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Note

The hydrolysis of L-fucose from some naturally occurring compounds by purified human-liver α-L-fucosidase

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Human liver contains an α-L-fucosidase which can hydrolyse *p*-nitrophenyl and 4-methylumbelliferyl α-L-fucosides[1,2]. The enzyme has been substantially purified[3] and can also hydrolyse 1- and 2-naphthyl α-L-fucosides[4].

The ability of the enzyme to hydrolyse L-fucose from naturally occurring compounds has not been reported, and a purification procedure based on ability to hydrolyse an artificial substrate may not yield an enzyme which is active on naturally occurring compounds. This possibility may be important when postulating a physiological role for the enzyme, and considering its relevance to the disease fucosidosis.

We now report that the enzyme was unable to release L-fucose from glycoproteins derived from human saliva, gastric secretion, or liver carcinoma, but could release the sugar from a series of oligosaccharides derived from liver carcinoma, which possessed H blood-group specificity (see Table I). Oligosaccharides derived from human gastric secretion (of Le^a blood-group specificity) were also hydrolysed (see Table II). Increase in either incubation time or in amount of enzyme caused greater release of L-fucose.

The results suggest that the ability of the purified enzyme to cleave L-fucose from oligosaccharides decreases as the molecular weight of the oligosaccharide increases. With relatively large oligosaccharides (10 sugar residues), hydrolysis of L-fucose proceeds slowly. By increasing the substrate and enzyme concentrations, the α-L-fucosidase was able to hydrolyse all of the L-fucose from the Le^a decasaccharide:

L-Fucose released (%)	37	52	63	71	79	~100
Time of incubation at 37° (days)	0.67	2	7	14	28	56

TABLE I

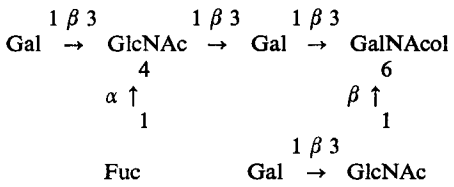
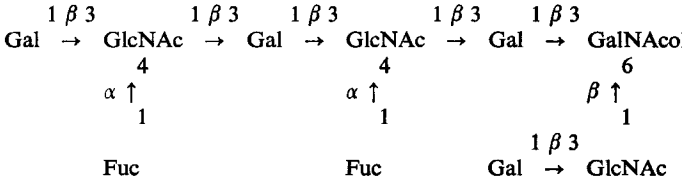
HYDROLYSIS OF L-FUCOSE FROM BLOOD-GROUP H SPECIFIC OLIGOSACCHARIDES BY PURIFIED HUMAN-LIVER α-L-FUCOSIDASE[a]

Substrate	*L-Fucose released (%)*
Fuc(1α2)Gal(1β3)GalNAcol	100
Fuc(1α2)Gal(1β3)GalNAcol(6β1)GlcNAc(3β1)Gal	100
Fuc(1α2)Gal(1β3)GalNAcol(6β1)GlcNAc(3β1)Gal + Fuc(1α2)Gal(1β3 or 4)GlcNAc(1β3)Gal(1β3)GalNAcol(6β1)GlcNAc(3β1)Gal	70
Fuc(1α2)Gal(1β3)GlcNAc(1β3)Gal(1β3)GalNAcol(6β1)GlcNAc(3β1)Gal with some Fuc(1α4)GlcNAc and Fuc(1α2)Gal(1β3 or 4)GlcNAc(1β3)Gal(1β3)GalNAcol(6β1)GlcNAc(3β1)Gal	50
Fuc(1α2)Gal(1β3)GlcNAc(1β3)Gal(1β3)GlcNAc(1β3)Gal(1β3)GalNAcol(6β1)GlcNAc(3β1)Gal with some Fuc(1α4)GlcNAc	Traces only

[a]Incubation time was 16 h.

TABLE II

HYDROLYSIS OF L-FUCOSE FROM BLOOD-GROUP Le^a SPECIFIC OLIGOSACCHARIDES BY PURIFIED HUMAN-LIVER α-L-FUCOSIDASE

Substrate	*Amount of enzyme added*[a] *(ml)*	*Incubation time (h)*	*L-Fucose released (%)*
	0.05	16	15
As above	0.1	16	30
As above	0.1	80	57
As above	0.3	80	80
As above	0.5	170	100
	0.05	16	23
As above	0.5	170	52

[a]Final incubation volume was 2 ml

Similar results were found with a larger Le^a-active oligosaccharide (13 sugar residues).

The inability of the purified human-liver α-L-fucosidase to hydrolyse L-fucose from glycoproteins agrees with the results found previously for partially purified, pig-kidney α-L-fucosidase[5] and the crude rat-epididymal enzyme[6]. This result could be due to the relative inaccessibility of the L-fucose residues of the glycoprotein, or to steric hindrance of the active site of the enzyme by the glycoprotein molecule. The ability of protozoal α-L-fucosidase to cleave L-fucose from such large molecules[7] implies that the L-fucose residues are accessible to these enzymes. α-L-Fucosidase derived from *Trichomonas foetus* (H and Le^a blood-group destroying enzymes) did not bind to the Sepharose 4B *N*-(ε-aminocaproyl)-L-fucosylamine column used in the purification of human liver enzyme, and was unable to hydrolyse aryl α-L-fucosides. This result suggests that the substrate requirements of the protozoal and human enzymes are considerably different.

The purified human-liver enzyme was able to hydrolyse L-fucose relatively easily from oligosaccharides of low molecular weight, and in this respect is similar to the α-L-fucosidase derived from pig kidney[5].

The results show that L-fucose is cleaved by the enzyme from H and Le^a blood-group oligosaccharides (2-*O*-α-L-fucosyl-D-galactose and 2-acetamido-2-deoxy-4-*O*-α-L-fucosyl-D-glucose, respectively). The rate of hydrolysis of L-fucose from the oligosaccharides decreases with increasing molecular weight. Total release of L-fucose was possible from an oligosaccharide consisting of 13 sugar residues, but long periods of incubation and a high concentration of enzyme were required.

The results suggest that, if the physiological function of the enzyme is concerned with catabolism of macromolecules such as glycoproteins, preliminary depolymerisation would be necessary before the α-L-fucosidase is able to function.

EXPERIMENTAL

Human liver α-L-fucosidase was purified as previously reported[3]. Glycoproteins and oligosaccharides derived from human liver carcinoma, gastric secretion, and saliva were isolated as previously described[8].

Enzymic hydrolysis of substrates. — Purified α-L-fucosidase (0.05–0.3 ml) was incubated at 37° for various periods with the substrates (~0.08mM) in 0.1M sodium acetate buffer (pH 5.5); the final volume was 2–6 ml. For larger oligosaccharides, modified reaction conditions were used, involving increased substrate and enzyme concentrations. Thus, the decasaccharide (1.5 mg) derived from gastric secretion (Le^a-active, see Table II for structure) was dissolved in aqueous, purified α-L-fucosidase (0.3 ml), and 0.1M sodium acetate buffer (0.02 ml, pH 5.5) was added. Incubation was carried out at 37°. A similar experiment was carried out with an Le^a-active oligosaccharide possessing 13 sugar residues.

Acid hydrolysis of substrates. — Substrates were hydrolysed with 2M HCl at 100° for 1.75 and 2 h. L-Fucose was detected in the hydrolysates by g.l.c. with a Pye series 104 chromatograph[9]. After addition of galactitol (the internal standard) to the hydrolysates, deacidification (where necessary) and trimethylsilylation were carried out as previously described[9].

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REFERENCES

1 D. Robinson and R. Thorpe, *Clin. Chim. Acta*, 47 (1973) 403–407.
2 D. Robinson and R. Thorpe, *Clin. Chim. Acta*, 55 (1974) 65–69.
3 D. Robinson and R. Thorpe, *FEBS Lett.*, 45 (1974) 191–193.

4 R. THORPE, unpublished results.
5 G. YA. WIEDERSCHAIN AND E. L. ROSENFELD, *Bull. Soc. Chim. Biol.*, 51 (1969) 1075–1083.
6 R. B. CARLSON AND J. G. PIERCE, *J. Biol. Chem.*, 247 (1972) 23–32.
7 W. M. WATKINS, *Biochem. J.*, 71 (1959) 261–274.
8 M. D. G. OATES, A. C. ROSBOTTOM, AND J. SCHRAGER, *Carbohydr. Res.*, 34 (1974) 115–137.
9 M. D. G. OATES AND J. SCHRAGER, *J. Chromatogr.*, 28 (1967) 232–245.