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Substrate specificity of human liver neutral α-mannosidase

Samer AL DAHER,* Rita DE GASPERI,†‡ Peter DANIEL,§ Shirish HIRANI,‖ Christopher WARREN† and Bryan WINCHESTER*¶

* Enzymology Unit, Division of Biochemistry and Metabolism, Institute of Child Health (University of London), 30 Guilford Street, London WC1N 1EH, U.K., † Carbohydrate Unit, Lovett Laboratories, Harvard Medical School and Massachusetts General Hospital, Charlestown, MA 02129, U.S.A., § Eunice Kennedy Shriver Center for Mental Retardation, Waltham, MA 02254, U.S.A., and ‖ Genzyme, One Kendall Square, Cambridge, MA 02139, U.S.A.

The digestion of radiolabelled natural oligosaccharide substrates by human liver neutral α-mannosidase has been studied by h.p.l.c. and h.p.t.l.c. The high-mannose oligosaccharides $Man_9GlcNAc$ and $Man_8GlcNAc$ are hydrolysed by the enzyme by two distinct non-random routes to a common product of composition $Man_6GlcNAc$, which is then slowly converted into a unique $Man_5GlcNAc$ oligosaccharide, Manα(1 → 2)Manα(1 → 2)Manα(1 → 3)[Manα(1 → 6)] Manβ(1 → 4)GlcNAc. These pathways are different from the processing and lysosomal catabolic pathways for these structures. In particular, the α(1 → 2)-linked mannose residues attached to the core α(1 → 3)-linked mannose residue are resistant to hydrolysis. The key processing intermediate, Manα(1 → 3)[Manα(1 → 6)]Manα(1 → 6)[Manα(1 → 3)] Manβ(1 → 4)GlcNAc, is not produced in the digestion of high-mannose glycans by the neutral α-mannosidase, but it is hydrolysed by the enzyme by a non-random route to Manβ(1 → 4)GlcNAc via the core structure Manα(1 → 3)[Manα(1 → 6)]Manβ(1 → 4)GlcNAc. In contrast with its ready hydrolysis by lysosomal α-mannosidase, the core α(1 → 3)-mannosidic linkage is quite resistant to hydrolysis by neutral α-mannosidase. The precise specificity of neutral α-mannosidase towards high-mannose oligosaccharides suggests that it has a role in the modification of such structures in the cytosol.

INTRODUCTION

The enzyme α-D-mannosidase (EC 3.2.1.24) exists in mammalian cells as structurally and genetically distinct multiple forms with different subcellular locations, physicochemical and kinetic properties and functions (Winchester, 1984). The lysosomal form of the enzyme, which has been given the gene symbol MAN_B located on chromosome 19pter → q13 (Champion *et al.*, 1978*a*), is involved in the catabolism of asparagine-linked glycans of glycoproteins (Michalski *et al.*, 1990; Al Daher *et al.*, 1991; De Gasperi *et al.*, 1991). It has an acidic pH optimum and catalyses the hydrolysis of α(1 → 2)-, α(1 → 3)- and α(1 → 6)-mannosidic linkages in oligosaccharides derived from asparagine-linked glycans. The catabolic route for a particular glycan is highly specific and non-random and is determined by its structure (Michalski *et al.*, 1990; Al Daher *et al.*, 1991; De Gasperi *et al.*, 1991). A genetic deficiency of this activity in cats, cattle or humans leads to the lysosomal storage disease α-mannosidosis (Jolly *et al.*, 1981).

The membrane-bound forms of α-mannosidase located in the endoplasmic reticulum and Golgi apparatus are involved in the processing of the carbohydrate moieties of glycoproteins (Kornfeld & Kornfeld, 1985).

In addition, many mammalian cells have also been shown to contain a soluble α-mannosidase, which has a neutral pH optimum and is located in the cytosol (Suzuki *et al.*, 1969; Okumura & Yamashina, 1970; Marsh & Gourlay, 1971; Carroll *et al.*, 1972; Suzuki & Kushida, 1973; Phillips *et al.*, 1974*a*; Shoup & Touster, 1976). This activity has been designated MAN_A and has been localized to the q11 → qter region of chromosome 15 in man (Champion *et al.*, 1978*b*). The function of this activity is not known. The substrate specificity of the soluble neutral α-mannosidase activity in rat liver (Opheim & Touster, 1978; Bischoff & Kornfeld, 1986; Haeuw *et al.*, 1991) and kidney (Tulsiani & Touster, 1987) has been investigated. It is distinct from that of any of the membrane-bound processing α-mannosidases (Kornfeld & Kornfeld, 1985), although the enzyme has been reported to be structurally related to an endoplasmic reticulum α-mannosidase (Bischoff & Kornfeld, 1986). The neutral α-mannosidase in human liver has distinct kinetic and physicochemical properties from the other human α-mannosidases (Phillips *et al.*, 1974*b*; Hirani & Winchester, 1979). In this paper the specificity of partially purified human neutral α-mannosidase towards a series of natural oligosaccharide substrates has been investigated to try to understand its metabolic function.

MATERIALS AND METHODS

Preparation of human liver neutral α-mannosidase

Human liver neutral α-mannosidase was partially purified from post-mortem liver, which had been stored at −20 °C until required, essentially as described by Phillips *et al.* (1976). Briefly, liver was homogenized (50%, w/v) in 10 mM-sodium phosphate buffer, pH 6.0, and centrifuged at 30000 *g* in an MSE Prepspin centrifuge at 4 °C for 30 min. The supernatant was filtered through glass wool and the material precipitating between 0% and 35% saturation with $(NH_4)_2SO_4$ was collected by centrifugation. The pellet was resuspended in 10 mM-sodium phosphate buffer, pH 6.8, containing 0.1 mM-$CaCl_2$, 0.1 mM-$MnCl_2$ and 0.1 mM-$MgCl_2$ and the solution passed through a column (2 cm × 0.6 cm) of concanavalin A–Sepharose. α-Mannosidase activity with a neutral pH optimum was not retained on the column, whereas the soluble lysosomal α-mannosidase, with a pH optimum of pH 4.0, could only be eluted with buffer containing 0.5 M-methyl α-D-mannopyranoside. The unbound fraction

‡ Present address: Department of Neurology, New York University Medical School, 550 First Avenue, New York, NY 10016, U.S.A.
¶ To whom correspondence should be addressed.

Table 1. Structures, abbreviations, retention times and origins of the oligosaccharide substrates and standards

The linkages in the oligosaccharide structures are $\alpha(1 \rightarrow 2)$ ●–● , $\alpha(1 \rightarrow 3)$, $\alpha(1 \rightarrow 6)$ and $\beta(1 \rightarrow 4)$ ●–□ . The retention times are representative values obtained from the h.p.l.c. analysis of a mixture of standards. Key to origin: (i) commercial (Genzyme Corporation); (ii) obtained by treatment with endohexosaminidase H of compounds described in Warren *et al.* (1988*a*); (iii) isolated as described in Warren *et al.* (1988*b*), and for the oligosaccharide (**3a**) after treatment with endohexosaminidase D; (iv) isolated as described in Warren *et al.* (1983); (v) prepared by treatment of compound (**2b**) with endoglucosaminidase isolated from human tissues (De Gasperi *et al.*, 1989). Numbering of the oligosaccharides is based on the number of mannose residues. To distinguish the isomeric structures, a, b, c etc. were arbitrarily assigned.

Substrate	Abbreviation and no.	Retention time (min)	Origin
	$Man_9GlcNAc_1$ (**9**)	60.55	(i)
	$Man_8GlcNAc_1$ (**8a**)	58.92	(i)
	$Man_7GlcNAc_1$ (**7a**)	56.23	(i)
	$Man_7GlcNAc_1$ (**7b**)	56.23	(i)
	$Man_6GlcNAc_1$ (**6a**)	53.43	(i)
	$Man_5GlcNAc_1$ (**5a**)	50.14	(ii)
	$Man_5GlcNAc_1$ (**5b**)	48.17	(iii)
	$Man_4GlcNAc_1$ (**4a**)	45.08	(ii)
	$Man_4GlcNAc_1$ (**4b**)	42.92	(iii)
	$Man_4GlcNAc_1$ (**4c**)	42.10	(iv)
	$Man_3GlcNAc_1$ (**3a**)	36.97	(ii)
	$Man_3GlcNAc_1$ (**3b**)	36.97	(ii)
	$Man_3GlcNAc_1$ (**3c**)	35.48	(iv)
	$Man_2GlcNAc_1$ (**2a**)	22.90	(iv)
	$Man_2GlcNAc_1$ (**2b**)	22.90	(v)

was dialysed against 50 mM-Tris/HCl, pH 6.0, at 4 °C. This preparation of α-mannosidase was used for the specificity studies. It had a sharp activity–pH profile with an optimum at pH 6.5. It was inhibited by 1 mM-deoxymannojirimycin (33%), swainsonine (K_i, 5 μM; I_{50} 5×10^{-5} M at 0.2 mM substrate) and 0.5 mM-Zn^{2+} (25%) but activated by 0.5 mM-Co^{2+} (20%). Gel filtration on Sepharose 6B showed no evidence of α-mannosidase activity with an M_r of 130000 and an intermediate pH optimum of 5.6, characteristic of the human membrane-bound Golgi α-mannosidase (Hirani & Winchester, 1979). The lack of detergent in the extraction buffer, chromatography on concanavalin A–Sepharose and Sepharose 6B and the sharpness of the activity–pH profile exclude the presence of other membrane-bound α-mannosidases that are active towards the synthetic substrate, 4-methylumbelliferyl α-mannoside. The presence in the preparation of a previously undescribed activity towards oligosaccharides cannot be completely excluded but the properties of the activity are consistent with those previously reported for human neutral α-mannosidase (MAN_A) (Phillips *et al.*, 1974*b*; Hirani & Winchester, 1979; Cenci di Bello *et al.*, 1989).

Isolation, purification and labelling of oligosaccharide substrates

The origin, purification and radiolabelling of the oligosaccharide substrates were exactly as described previously (Al Daher *et al.*, 1991). Their structures and abbreviations are shown in Table 1. The purity of all the modified oligosaccharides was checked by h.p.l.c. and h.p.t.l.c. Except in some preliminary experiments reduced oligosaccharides were used as substrates. The suffix -ol is omitted from the structures of the oligosaccharide substrates for clarity, but it is implied throughout unless specifically stated otherwise.

Incubation conditions

Routinely 15–20 nmol of substrate was incubated with 0.1–0.2 unit of neutral α-mannosidase activity in 100 μl of sodium phosphate/citrate buffer, pH 6.5. At different intervals of time, samples were removed for analysis by h.p.l.c. and h.p.t.l.c. The reaction was stopped by addition of an equal volume of ethanol (95%) followed by boiling the sample for 3 min. After centrifugation at 8000 *g* for 3 min to remove the protein, the supernatant was passed through a Duolite mixed-bed resin MB 6113, a mixture of Duolite C225 in the H^+ form and A 101 D in the OH^- form, with the inclusion of an indicator. The neutral oligosaccharides were eluted with deionized water. During long incubations, extra enzyme (0.1–0.2 unit) was added every 8 h because of the lability of the neutral α-mannosidase.

Analysis of the digestion products and substrates by h.p.l.c., h.p.t.l.c. and acetolysis

Samples of the digestion mixtures or substrates were analysed by h.p.t.l.c., h.p.l.c. and acetolysis as described previously (Al Daher *et al.*, 1991).

RESULTS AND DISCUSSION

Digestion of high-Mannose glycans

$Man_9GlcNAc$. The largest potential substrate in glycoproteins for neutral α-mannosidase would be the *N*-linked glycan $Man_9GlcNAc_2$. The corresponding oligosaccharide, $Man_9GlcNAc$ (**9**), which would be derived from the $Man_9GlcNAc_2$ glycan *in vivo* by the successive action of aspartoamidohydrolase and endo-*N*-acetyl-β-glucosaminidase (De Gasperi *et al.*, 1989) was incubated with the human neutral α-mannosidase. The digestion mixture was analysed by h.p.l.c. (Fig. 1*a*). This showed that it was broken down by a non-random pathway, predominantly to an oligosaccharide of composition $Man_8GlcNAc$, which was only very slowly converted into $Man_5GlcNAc$ (Scheme 1). The structures of the apparently homogeneous intermediates were determined by comparison of their retention times with those for standards and by acetolysis of pure samples isolated from the reaction mixture by h.p.l.c. (Figs. 1*b*, 1*c* and 1*d*). The $Man_8GlcNAc$ intermediate had a different structure (**8b**) from the predominant oligosaccharide of composition $Man_8GlcNAc$ (**8a**) found on intact glycoproteins and in the storage products in α-mannosidosis. However, it did have the same structure as the first digestion intermediate

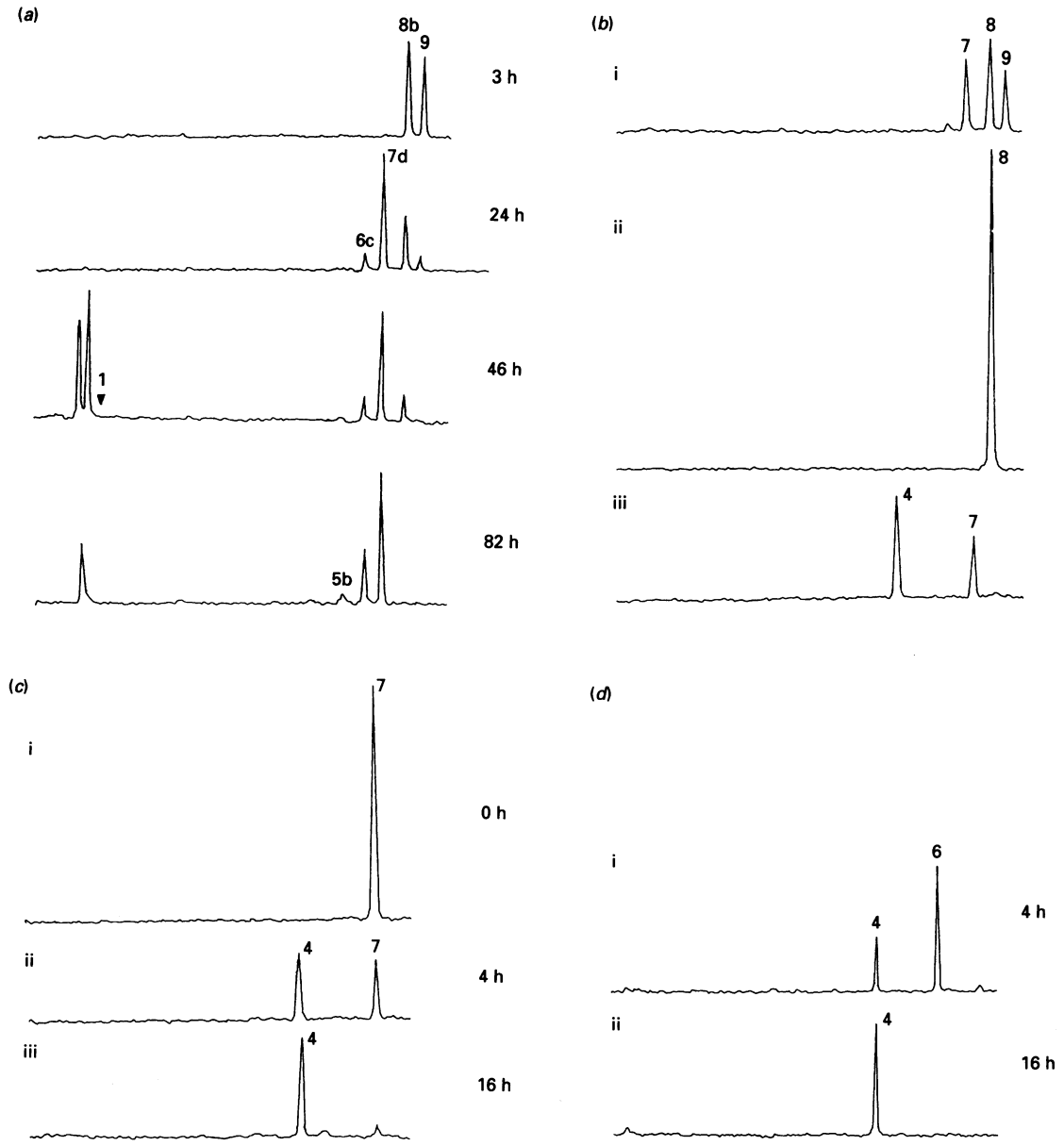


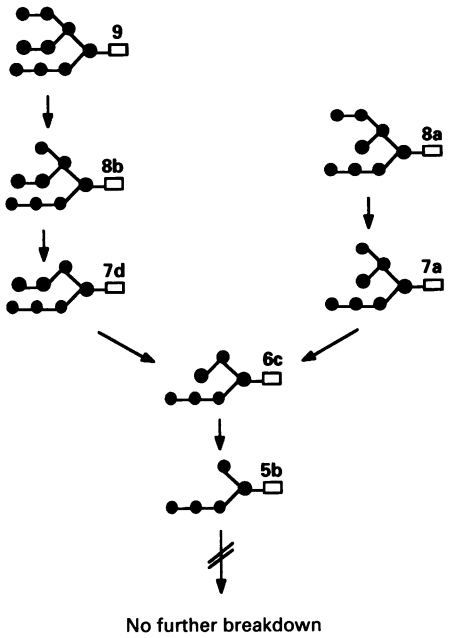
Fig. 1. Analysis of the digestion of $Man_9GlcNAc$ (9) by neutral α-mannosidase

(*a*) H.p.l.c. of the digestion mixture at different time-intervals. Fresh enzyme was added every 8 h. **1** = Manβ(1 → 4)GlcNAc. (*b*) H.p.l.c. of (i) digestion mixture after 6 h; (ii) $Man_8GlcNAc$ isolated from digestion mixture after 6 h; (iii) $Man_8GlcNAc$ intermediate after acetolysis for 4 h. (*c*) (i) $Man_7GlcNAc$ intermediate isolated from digestion mixture after 6 h; (ii) and (iii) $Man_7GlcNAc$ intermediate after acetolysis for 4 and 16 h respectively. (*d*) Acetolysis of $Man_6GlcNAc$ intermediate from digestion mixture at 6 h after acetolysis for (i) 4 and (ii) 16 h.

produced by the action of human lysosomal α-mannosidase on $Man_9GlcNAc$ (**9**) (Al Daher *et al.*, 1991).

The $Man_7GlcNAc$ intermediate was shown to consist predominantly of a single compound (**7d**) by acetolysis (Fig. 1*c*), with the $Man_4GlcNAc$ generated by acetolysis having the same retention time as an authentic sample of Manα(1 → 2)Manα(1 → 2)Manα(1 → 3)Manβ(1 → 4)GlcNAc (**4c**). The formation of $Man_6GlcNAc$ (**6c**) was very slow, and further digestion to $Man_5GlcNAc$ (**5b**) was very limited, even after the addition of fresh enzyme and prolonged incubation. The structure of the limit digestion product was confirmed by comparison of its retention time with that of an authentic sample of $Man_5GlcNAc$ (**5b**). Confirmation that this oligosaccharide was not susceptible to digestion by the neutral α-mannosidase was obtained by directly incubating some of the authentic sample with the enzyme and showing by h.p.t.l.c. that no breakdown occurred.

$Man_8GlcNAc$. The oligosaccharide $Man_8GlcNAc$ (**8a**), which corresponds to the most abundant $Man_8GlcNAc$ structure in intact glycans and in the storage products in α-mannosidosis, was also only partially digested by the neutral α-mannosidase (Fig. 2*a*). Although the limit digestion product $Man_5GlcNAc$ (**5b**) was the same as that obtained from $Man_9GlcNAc$, the route and the intermediates were different (Scheme 1). The $Man_7GlcNAc$ intermediate had the same retention time as the authentic mixture (**7a/b**), whose structures had been established by n.m.r. Analysis by acetolysis (Fig. 2*b*) showed that it consisted exclusively of compound (**7a**). Subsequent removal of the peripheral α(1 → 6)-linked residue led to the formation of Man_6-GlcNAc (**6c**), the same intermediate obtained in the digestion of



Scheme 1

$Man_9GlcNAc$ (**9**). This was again only slowly broken down to $Man_5GlcNAc$ (**5b**), the identity of which was confirmed by co-elution on h.p.l.c. after co-injection with a sample of the authentic compound. When the whole digestion mixture was subjected to prolonged acetolysis, the predominant product was Manα(1 → 2)Manα(1 → 2)Manα(1 → 3)Manβ(1 → 4)GlcNAc (**4c**). This indicated that the α(1 → 2) linkages on the branch attached to the core α(1 → 3)-linked mannose residue are resistant to hydrolysis by the neutral enzyme and provided further support for the structures of the $Man_{6-7}GlcNAc$ intermediates shown in Scheme 1.

The pathways for the breakdown of $Man_9GlcNAc$ (**9**) and $Man_8GlcNAc$ (**8a**) are different from the pathways for the processing of asparagine-linked high-mannose glycans, catalysed by an endoplasmic-reticulum α-mannosidase and Golgi α-mannosidases I and II (Kornfeld & Kornfeld, 1985) and for their catabolism by lysosomal α-mannosidase (Al Daher *et al.*, 1991). In particular, the α(1 → 2)-linked mannose residues on the branch attached to the core α(1 → 3)-linked mannose are not removed by the neutral enzyme, whereas the α(1 → 3)- and α(1 → 6)-linked mannose residues on the core α(1 → 6) branch are removed. This suggests that this enzyme is not involved in the processing of glycoproteins that pass through the Golgi en route to extracellular and intracellular destinations.

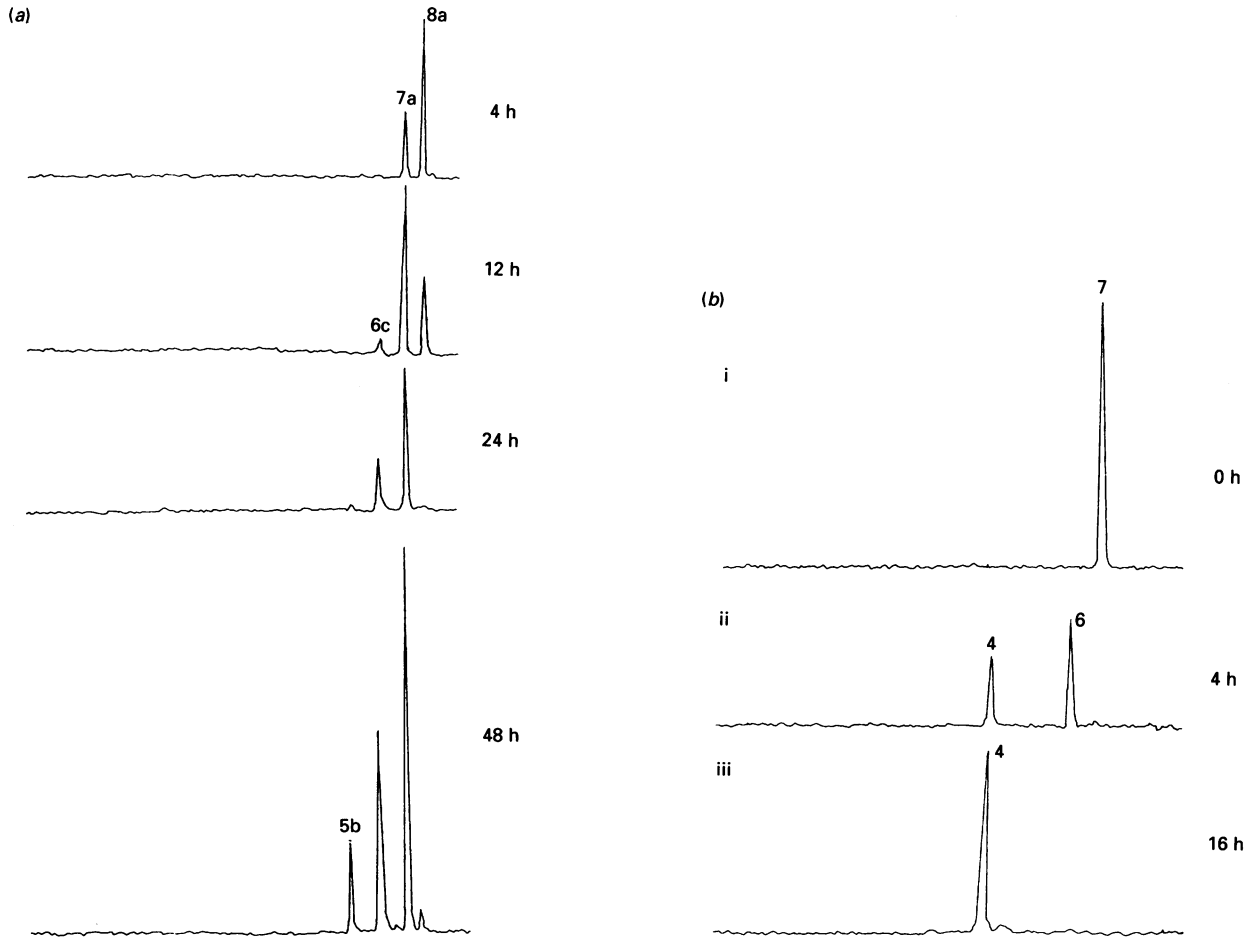


Fig. 2. Analysis of the digestion of $Man_8GlcNAc$ (8a) by neutral α-mannosidase

(*a*) H.p.l.c. of the digestion mixture at various time-intervals. (*b*) (i) H.p.l.c. of the $Man_7GlcNAc$ intermediate isolated from the digestion mixture at 12 h and after acetolysis for 4 h (ii) and 16 h (iii).

Digestion of processing intermediates: $Man_5GlcNAc$ (5a) and $Man_3GlcNAc$ (3a)

The end product of the digestion of high-mannose glycans by the neutral α-mannosidase, $Man_5GlcNAc$ (**5b**), does not lie on the major pathway for the processing of asparagine-linked glycans (Kornfeld & Kornfeld, 1985). The $Man_5GlcNAc$ isomer that does occur on this pathway is structure (**5a**). This isomer is not digested by Golgi α-mannosidase II without the prior addition of an *N*-acetylglucosamine residue on the core α(1 → 3)-linked mannose residue, catalysed by *N*-acetylglucosaminyltransferase I. However, $Man_5GlcNAc$ (**5a**) was completely digested by the neutral α-mannosidase (Fig. 3 and Scheme 2). Two $Man_4GlcNAc$ intermediates were formed in about equal proportions. Their retention times were different from those for the standards, (**4a**), (**4b**) and (**4c**). The apparently homogeneous $Man_3GlcNAc$ intermediate had the same retention time as the two authentic standards, (**3a**) and (**3b**), which are not resolved under the conditions used. Therefore it was isolated by h.p.l.c. and subjected to acetolysis. As only $Man_2GlcNAc$ was produced, the structure of the $Man_3GlcNAc$ intermediate must be (**3a**). From this, it was deduced that the structures of the two $Man_4GlcNAc$ intermediates were (**4e**) and (**4f**). The nature of the heterogeneous $Man_2GlcNAc$ intermediate in the catabolic pathway was elucidated by h.p.t.l.c. (Fig. 4*a*), because the trisaccharides, (**2a**) and (**2b**), are not fully resolved by h.p.l.c. Densitometric scanning showed that the core α(1 → 3)-mannosidic linkage on $Man_3GlcNAc$ (**3a**) was hydrolysed preferentially to give a mixture of (**2a**) and (**2b**) in the proportions 3:17. Finally both trisaccharides were broken down to Manβ(1 → 4)GlcNAc. Confirmation of the final stages in the pathway was obtained by direct digestion of an authentic sample of $Man_3GlcNAc$ (**3a**), followed by analysis of the digestion mixture by h.p.t.l.c. (Fig. 4*b*).

Although the breakdown of high-mannose glycans by the neutral α-mannosidase stops at $Man_{5-6}GlcNAc$ (**5b–6c**), it can catalyse the removal of all the α-linked mannose residues from the oligosaccharide (**5a**). The route of breakdown differs significantly from the lysosomal catabolic route (Al Daher *et al.*, 1991). The core α(1 → 3)-mannosidic linkage is the most susceptible to the lysosomal enzyme, but it is resistant to hydrolysis by the neutral enzyme, with the result that the core structure (**3a**) is generated. The α(1 → 3)-mannosidic linkage in this structure (**3a**)

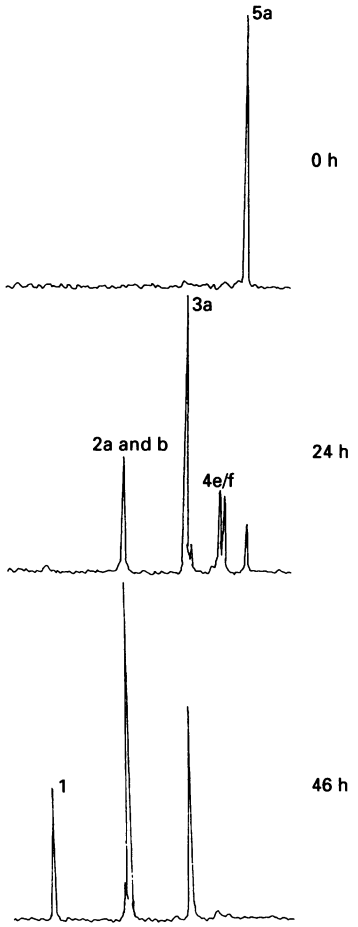
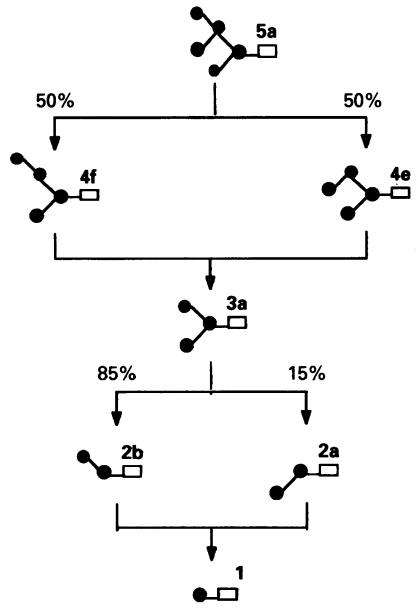


Fig. 3. Analysis by h.p.l.c. of the digestion of $Man_5GlcNAc$ (5a) by neutral α-mannosidase

H.p.l.c. of digestion mixture at different intervals of time.



Scheme 2

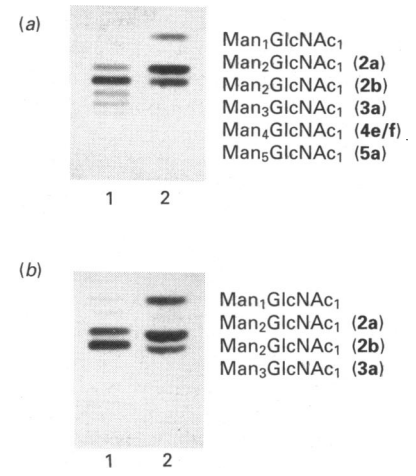


Fig. 4. Analysis by h.p.t.l.c. of the digestion of $Man_5GlcNAc$ (5a) and $Man_3GlcNAc$ (3a) by neutral α-mannosidase

H.p.t.l.c. of the digestion of (*a*) $Man_5GlcNAc$ (**5a**) and (*b*) $Man_3GlcNAc$ (**3a**) after 12 h (lane 1) and 24 h (lane 2).

Table 2. Initial rates of hydrolysis of some of the natural substrates by neutral α-mannosidase

Substrates (15 nmol) were incubated with 100 munits of enzyme activity at 37 °C for a period of 2 h in phosphate/citrate buffer, pH 6.5. The reactions were stopped and the samples were processed as described in the Materials and methods section and analysed by h.p.l.c. The values are the percentage amounts of intermediates formed in the reaction after 2 h. M, Man; G, GlcNAc; n., not detected.

Starting material	Percentage of substrate and intermediates in reaction mixture								
	M_9G_1	M_8G_1	M_7G_1	M_6G_1	M_5G_1	M_4G_1	M_3G_1	M_2G_1	M_1G_1
$Man_9GlcNAc_1$ (**9**)	64	36	n.	n.	n.	n.	n.	n.	n.
$Man_8GlcNAc_1$ (**8a**)	–	69	31	n.	n.	n.	n.	n.	n.
$Man_5GlcNAc_1$ (**5a**)	–	–	–	–	83	17	n.	n.	n.
$Man_5GlcNAc_1$ (**5b**)	–	–	–	–	100*	n.	n.	n.	n.

* For this substrate no intermediates were detected even after 48 h incubations.

is then hydrolysed preferentially by the neutral enzyme, but the α(1 → 6) linkage is also appreciably broken. In contrast, the lysosomal enzyme acts on the α(1 → 6) linkage in structure (**3a**) only after the α(1 → 3) linkage has been hydrolysed (Al Daher *et al.*, 1991). An α-mannosidase that does not require the prior action of *N*-acetylglucosaminyltransferase I to convert $Man_5GlcNAc_2Asn$ into $Man_3GlcNAc_2Asn$ is present in baby-hamster kidney cells and rat liver (Monis *et al.*, 1987). This activity differs from the neutral α-mannosidase in that it cannot hydrolyse the core α(1 → 3)- and α(1 → 6)-mannosidic linkages and is not inhibited by swainsonine. Current ideas on the biosynthesis and intracellular transport of glycoproteins suggest that a cytosolic α-mannosidase is unlikely to encounter asparagine-linked glycans of composition $Man_{3-5}GlcNAc_2$. Therefore its catabolic effect on these oligosaccharides could be incidental. Tulsiani & Touster (1987) have suggested that the cytosolic α-mannosidase is involved in the extralysosomal catabolism of the glycans on glycoproteins because of the accumulation of linear oligosaccharides such as $Man_4GlcNAc$ (**4c**), $Man_3GlcNAc$ (**3c**) and $Man_2GlcNAc$ (**2a**) in human mannosidosis and the reported accumulation of storage products in the cytosol of kidneys of rats treated with swainsonine. We have postulated that a residual lysosomal α(1 → 6)mannosidase activity is responsible for the formation of the linear oligosaccharide storage products in mannosidosis (Al Daher *et al.*, 1991). Furthermore we have shown that in both human genetic and swainsonine-induced mannosidosis fibroblasts, all storage products are located predominantly in the lysosomes (S. Al Daher, P. Daniel, C. Warren & B. Winchester, unpublished work).

The neutral α-mannosidase activity in a crude cytosolic fraction of rat liver has the same specificity towards $Man_9GlcNAc$ (**9a**) as the human neutral α-mannosidase (Haeuw *et al.*, 1991). The structures of the digestion intermediates produced by the rat enzyme were determined by n.m.r. In contrast, the purified rat liver neutral α-mannosidase hydrolyses the same substrate in a random manner (Bischoff & Kornfeld, 1986), and the membrane-bound endoplasmic reticulum α-mannosidase, from which the cytosolic enzyme appears to be derived by proteolysis, specifically converts compound (**9a**) into (**8a**) intracellularly (Bischoff *et al.*, 1986). These observations suggest that the specificity of a common active site may depend on the physical form of the enzyme. It is possible that the soluble neutral α-mannosidase is a proteolytic artifact of cell fractionation or disruption and that its real function is as the catalytic domain of a membrane-bound α-mannosidase. This does not preclude it being synthesized as an active membrane-bound precursor, which releases a functional catalytic domain into the cytosol on proteolysis.

The function of the neutral α-mannosidase is not clear. Its substrate specificity is different from that of mammalian membrane-bound α-mannosidases involved in the processing of glycoproteins (Kornfeld & Kornfeld, 1985). In particular, the enzyme can catalyse the hydrolysis of the core α(1 → 3)- and α(1 → 6)-mannosidic linkages in compound (**3a**), suggesting that it is not on the main glycoprotein-processing pathway. The α(1 → 2) linkages in high-mannose glycans such as (**9**) and (**8a**) are hydrolysed much faster than the α(1 → 3) and the α(1 → 6) linkages in $Man_5GlcNAc$ (**5a**) (Table 2), suggesting that the trimming of high-mannose glycans may be part of the function of the enzyme. We speculate that it has a specific catalytic function outside the major processing and catabolic pathways in modifying mannose-containing glycans. These glycans could form part of molecules other than glycoproteins, e.g. dolichol-pyrophosphoryl oligosaccharides or the glycosylphosphatidyl-inositol anchor. The structure of the limiting product from the digestion of high-mannose glycans by neutral α-mannosidase, $Man_5GlcNAc_1$ (**5b**), has the same structure as the $Man_5GlcNAc_2$-oligosaccharide formed on dolichol pyrophosphate by the transfer of mannose from GDP-mannose in the cytosol. Perhaps the cytosolic α-mannosidase has an editing function. It could ensure that only the correctly formed dolicholpyrophosphoryl oligosaccharide is translocated across the endoplasmic-reticulum membrane from the cytosolic face to the luminal face, where it is elongated by the transfer of mannose and glucose from dolichol-phosphoryl-mannose and -glucose to form the common precursor. Studies on the biosynthesis of the neutral α-mannosidase should throw light on its function.

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