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Molecular forms of β-hexosaminidase and cathepsin D in serum and urine of healthy subjects and patients with elevated activity of lysosomal enzymes

Michael ZÜHLSDORF, Michael IMORT, Andrej HASILIK and Kurt von FIGURA
Physiologisch-Chemisches Institut, Universität Münster, Waldeyerstrasse 15, D 4400 Münster, Federal Republic of Germany

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A procedure is described that allows the characterization of the molecular forms of β-hexosaminidase and cathepsin D in controls and pathological specimens of human serum and human urine. The following observations were made. (1) In human serum, β-hexosaminidase (α- and β-chain) and cathepsin D are present predominantly in their high-molecular-weight precursor forms. In human urine, these enzymes exist as both precursor and mature forms. (2) Cathepsin D precursor from serum and urine differs in the number of oligosaccharides that are sensitive to endo-β-*N*-acetylglucosaminidase H. Therefore the urine enzyme is not likely to originate from the serum. (3) The presence exclusively of precursors of β-hexosaminidase and of cathepsin D in the sera of patients with hepatitis suggests that in hepatitis secretion of lysosomal enzymes is elevated, rather than the enzymes leaking from damaged cells. (4) In the urine of patients with nephrotic syndrome, β-hexosaminidase and cathepsin D are present in grossly elevated amounts, but do not differ in the polypeptide patterns from controls. (5) In urine from a patient with mucolipidosis II, the elevated activity of β-hexosaminidase is accounted for mainly by the precursor forms. Mature β-chain of β-hexosaminidase is lacking, and incompletely processed β-hexosaminidase polypeptides are present. Both the precursor and the mature forms of cathepsin D are increased. They contain only complex oligosaccharides.

Lysosomal enzymes are synthesized as precursors of higher molecular weight and subsequently processed to mature forms, presumably by proteinases [for reviews see Hasilik (1980) and Hasilik & von Figura (1983)]. Maturation of lysosomal enzymes occurs after their segregation from the secretory pathway and is accomplished, at least partly, within the lysosomes (Gieselmann *et al.*, 1983). In cultured cells a small fraction of the newly synthesized lysosomal enzymes, usually less than 20% of the total, escapes from segregation into lysosomes and is secreted into the medium (Hasilik & Neufeld, 1980). In the medium they remain in the precursor forms. In fibroblasts and several other cell types (smooth-muscle cells, endothelial cells, lymphocytes, hepatocytes) no mature forms of lysosomal enzymes are secreted (Hasilik *et al.*, 1981; Rosenfeld *et al.*, 1982; Imort *et al.*, 1983). Macrophages, however, are known to release mature lysosomal enzymes upon stimulation (Imort *et al.*, 1983). Another source of mature forms of lysosomal enzymes might be damaged cells.

Abbreviation used: SDS, sodium dodecyl sulphate.

Lysosomal enzymes are common in human body fluids, such as serum, urine and tears. The cellular sources and the pathways of release of these enzymes remain to be established. The activities of lysosomal enzymes are elevated in certain acquired or genetic diseases. Increase in β-hexosaminidase in serum and urine is reported for inflammatory diseases of liver (Calvo *et al.*, 1982) and kidney (Dance *et al.*, 1970). In all body fluids of patients with mucolipidosis II (I-cell disease) the activities of lysosomal enzymes may be more than 10-fold above the norm (for review see McKusick *et al.*, 1978). In this disease, in certain cells the segregation of lysosomal enzymes is defective and the majority of newly synthesized lysosomal enzymes are secreted as their precursors, as demonstrated for fibroblasts (Hasilik & Neufeld, 1980).

Characterization of the molecular forms of lysosomal enzymes in body fluids may provide information on mechanisms of release of lysosomal enzymes into these fluids under normal and pathological conditions. In the present study we describe the molecular forms of two lysosomal enzymes in serum and urine of healthy subjects and of patients

with nephrotic syndrome, hepatitis and mucolipidosis II. Part of these results has been presented in preliminary form (Hasilik *et al.*, 1982).

Materials and methods

Materials

Cathepsin D and β-hexosaminidase purified from human placenta and antisera raised in goat or rabbit against these enzymes were those previously described (Hasilik & Neufeld, 1980; von Figura & Weber, 1978). 2-Acetamido-*N*-(ε-aminohexanoyl)-2-deoxy-β-D-glucopyranosylamine was kindly provided by Dr. N. Sharon, Rehovot, Israel. Molecular-weight standards were obtained from Bio-Rad, München, Germany, and [^{14}C]methylated standards were from NEN, Dreieich, Germany. SDS was from Sigma, München, Germany. Endo-β-*N*-acetylglucosaminidase H was obtained from Health Research Inc., Albany, NY, U.S.A.

Subjects

Serum and urine were obtained from five control subjects, one patient with mucolipidosis II, two patients with nephrotic syndrome and six patients with liver diseases (acute viral hepatitis, chronic active hepatitis and cirrhosis).

Preparation of β-hexosaminidase from human serum

Serum (50ml) was dialysed for 13h against 5 litres of 20mM-sodium phosphate, pH6.0, cleared by centrifugation for 15min at 6000*g* and affinity-purified on a column (0.9cm × 2.7cm) of 2-acetamido-*N*-(ε-aminohexanoyl)-2-deoxy-β-D-glucopyranosylamine-Sepharose 4B (Geiger & Arnon, 1978). The column was washed with 12ml of 20mM-sodium phosphate, pH6.0, and eluted with 10ml of 0.15M-NaCl in 20mM-sodium phosphate, pH6.8, and 8ml of 0.15M-NaCl in 1% (w/v) glycine/20mM-Tris/HCl (pH8.6). The fractions eluted at pH6.8 and 8.6 were pooled, concentrated by ultrafiltration and dialysed against 0.15M-NaCl in 50mM-Tris/HCl, pH7.4. β-Hexosaminidase was purified by this step 500-fold to a specific activity of 0.14unit/mg, with a yield of 35%. Anti-β-hexosaminidase immunoglobulins immobilized on Sepharose 4B were added in an amount sufficient for complete binding of β-hexosaminidase. After incubation for 6h at 4°C, the gel was pelleted by centrifugation for 2min in an Eppendorf model 5412 centrifuge and washed thoroughly (Hasilik & Neufeld, 1980). β-Hexosaminidase was solubilized by heating the gel for 2min at 95°C in 125mM-Tris/HCl (pH6.8)/1% (w/v) SDS/10% (v/v) glycerol. The sample was centrifuged (Eppendorf) and the supernatant heated for 6min at 95°C after being adjusted to 10mM-dithiothreitol. In later experiments (see Fig. 5) β-hexosaminidase was directly precipitated from serum with the aid of anti-(β-hexosaminidase) immunoglobulin–Sepharose 4B. The latter was prepared by coupling immunoglobulins to CNBr-activated Sepharose 4B (1mg of immunoglobulin/ml wet gel) and bound 20μg of placental β-hexosaminidase per ml of wet gel. The immunoglobulins were prepared by adsorption of goat anti-β-hexosaminidase serum to β-hexosaminidase–Sepharose 4B and sequential elution of immunoglobulins with 2M-$MgCl_2$ and 0.1M-glycine/HCl, pH2.6. In a separate experiment (see Fig. 1) 800munits of β-hexosaminidase affinity-purified from 150ml of serum were immunoprecipitated with goat anti-β-hexosaminidase serum (Hasilik & Neufeld, 1980) and solubilized in 0.125mM-Tris/HCl (pH6.8)/1% SDS/10% glycerol as above.

Preparation of β-hexosaminidase from human urine

Fresh human urine was mixed with $(NH_4)_2SO_4$ (0.5g/ml of urine). Proteins were extracted from the precipitate with 0.15M-NaCl as described previously (von Figura, 1977), dialysed against 20mM-sodium phosphate, pH6.0, and affinity-purified as described for serum β-hexosaminidase. The fractions eluted at pH6.8 and 8.6 were pooled, dialysed against 20mM-sodium phosphate and rechromatographed on the same column. The fractions eluted at pH8.6 were dialysed against 20mM-Tris/HCl, pH7.0, and concentrated by ultrafiltration. The final preparation, containing 7% of the starting β-hexosaminidase activity with a specific activity of 20units/mg of protein, was heated for 6min at 95°C in 0.125mM-Tris/HCl (pH6.8)-1% SDS/10mM-dithiothreitol/10% glycerol. In later experiments (see Fig. 5) β-hexosaminidase was directly precipitated, with the aid of anti-(β-hexosaminidase) immunoglobulin–Sepharose 4B, from urine proteins extracted as described above.

Preparation of β-hexosaminidase from human liver

Foetal human liver (1.1g) was homogenized in 2ml of 20mM-sodium phosphate (pH6.0) containing 1mM-phenylmethanesulphonyl fluoride and centrifuged at 40000*g* for 30min. The supernatant was dialysed against 0.15M-NaCl/20mM-sodium phosphate, pH6.0, cleared by centrifugation and applied to the affinity column described above. The fractions eluted at pH8.6 contained 80% of the starting β-hexosaminidase activity, with a specific activity of 3–4units/mg (160-fold purification). A sample (250munits) of β-hexosaminidase was immunoprecipitated with goat anti-β-hexosaminidase serum and solubilized as described by Hasilik & Neufeld (1980).

Preparation of cathepsin D from human serum and urine

Serum was adjusted to 0.1% (v/v) Triton X-100 and centrifuged for 1h at 50000*g*. From the supernatant cathepsin D was precipitated by adding 0.4 vol. of a goat antiserum against cathepsin D from human placenta (precipitating 10μg of placental cathepsin D/ml of antiserum). Washing and solubilization of the immune precipitate for gel electrophoresis were as previously described (Hasilik & Neufeld, 1980).

Urine proteins obtained by precipitation with $(NH_4)_2SO_4$ and extraction with 0.15M-NaCl as described above were reprecipitated with 70%-satd. $(NH_4)_2SO_4$ and suspended in water. After dialysis against 20mM-Tris/HCl, pH7.0, the samples (300-fold concentrated compared with the starting urine) were adjusted to 0.1% Triton X-100 and cleared by centrifugation at 50000*g* for 1h. Cathepsin D was immunoprecipitated from the supernatant by using 0.2ml of antiserum per ml of supernatant and processed for gel electrophoresis as described by Hasilik & Neufeld (1980).

Treatment with endo-β-N-acetylglucosaminidase H

Immunoprecipitates of cathepsin D were solubilized in 20mM-sodium acetate, pH5.8, containing 1% SDS and heated for 6min at 95°C. The solubilized samples were diluted with 2vol. of water and dialysed for 12h against 20mM-sodium acetate, pH5.8, at room temperature. Endo-β-*N*-acetylglucosaminidase H (1unit/ml of 20mM-sodium acetate, pH5.8) was added to give a final concentration of 30units/ml, and dialysis was continued for 24h at 37°C. Controls were treated likewise, but in the absence of endo-β-*N*-acetylglucosaminidase H. The samples were mixed with 1vol. of 0.25M-Tris/HCl, pH6.8, containing 2% SDS, 20mM-dithiothreitol and 20% glycerol, and heated for 6min at 95°C.

Polyacrylamide-gel electrophoresis and detection of β-hexosaminidase and cathepsin D after electroblotting

Polyacrylamide-gel electrophoresis in the presence of SDS was performed as described by Laemmli (1970), and electrophoretic transfer of separated polypeptides to nitrocellulose (BA 83; Schleicher und Schüll, Dassel, Germany) as described by Towbin *et al.* (1979), at 20V/cm for 4h at 22–24°C. Under these conditions the percentages of transferred [^{14}C]methylated standards were as follows: phosphorylase *b* (92kDa), 40%; bovine serum albumin (69kDa), 86%; ovalbumin (46kDa), 77%; carbonic anhydrase (30kDa), 72%; and cytochrome *c* (12.3kDa), 92%. After electroblotting, the part of the nitrocellulose sheet with the standard proteins was cut off and stained with Coomassie Blue. The nitrocellulose sheets were washed once with 0.15M-NaCl and incubated for 4h at 37°C in 0.15M-NaCl/50mM-Tris/HCl (pH7.0)/3% (w/v) bovine serum albumin (buffer A). Then the sheets were incubated at 4°C in antisera diluted in buffer A (25-fold diluted goat anti-β-hexosaminidase serum, 75-fold diluted rabbit anti-cathepsin D serum). Controls were incubated with identically diluted preimmune sera. For detection of β-hexosaminidase the incubation was done for 12h, and for that of cathepsin D for 4h. The sheets were washed for 1h with five changes of 0.15M-NaCl and incubated at 4°C for 2h with anti-(goat immunoglobulin G)–peroxidase conjugate or anti-(rabbit immunoglobulin G)–peroxidase conjugate (both from Miles, Rehovot, Israel) 500-fold diluted in buffer A. After washing for 1h with five changes of 0.15M-NaCl, the nitrocellulose sheets were incubated at room temperature with 50mM-Tris/HCl, pH7.4, contain-

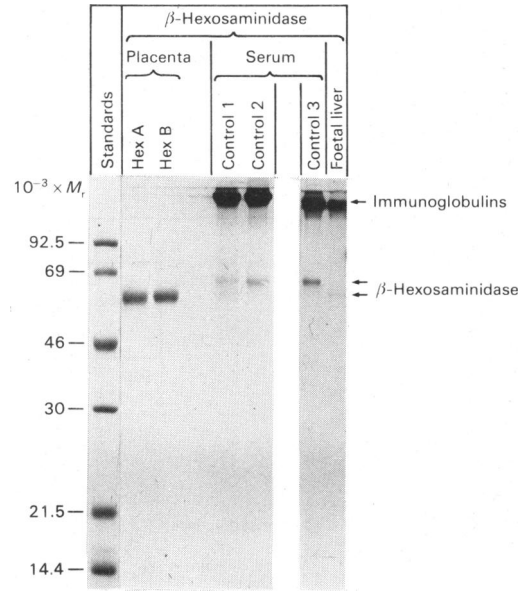

Fig. 1. *Gel electrophoresis of β-hexosaminidase from human serum and human liver*

β-Hexosaminidase was purified from 150ml of human serum from three healthy subjects, and from 0.1g of human foetal liver, by affinity chromatography and immunoprecipitation. The immunoprecipitates were solubilized in the absence of dithiothreitol and subjected to gel electrophoresis in the presence of SDS. The gel was stained with Coomassie Blue. Arrows indicate the migration of immunoglobulins and of precursor (63kDa) and mature (58kDA) forms of β-hexosaminidase from human fibroblasts. For comparison, β-hexosaminidases A (Hex A) and B (Hex B) purified as mature forms from human placenta (Hasilik & Neufeld, 1980) are also shown.

ing 0.03% (w/v) 2,3-diaminobenzidine (EGA-Chemie, Steinheim, Germany) (care: possibly carcinogenic) and 0.005% H_2O_2. After 1–5 min the reaction was stopped by washing the sheets in 7% (v/v) acetic acid and several changes of water.

Other methods

Activity of β-hexosaminidase was determined with *p*-nitrophenyl-β-D-*N*-acetylglucosaminide as substrate (von Figura, 1977). One unit is defined as the amount of enzyme splitting 1 μmol of substrate/min. Protein was determined as described by Lowry *et al.* (1951), with bovine serum albumin as standard.

Results

β-Hexosaminidase and cathepsin D in serum and urine

In a preliminary experiment β-hexosaminidase was purified from serum and liver by a combination of affinity chromatography and immunoprecipitation. The immunoprecipitate was solubilized under non-reducing conditions in the presence of SDS, separated by gel electrophoresis and stained for proteins (Fig. 1). The major fraction of serum β-hexosaminidase had an apparent molecular mass of 63 kDa, which is characteristic of the precursor form of β-hexosaminidase (Hasilik & Neufeld, 1980). A small fraction (less than 20%, as estimated by densitometry) behaved as the mature β-hexosaminidase (apparent molecular mass 58 kDa), which is the only form detectable in foetal human liver and behaves like isoenzymes A and B from human placenta. Under non-reducing conditions the α- and β-chains of β-hexosaminidase co-migrate in gel electrophoresis.

In further experiments β-hexosaminidase and cathepsin D were immunoprecipitated from serum and urine either directly or after partial purification (see the Materials and methods section). The immunoprecipitates were solubilized in the presence of SDS and dithiothreitol, separated by gel electrophoresis and transferred to nitrocellulose by electroblotting. The polypeptides related to β-hexosaminidase and cathepsin D were detected on the nitrocellulose by the peroxidase technique. This procedure had several advantages as compared with the protein staining: (i) less material was required, (ii) reducing conditions could be employed, allowing a better resolution of precursor and mature forms, and (iii) immunoglobulin G and proteins contaminating the immunoprecipitates were stained, but only weakly.

In Fig. 2 the sensitivity of the procedure for

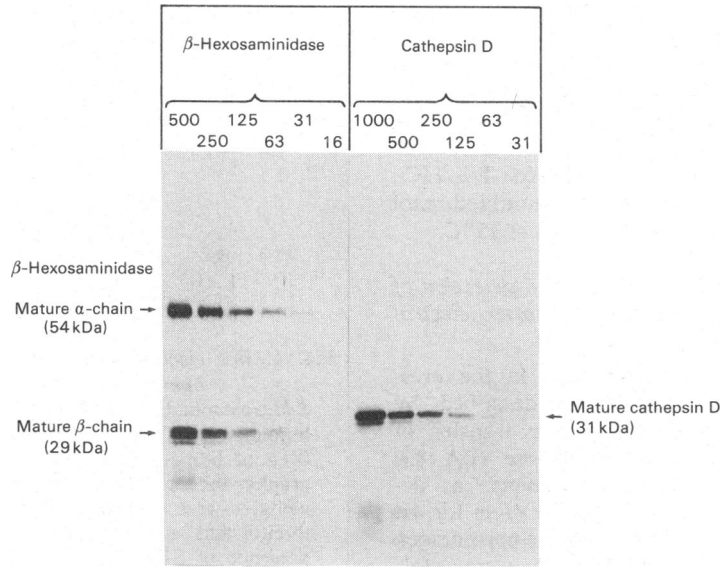

Fig. 2. *Immunochemical detection of β-hexosaminidase and cathepsin D from human placenta*

β-Hexosaminidase (16–500 ng) and cathepsin D (31–1000 ng) were separated by gel electrophoresis in the presence of SDS, electro-blotted on to nitrocellulose and immunochemically detected as described in the Materials and methods section. The amount (ng) of lysosomal enzyme protein applied is indicated above the lanes. The migration of the major β-hexosaminidase and cathepsin D polypeptides is indicated, with the molecular weights as determined previously (Hasilik & Neufeld, 1980). For the minor polypeptides see Hasilik & von Figura (1981).

β-hexosaminidase and cathepsin D purified from human placenta is shown. As little as 31 ng of β-hexosaminidase and 63 ng of cathepsin D were detectable.

In serum the major polypeptides of β-hexosaminidase (Fig. 3) and cathepsin D (Fig. 4) had the molecular masses characteristic of the respective precursor forms (apparent values 67 and 63 kDa for α- and β-chains of β-hexosaminidase and 53 kDa for cathepsin D). A small fraction of β-hexosaminidase-related polypeptides behaved as mature α- and β-chains (apparent molecular masses 54 and 29 kDa respectively). Mature cathepsin D (apparent molecular mass 31 kDa) was hardly detectable.

In urine both the precursor and mature forms of β-hexosaminidase (Fig. 3) and of cathepsin D (Fig. 4) were detectable. The mature chains represented the major fraction of β-hexosaminidase, whereas the precursor of cathepsin D was the predominant form.

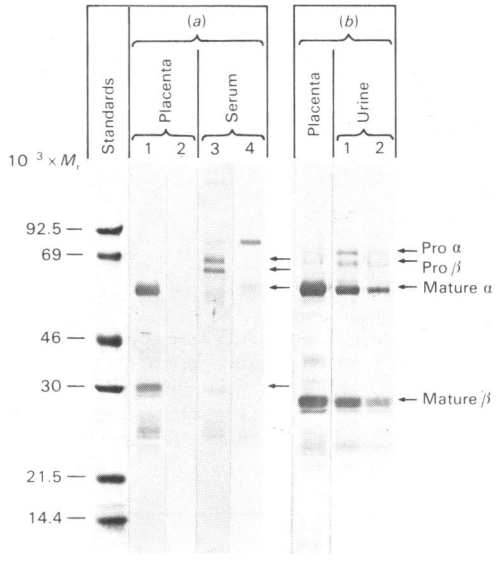

Fig. 3. *β-Hexosaminidase from human serum (a) and human urine (b)*

(*a*) β-Hexosaminidase purified and immunoprecipitated from 12 ml of serum and 1 μg of placental β-hexosaminidase were subjected to gel electrophoresis, electro-blotting and immunochemical staining. Lanes 1 and 3 were incubated with anti-β-hexosaminidase serum, and lanes 2 and 4 with preimmune serum. Some extra bands were observed in the β-hexosaminidase immunoprecipitated from serum, which were stained after incubation with either preimmune serum or antiserum. These bands are not considered to be related to β-hexosaminidase. Some are believed to derive from heavy (50–55 kDa) and light (approx. 25 kDa) chains of immunoglobulin. A high-molecular-weight polypeptide (87 kDa) stained consistently more intensively in the controls incubated with preimmune serum. The standards (phosphorylase *b*, 92.5 kDa; bovine serum albumin, 69 kDa; ovalbumin, 46 kDa; carbonic anhydrase, 30 kDa; soya-bean trypsin inhibitor, 21.5 kDa; lysozyme, 14.4 kDa) were stained with Coomassie Blue. (*b*) β-Hexosaminidase immunoprecipitated from 240 and 100 ml of urine (lanes 1 and 2) and 1 μg of placental β-hexosaminidase were subjected to gel electrophoresis. The migrations of the precursor (Pro) and mature forms of the α- and β-chains are indicated by arrows.

In urine both the precursor and mature forms of β-hexosaminidase (Fig. 3) and of cathepsin D (Fig. 4) were detectable. The mature chains represented the major fraction of β-hexosaminidase, whereas the precursor of cathepsin D was the predominant form.

β-Hexosaminidase and cathepsin D in pathological specimens

In the serum of patients with different forms of hepatitis, the activity of β-hexosaminidase was elevated 4–6-fold. The amounts of β-hexosaminidase and cathepsin D as determined by the peroxidase technique were elevated to a similar extent (shown in Figs. 5 and 6 for serum from a patient with acute viral hepatitis). Only the precursor forms of both enzymes were detectable, suggesting that a secretion and not a release from damaged cells is the cause of the elevated activities of β-hexosaminidase and cathepsin D in hepatitis.

In the urine of two patients with nephrotic syndrome, β-hexosaminidase activity was elevated 8–40-fold and the daily excretion 8–12-fold. The amounts of β-hexosaminidase and cathepsin D detectable by the immune-peroxidase procedure were similarly increased. The polypeptide patterns of β-hexosaminidase and cathepsin D did not differ from those in controls (Figs. 5 and 6).

The catalytic activity of β-hexosaminidase as well as concentrations of β-hexosaminidase and cathepsin D were elevated in the urine of a mucolipidosis-II patient (5–10-fold). The increase in β-hexosaminidase was accounted for by its precursor forms (Fig. 5). In addition, a partially processed 58 kDa polypeptide of β-hexosaminidase was present that was not found in the control urine. Treatment with endo-β-*N*-acetylglucosaminidase H (Fig. 6) showed that urinary cathepsin D of a patient with mucolipidosis II contains only complex oligosaccharides, in contrast with urinary cathepsin D of controls, which contains mainly cleavable oligosaccharides (see below).

Oligosaccharides in cathepsin D in serum and urine

Human cathepsin D contains two oligosaccharides which may be cleavable by (high-mannose and hybrid type) or resistant to (complex type) endo-β-*N*-acetylglucosaminidase H (Hasilik & von Figura, 1981). Analysis of the oligosaccharides in cathepsin D showed a clear difference between cathepsin D from serum and urine. In the serum of controls and of patients with hepatitis or nephrotic syndrome, most of the molecules of cathepsin D precursor contain two resistant oligosaccharides.

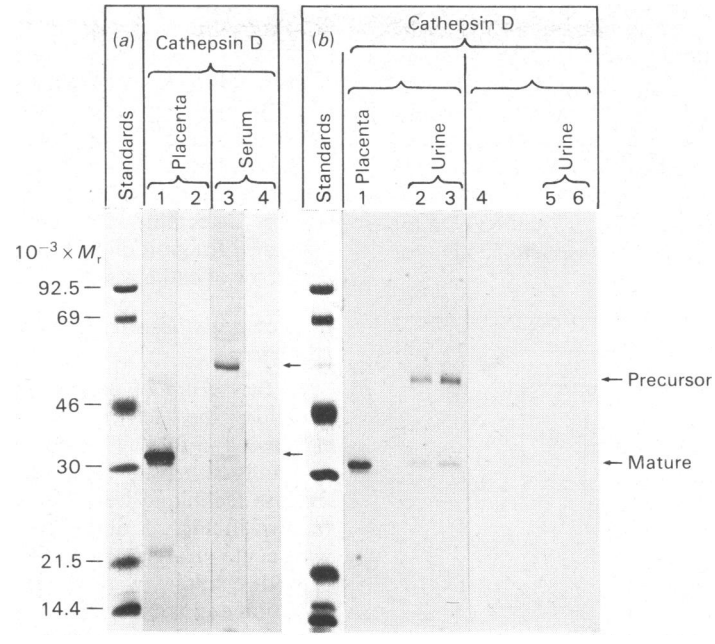

Fig. 4. *Cathepsin D from human serum (a) and human urine (b)*
(*a*) Cathepsin D immunoprecipitated from 0.5 ml of serum and 500 ng of placental cathepsin D were applied. Lanes 1 and 3 were incubated with anti-(cathepsin D) rabbit serum, and lanes 2 and 4 with preimmune serum. (*b*) Cathepsin D purified and immunoprecipitated from 30 ml (lanes 2 and 5) and 60 ml (lanes 3 and 6) of urine and 500 ng of placental cathepsin D (lanes 1 and 4) were applied. The left part was incubated with anti-(cathepsin D) rabbit serum, and the right part with preimmune serum. The migrations of the precursor (53 kDa) and mature forms (31 kDa) of cathepsin D are indicated by arrows (for standards see Fig. 3).

The minor form contains one cleavable oligosaccharide. In contrast, the major part of the precursor and mature forms of cathepsin D in the urine of controls and patients with nephrotic syndrome contains one or two cleavable oligosaccharides. Thus cathepsin D in serum and urine differ in their carbohydrate side chains.

Discussion

The present paper demonstrates that under normal conditions most of the β-hexosaminidase and cathepsin D present in human serum appears to consist of precursors, on the basis of their molecular size, which is the same as of precursors identified in fibroblasts (Hasilik & Neufeld, 1980), endothelial cells, smooth-muscle cells (Hasilik *et al.*, 1981), macrophages (Imort *et al.*, 1983), lymphocytes and granulocytes (M. Imort & K. von Figura, unpublished work). Under culture conditions these cells secrete the fraction of lysosomal enzymes that escapes segregation into the lysosomes into the culture medium as high-molecular-weight precursors. It is thus suggested that this is the origin of the lysosomal enzymes present in the serum. In human urine, both the precursor and the mature forms of β-hexosaminidase and cathepsin D are found. We assume that the precursor forms represent a fraction of newly synthesized lysosomal enzymes that escapes segregation to the lysosomes and is secreted into urine by cells lining the urogenital tract. The origin of the mature forms is less clear. They may originate from macrophage-type cells, which release mature forms upon stimulation (Imort *et al.*, 1983), or they may leak from the lysosomal apparatus of damaged cells. In a control experiment we were unable to demonstrate conversion of the precursor of β-hexosaminidase and cathepsin D into mature forms by incubation *in vitro* with urine. It is therefore unlikely that mature forms are generated within the urine after release by the cells.

A relationship between lysosomal enzymes in serum and urine seems rather unlikely. They differ in their carbohydrate and the pattern of the molecular forms. The daily excretion of cathepsin D and β-hexosaminidase corresponds to less than 5% of the enzymes present in the serum. There is a remote possibility that the kidney clears selectively from the serum and transcytoses into urine lysosomal-enzyme precursors rich in high-mannose oligosaccharides. In nephrotic syndrome, however,

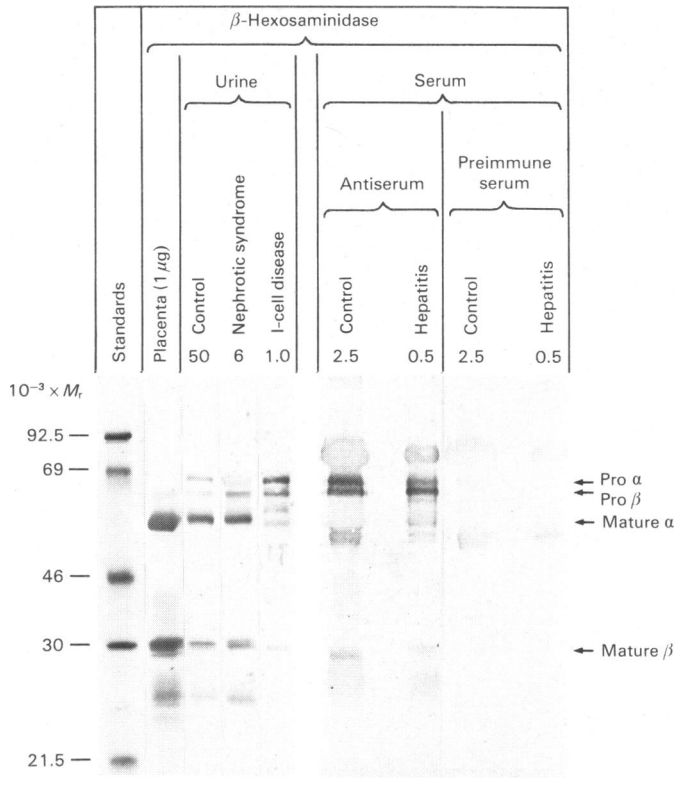

Fig. 5. *β-Hexosaminidase in pathological urine and serum*
The amounts (ml) of urine and serum from which β-hexosaminidase was isolated is indicated. For details and standards see Fig. 3.

the daily excretion of cathepsin D and β-hexosaminidase corresponded closely to 50% of the enzyme present in circulation. In this condition the difference in carbohydrate content makes it rather improbable that most of the enzyme in the urine originates from the serum.

Various genetic and acquired diseases are known in which lysosomal-enzyme activities are increased in serum and/or urine. In fibroblasts from patients with mucolipidosis II and III the segregation of lysosomal enzymes from the secretory pathway is impaired, and the newly synthesized lysosomal enzymes become largely secreted into the medium as precursors. Similar processes occur *in vivo*, since body fluids of patients with mucolipidosis II or III contain grossly elevated lysosomal-enzyme activities. Analysis of the molecular forms of β-hexosaminidase and cathepsin D in the urine of such a patient showed that these enzymes are secreted as precursors. This could be expected from the pathogenesis of the disorder and from previous cell-culture studies (Hasilik & Neufeld, 1980; Imort *et al.*, 1983). Moreover, the oligosaccharides in cathepsin D are exclusively of the complex type, as was found previously for cathepsin D secreted by mucolipidosis-II fibroblasts (for interpretation see Hasilik & von Figua, 1981). Our observations on the shift in the oligosaccharide pattern are consistent with a report on β-hexosaminidase A (Kress *et al.*, 1982), and contrast with another on β-hexosaminidase B (Hirani *et al.*, 1982), from mucolipidosis-III urine.

In diseases with extensive destruction of cells one would expect release of the mature forms of lysosomal enzymes. It therefore came as a surprise that the β-hexosaminidase and cathepsin D in serum of a patient with acute viral hepatitis, a disease with significant necrosis of liver cells, behaved as precursor forms, suggesting their secretion by intact cells. Clearly further analysis of the molecular forms of lysosomal enzymes present in serum and other body fluids under various conditions are required to establish the validity of these parameters for the prediction of the origin of lysosomal enzymes. Furthermore it must be kept in mind that the pattern of lysosomal enzyme forms is affected not only by secretion and leakage but also by uptake by the cells present in or lining the fluid compartment.

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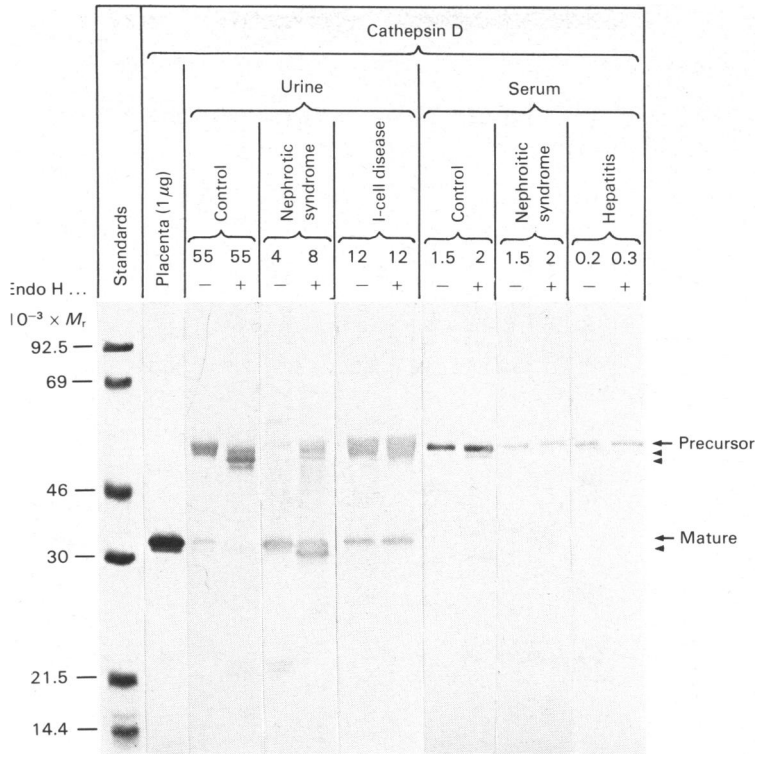

Fig. 6. *Effect of endo-β-N-acetylglucosaminidase H on cathepsin D of urine and serum from controls and patients*
The amounts (ml) of urine and serum from which cathepsin D was isolated are indicated. For details of the treatment with endo-β-*N*-acetylglucosaminidase H (Endo H) see the Materials and methods section. The migration of the precursor and mature forms of cathepsin D is indicated by arrows. Arrow-heads mark the positions of migration of cathepsin D forms that lost one (29 kDa and 51 kDa) or two (49 kDa) oligosaccharides on treatment with endo-β-*N*-acetylglucosaminidase H. For standards see Fig. 3.

obtaining samples from their patients, and of Dr. U. Klein in purifying β-hexosaminidase from foetal liver. This work was supported by the Deutsche Forschungsgemeinschaft (SFB 104) and the 'Fonds der Chemischen Industrie'.

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