Affinity purification and subunit structures of β -*N*-acetylhexosaminidases A and B from boar epididymis

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β -*N*-Acetylhexosaminidase from boar epididymis was separated into two forms, A and B, on DEAE-cellulose. Both these forms were excluded from Sepharose S-200 and had apparent M_r values of 510 000 on gradient gel electrophoresis under non-denaturing conditions. Affinity chromatography on 2-acetamido-*N*-(6-aminohexanoyl)-2-deoxy- β -D-glucopyranosylamine coupled to CNBr-activated Sepharose 4B was used to separate and purify β -*N*-acetylhexosaminidases A and B that had specific activities of 115 and 380 μ mol/min per mg of protein respectively. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of denatured β -*N*-acetylhexosaminidase A gave a single major component of M_r 67 000. β -*N*-Acetylhexosaminidase B also had this component, and in addition had polypeptides of M_r 29 000 and 26 000. All these polypeptides were glycosylated. Antiserum to the B form precipitated form A from solution and reacted with the 67 000- M_r component or form A after electrophoretic transfer from sodium dodecyl sulphate/polyacrylamide gels to nitrocellulose sheets. The 67 000- M_r components of forms A and B yielded identical peptide maps when digested with *Staphylococcus aureus* V8 proteinase, and the 29 000- M_r and 26 000- M_r components in form B may be related to the 67 000- M_r polypeptide.

In their survey of β -*N*-acetylhexosaminidase activities in mammalian tissues, Conchie *et al.* (1959) showed that the enzyme was present in most tissues and in general exhibited no remarkable changes with maturation of the animal. Epididymis is an exception to this, activities being low in infantile tissue, but increasing in adult males to surpass by far the activities of other tissues (Conchie *et al.*, 1959). The richest mammalian source of β -*N*-acetylhexosaminidase is boar epididymis (Findlay & Levvy, 1960), a tissue with a β -*N*-acetylhexosaminidase activity over 100 times that of kidney, which is the next most active tissue in the pig. The most active part of the caput epididymis had an activity per g wet wt. 5 times that of the epididymis as a whole (Findlay & Levvy, 1960).

Pig epididymal β -*N*-acetylhexosaminidase can be separated into acidic and basic forms by chromatography on DEAE-cellulose, and in that respect they resemble the human hexosaminidases (Leaback & Walker, 1967). Findlay & Levvy (1960) purified the pig epididymal β -*N*-acetylhexos-

aminidase by a combination of $(\text{NH}_4)_2\text{SO}_4$ and solvent fractionations, but they were unaware of the existence of two forms. Bamberg *et al.* (1975) purified the enzyme by affinity chromatography, but the product was heterogeneous with respect to its behaviour on electrophoresis and gel-filtration columns.

Our interest in the biosynthesis of mammalian β -*N*-acetylhexosaminidase has led us to pig epididymis as a source of mRNA and a consideration of the properties of the enzymes from this tissue. In the present paper we describe the purification of the acidic (A) and basic (B) forms of β -*N*-acetylhexosaminidase from pig epididymis by affinity chromatography and report on their subunit compositions.

Experimental

Materials

CNBr-activated Sepharose 4B and Sephacryl S-200 (superfine grade) were purchased from Pharmacia, Hounslow, Middx., U.K. *p*-Nitrophenyl 2-acetamido-2-deoxy- β -D-glucopyranoside and

Abbreviation used: SDS, sodium dodecyl sulphate.

Naphthol AS-B1 2-acetamido-2-deoxy- β -D-glucopyranoside were from Koch-Light Laboratories, Colnbrook, Bucks., U.K. Bovine serum albumin (Fraction V), Diazo Red RC, Coomassie Brilliant Blue R-250 and horseradish peroxidase (type VI) were from Sigma Chemical Co., Poole, Dorset, U.K. DEAE-cellulose was from Whatman Biochemicals, Maidstone, Kent, U.K. Acrylamide and *NN'*-methylenebisacrylamide were supplied by Bio-Rad Laboratories, Watford, Herts., U.K. All other reagents were purchased from Fisons Scientific Apparatus, Loughborough, Leics., U.K.

Preparation of homogenate and $(\text{NH}_4)_2\text{SO}_4$ fractionation

Testes from mature boars with epididymides attached were obtained at slaughter and stored at -20°C . Partially unfrozen epididymides (50g) were dissected from the testes, cut into small pieces with scissors and homogenized in 50mM-sodium citrate buffer, pH 4.5 (150ml), in a top-drive macerator. The homogenate was filtered through two thicknesses of muslin to remove undisrupted tissue, and then centrifuged at 10000g for 20min at 4°C in the $6 \times 250\text{ml}$ head of an IEC 20A centrifuge. $(\text{NH}_4)_2\text{SO}_4$ was added to the clear supernatant (155ml) to 20% saturation, and the mixture was left being stirred overnight at 4°C . The supernatant was recovered by centrifugation at 10000g for 20min as before, adjusted to 70% saturation with $(\text{NH}_4)_2\text{SO}_4$ and stirred at 4°C for 3h. The suspension was centrifuged at 10000g for 20min, and the precipitate was dissolved in 10mM-sodium phosphate buffer, pH 6.8 (final volume 85ml).

Affinity chromatography on 2-acetamido-N-(6-aminohexanoyl)-2-deoxy- β -D-glucopyranosylamine coupled to CNBr-activated Sepharose 4B

Affinity chromatography was performed on 2-acetamido-N-(6-aminohexanoyl)-2-deoxy- β -D-glucopyranosylamine coupled to CNBr-activated Sepharose 4B prepared by the method of Koshy *et al.* (1975). The gel (6ml) was packed in the barrel of a 10ml disposable syringe fitted with a disc of filter paper to support the gel bed, and equilibrated with 10mM-sodium phosphate buffer, pH 6.8, containing 70mM-NaCl. The 20–70%-satn. $(\text{NH}_4)_2\text{SO}_4$ enzyme preparation was adjusted to 70mM-NaCl and applied to the affinity column at 60ml/h.

The column was kept at 0°C throughout the run. Fractions were collected by drop-counting and were approx. 4ml. After application of the sample, the column was washed with 10mM-sodium phosphate buffer, pH 6.8, containing 70mM-NaCl (45ml), and then with 10mM-sodium phosphate buffer, pH 6.8, containing 10mM-NaCl until the A_{280} read on a Cary 219 spectrophotometer was

zero (40ml). β -N-Acetylhexosaminidase B was eluted with 10mM-sodium phosphate buffer, pH 6.8, containing 10mM-NaCl and 60mM-N-acetylglucosamine (80ml). In preparation for elution of β -N-acetylhexosaminidase A, the column was washed with 10mM-sodium phosphate buffer, pH 6.8, containing 10mM-NaCl (20ml), and then the same buffer containing 100mM-NaCl until the A_{280} was zero (90ml). β -N-Acetylhexosaminidase A was eluted with 10mM-sodium phosphate buffer, pH 6.8, containing 100mM-NaCl and 60mM-N-acetylglucosamine (30ml), and finally the column was eluted with 10mM-sodium phosphate buffer, containing 1.0M-NaCl.

DEAE-cellulose chromatography

Chromatography on DEAE-cellulose was performed on columns ($11\text{cm} \times 1.2\text{cm}$ diam.) equilibrated with 10mM-sodium phosphate buffer, pH 6.8. Enzyme activity retained by the column was eluted in a linear gradient of NaCl that reached a concentration of 0.1M in 120ml of buffer.

Gel filtration

Samples of the dialysed $(\text{NH}_4)_2\text{SO}_4$ fraction and purified β -N-acetylhexosaminidases A and B were analysed on Sephacryl S-200 (superfine grade) columns ($40\text{cm} \times 2.6\text{cm}$ diam.) equilibrated in 10mM-sodium phosphate buffer, pH 6.8, containing 0.1M-NaCl.

Enzyme assays and determination of the specific activities of β -N-acetylhexosaminidases A and B

β -N-Acetylhexosaminidase activity was assayed by incubating a suitably diluted sample of the enzyme (10 μl) with 200 μl of 3mM-p-nitrophenyl 2-acetamido-2-deoxy- β -D-glucopyranoside in 100mM-sodium citrate buffer, pH 4.5, containing 0.1mM-NaCl and 0.01mg of bovine serum albumin (hexosaminidase-free)/ml for 5min at 37°C . The reaction was stopped by adding 2ml of 1M- Na_2CO_3 and the A_{400} read.

Protein was assayed by the method of Lowry *et al.* (1951), with bovine serum albumin as a standard.

Gradient gel electrophoresis

Electrophoresis of β -N-acetylhexosaminidases under non-denaturing conditions was performed in polyacrylamide-gradient gels purchased from Uniscil, Southend, Essex, U.K. Immediately before use the gels were placed in the electrophoresis tank (Uniscil) containing buffer (10.75g of Tris, 5g of boric acid and 930mg of EDTA in 1 litre of water and run at 125V for 15min. Samples (20 μl) containing 10% (w/v) sucrose were applied, and the gel was run at 90V until the samples had left the

applicators and entered the gel. Electrophoresis was continued at 125 V for 18 h at 10°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), with Naphthol AS-B1 β -N-acetylglucopyranoside as the substrate and Diazo Red RC as the coupling dye.

SDS/polyacrylamide-gel electrophoresis

Polyacrylamide-gel electrophoresis in the presence of SDS was performed in 10% gels with the buffer system described by Laemmli (1970). Proteins were detected in SDS/polyacrylamide gels by the silver staining method of Eschenbruch & Bürk (1982) or by staining with a 0.125% solution of Coomassie Brilliant Blue R-250 in 10% (v/v) acetic acid/20% (v/v) methanol. Destaining was in the acetic acid/methanol mixture until the background was clear. Glycoproteins were detected by the method of Parish *et al.* (1978).

Peptide mapping of β -N-acetylhexosaminidases A and B

Polypeptide chains of β -N-acetylhexosaminidases A and B were stained lightly with Coomassie Blue and excised from 10%-polyacrylamide gels. These bands were then inserted into sample wells of a second (15%) gel and overlaid with 1 μ g (10 μ l) of *Staphylococcus aureus* V8 proteinase, as described by Cleveland *et al.* (1977). Electrophoresis was at 20 mA until the Bromophenol Blue tracking dye entered the resolving gel and then at 30 mA for the remainder of the run. Peptides were stained first with Coomassie Blue and then by the silver staining method of Eschenbruch & Bürk (1982).

Immunological techniques

Antiserum to purified β -N-acetylhexosaminidase B was raised by injecting 0.5 mg of the enzyme emulsified in 2 ml of Freund's complete adjuvant (Difco Laboratories, Detroit, MI, U.S.A.) subcutaneously into ten sites on the back of a Half-lop New Zealand White rabbit (Ranch Rabbits, Crawley, Sussex, U.K.). The rabbit was challenged with 0.5 mg of enzyme in 2 ml of Freund's complete adjuvant (Difco Laboratories) after 4 weeks, and the animal was bled after a further 3 weeks.

Rabbit anti-(boar epididymal hexosaminidase B) serum was tested in double-diffusion analysis against epididymal hexosaminidases A and B by the method of Ouchterlony (1958).

Immunoprecipitates formed in this way were tested for enzyme activity by covering the surface of the agar gel with 2 mM-methylumbelliferyl 2-acetamido-2-deoxy- β -D-glucopyranoside in 0.4 M-sodium citrate buffer, pH 4.5. 4-Methylumbellifer-

one liberated from the substrate was detected by its fluorescence under u.v. light at 365 nm.

The capacity of antisera to precipitate β -N-acetylhexosaminidase from solution was assessed by incubating serum diluted with 10 mM-sodium phosphate buffer, pH 6.8, containing 0.15 M-NaCl (50 μ l) with 50 μ l of β -N-acetylhexosaminidase (activity approx. 0.03 μ mol/min) and 50 μ l of 10 mM-sodium phosphate buffer, pH 6.8, containing 0.1 M-NaCl and 0.01% bovine serum albumin, at 4°C for 18 h in Eppendorf micro-centrifuge tubes. Immunoprecipitates were sedimented by centrifugation in an Eppendorf micro-centrifuge for 5 min, and the supernatants were assayed for hexosaminidase activity.

Electrophoretic transfer of polypeptides from SDS/polyacrylamide gels to nitrocellulose sheets and detection of antigens by using immunoglobulin G-peroxidase conjugates was by the method of Burnette (1981). Coupling of rabbit anti-(boar epididymal β -N-acetylhexosaminidase B) immunoglobulin G to horseradish peroxidase was by the method of Engvall (1980).

Results

Behaviour of epididymal β -N-acetylhexosaminidases on DEAE-cellulose and Sephacryl S-200

When a sample of the dialysed $(\text{NH}_4)_2\text{SO}_4$ fraction was applied to DEAE-cellulose equilibrated with 10 mM-sodium phosphate buffer, pH 6.8, 70% of the β -N-acetylhexosaminidase activity was unretained (form B) and the rest of the activity was eluted in the salt gradient (form A). Application of a similar sample to a column (40 cm $\times$ 2.6 cm diam.) of Sephacryl S-200 equilibrated with 10 mM-sodium phosphate buffer, pH 6.8, containing 0.1 M-NaCl gave a single peak of β -N-acetylhexosaminidase which was eluted with the void volume. β -N-Acetylhexosaminidases A and B, purified by affinity chromatography, also ran as single excluded peaks under these conditions. This behaviour was not observed when salt was omitted from the column buffer; then a small retarded peak of β -N-acetylhexosaminidase was found, and most of the activity was adsorbed on the nylon mesh of the column applicator.

Purification of β -N-acetylhexosaminidases A and B by affinity chromatography

Affinity chromatography was successfully applied to the purification of forms A and B, which differ in conditions required to elute them from the affinity gel, thus making possible the purification of both forms from the $(\text{NH}_4)_2\text{SO}_4$ fraction in a single passage through the column (Fig. 1). This simple procedure (Table 1) gave a 17-fold purification of form A and 58-fold purification of form B,

Table 1. Purification of β -N-acetylhexosaminidases A and B from boar epididymis
For experimental details see the text.

Purification step	Volume (ml)	Protein (mg)	Total activity (units)	Specific activity (units/mg)	Recovery (%)	Purification (fold)
Supernatant	155	1850	12300	6.6	100	1
(NH ₄) ₂ SO ₄ fraction	85	1260	7940	6.3	65	0.95
Affinity chromatography						
Form A	30	9.6	1100	115	30*	17
Form B	20	8.9	3410	380	40*	58

* Calculated on the basis of 30% form A and 70% form B in the supernatant.

Fig. 1. Elution profile of β -N-acetylhexosaminidase activity (○) and protein (A_{280}) (●) from an affinity column (6 ml) prepared by coupling 2-acetamido-N-(6-amino-hexanoyl)-2-deoxy- β -D-glucopyranosylamine to CNBr-activated Sepharose 4B

Enzyme was applied in 10mM-sodium phosphate buffer, pH 6.8, containing 70mM-NaCl. β -N-acetylhexosaminidase B was eluted with 60mM-N-acetylglucosamine in 10mM-sodium phosphate buffer, pH 6.8, containing 10mM-NaCl (arrow B), and form A with the same concentration of N-acetylglucosamine but in buffer containing 100mM-NaCl (arrow A). Residual protein and enzyme activity was eluted with 1.0M-NaCl in phosphate buffer. A full description is given in the Experimental section.

which had specific activities of 115 and 380 μ mol/min per mg of protein respectively. This difference in the specific activities of the two forms of β -N-acetylhexosaminidase could not be accounted for by the presence of contaminating proteins in the preparation of the A form, which we estimate to be about 3% of total protein. This is the proportion of protein contributed by non-specific elution from the column in 10mM-sodium phosphate buffer,

pH 6.8, containing 100mM-NaCl, and was not lessened by protracted elution of the column.

Coomassie Blue staining of SDS/polyacrylamide gels did not reveal the presence of contaminating polypeptides when 10 μ g of β -N-acetylhexosaminidases A and B were loaded (Fig. 2). Prolonged silver staining showed that the A preparation had small amounts of contaminating polypeptides over the whole M_r range covered by the gel track. In contrast, the B preparation was completely free from these contaminants.

We made no attempt to saturate the affinity column with enzyme, but even after the application of 8000 units of activity to the 6 ml column over 95% of the applied activity was still retained (Fig. 1). β -N-Acetylhexosaminidases A and B were recovered separately from the column by adjusting the salt concentrations of the N-acetylglucosamine solution used for elution.

Recovery of enzyme activity from the affinity column was only 57%. This is likely to be due to instability of the purified enzyme in solution (Findlay & Levvy, 1960), rather than to retention of enzyme by the column, which was free from discoloration at the end of the run.

Gradient gel electrophoresis of the native β -N-acetylhexosaminidases

Attempts to obtain M_r values for the β -N-acetylhexosaminidases by electrophoresis in polyacrylamide gels were only partially successful. Most of the protein (about 80%) ran with an apparent M_r value of about 510000, but even after 18h of electrophoresis the bands were broad and not well-defined, unlike the M_r markers run on the same gel. Bands of lower intensity were detected with mobilities corresponding to M_r 180000 and 100000. Only weak staining was obtained with the histochemical substrate, and very much less than expected from the amount of activity run on the gel (at least 1 μ mol/min per track). This behaviour is quite unlike that of β -N-acetylhexosaminidase A from human placenta and liver, which forms a sharp band at its exclusion limit in the gel (M_r ,

Fig. 2. SDS/polyacrylamide-gel electrophoresis of purified β -N-acetylhexosaminidases A and B

Gels were prepared and run as described in the Experimental section; polypeptides were stained with Coomassie Blue. M_r values are those of commercial standards run on the same gel. Lane 1, β -N-acetylhexosaminidase A; lane 2, β -N-acetylhexosaminidase B.

100000), but the B form from these sources runs as a diffuse band of about M_r 200000 (L. C. B. B. Coelho & J. L. Stirling, unpublished work).

SDS/polyacrylamide-gel electrophoresis of β -N-acetylhexosaminidases A and B

Samples of affinity-chromatography-purified β -N-acetylhexosaminidases A and B were denatured by boiling them in 2% SDS in the presence of 2-mercaptoethanol and run on 10%-polyacrylamide gels (Fig. 2). β -N-Acetylhexosaminidase A ran as a single major polypeptide with M_r 67000. This component was the most intense band in the B preparation, which had in addition bands of M_r 29000 and 26000. Identical patterns were obtained when mercaptoethanol was omitted from the sample buffer.

The single polypeptide component in form A and all three components in the B preparation stained positively in the concanavalin A/peroxidase method for detecting glycoproteins under conditions where non-glycosylated M_r standards were undetected.

Fig. 3. Immunoprecipitation of β -N-acetylhexosaminidase by rabbit anti-(boar β -N-acetylhexosaminidase B) serum

Samples containing 0.03 unit of β -N-acetylhexosaminidase were incubated overnight at 4°C with dilutions of rabbit anti-(boar β -N-acetylhexosaminidase B) and then centrifuged, and the supernatants were tested for enzyme activity as described in the Experimental section. ●, Purified β -N-acetylhexosaminidase B; ▲, crude extract of epididymis; ○, crude extract of kidney.

Immunological relationship of pig β -N-acetylhexosaminidases

β -N-Acetylhexosaminidases A and B each gave single immunoprecipitin lines when allowed to react with rabbit anti-(boar β -N-acetylhexosaminidase B) serum in double-diffusion analysis; both were enzymically active and fused, indicating that the enzymes were antigenically identical. An immunoglobulin G-peroxidase conjugate prepared with this serum reacted with all three polypeptides in the β -N-acetylhexosaminidase B preparation and the 67000- M_r component of form A after their transfer from an SDS/polyacrylamide gel to nitrocellulose.

Anti-(β -N-acetylhexosaminidase B) serum (diluted 1:16) gave complete precipitation of 0.03 unit of purified β -N-acetylhexosaminidase B and of the enzyme activity (0.01 unit) represented by an equivalent amount of β -N-acetylhexosaminidase A

protein. When 0.03 unit of β -N-acetylhexosaminidase A was used in the immunoprecipitation reaction, 72% of the activity was precipitated at a 1:16 dilution of the antiserum, and complete precipitation was obtained at a dilution of 1:8.

Incomplete precipitation was observed when various dilutions of antiserum were incubated with 0.03 unit of β -N-acetylhexosaminidase in buffer extracts of epididymis and kidney (Fig. 3), in contrast with the behaviour of purified β -N-acetylhexosaminidase B, which is shown for comparison.

Peptide mapping of polypeptides from β -N-acetylhexosaminidase

With the use of the method of Cleveland *et al.* (1977), polypeptides excised from SDS/polyacrylamide gels were digested with *S. aureus* proteinase V8 and separated on 15%-polyacrylamide gels run in SDS (Fig. 4a). Indistinguishable patterns of peptides were yielded by the 67000- M_r polypeptide present in β -N-acetylhexosaminidases A and B. One of the two most abundant of these peptides

Fig. 4. Peptides generated by treatment of β -N-acetylhexosaminidase polypeptides with *S. aureus* V8 proteinase

Polypeptides were excised from SDS/polyacrylamide gels and loaded in sample wells of a 15%-polyacrylamide gel, overlaid with 1 μ g (10 μ l) of *S. aureus* V8 proteinase and run as described in the Experimental section. Peptides were detected by staining with Coomassie Blue and then by silver staining. (a) Digestion products from: lane 1, the 67000- M_r polypeptide from β -N-acetylhexosaminidase A; lane 2, the 67000- M_r polypeptide from form B. (b) Digestion products from: lane 1, the 67000- M_r polypeptide from form B; lane 2, 29000- M_r polypeptide from form B; lane 3, 26000- M_r polypeptide from form B; lane 4, as lane 1, but with a lower loading.

was also present in digests of the 29000- M_r component of form B (Fig. 4b). There was some similarity in the pattern of peptides generated from the 26000- M_r component of form B and those from the 67000- M_r bands, but the most abundant peptide from this component was present in only small amounts in digests of the 67000- M_r and 29000- M_r bands. These observations on relative abundance are based on the intensity of staining with Coomassie Blue and not silver staining, as shown in Figs. 4(a) and 4(b).

Discussion

β -N-Acetylhexosaminidase is an abundant enzyme in extracts of boar epididymis (Findlay & Levvy, 1960), and on the basis of our estimates of the specific activities of the two forms we calculate that it accounts for 2–3% of the extracted epididymal protein. This is likely to be an overestimate, because the purified enzymes are known to be unstable, and consequently our estimates of their specific activities may be low, but nevertheless the abundance of the combined amounts of the A and B forms is at least 1% of the extracted protein.

We have followed the convention of others (Leaback & Walker, 1967; Wetmore & Verpoorte, 1972; Kyosaka *et al.*, 1980) in referring to the more acidic of the pig β -N-acetylhexosaminidases as the A form and the more basic as the B form. This convention is also used with the human β -N-acetylhexosaminidases, but this similarity of the human and pig enzymes, based on their behaviour on ion-exchange columns, is a superficial one, and does not appear to have the same structural basis. Human β -N-acetylhexosaminidases A and B both have M_r about 55000 when denatured by boiling in SDS and run in SDS/polyacrylamide-gel electrophoresis. Smaller- M_r polypeptides (M_r 29000 and smaller) are observed when the enzyme is denatured in the presence of mercaptoethanol or dithiothreitol. Under reducing conditions, the A form is resolved into α (M_r 53000) and β (M_r 29000) subunits, whereas the B form is composed of β subunits only. These reducing agents have no effect on the electrophoretic behaviour of boar epididymal hexosaminidases A and B in SDS/polyacrylamide gels, the A form giving a single polypeptide (M_r 67000) and the B form polypeptides of M_r 67000, 29000 and 26000.

All three polypeptides from form B and the single polypeptide from form A appear to be glycosylated, and in that respect they resemble the subunits of human β -N-acetylhexosaminidases (L. C. B. B. Coelho & J. L. Stirling, unpublished work). The 67000- M_r polypeptide in form A is immunologically related to at least one of the polypeptides that comprise form B, since it gave a

positive reaction with an antiserum raised against form B. Further evidence for a close structural relationship between the 67000- M_r components of forms A and B was revealed by peptide mapping, both giving an identical pattern of peptides after digestion with *S. aureus* V8 proteinase. Although the evidence is less good, the 29000- M_r and 26000- M_r polypeptides appear to be related to the 67000- M_r subunit, since similar peptides are generated by treatment with *S. aureus* V8 proteinase.

On the basis of these results, we suggest that both β -*N*-acetylhexosaminidases A and B may be the products of the same gene, and that the smaller- M_r polypeptides in preparation B are derived from the 67000- M_r component by proteolytic cleavage *in vivo* or during the extraction of the enzymes. We have not established whether the smaller- M_r polypeptides are present in stoichiometric amounts in form B, but from their appearance in SDS/polyacrylamide gels stained with Coomassie Blue they appear to be minor components.

In their native states both β -*N*-acetylhexosaminidases are likely to exist as glycoproteins comprising at least four 67000- M_r subunits, judged from their behaviour on gel filtration in the presence of 100 mM-NaCl. At lower salt concentrations they showed a strong tendency to aggregate, and under the conditions used for gradient gel electrophoresis the activity was detected on a band corresponding to an M_r of 510000.

Undoubtedly the tendency of β -*N*-acetylhexosaminidases A and B to aggregate under conditions of low ionic strength has consequences for their behaviour on ion-exchangers, and the proportions of the A to B forms may be influenced by the state of aggregation when loaded on to the column. Preparations of form B were found to contain up to 30% of form A on storage for several months at 4°C, indicating that subunits from the two forms may be in equilibrium with each other.

β -*N*-Acetylhexosaminidase A is the predominant form in pig kidney (Kyosaka *et al.*, 1980), an observation that we confirm, but in its subunit composition it differs in some respects from the corresponding enzyme extracted from epididymis. Both have a component in the region of M_r 60000–70000, but the kidney enzyme also has polypeptides of M_r 31000 and 9000 (Kyosaka *et al.*, 1980), which were not present in epididymal β -*N*-acetylhexosaminidase A. The kidney and epididy-

mal enzymes appear to share immunological determinants, since an antiserum raised to the epididymal form B was able to precipitate some of the kidney enzyme present in a crude extract. Thus it seems likely that the epididymal and kidney β -*N*-acetylhexosaminidases are the products of the same structural gene. This raises interesting questions about the expression of the gene for β -*N*-acetylhexosaminidase during the maturation of boars. Whether the massive increase in β -*N*-acetylhexosaminidase, which is greatest in the caput epididymis, results from a generalized effect of testosterone on the metabolism of the tissue or is a more specific effect on the expression of the β -*N*-acetylhexosaminidase gene remains a fascinating avenue to be explored.

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