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Structure of the lysosomal neuraminidase–β-galactosidase–carboxypeptidase multienzymic complex

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Lysosomal neuraminidase (sialidase; EC 3.2.1.18) and β-galactosidase (EC 3.2.1.23), together with a carboxypeptidase, the so-called 'protective protein', were co-purified from the human placenta by affinity chromatography on a concanavalin A–Sepharose column followed by a thiogalactoside–agarose affinity column for β-galactosidase. Analysis of the purified material by gel-filtration h.p.l.c. revealed three distinct molecular forms, all with high β-galactosidase specific activity, but only the largest one expressed neuraminidase activity. Rechromatography of each individual species separately indicated that all three are in fact part of an equilibrium system (the neuraminidase–β-galactosidase–carboxypeptidase complex or NGC-complex) and that these species undergo slow conversion into one another through dissociation and association of protomeric components. Each species was sufficiently stable for the determination of their hydrodynamic properties by gel-filtration h.p.l.c. and sedimentation velocity. The largest species had an apparent sedimentation coefficient, $s_{20,w}$, of 18.8 S and a Stokes' radius of 8.5 nm, giving a molecular mass of 679 kDa and a fractional ratio, $f/f_{min.}$, of 1.47. The latter value indicates that the macromolecule is asymmetric or highly hydrated. This large species is composed of four types of polypeptide chains of molecular mass 66 kDa (neuraminidase), 63 kDa (β-galactosidase), 32 kDa and 20 kDa (carboxypeptidase heterodimer). The 32 kDa and 20 kDa protomers are linked together by a disulphide bridge. Glycopeptidase F digestion of the NGC-complex transformed the diffuse 66–63 kDa band on the SDS gel into two close but sharp bands at 58 and 56 kDa. The two smaller species which were separated on the h.p.l.c. column correspond to tetrameric and dimeric forms of the 66–63 kDa protomers and express exclusively β-galactosidase activity. Treatment of the NGC-complex with increasing concentrations of guanidinium hydrochloride up to 1.5 M also resulted in dissociation of the complex into the same smaller species mentioned above plus two protomers of molecular mass around 60 and 50 kDa. A model of the largest molecular species as a hexamer of the 66–63 kDa protomers associated to five carboxypeptidase heterodimers (32 kDa and 20 kDa) is proposed.

INTRODUCTION

The hydrolases neuraminidase (sialidase; EC 3.2.1.18) and β-galactosidase (EC 3.2.1.23) are part of a lysosomal multienzymic complex that also contains a 'protective protein' [1,2]. This protein is necessary for the multimerization of β-galactosidase but also for the stabilization and expression of neuraminidase activity in the complex [2]. The protective protein expresses carboxypeptidase activity (J. Tranchemontagne & M. Potier, unpublished work) as expected from its sequence similarity with the yeast proteases carboxypeptidase Y and KEX1 gene product [3].

The neuraminidase–β-galactoside–carboxypeptidase complex (NGC-complex) has been purified from bovine testis [1] and human placenta [2] by affinity chromatography on a *p*-aminophenyl-β-D-thiogalactoside–CH-Sepharose column by virtue of its β-galactosidase content. In the NGC-complex, neuraminidase corresponds to a protomer of molecular mass 66 kDa and β-galactosidase to a 63 kDa protomer, and the carboxypeptidase is a heterodimer of 32 and 20 kDa protomers, linked together by a disulphide bridge [3,4]. The deficiency of each of these proteins in the NGC-complex causes a lysosomal storage disease in man: sialidosis for neuraminidase deficiency [5], G_{M1}-gangliosidosis for lack of β-galactosidase [6] and galactosialidosis (both neuraminidase and β-galactosidase are affected) for the carboxypeptidase deficiency [7].

To understand structure–function relationships in the NGC-complex, it is necessary to establish how the various protomers interact and how the structure of this complex is organized. We purified the NGC-complex from human placenta and measured its molecular mass by hydrodynamic methods, and also determined the stoichiometry of its protomeric components. This information is used to propose a structural model of the NGC-complex.

MATERIAL AND METHODS

Purification of the NGC-complex from human placenta

The NGC-complex was purified by a modification of the method of Verheijen *et al.* [1,2]. Briefly, fresh human placentae were obtained at caesarean section, perfused with physiological saline and heparin to remove as much blood as possible and kept at −78 °C until use. Five placentae [about 2 kg of tissue in 3 litres of 20 mM-sodium phosphate buffer (pH 6)/leupeptin (5 μg/ml)] were homogenized in a cold Waring blender and centrifuged at 25000 *g* for 30 min. The supernatant was applied to a concanavalin A–Sepharose (Pharmacia) column (2.5 cm × 40 cm) equilibrated with 20 mM-sodium phosphate buffer (pH 6). The column was then washed with the equilibration buffer containing first 0.1 M-NaCl and then 1 M-NaCl until the A_{280} dropped to about 0.1. The glycoprotein fraction was eluted with a mixture of 0.5 M-methyl-α-D-mannoside and 0.5 M-methyl-α-D-glucoside in the elution buffer. The glycoprotein fraction was kept on ice in the presence of 4 μg of leupeptin/ml and concentrated in an Amicon ultrafiltration cell (PM-10 membrane) to a final concentration of about 40 mg/ml. The preparation was dialysed against 20 mM-sodium acetate buffer (pH 5.2)/0.1 M-NaCl, and the neuraminidase was stabilized by incubation at 37 °C for 90 min.

Abbreviations used: NGC-complex, neuraminidase–β-galactosidase–carboxypeptidase complex; GdmHCl, guanidinium hydrochloride.

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The preparation was then applied to a 10 ml column of *p*-aminophenyl-β-D-thiogalactoside–CH-Sepharose (Sigma) equilibrated with 20 mM-sodium acetate buffer (pH 5.2)/0.1 M-NaCl. The column was washed with 50 ml of the equilibration buffer at a flow rate of 30 ml/h. The NGC-complex was eluted with 10 mM-tris(hydroxymethyl)aminomethane buffer (pH 7)/0.1 M-NaCl/0.5 M-γ-D-galactonolactone. The preparation was then dialysed twice against 2 litres of 20 mM-sodium acetate buffer (pH 5.2)/0.1 M-NaCl and kept frozen at −20 °C. The specific activities of neuraminidase and β-galactosidase in the purified preparation were 160 and 10550 nmol/min per mg of protein respectively.

The neuraminidase and β-galactosidase activities were assayed with the corresponding 4-methylumbelliferylglycoside derivatives as substrates according to published procedures [6,8,9]. Proteins were assayed according to Bradford [10] or Lowry *et al.* [11], using bovine serum albumin as standard.

Gel-filtration analysis

H.p.l.c. of standard proteins and purified complex was performed on a Waters Associates instrument model 201A according to le Maire *et al.* [12,13] using 20 mM-sodium acetate buffer (pH 5.2)/0.1 M-NaCl as eluant. The chromatographic column (4 mm × 300 mm) was a Waters Associates SW 300 gel-filtration type eluted at a flow rate of 0.8 ml/min. The following standard proteins with known Stokes' radii were used to calibrate the column: thyroglobulin (8.6 nm), *Escherichia coli* β-galactosidase (6.9 nm), ferritin (6.3 nm), catalase (5.2 nm), aldolase (4.6 nm), albumin (3.5 nm), trypsin inhibitor (2.2 nm) and ribonuclease A (1.8 nm). The void and total volumes of the column were measured using cytochrome oxidase and $NaNO_3$ respectively. As proposed by le Maire *et al.* [13], the Stokes' radius of standard proteins was plotted against the partition coefficient.

The NGC-complex was denatured at 25 °C with various concentrations of guanidinium hydrochloride (GdmHCl) of between 0.5 and 1.5 m in 50 mM-sodium phosphate buffer (pH 6.8)/0.1 M-NaCl and analysed by h.p.l.c. using the same buffer as eluant. Some of the standard proteins mentioned above, after being denatured, reduced and alkylated, were used to calibrate the column for molecular mass determination in the presence of GdmHCl in the eluant.

SDS/polyacrylamide-gel electrophoresis

Protomer composition of the NGC-complex species was analysed on a 11% (w/v) polyacrylamide gel by the method of Laemmli [14]. Proteins were detected with a silver stain [15]. Densitometry and quantification of the bands were done with a Zeineh scanning photometer (Biomed Instruments Inc., Fullerton, CA, U.S.A.) with a laser light source. The relationship between photometer response and quantity of protein was linear in the range 0.05–2 μg of protein.

Molecular mass determination

The $s_{20,w}$ values of the various fractions of the NGC-complex, separated by h.p.l.c. according to size, were determined by the method of Martin & Ames [16] in a 5–20% sucrose-density gradient (11 ml) in 20 mM-sodium acetate buffer (pH 5.2)/0.1 M-NaCl. The tubes were centrifuged at 160000 *g* for 14 h at 4 °C in the presence of standard proteins of known $s_{20,w}$: thyroglobulin (19.1 S) and catalase (11.1 S). After centrifugation, fractions (0.2 ml) were collected from the bottom of the tube to determine absorbance at 230 nm (thyroglobulin), and the activities of catalase and β-galactosidase. The relationship between the $s_{20,w}$ of standard proteins and the distance migrated in the sucrose gradient was plotted, and the apparent $s_{20,w}$ values of the various fractions of the NGC-complex were obtained from this calibration line.

The Stokes radius (R_s) of each species separated by h.p.l.c. was used with the apparent sedimentation coefficient ($s_{20,w}$) to calculate the molecular mass (M) using the following equation:

$$M = 6\pi\eta N R_S s_{20,w}/(1-\bar{v}\rho) \quad (1)$$

where N is Avogadro's number, η is the viscosity, ρ is the density of the solution and $\bar{v}$ is the partial specific volume of the protein.

The frictional ratio ($f/f_{min.}$) of each species was determined according to the equation:

$$f/f_{min.} = R_S/(3M\bar{v}/4\pi N)^{\frac{1}{3}} \quad (2)$$

Glycopeptidase digestion

Glycopeptidase F was purchased from Genzyme Corp. (Boston, MA, U.S.A.). The purified NGC-complex was dialysed against deionized water and lyophilized to dryness. Enzyme treatment was carried out according to Tarentino *et al.* [17].

RESULTS

The h.p.l.c. chromatographic profile of the purified NGC-complex is shown in Fig. 1(*a*). Three distinct protein peaks were separated on the 300SW gel-filtration column according to size. The assay of neuraminidase and β-galactosidase activities in each peak indicated that the first peak, which was eluted close to the void volume, contained both neuraminidase (238 nmol/min per mg of protein) and β-galactosidase (9300 nmol/min per mg), activities whereas peaks 2 and 3 contained exclusively β-galactosidase activity (17100 and 16200 nmol/min per mg of protein respectively). The fourth peak contained no enzymic activity and probably corresponds to an impurity. Rechromatography of each peak separately on the same h.p.l.c. column showed that the three species are in a dynamic equilibrium. Peak 1 yielded the peaks 2 and 3 in identical proportions to those of the initial chromatography (Fig. 1*b*). The isolated peak 2 was more stable and yielded minimal amounts of peaks 1 and 3 after rechromatography (Fig. 1*c*). Peak 3 gave a relatively large peak 2 and a small peak 1 (Fig. 1*d*).

Because of the nature of the multienzymic complex and to clarify the terminology, we propose to use the term 'NGC-complex' to designate the preparation obtained from the thiogalactoside–agarose affinity column (see the Materials and methods section) which represents the whole equilibrium system. The different species which are part of this equilibrium and which were separated on the h.p.l.c. column will be designated as 'NGC complex species 1, 2 and 3' in order of decreasing size.

The stability of the equilibrium system was tested by diluting the NGC-complex by 40-fold and reapplying the sample on to the same h.p.l.c. column. Dilution did not affect the proportion of peaks obtained by h.p.l.c. This result is consistent with an equilibrium system where association and dissociation both occur at a relatively slow rate as compared with the rate of separation on the h.p.l.c. column [18]. This equilibrium can be progressively displaced towards peaks 2 and 3 by addition of GdmHCl in the elution buffer (Fig. 1*e*) but not with NaCl at concentrations as high as 5 M, suggesting that the NGC-complex structure is maintained by hydrophobic interactions with little participation of inter-protomer salt bridges. In 1.5 M-GdmHCl, peak 1 was diminished with concomitant increases in peaks 2 and 3 (Fig. 1*e*). Smaller molecular species corresponding to approx. 60 kDa (peak 4) and 50 kDa (peak 5) were also detected, suggesting partial dissociation by GdmHCl of the NGC-complex into its constitutent protomers (see below).

The protomer composition of each species implicated in the formation of the NGC-complex was determined by SDS/

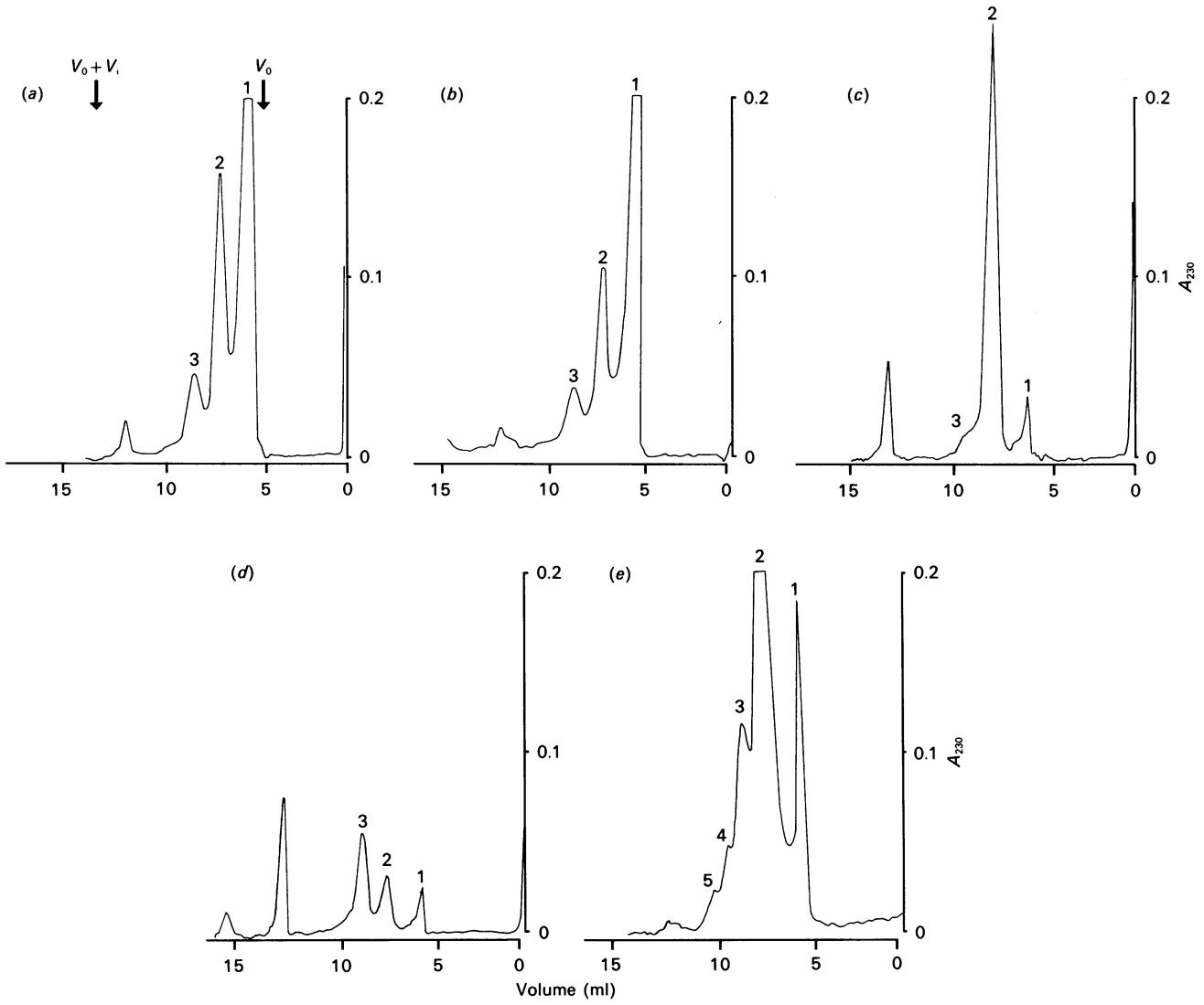

Fig. 1. H.p.l.c. of purified NGC-complex

Chromatogram of the purified NGC-complex on a 300SW h.p.l.c. column (*a*), and rechromatography of peak 1 (*b*), peak 2 (*c*) and peak 3 (*d*). (*e*) Chromatography of the NGC-complex in the presence of 1.5 M-GdmHCl. V_0 = void volume; V_0+V_i = void volume plus volume inside the gel beads.

polyacrylamide-gel electrophoresis (Fig. 2). The NGC-complex preparation revealed five major components: a sharp band corresponding to a molecular mass of 76 kDa identified previously by other authors [4] as the IgM heavy chain, which co-purifies with the NGC-complex, a diffuse band at 66–63 kDa (probably composed of at least two different protomers; see below for the effect of deglycosylation), and a sharper band at 54–52 kDa which may represent the precursor of the two smaller bands at 32 and 20 kDa [3]. In some purified NGC-complex preparations, little or no 76 kDa and 54–52 kDa bands were present (see Fig. 3). The NGC-complex species 1 (Fig. 2, lane c) has a protomeric composition similar to that of the whole NGC-complex (Fig. 2, lane a), whereas species 2 and 3 (lanes d and e) contained little 32 and 20 kDa protomers. The 54–52 kDa band seen in the NGC-complex preparation is found in species 3 exclusively.

Since different proteins often exhibit different reactivity to silver stain, we verified the linearity of the relationship between known amounts of the 66–63 kDa and 32 and 20 kDa protomers applied on the gel and quantified the intensity of the stained bands by laser microdensitometry. Since many preparations of the NGC-complex species 2 contain a majority of the band at 66–63 kDa (Figs. 2 and 3), a calibration was first made with such a preparation. The NGC-complex species 1 contains a mixture of 66–63 kDa and 32 kDa/20 kDa protomers and thus the first calibration made with species 2 was used to determine the amount of protein in species 1 that comes from the 66–63 kDa protomers. The rest of the protein was attributed to the 32 kDa and the 20 kDa protomers together and was used to trace a second calibration line for these latter protomers. From these calibration lines, we concluded that the molar ratio of the 66–63 kDa protomers and the 32 kDa and 20 kDa protomers, the two latter being taken together, is 1.27 ± 0.15 (means ± S.D., $n = 3$) in species 1. The molar ratio of the 32 kDa to the 20 kDa protomer cannot be determined because we have not established calibration lines with each protein independently. However, it appears that the two protomers are in a 1:1 molar ratio in the NGC-complex species 1 and that they are linked together by a disulphide bridge. That is, when the purified complex was run on a SDS/polyacrylamide gel without treatment with β-mercapto-

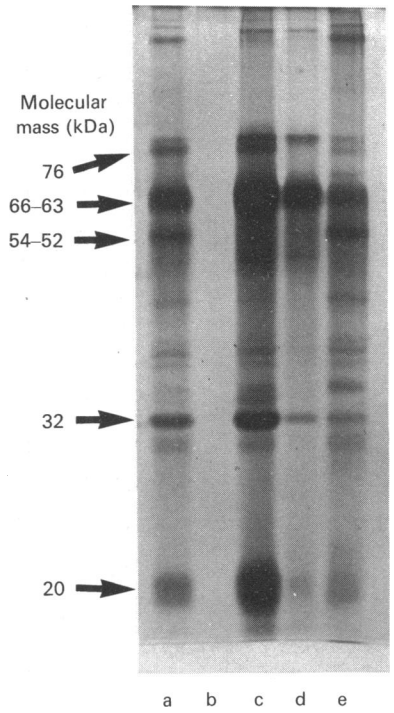

Fig. 2. SDS/polyacrylamide-gel electrophoresis of NGC-complex proteins under reducing conditions

Lane a, whole NGC-complex (15 μg of protein); b, control without protein; c, species 1 (37 μg); d, species 2 (10 μg); e, species 3 (19 μg). The major protomer bands are indicated by arrows. Proteins were revealed by silver staining.

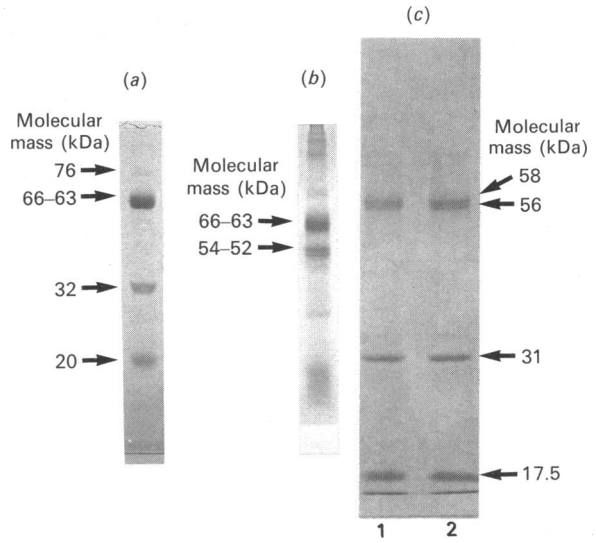

Fig 3. SDS/polyacrylamide-gel electrophoresis of NGC-complex: effects of β-mercaptoethanol and glycopeptidase treatment

(*a*) Purified NGC-complex species 1 under reducing conditions (5 μg); (*b*) without treatment with β-mercaptoethanol; and (*c*) after glycopeptidase digestion: lane 1, 0.3 enzyme units; lane 2, 3 enzyme units. Proteins were revealed by silver staining.

ethanol, the two bands at 32 kDa and 20 kDa disappeared and were replaced by a 54–52 kDa doublet band (Fig. 3*b*). The doublet was not consistently observed in different preparations and is probably caused by heterogeneity of sugar composition. This effect of β-mercaptoethanol confirms that, in the NGC-complex, the 32 and 20 kDa protomers form a heterodimer of equimolar ratio of the two protomers [3].

Fig. 3(*c*) shows a decrease in the apparent molecular mass of the protomers on the SDS/polyacrylamide-gel after deglycosylation by treatment with glycopeptidase F. After treatment, the diffuse band at 66–63 kDa was resolved into two close but clearly distinct bands at 58 and 56 kDa, possibly representing the two proteins neuraminidase and β-galactosidase. The 32 kDa band decreased only slightly in molecular mass to 31 kDa and the 20 kDa band was converted to a 17.5 kDa band. We assumed that the glycopeptidase digestion is complete, since increasing by 10-fold the amount of glycopeptidase in the incubation medium did not affect the band pattern (Fig. 3*c*). The decrease of molecular mass after glycopeptidase digestion was assumed to be due to complete carbohydrate removal, and was used to estimate the percentage of sugar linked to each protomer (Table 1). The partial specific volume, $\bar{v}$, of each species was then computed from their protomer content assuming a $\bar{v}$ of 0.74 ml/g for the protein core and 0.63 ml/g for the carbohydrate portion of each protomer (Table 1). It must be pointed out that the assumption of complete carbohydrate removal from all protomers by glycopeptidase digestion is unwarranted. However, the change in the partial specific volume due to an error in the estimation of associated sugar is relatively small and the calculated molecular mass of each species will only be minimally affected (Table 1).

The sedimentation coefficients, $s_{20,w}$, of the various species separated on the h.p.l.c. column were determined by the method of Martin & Ames [16], and a typical experimental result is presented in Fig. 4 for NGC-complex species 1. Dissociation of the NGC-complex species is also observed by the formation of a β-galactosidase activity peak corresponding to an $s_{20,w}$ of around 8.3 S; this is probably a mixture of NGC-complex species 2 and 3 which have similar $s_{20,w}$ values (Table 1).

The molecular masses and the frictional ratios, $f/f_{min.}$, of the three species separated by h.p.l.c. are given in Table 1 as obtained from experimentally determined Stokes' radius and $s_{20,w}$ values using eqns. (1) and (2). Given their calculated molecular mass and protomer composition, species 2 and 3 are probably

Table 1. Hydrodynamic properties and sugar content of the NGC-complex species separated by gel-filtration h.p.l.c.

The $\bar{v}$ of each species was computed from their respective protomer composition and sugar content of each protomer as determined by glycopeptidase digestion, assuming a $\bar{v}$ of 0.74 ml/mg for the protein core and 0.63 ml/mg for the carbohydrate part. The values of Stokes' radius (R_s) and $s_{20,w}$ represent the means ± S.E.M. of three determinations.

Species	R_s (nm)	$s_{20,w}$ (S)	$\bar{v}$ (ml/g)	Molecular mass (kDa)	$f/f_{min.}$	Sugar content (%)
1	8.5±0.2	18.8±0.8	0.730	679	1.47	9.0
2	6.5±0.2	8.6±0.3	0.728	235	1.60	10.8
3	4.5±0.1	7.9±0.4	0.728	147	1.29	10.8

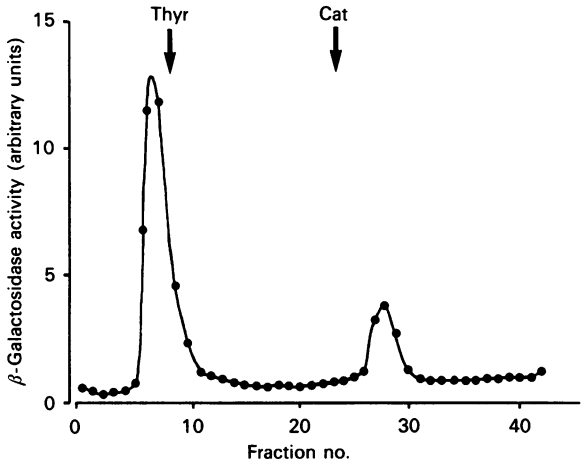

Fig. 4. Sedimentation analysis of the NGC-complex species 1

Thyr and Cat indicate the positions of sedimentation of thyroglobulin and catalase respectively.

tetramers and dimers respectively of 66–63 kDa protomers. Given the molecular mass of the NGC-complex species 1 (679 kDa) and the proportion of each protomer in this species, we calculated that it would contain 6.47 66–63 kDa protomers and 5.09 52 kDa heterodimers (32 kDa plus 20 kDa protomers linked by a disulphide bridge).

The frictional ratio of a protein reflects its asymmetry and hydration. The relatively high $f/f_{min.}$ ratio of the NGC-complex species may be explained on the basis of the carbohydrate content of the NGC-complex, which will increase the hydration and/or assymetry of the multienzyme complex.

DISCUSSION

H.p.l.c. analysis of the thiogalactoside-affinity-purified preparation of the NGC-complex showed that the molecular species so purified are part of an equilibrium mixture of related species. Rechromatography of the molecular species which was eluted as peak 1 on the h.p.l.c. column gave two new peaks corresponding to species 2 and 3 on the chromatogram (Fig. 1*b*). Rechromatography of peaks 2 and 3 separately (Figs. 1*c* and 1*d*) yielded small quantities of peak 1, as expected from the small amount of 32 and 20 kDa protomers found in these peaks (Fig. 2) and by previous work by van der Horst *et al.* [4] suggesting that these protomers are needed for the formation of the NGC-complex. Thus the heterodimer of the 32 and 20 kDa protomers seems to have strong affinity for species 1 and stays associated with it, perhaps through a rearrangement of the structure. Species 2, which represents a tetramer of 66–63 kDa protomers, is more stable than the other species whereas species 3 (dimer) recombined into species 2.

Although the hydrodynamic methods inform us on the NGC-complex structure, it is not known if this association–dissociation phenomenon represents the situation *in vivo* because relatively high hydrostatic pressures are attained during the h.p.l.c. procedure as well as during sedimentation analysis. Dissociation constants at some protein complex have been found to be pressure-sensitive [19]. However, other authors have also reported the existence of several forms of lysosomal β-galactosidase differing in molecular size [20–23].

Our data may have some significance to the situation *in vivo* where this association–dissociation system would constitute a simple way of regulating the degradation of complex oligosaccharides in the lysosome. Sialic acid residues being at the non-reducing end of complex oligosaccharides, the removal of these residues is the first step and the key to this degradation pathway composed of a series of glycosidases which successively remove sugar residues from the non-reducing end of complex oligosaccharide chains. Therefore, by regulating neuraminidase activity, one controls the whole degradation of these oligosaccharides. It has been shown recently that interaction with both β-galactosidase and the carboxypeptidase is necessary for the expression of neuraminidase activity and also to maintain the structure of the complex [4]. The regulation can thus be done in three ways: by controlling the level of neuraminidase directly at the gene level, or through β-galactosidase and/or carboxypeptidase. Since the lysosomal enzymes are themselves glycoproteins containing complex oligosaccharides, some regulation mechanisms may be needed to protect the lysosomal enzymes against excessive degradation.

The effect of digestion by glycopeptidase on the purified NGC-complex reported here differs from that reported by van der Horst *et al.* [4]. Instead of our two bands at 58 kDa and 56 kDa (Fig. 3*c*), they found a 38 kDa band which was detected with a neuraminidase-specific antibody, and a 57 kDa band which reacted with an anti-β-galactosidase immunoserum. This discrepancy may be due to proteolytic degradation by contaminating proteases in their glycopeptidase preparation. With our glycopeptidase preparation, no proteolytic degradation was observed on the SDS/polyacrylamide-gel after digestion under various conditions of incubation time and amount of enzyme.

The molecular masses reported previously for the NGC-complex or β-galactosidase multimeric forms in various tissues were usually obtained by gel filtration using standard proteins of known molecular mass to calibrate the column [20–23]. These values must be considered as approximate since we found that the NGC-complex is highly hydrated and/or asymmetric, a situation which could lead to relatively large systematic errors in molecular mass determinations by gel filtration, which assumes that all proteins behave as a non-hydrated sphere [24]. We determined the Stokes' radii of the NGC-complex species using a calibrated gel-filtration column, and apparent $s_{20,w}$ values for the NGC-complex species were obtained independently. This approach also allows us to obtain information on hydration and shape of the NGC-complex.

The determination of the molecular mass of the NGC-complex and stoichiometry of protomer components allows us to propose a structural model for the NGC-complex species. Knowing that the molar ratio of the 66–63 kDa protomers to the 52 kDa protomer is 1.27 in species 1, the best fit of an integer number of protomers to get a molecular mass close to the experimental value of 679 kDa was five protomers of 52 kDa and six protomers of 66–63 kDa (Fig. 5). This yields a calculated molecular mass of 644 kDa, a difference of only 5% from our measured value. It is interesting to note that a core hexamer has five faces, each of which may be occupied by a 52 kDa heterodimer. This asymmetric hexamer, which would be made of both neuraminidase and β-galactosidase protomers, is in equilibrium with a tetramer, peak 2 on the h.p.l.c. column, and a dimer, peak 3 (Fig. 1*a*). In this respect, it is interesting that radiation-inactivation experiments conducted in our laboratory are consistent with the idea either that neuraminidase and β-galactosidase each exist in dimeric form as a basic structural element of the NGC-complex, or that interaction between the two protomers are required for expression of each enzyme activity [25]. The proposed structure also takes into account the finding that the 52 kDa heterodimer is probably in contact with both neuraminidase and β-galactosidase protomers in the core hexamer; an ideal arrangement to stabilize neuraminidase and to ensure multimerization of β-

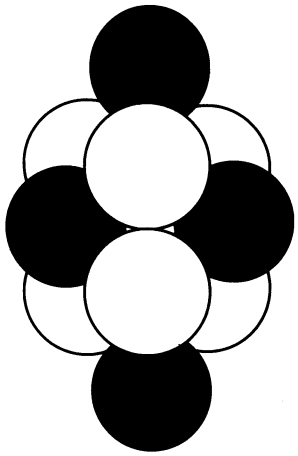

Fig. 5. Model of the NGC-complex species 1

The species is represented as a core hexamer of neuraminidase and β-galactosidase protomers (proportions unknown) surrounded by five carboxypeptidase heterodimers on each face of the hexamer.

galactosidase, the previously proposed function of this protein [4]. The 52 kDa heterodimer would then act as a bridge between the protomers of the core hexamer.

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