrow 1), then with 50 mM-Hepes/NaOH buffer, pH 8.5, containing 100 mM-sorbitol (arrow 2) and then with 100 mM-Tris/HCl buffer, pH 8.5 (arrow 3). Fractions were tested for activity towards 4-MUGlcNAc (●) and 4-MUGlcNAc-6-SO₄ (○).

normal granulocytes prepared in an identical way (Orlacchio *et al.*, 1984). Elution of the column with 50 mM-Hepes/NaOH buffer, pH 8.5, containing 15 mM-EDTA gave a peak that accounted for 28 % of applied activity. Further elution with 50 mM-Hepes/NaOH buffer, pH 8.5, containing 100 mM-sorbitol gave a small peak with 1 % of applied activity. Elution with 100 mM-Tris/HCl buffer, pH 8.5, produced a peak of protein but no further enzyme. The overall recovery was thus only 59 %.

Assay of the column fractions with 4-MUGlcNAc-6-SO₄ (Fig. 1) resulted in the detection of only two peaks of β -N-acetylhexosaminidase activity, that not retained by the column and that eluted with 50 mM-Hepes/NaOH buffer, pH 8.5, containing 15 mM-EDTA. These accounted for 29 % and 15 % of applied activity respectively.

Separation of β -N-acetylhexosaminidase from HL-60 cells and normal granulocytes by DEAE-cellulose chromatography

When extracts of HL-60 cells were applied to columns of DEAE-cellulose and the fractions were assayed with 4-MUGlcNAc, there was a small non-retained peak of β -N-acetylhexosaminidase accounting for less than 1 % of applied activity. The behaviour of this form is consistent with it being β -N-acetylhexosaminidase B. Two further peaks of activity were eluted by the salt gradient (Fig. 2a). The first of these represented 54 % and the second represented 32 % of the applied activity. Assay with 4-MUGlcNAc-6-SO₄ detected only the peaks in the salt gradient, the first representing 18 % and the second representing 28 % of the applied activity, an overall recovery of 46 %.

β -N-acetylhexosaminidase activity in the extract of granulocytes was resolved into two peaks when assayed with 4-MUGlcNAc (Fig. 2b). The non-retained peak represented 14 %

Fig. 2. Separation of β -N-acetylhexosaminidase from HL-60 cells and normal granulocytes by DEAE-cellulose chromatography

Cell lysates (1 ml) of (a) HL-60 cells and (b) normal granulocytes were dialysed against 10 mM-sodium phosphate buffer, pH 6.0, and applied to a DEAE-cellulose column (3 ml) equilibrated with the same buffer. Enzyme activity retained by the column was eluted in a linear gradient of 0–0.5 M-NaCl in 120 ml of buffer. Fractions were assayed for activity towards 4-MUGlcNAc (●) and 4-MUGlcNAc-6-SO₄ (○).

and the peak in the salt gradient represented 62 % of the activity applied to the column. Assay with 4-MUGlcNAc-6-SO₄ detected only the peak eluted in the salt gradient.

Analysis on PBA columns of the β -N-acetylhexosaminidase peaks separated by DEAE-cellulose chromatography

The first peak of activity eluted from DEAE-cellulose by the salt gradient was applied to a column of Matrex gel PBA-30. All the activity was retained by the column and was recovered as a single peak (68 % of applied activity) when the column was eluted with 50 mM-Hepes/NaOH buffer, pH 8.5, containing 15 mM-EDTA (Fig. 3a). From its chromatographic behaviour and its ability to hydrolyse 4-MUGlcNAc-6-SO₄ we tentatively identify this form as β -N-acetylhexosaminidase A although it is eluted at a lower salt concentration than is usual for this isoenzyme. When the second peak from DEAE-cellulose was similarly applied to Matrex gel PBA-30 a high proportion (42 % of that applied) was not retained and a further 18 % was recovered in the 50 mM-Hepes/NaOH buffer, pH 8.5, containing 15 mM-EDTA eluate (Fig. 3b). Thus this peak, although largely composed of the 'extra' form of β -N-acetylhexosaminidase, is not entirely free from contamination by a form of the enzyme that behaves in a similar manner to β -N-acetylhexosaminidase A on the Matrex gel PBA-30 column. In view of this we used the 'extra' form prepared by Matrex gel PBA-30 chromatography for analysis of its properties.

Fig. 3. Analysis on Matrex gel PBA-30 columns of the β -N-acetylhexosaminidase peaks separated by DEAE-cellulose chromatography

(a) First peak of β -N-acetylhexosaminidase activity eluted in the salt gradient from DEAE-cellulose chromatography of HL-60 cell extracts was applied to a column of Matrex gel PBA-30 (1 ml) equilibrated with 50 mM-Hepes/NaOH buffer, pH 8.5, containing 10 mM-MgCl₂. After 30 min preincubation, elution was continued with 50 mM-Hepes/NaOH buffer, containing 15 mM-EDTA (arrow 1), then with 50 mM-Hepes/NaOH buffer, pH 8.5, containing 100 mM-sorbitol (arrow 2). Fractions were assayed for activity towards 4-MUGlcNAc. (b) Second peak of β -N-acetylhexosaminidase activity eluted in the salt gradient from DEAE-cellulose chromatography of HL-60-cell extracts was applied to a column of Matrex gel PBA-30 (1 ml) and chromatographed as above.

Analysis of the A and 'extra' forms of β -N-acetylhexosaminidase from HL-60 cells by automated chromatofocusing

Automated chromatofocusing profiles of the A and 'extra' forms of β -N-acetylhexosaminidase from HL-60 cells separated on Matrex gel PBA-30 (Fig. 4) showed that the peak that we tentatively identified as β -N-acetylhexosaminidase A also behaves as the A form on this separation method. The 'extra' peak was completely retained by the Polybuffer Exchanger PBE-94 gel and was not eluted in the pH gradient. The activity was recovered, however, when the column was washed with 1 M-NaCl at the end of the run.

Ratios of activities towards 4-MUGlcNAc-6-SO₄ and 4-MUGlcNAc of the chromatographic peaks eluted from Matrex gel PBA-30 and DEAE-cellulose

Activities of the peak fractions of hexosaminidases eluted from Matrex gel PBA-30 and DEAE-cellulose were expressed as ratios and compared with the ratio of activities found in normal granulocytes (Table 1).

Fig. 4. Analysis by automated chromatofocusing of β -N-acetylhexosaminidase peaks separated by Matrex gel PBA-30 chromatography

(a) Elution profile of the peak eluted from a Matrex gel PBA-30 column by 50 mM-Hepes/NaOH buffer, pH 8.5, containing 15 mM-EDTA (A form). (b) Elution profile of the peak eluted from a Matrex gel PBA-30 column by 50 mM-Hepes/NaOH buffer, pH 8.5, containing 10 mM-MgCl₂ ('extra' form). Samples (100 μ l) in 25 mM-piperazine/HCl buffer, pH 5.5, were applied to a Polybuffer Exchanger PBE-94 microcolumn (4 mm diam. $\times$ 70 mm) equilibrated with the same buffer. Elution was continued with this buffer for 20 min, then Polybuffer 74, pH 4.9 (0.15 mmol/pH unit per ml), was pumped through the column (arrow 1). After a further 30 min Polybuffer 74, pH 4.0 (0.15 mmol/pH unit per ml), was pumped through the column and this was continued for 30 min (arrow 2). Finally, the column was washed with 1.0 M-NaCl for 15 min (arrow 3). Enzyme activity eluted from the column was continuously detected by automated assay.

Concanavalin A-Sepharose chromatography

All the β -N-acetylhexosaminidase in the HL-60 cell extract was retained on concanavalin A-Sepharose, and was eluted as a single peak of activity (88% of the total activity loaded) by a linear gradient of methyl α -mannoside.

Gel filtration of β -N-acetylhexosaminidase on Sphergel TSK SW 4000

Estimates of M_r values of β -N-acetylhexosaminidase 'extra', A and B forms separated by Matrex gel PBA-30 chromatography were 130 000, 100 000 and 94 000 respectively.

Isoelectric focusing of β -N-acetylhexosaminidases from HL-60 cells

Flat-bed isoelectric focusing was used to compare the pI values of the 'extra' and A forms of β -N-acetylhexosaminidase isolated from HL-60-cell extracts by Matrex gel PBA-30 chromatography. The 'extra' form was resolved into several components with pI values in the range 4.3–4.6; the A preparation was separated into two groups of components with pI values of 4.7–5.0 and 5.1–5.3 (Fig. 5). Normal granulocytes, lymphocytes and monocytes were completely lacking the pI 4.3–4.6 components that appeared so prominently in the extract of HL-60 cells (results not shown).

Table 1. Ratios of β -N-acetylhexosaminidase activities towards 4-MUGlcNAc-6-SO₄ and 4-MUGlcNAc in the chromatographic peaks eluted from Matrex gel PBA-30 and DEAE-celluloseRatios of activities were measured with 0.3 mM-4-MUGlcNAc-6-SO₄ and 3 mM-4-MUGlcNAc.

Sample	Ratio of activities towards 4-MUGlcNAc-6-SO ₄ / 4-MUGlcNAc
HL-60 cells	
Matrex gel PBA-30 chromatography	
Non-retained peak	0.130
Peak eluted with Hepes/EDTA	0.047
Peak eluted with Hepes/sorbitol	0
DEAE-cellulose chromatography	
Non-retained peak	0
First peak eluted in NaCl gradient	0.045
Second peak eluted in NaCl gradient	0.130
Normal granulocytes	
DEAE-cellulose chromatography	
Non-retained peak (β -N-acetylhexosaminidase B)	0
Peak eluted in NaCl gradient (β -N-acetylhexosaminidase A)	0.060

Fig. 5. Isoelectric focusing of β -N-acetylhexosaminidase from HL-60 cells

Samples of 'extra' form (lane 1), A form (lane 2) and a crude extract of HL-60 cells (lane 3) were analysed by analytical isoelectrofocusing on horizontal thin-layer gels containing 5.0 % (w/v) polyacrylamide and 3 % Ampholine mixture (final pH range 3.5–9.5). Runs were performed at 5 °C for 1.5 h at 25 W constant power. Isoenzymes were located on the gels as described in the Experimental section.

Kinetic properties and thermal stabilities of β -N-acetylhexosaminidases from HL-60 cells

β -N-Acetylhexosaminidase A, B and 'extra' forms had very similar K_m values with 4-MUGlcNAc as the substrate (1.0, 0.9 and 1.2 mM respectively), and K_i values for *N*-acetylglucosamine were also similar (5.0, 4.5 and 4.3 mM respectively). With 4-MUGlcNAc-6-SO₄ as the substrate the 'extra' form had a lower K_m (0.30 mM) than the A form (0.55 mM).

Thermal denaturation of hexosaminidase A, B and 'extra' forms at 50 °C showed that, although the A form was inactivated in a fairly characteristic manner ($t_{1/2}$ 13 min), the B form was more thermolabile than its counterpart in normal tissues ($t_{1/2}$ 23 min). The 'extra' form was the least stable of the three ($t_{1/2}$ 8.5 min).

Western blotting and immunodetection of the polypeptides in the 'extra' form of β -N-acetylhexosaminidase from HL-60 cells

Immunological detection of polypeptides from the 'extra' form of β -N-acetylhexosaminidase, after SDS/PAGE and Western blotting with an anti-(β -N-acetylhexosaminidase A) serum,

Fig. 6. Immunodetection of β -N-acetylhexosaminidase subunits in the 'extra' form

Samples of placental β -N-acetylhexosaminidase A (lane 1) and the HL-60-cell 'extra' form (lane 2) were subjected to SDS/PAGE under reducing conditions. Polypeptides were transferred to nitrocellulose by Western blotting and the subunits were detected with an anti-(human placental β -N-acetylhexosaminidase A) serum by the procedure cited in the Experimental section. The locations of the α - and β -subunits of placental β -N-acetylhexosaminidase are indicated.

showed two closely spaced bands with M_r values similar to that of the mature α -subunit of human placental β -N-acetylhexosaminidase A. There was no band corresponding to the mature β -subunit of human placental β -N-acetylhexosaminidase A (Fig. 6). Confirmation that the 'extra' form contained α - but not β -subunits came from immunoprecipitation experiments. Whereas the A form was precipitated by both anti-(β -N-acetylhexosaminidase α -subunit) and anti-(β -N-acetylhexosaminidase β -subunit) sera, the 'extra' form was precipitated by anti-(α -subunit) serum but not by anti-(β -subunit) serum. A sample of placental β -N-acetylhexosaminidase B was precipitated by the anti-(β -subunit) serum but not by the anti-(α -subunit) serum (Fig. 7). Attempts to use the specific anti-(α -subunit) and anti-(β -subunit) sera for immunodetection following Western blotting were unsuccessful.

Fig. 7. Immunoprecipitation of A and 'extra' isoenzymes of β -N-acetylhexosaminidase from HL-60 cells and of β -N-acetylhexosaminidase B from human placenta by anti-(β -N-acetylhexosaminidase α -subunit) and anti-(β -N-acetylhexosaminidase β -subunit) sera

Isoenzyme samples were mixed with appropriate dilutions of antisera and incubated for 20 h at 4 °C. The immunoprecipitates were sedimented by centrifugation at 12000 *g* for 10 min. Precipitation was evaluated by measuring the enzyme activity of the supernatants. (a) Anti-(β -N-acetylhexosaminidase α -subunit) serum; (b) anti-(β -N-acetylhexosaminidase β -subunit) serum. ○, A form; ●, 'extra' form; ▲, placental β -N-acetylhexosaminidase B.

DISCUSSION

The promyelocytic cell line HL-60 expresses a stable pattern of β -N-acetylhexosaminidase isoenzymes when analysed by isoelectric focusing (Moss *et al.*, 1985; Drexler *et al.*, 1986) or chromatofocusing (Orlacchio *et al.* 1986b). We have previously described the presence of a particular type of β -N-acetylhexosaminidase in leukaemic cells of lymphoid and myeloid origins, distinguished by its behaviour on PBA chromatography, that is absent from normal lymphocytes and myelocytes and is distinct from β -N-acetylhexosaminidases A and B (Orlacchio *et al.*, 1984). This 'extra' form is particularly prominent in extracts of HL-60 cells, and we confirm here that it is lacking in granulocytes, a differentiation end product of the myeloid line.

We have considered the various ways in which the 'extra' form may arise. One possibility is that the enzyme is unrelated to the A and B forms, but this seems unlikely because anti-(β -N-acetylhexosaminidase α -subunit) serum was able to precipitate 90% of the 'extra' form. Altered behaviour of β -N-acetylhexosaminidase isoenzymes in leukaemic cells has been ascribed to post-translational modifications involving glycosylation (Swallow *et al.*, 1977; Dewji *et al.*, 1981; Moss & Besley, 1985; Drexler *et al.*, 1986). At first sight this would appear to be the case for the β -N-acetylhexosaminidase of HL-60 cells because of their behaviour on Matrex gel PBA-30 chromatography. However, the isoenzymes are eluted in decreasing order of their isoelectric points and it seems likely that the predominant effect is one based on electrostatic interaction rather than specific interactions involving the *cis*-diol groups of the glycoproteins (Emiliani *et al.*, 1988). That the 'extra' form is a glycoprotein is evident from its binding to concanavalin A-Sepharose, but we have no other evidence that it differs in its glycosylation from the A and B forms.

A noticeable feature of the 'extra' form is its activity towards 4-MUGlcNAc-6-SO₄, which is characteristic of those forms of β -N-acetylhexosaminidase containing the α -subunit (Kytzia & Sandhoff, 1985). It was therefore decided to compare the behaviour of the 'extra' form with that of β -N-acetylhexosaminidase A on DEAE-cellulose. The peak of activity that was eluted at a higher concentration of NaCl than the A form was largely composed of the 'extra' form (judged from its

behaviour on Matrex gel PBA-30 and also from its ratio of activities towards 4-MUGlcNAc-6-SO₄ and 4-MUGlcNAc). The A and 'extra' forms had pI values of 4.7–5.3 and 4.3–4.6 respectively. These are similar to pI values of 4.9 previously reported for β -N-acetylhexosaminidase A and 4.2 for β -N-acetylhexosaminidase S (Mahuran & Lowden, 1980).

Consistent with its behaviour on isoelectric focusing, the 'extra' form was not eluted in the pH gradient on chromatofocusing, although it could be recovered by elution of the column with 1.0 M-NaCl. In this respect there seems to be similarity between the behaviour of the 'extra' form in HL-60-cell extracts and β -N-acetylhexosaminidase S (O'Dowd *et al.*, 1986). Both have acidic pH optima, are bound to concanavalin A-Sepharose, are more thermolabile than β -N-acetylhexosaminidase A, have similar native *M_r* values and have *K_m* values for 4-MUGlcNAc-6-SO₄ of 0.3 mM. In the first two of these properties the 'extra' form and β -N-acetylhexosaminidase S are clearly distinguished from β -N-acetylglucosaminidase C, an isoenzyme with which β -N-acetylhexosaminidase S is sometimes confused because of their similar electrophoretic mobilities (Hooghwinkel *et al.*, 1972). Immunoprecipitation studies were consistent with the absence of β -subunits from the 'extra' form and Western blotting demonstrated that mature β -subunits were absent, so that it would seem likely that the 'extra' form in HL-60 cells is β -N-acetylhexosaminidase S.

This work was supported by Italian Centro Nazionale delle Ricerche and Ministero Pubblica Istruzione and by the U.K. Medical Research Council. A grant from Associazione Italiana Ricerche sul Cancro to C. E. is gratefully acknowledged.

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Received 6 October 1989; accepted 24 October 1989