SUB-UNITS OF *N*-ACETYLGLUCOSAMINIDASES

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1. Introduction

In addition to β-*N*-acetylglucosaminidase (EC 3.2.1.30) the digestive gland of the limpet *Patella vulgata* (L.) contains an α,β-*N*-acetylglucosaminidase which hydrolyses both α- and β-glycosides of *N*-acetylglucosamine [1, 2]. In this paper we describe the first direct demonstration that *N*-acetyl-glucosaminidases contain two kinds of sub-units and that the composition of the corresponding sub-units of each enzyme is very similar.

2. Materials and methods

α,β-*N*-Acetylglucosaminidase and β-*N*-acetylglucosaminidase were prepared as described [1, 2]. Sub-units of each enzyme were prepared by dialysing at 4°C against 0.1 M phosphate buffer, pH 7.2, containing 1% (w/v) 2-mercaptoethanol, 4 M urea and 0.1% (w/v) sodium dodecyl sulphate with two changes for 24 hr. The dialysed enzyme solution was then incubated at 45°C for 2 hr. The incubation mixture was applied to a Sephadex G-150 (Pharmacia Fine Chemicals AB) column, 1.8 × 95 cm, equilibrated with 0.1 M phosphate buffer, pH 7.2, containing 4 M urea and 0.1% (w/v) sodium dodecyl sulphate. The column was eluted with the equilibrating buffer at a flow rate of 5 ml/hr at room temperature. 2 ml fractions were collected and the column effluent was monitored by reading the absorbance at 280 nm on a Pye-Unicam SP 3000 Spectrophotometer.

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Sub-unit molecular weights were determined by the method of Dunker and Rueckery [3]. Phosphorylase *a* (92 500), bovine serum albumin (66 000), L-glutamic acid dehydrogenase (53 000), egg albumin (45 000) and deoxyribonuclease I (31 000) were used as markers.

Hydrolysis of the isolated sub-units was carried out in evacuated sealed tubes containing 6 N HCl at 105°C for 24 hr. The hydrolysate was analysed on a JLC-6HA Amino Acid Analyzer using a 0.9 × 50 cm column for acidic and neutral amino acids and a 0.9 × 25 cm column for basic amino acids utilising the method of Speckman et al. [4]. Hexose was determined using sulphonated α-naphthol [5] and phenol–sulphuric acid [6] as the colour reagent.

3. Results and discussion

Both α,β-*N*-acetylglucosaminidase and β'-*N*-acetylglucosaminidase were found to consist of two types of sub-units (figs. 1 and 2). These were designated L and S and the area of the peak absorbing at 280 nm due to the L sub-unit was found to be approximately twice that of the S sub-unit for the α,β-enzyme (fig. 1) but the peak areas of the L and S sub-units for the β-enzyme (fig. 2) were found to be very similar. The molecular weight of the sub-units showed that the L sub-unit for both enzymes has a mol. wt. of 82 000 and the S sub-unit for both enzymes had a mol. wt. of 54 000 (fig. 3). Since the molecular weight of the α,β-enzyme was found to be 217 000 [1], the result obtained implies that α,β-*N*-acetylglucosaminidase contains two sub-units of mol. wt. 82 000 and one sub-unit of mol. wt. 54 000. Similarly, since the molecular weight of the β-enzyme is 136 000 [2], it appears that β-*N*-acetylglucosaminidase contains one sub-unit of mol. wt. 82 000 and one sub-unit of mol. wt. 54 000.

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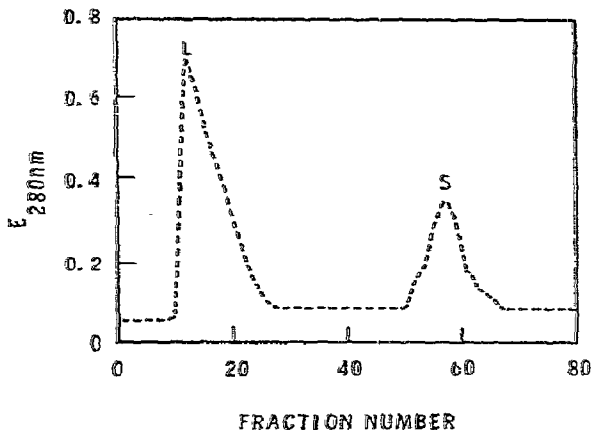

Fig. 1. Chromatographic separation on Sephadex G-150 of sub-units derived from α,β-*N*-acetylglucosaminidase.

Amino acid analysis and carbohydrate estimations of the isolated sub-units showed that the composition of the sub-units of mol wt in both 54 000 enzymes is identical within experimental error (tables 1 and 2). Similarly the amino acid content and the carbohydrate content of the two 82 000 sub-units present in the α,β-enzyme is almost exactly the same as that of the single 82 000 sub-unit of the β-enzyme (tables 1 and 2). These results suggest that the corresponding sub-units of both enzymes are similar and may be identical.

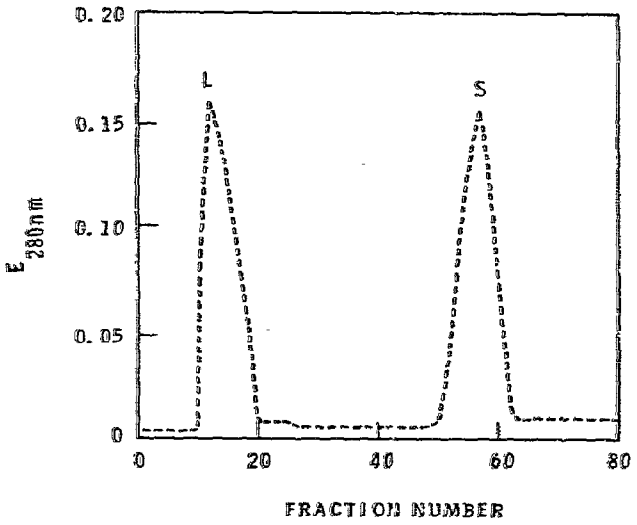

Fig. 2. Chromatographic separation on Sephadex G-150 of sub-units derived from β-*N*-acetylglucosaminidase.

Table 1
Composition of α,β-*N*-acetylglucosaminidase sub-units.

Constituent	Number of residues per sub-unit (mol wt 82 000)	Number of residues in sub-unit (mol wt 54 000)
Aspartic acid	83	53
Threonine	27	26
Serine	27	24
Glutamic acid	54	9
Proline	33	12
Glycine	27	26
Alanine	20	25
Valine	37	13
Methioine	18	0
Isoleucine	27	11
Leucine	33	30
Tyrosine	29	5
Phenylalanine	23	10
Lysine	22	15
Histidine	10	10
Ammonia	68	68
Arginine	25	0
Half-cystine	7	25
Tryptophan	15	7
Glucosamine	7	0
Hexose	47	41

Serine, threonine and glucosamine were not corrected for loss during hydrolysis. Tryptophan was determined spectrophotometrically [9].

This conclusion has been verified by immunodiffusion. In these experiments it was shown that the native enzymes posses antigenic determinants in common.

These results provide the first direct evidence for the existence of sub-units in *N*-acetylglucosaminidases. Verpoorte [7] found that dithiothreitol greatly reduced the molecular weight of the A and B forms of β-*N*-acetylglucosaminidase from bovine spleen and in consequence suggested that both enzymes contained sub-units. Recently Srivastava and Beutler [8] concluded that the most probable explanation of their genetic and immunological studies on β-*N*-acetylglucosaminidase A and B from human placenta was that the A form contained two different sub-units whilst the B form contained only one of these sub-units.

Table 2
Composition of β-*N*-acetylglucosaminidase sub-units.

Constituent	Number of residues in sub-unit (mol wt 82 000)	Number of residues in sub-unit (mol wt 54 000)
Aspartic acid	83	53
Threonine	28	24
Serine	28	22
Glutamic acid	52	9
Proline	33	12
Glycine	25	28
Alanine	20	25
Valine	34	19
Methionine	12	0
Isoleucine	27	11
Leucine	33	30
Tyrosine	29	5
Phenylalanine	23	10
Lysine	22	15
Histidine	10	10
Ammonia	68	68
Arginine	25	0
Half-cystine	7	22
Tryptophan	15	7
Glucosamine	4	0
Hexose	47	41

Threonine, serine and glucosamine were not corfected for loss during hydrolysis. Tryptophan was determined spectrophotometrically [9].

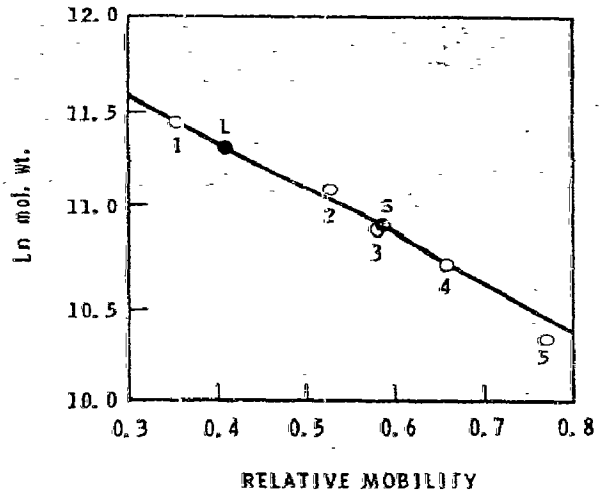

Fig. 3. Plot of Ln molecular weight against relative mobility (mobility relative to bromophenol blue). 1 = Phosphorylase *a*, 2 = Bovine serum albumin, 3 = L-glutamic acid dehydrogenase 4 = egg albumin and 5 = deoxyribonuclease I. L and S are the sub-units derived from α,β-*N*-acetylglucosaminidase and β-*N*-acetylglucosaminidase.

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