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PURIFICATION AND KINETIC STUDIES OF AN α-L-FUCOSIDASE OF *Venus mercenaria**

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ABSTRACT

An α-L-fucosidase activity has been isolated from the liver (hepatopancreas) of the common edible clam, *Venus mercenaria*, and has been purified approximately 300-fold (11% yield) by affinity chromatography on agarose–ε-aminocaproylfucosamine. Isoelectric focusing profiles were heterogeneous, revealing several isoenzymes. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis indicated the presence of a single subunit of M_r 50 000. The purified enzyme preparation contained only trace amounts of other α- and β-D-glycosidases tested. In addition to *p*-nitrophenyl α-L-fucopyranoside, the enzyme hydrolyzed natural substrates such as fucose-containing milk pentasaccharides, thyroglobulin glycopeptides, human salivary glycoproteins, and blood-group-active glycosphingolipids. The enzyme preparation had a broad pH optimum range between 4.5 and 5.5. The apparent K_m value with respect to *p*-nitrophenyl α-L-fucopyranoside was 0.26mM.

INTRODUCTION

Many L-fucose-containing glycoconjugates have important biological functions in solution or at cellular membrane surfaces. These functions include growth regulation, receptor function, cell-cell interactions, and antigenicity. In order to elucidate completely the mechanisms of biological activity, however, the exact structure of glycoconjugate carbohydrate chains must be determined. Such studies have been hindered by the limitations of chemical methods of sequencing. Enzymes that specifically degrade oligosaccharide chains under mild conditions are of great utility in structural studies, as they reveal the sequence of sugars as well as provide information regarding anomeric configurations.

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α-L-Fucosidases (EC 3.2.1.51) are broadly classified into three groups based on their aglycon specificity: bacterial and mold α-L-fucosidases, mammalian α-L-fucosidases, and mollusc α-L-fucosidases (see ref. 2 for a review). In addition, almond emulsin[3,4] contains two α-L-fucosidases which hydrolyze the α-L-Fuc*p*-(1→3)-β-D-Glc*p*NAc→R and the α-L-Fuc*p*-(1→4)-β-D-Glc*p*NAc→R linkages present in milk oligosaccharides, and the α-L-Fuc*p*-(1→2)-β-D-Gal*p*→R linkage present in 2′-fucosyllactose.

The present communication describes the purification and specificities of (1→2)-, (1→3)-, and (1→4)-α-L-fucosylhydrolases isolated from the common edible clam, which are valuable aids in structural studies of L-fucose-containing biosynthetic products.

EXPERIMENTAL

Preparation of L-fucose-containing glycopeptides from thyroglobulin. — L-Fucose-containing glycopeptides from bovine and porcine thryoglobulin were obtained by Pronase digestion according to a modified procedure of Fukuda and Egami[5,6] as follows. Thyroglobulin (20 mg) was dissolved in 0.05M Tris·HCl (pH 7.6) containing 10mM $CaCl_2$ and was incubated with Pronase (20 mg) for 24 h at 37°. Additional Pronase (5 mg) was added and the incubation was continued for a further 72 h. The reaction was stopped by heating at 100° for 5 min. After centrifugation at 12 000 *g* for 10 min, the supernatant was dialyzed against water and lyophilized.

Chemical synthesis of α-L-fucopyranosylamine. — α-L-Fucopyranosylamine was prepared by the method of Isbell and Frush[7]. To methanol (8 mL) were added L-fucose (3 g, 1.8 mmol) and NH_4Cl (0.08 g, 1.5 mmol) in a 100-mL round-bottomed flask. The mixture was treated with NH_3 gas at 0° until the sugar had dissolved and was stored in a closed flask at 4°. After 48 h, the crystalline α-L-fucopyranosylamine was separated, washed with cold 1:1 (v/v) methanol–ether and dried in the presence of NaOH in an atmosphere of NH_3.

Activation of Sepharose 4B by CNBr. — A simplified method[8] was used for the activation of Sepharose 4B. A slurry of washed agarose beads (1 vol.) consisting of equal volumes of gel and water was added to 2M Na_2CO_3 (1 vol.) with stirring, and an acetonitrile solution (0.05 vol.) of CNBr (2 g of CNBr/mL of acetonitrile) was added all at once. The slurry was stirred vigorously for 2 min, poured into a coarse sintered-glass funnel, and washed with 10 vol. each of 0.1M $NaHCO_3$ (pH 9.5), water, and 0.2M $NaHCO_3$ (pH 9.5). After the last wash, the slurry was filtered under vacuum to a moist cake and transferred to a beaker containing 0.2M $NaHCO_3$ (1 vol.) (pH 9.5).

Preparation of ε-aminocaproic acid–Sepharose 4B. — To freshly prepared, activated agarose beads (10 mL) was added ε-aminocaproic acid (1.31 g, 10 mmol). The coupling reaction was allowed to proceed for 20 h at 4° with shaking. Tris·HCl was then added to the slurry until a 1M solution was reached, the pH was adjusted

to 9.0, and shaking was continued for 4 h at room temperature. Finally, the gel was washed with 10 vol. each of: 0.05M Tris·HCl (pH 8.0) containing 0.5M NaCl, 0.05M sodium formate buffer (pH 4.0) containing 0.5M NaCl, and water.

*Preparation of the agarose–1-*N*-(ε-caproyl)-α-*L*-fucopyranosylamine conjugate (ACA-FA–Sepharose 4B).* — The coupling procedure was basically that recommended by Pharmacia Fine Chemicals, Inc. (Piscataway, New Jersey). The ε-aminocaproyl–Sepharose 4B gel (10 g), prepared as described above, was suspended in water to make a slurry of 25 mL and stirred at room temperature. α-L-Fucopyranosylamine (0.1 g) was added and the pH of the slurry was adjusted to 4.5 with 0.1M NaOH. Solid 3-(3-dimethylaminopropyl)-1-ethylcarbodiimide hydrochloride (0.5 g) was added over a period of 5 min, the pH being maintained at 4.5. The mixture was stirred for 1 h and then kept at room temperature for 20 h without stirring. The gel was successively washed with M NaCl, 0.1M Tris·HCl buffer (pH 8.6) containing M NaCl, 0.05M sodium acetate buffer (pH 3.0) containing M NaCl, 0.1M Tris·HCl buffer (pH 8.6), water, and 10mM sodium citrate–NaH_2PO_4 buffer (pH 5.5) containing 0.02% (w/v) NaN_3 (NaCPA).

Enzyme assay conditions. — (*A*) p-*Nitrophenyl α-L-fucopyranoside assay.* The standard assay mixture contained *p*-nitrophenyl α-L-fucopyranoside [α-L-Fuc*p*$OC_6H_4NO_2(p)$; 0.15 μmol], sodium citrate–dihydrogen phosphate buffer (15 μmol; pH 5.0 or 5.5), bovine serum albumin (75 μg), and enzyme protein (0.05–0.3 μg) in a final volume of 0.15 mL. Enzyme and substrate blanks were run as appropriate. The mixture was incubated at 37° for 15 min, 0.2M Na_2CO_3 (1 mL) was added to stop the reaction. The yellow chromogen, formed by *p*-nitrophenol under alkaline conditions, was extracted with butanol (2 mL) and then determined spectrophotometrically at 400 nm with a Gilford 240 spectrophotometer. Under these conditions, the molar extinction coefficient of *p*-nitrophenol was $1.8 \cdot 10^4 \cdot M^{-1} \cdot cm^{-1}$. The reaction rate was constant with respect to time and proportional to protein concentration. One unit of enzyme activity was defined as the amount of enzyme that hydrolyzes 1 μmol of α-L-Fuc*p*$OC_6H_4NO_2(p)$/min at 37°. Specific activity was expressed as units/mg of protein.

(*B*) *Coupled* L-*fucose dehydrogenase assay.* The amount of L-fucose liberated from natural glycoconjugates by the action of the clam α-L-fucosidase preparation was determined by the L-fucose dehydrogenase-spectrophotometric method[9]. The fucose-containing glycoconjugate substrate (0.1–0.3 mg) was dissolved in 0.02 mL of 0.1M citrate sodium–dihydrogen phosphate buffer (pH 5.5). α-L-Fucosidase (3 μg/0.03 mL) was then added and the mixture (0.05 mL total volume) was incubated for 48 h at 37°. The reaction mixture was subsequently incubated with 2.4mM NAD^+ and L-fucose dehydrogenase (0.9 mg; Miles) in 0.05M Tris·HCl buffer (pH 8.0) for 30 min at 37°, and the end-point absorbance at 340 nm was determined spectrophotometrically. The total incubation volume was 1.6 mL. For each series of incubations, a standard curve was established for L-fucose in a 0.03–0.15M concentration range.

Purification of clam α-L-fucosidase. — The clam α-L-fucosidase was purified

by a combination of acid precipitation, and ion-exchange and column chromatographic methods. All procedures were carried out at 0–6° unless otherwise stated.

(*A*) *Acid precipitation.* The livers (hepatopancreas) from 92 cherrystone clams were trimmed of excess surrounding tissue (109 g total, wet weight), washed with distilled water, and homogenized for 2 min in 10mM NaCPA buffer (pH 5.5; 4 vol., 400 mL) in a Waring Blender at low speed. After being kept for 1 h at 6°, an aliquot (280 mL) of the homogenate was centrifuged at 10 000 *g* for 60 min. The supernatant fraction I (250 mL) was dialyzed against 4-L changes of NaCPA buffer (pH 5.5) overnight. The dialyzed supernatant fraction was centrifuged at 10 000 *g* for 60 min. The supernatant fraction II was concentrated to 35 mL in an Amicon ultrafiltration apparatus (PM 30 membrane), dialyzed against three 200-mL changes of NaCPA buffer (pH 7.6) for 24 h, and centrifuged again at 10 000 *g* for 60 min.

(*B*) *Ion-exchange chromatography.* A portion of the supernatant fraction III (30 mL; 981 mg of protein) was applied to a DEAE-cellulose (DE-23) column (2.5 × 21 cm) previously equilibrated with 10mM NaCPA (pH 7.6). The column was washed with 10mM NaCPA (pH 7.6; ~700 mL) and then eluted with a linear (0–0.5M; 500 mL) gradient of NaCl in 10mM NaCPA (pH 7.6), followed by an additional 250 mL of 0.5M NaCl in 10mM NaCPA (pH 7.6). Fractions (6.6 mL) were collected in an ISCO model 1200 pup-fraction collector (Fig. 1). After being assayed, fractions (1–24, DE-1; and 25–102, DE-2) containing α-L-fucosidase activity were combined, concentrated (DE-1, 16 mL; DE-2, 21.5 mL) by ultrafiltration (PM 30) technique, and dialyzed against four 500-mL changes of 10mM NaCPA buffer (pH 5.5) (4 changes of 500 mL/change).

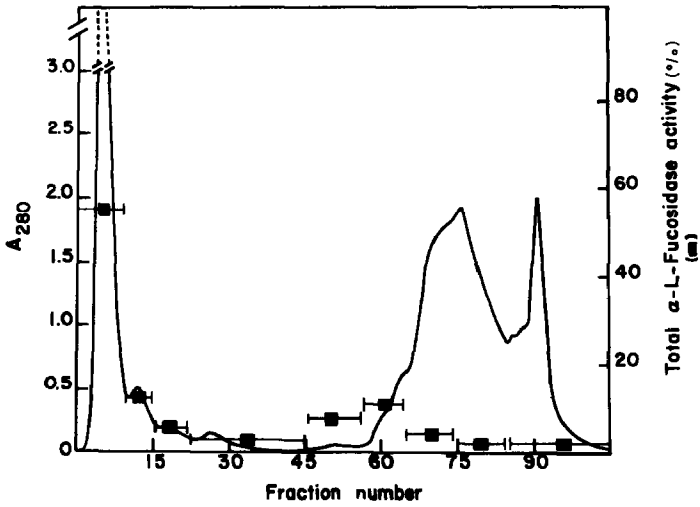

Fig. 1. Elution profile of α-L-fucosidase on DEAE-cellulose. The DE-23 column (2.5 × 21 cm) was washed with 10mM NaCPA buffer (pH 7.6), followed by a linear (0–0.5M) gradient of sodium chloride in 10mM NaCPA buffer (pH 7.6). See the Experimental section for details. Volume of each fraction was 6.6 mL.

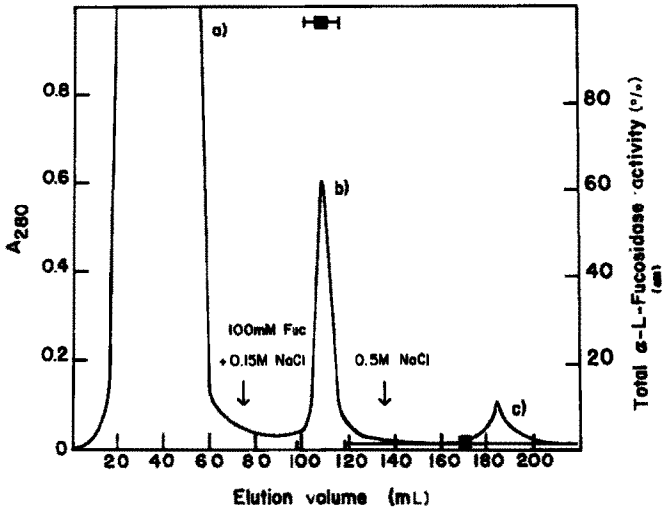

Fig. 2. Affinity chromatography of clam liver α-L-fucosidase on a column (1.1 × 35 cm) of agarose–1-(ε-caproyl)-α-L-fucopyranosylamine. The column was eluted as described in the Experimental section: (a) 10mM NaCPA (pH 5.5) wash peak; (b) L-fucose wash peak; and (c) sodium chloride wash peak.

(*C*) *Affinity column chromatography.* A portion (10 mL; 104 mg of protein) of the combined fractions of highest specific activity (DE-1) from the DEAE-cellulose column was applied to an ACA–FA–Sepharose 4B affinity column (1.1 × 35 cm) equilibrated with 10mM NaCPA buffer (pH 5.5). The column was washed with 10mM NaCPA buffer (pH 5.5; 75 mL) and then eluted sequentially with 10mM NaCPA buffer (pH 5.5) containing either 100mM L-fucose and 0.15M NaCl (60 mL) or 0.5M NaCl (100 mL) (see Fig. 2). The eluates (2 mL/tube) were combined in separate portions, (a) 0–100, (b) 101–116, and (c) 117–210 mL. Fractions (a) and (c) were concentrated to 8 and 5 mL, respectively. Fractions (b) and (c) were then dialyzed against 10mM NaCPA (pH 5.5) containing 0.15M NaCl (6 changes of 500 mL/change). All fractions were assayed for α-L-fucosidase activity. Fraction (b) (13 mL) was used as enzyme source in subsequent studies.

The enzyme was stored at −18° in 10mM NaCPA buffer (pH 5.5) containing 0.15M NaCl, and lost 20% of its activity after storage for one month.

RESULTS

Purification of α-L-fucosidase. — An α-L-fucosidase activity has been isolated from the liver of the cherrystone clam, *Venus mercenaria,* and has been purified approximately 300-fold by affinity chromatography on ACA–FA–Sepharose 4B. Table I summarizes the purification data.

The elution profile of α-L-fucosidase from the DEAE-cellulose column is shown in Fig. 1; 42% of the enzyme applied to the column was eluted with 10mM NaCPA buffer (pH 7.6) to yield a 6.5-fold purification. The 6.5-fold purified α-L-

TABLE I

PURIFICATION OF CLAM α-L-FUCOSIDASE BY AFFINITY CHROMATOGRAPHY

Fraction	*Total protein (mg)*	*Total activity (units)*[a]	*Specific activity (nmol·mg^{-1} of protein·min^{-1})*	*Fold*	*Yield (%)*
Homogenate	4284	92.1	21.5		100
Supernatant I (SI)	1225	66.2	54.0	2.5	72
Supernatant II (SII)	1145	63.4	55.4	2.6	69
DE-1	166	23.0	139.1	6.5	25
Affinity column	1.4	9.95	6106	330	11

[a]A unit of activity corresponds to the hydrolysis of 1 μmol of substrate/min at 37°.

fucosidase was then passed through an ACA–FA–Sepharose 4B affinity column (Fig. 2); 69% of the applied α-L-fucosidase activity was eluted with 10mM NaCPA (pH 5.5) containing 100mM L-fucose and 0.15M sodium chloride. The over-all recovery of activity was 11%.

Purity of α-L-fucosidase. — *(A) Polyacrylamide gels.* Polyacrylamide-gel electrophoresis of the purified native enzyme was performed on 7% polyacrylamide gels. Since the enzyme was unstable at pH >8.0, the running gel was polymerized at pH 7.5 and the electrophoresis performed in 50mM Tris–glycine buffer (pH 7.4) at 2mA/gel. After electrophoresis, one gel was sliced for assay of enzyme activity and another stained for protein with Coomassie Brilliant Blue R (0.25% containing 10% acetic acid and 45% methanol, v/v). The enzyme moved as a single broad band as indicated by both protein stain and activity determinations (Fig. 3).

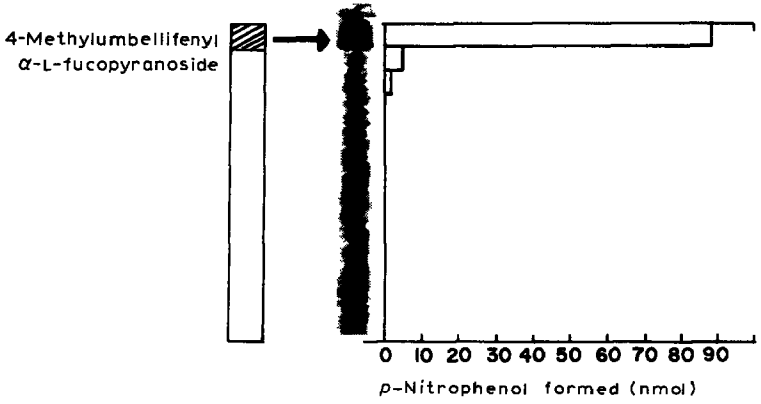

Fig. 3. Polyacrylamide-gel electrophoresis (pH 7.4) of clam α-L-fucosidase. Electrophoresis was carried out for 6 h at 2 mA/gel in 7% polyacrylamide gels. Left: the enzyme protein (30 μg) was isolated by affinity column chromatography and stained with Coomassie Blue. Right: enzyme activity profiles. Gel slices (5 mm) were incubated overnight at room temperature with *p*-nitrophenyl α-L-fucopyranoside and 4-methylumbelliferyl α-L-fucopyranoside under the standard assay conditions.

A duplicate gel was also incubated with the fluorescent substrate 0.4mM 4-methylumbelliferyl α-L-fucopyranoside under the standard assay conditions. Again the protein band appeared to be associated with α-L-fucosidase activity after exposure of the gel to long-wavelength u.v. light. The region of α-L-fucosidase activity appeared as a fluorescent band against a non-fluorescent background, as indicated schematically in Fig. 3.

(*B*) *Sodium dodecyl sulfate–polyacrylamide gels.* Although the purified enzyme preparation appeared to yield a single broad protein band, as indicated by polyacrylamide gel electrophoresis, $NaDodSO_4$–PAGE in the presence of 2-mercaptoethanol[10] indicated some heterogeneity (Fig. 4). In addition to the major broad band, four faint protein bands also were observed after staining with Coomassie Blue. No evidence is available at the present time to indicate whether the faint bands represent multiple forms of the same enzyme, or merely contaminants. Multiple forms of purified α-L-fucosidase have been observed previously[6,11–21].

The clam α-L-fucosidase is a glycoprotein, as evidenced by its positive periodic acid–Schiff stain[22]. One broad band was observed after staining instead of the major broad protein band and four faint bands observed after Coomassie Blue staining. The approximate M_r (50 000) of the broad protein band was determined as described by Weber and Osborn[23].

(*C*) *Isoelectric focusing in polyacrylamide gels.* The isoelectric focusing pattern of the purified clam α-L-fucosidase is shown in Fig. 5. The enzyme

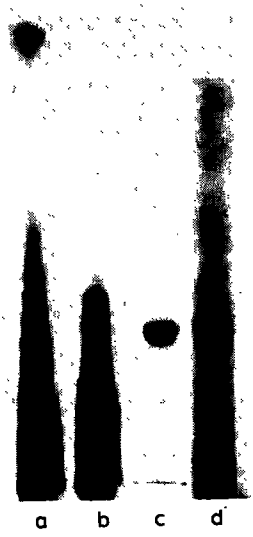

Fig. 4. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of clam α-L-fucosidase. The electrophoresis was carried out in 10% polyacrylamide gels at 3 mA/gel in the presence of 0.1% sodium dodecyl sulfate and 5% 2-mercaptoethanol: (a) Homogenate (0.8 mg of protein), (b) DE-1 fraction/(0.8 mg of protein), and (c) purified α-L-fucosidase (20 μg of protein); (a), (b), and (c) were stained with Coomassie Blue; (d) purified α-L-fucosidase stained with the periodic acid–Schiff reagent.

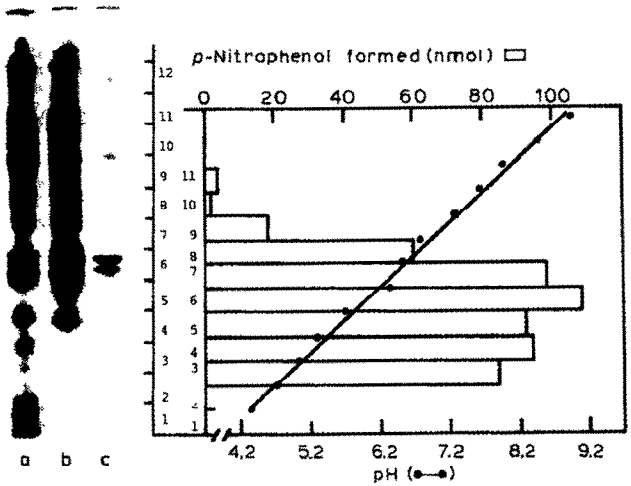

Fig. 5. Isoelectric-focusing pattern of clam α-L-fucosidase in polyacrylamide gels. Isoelectric focusing was performed in 5% polyacrylamide gels in the presence of 2% ampholytes and 8M urea at a constant current of 1 mA/gel until a voltage of 150 V was reached, and then at a constant voltage for 7 h longer[24]. The pH gradient was measured after incubating gel portions (7 mm; sliced with a fixed-blade slicer) in 2 mL of distilled water for 30 min. Enzyme activity was assayed as described in the text, after removal of ampholytes by transverse destaining for 15 min in 10mM NaCPA (pH 5.5) at 4°. Left, α-L-fucosidase fractions: (a) homogenate (1.5 mg of protein), (b) DE-1 fraction (1 mg of protein), and (c) affinity column fraction (55 μg of protein); all gels were stained with 0.02% Coomassie Brilliant Blue G-250 in 3.5% perchloric acid. Right, pH gradient and activity profile of duplicate gels containing purified enzyme.

preparation could be resolved into as many as 12 discrete bands[24]. The pI values of the protein bands ranged from 5.0 to 7.0. Enzymic activity with α-L-Fuc*p*$OC_6H_4NO_2$(*p*) as substrate was found in all bands. Further studies are necessary to determine whether the resolved proteins are all α-L-fucosidase isozymes.

(*D*) *Assay of α-L-fucosidase for contaminating glycosidases.* The following *p*-nitrophenyl glycosides were used as substrates in the standard incubation mixture: α-L- and β-D-fucopyranosides; α- and β-D-galactopyranosides, -glucopyranosides, and -mannopyranosides; α- and 2-acetamido-2-deoxy-α- and -β-D-glucopyranosides and 2-acetamido-2-deoxy-β-D-galactopyranoside. The α-L-fucosidase preparation contained α-D-galactosidase (4%) as a contaminant. The other α- and β-glycosidases tested were present in trace amounts (0–1%). It was also free from protease activity.

Characterization of purified α-L-fucosidase. — (*A*) *Effect of enzyme concentration, incubation time, and bovine serum albumin concentration.* The rate of hydrolysis of α-L-Fuc*p*$OC_6H_4NO_2$(*p*) was proportional to enzyme concentration between 0.4 and 4 μg of protein/mL of incubation volume and remained constant with the time of incubation for 20 min (Fig. 6). The α-L-fucosidase activity was increased by 15% when 75 μg of bovine serum albumin (BSA) were added to the standard assay mixture.

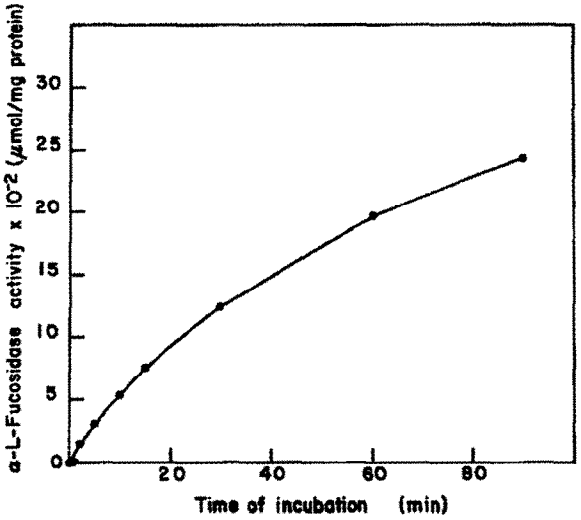

Fig. 6. Effect of incubation time on enzyme activity. The standard α-L-Fuc*p*$OC_6H_4NO_2$(*p*) assay method described in the Experimental section was used to estimate enzyme activity at pH 5.5. Samples containing 0.3 μg of purified α-L-fucosidase were incubated at 37° for the indicated time periods.

(*B*) *pH Activity profile.* The pH optimum of the purified α-L-fucosidase was determined as follows: enzyme protein (0.3 μg) and bovine serum albumin (75 μg) were added to 15 μL of M buffers of varying pH values (sodium citrate–citric acid buffer for pH 3.4–6.0; sodium acetate–acetic acid buffer for pH 3.6–5.8; and citrate–sodium dihydrogen phosphate buffer for pH 2.4–8.0). The reaction was initiated with 1.0mM α-L-Fuc*p*$OC_6H_4NO_2$(*p*). The final assay mixture volume was 0.15 mL. All incubations were carried out at 37° for 15 min. The pH-activity curve of the purified α-L-fucosidase acting on the *p*-nitrophenyl glycoside indicated maximal activity in a pH range of 4.5–5.5 in citrate–sodium dihydrogen phosphate or citrate buffer.

(*C*) *Heat stability.* The effect of partial heat inactivation was determined on the purified enzyme fraction obtained after affinity chromatography. The α-L-fucosidase (0.4 μg/mL of 0.1M citrate phosphate buffer, pH 5.5) was heated at 55° for periods up to 60 min in the presence and absence of bovine serum albumin (75 μg/0.15 mL of incubation volume), cooled in ice, and assayed for residual activity at pH 5.5 according to the standard α-L-Fuc*p*$OC_6H_4NO_2$(*p*) assay method. As shown in Fig. 7, the purified α-L-fucosidase was quite a heat stable enzyme. An initial increase in enzyme activity over the control value was observed, and more than 80% of the activity remained after heating at 55° for 60 min. Addition of bovine serum albumin to the preincubated mixture largely prevented loss of activity.

(*D*) *Effect of* p*-nitrophenyl* α-L*-fucopyranoside concentration on reaction rate.* An apparent K_m value (0.26mM) and maximal velocity (7.1 μmol·mg^{-1} of protein·min^{-1}) for the purified enzyme were determined graphically from a

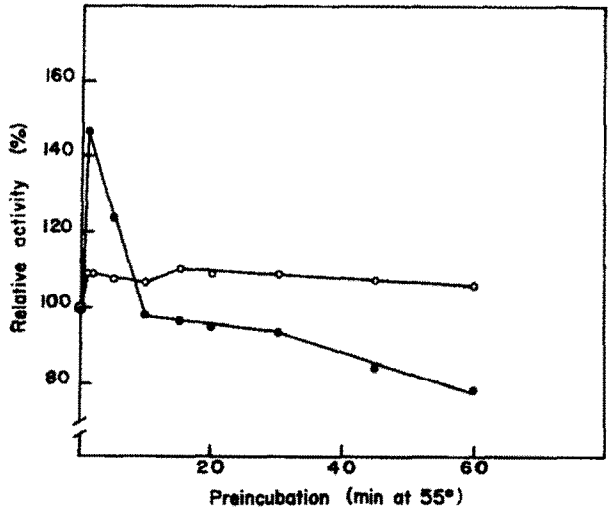

Fig. 7. Heat stability of α-L-fucosidase. Enzyme (55 pg) was heated at 55° for the indicated time periods, chilled at 0°, and asayed for residual activity according to the standard α-L-Fuc*p*$OC_6H_4NO_2(p)$ assay method. Results are expressed as percentages of residual activity, the control being an unheated sample: (●—●) α-L-fucosidase (55 pg), (○—○) α-L-fucosidase plus bovine serum albumin (75 μg).

Lineweaver–Burk plot[25] by use of α-L-Fuc*p*$OC_6H_4NO_2(p)$ as substrate. The purified α-L-fucosidase (0.3 μg) was incubated with various concentrations of substrate in 100mM citrate–sodium dihydrogen phosphate buffer (pH 5.0). Bovine serum albumin (75 μg) was also present in the 0.15-mL incubation mixture. All samples were incubated for 15 min at 37°.

(*E*) *Effect of 4-methylumbelliferyl α-L-fucopyranoside concentration on*

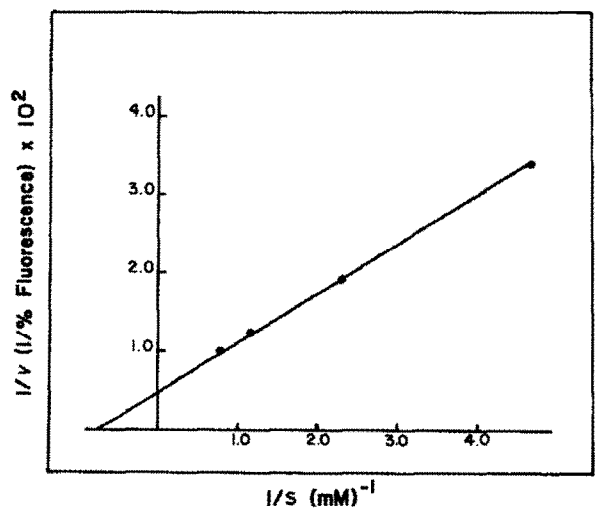

Fig. 8. Lineweaver–Burk plot for K_m determination using the 4-methylumbelliferyl α-L-fucopyranoside substrate. Incubation conditions are described in detail in the text.

TABLE II

GLYCOCONJUGATE SUBSTRATE SPECIFICITY OF CLAM α-L-FUCOSIDASE

Substrate	*Structure*	*Expected α-L-linkage*	*Hydrolysis (%)*
Blood group-related	[^{14}C]Fuc→GlcNAc→Gal→Glc→Cer	(1→3)[a]	79[b]
synthetic GSL compounds	[^{14}C]Fuc→Gal→GlcNAc→Gal→Glc→Cer	(1→2)[c]	81[b]
Human serum α_1-acid glycoprotein	[^{14}C]Fuc→Gal→GlcNAc→α_1AGP	(1→2)[c]	83[b]
	[^{14}C]Fuc→GlcNAc→α_1AGP	(1→3)[a]	70[b]
Lacto-*N*-fucopentaose II		(1→4)	31[d]
Bovine thyroglobulin glycopeptide (BTG)			19[e]
Human salivary glycoproteins (H-type; HSG)			30[e]

[a]Biosynthetic compound obtained by a purified FucT-3-catalyzed reaction[32–34]. [b]Assayed by 1% $Na_2B_4O_7$ electrophoresis[26]. [c]Biosynthetic compound obtained by a purified FucT-2-catalyzed reaction[32–34]. [d]Assayed by the L-fucose dehydrogenase method as described in the Experimental section. [e]Nmol of L-fucose released/mg of glycoprotein (assayed by L-fucose dehydrogenase).

reaction rate. The purified α-L-fucosidase was incubated with various concentrations of 4-methylumbelliferyl α-L-fucopyranoside (Fig. 8) in 70mM citrate buffer (pH 4.3) in a final volume of 0.14 mL. The samples were incubated for 15 min at 37° and the reaction was stopped with 2 mL of 0.2M sodium carbonate. Fluorescence was measured with a Perkin–Elmer fluorometer using an absorption wavelength of 360 nm and an emission wavelength of 450 nm. The apparent K_m determined for the 4-methylumbelliferyl glycoside was 1.3mM, almost five-fold higher than the K_m for the *p*-nitrophenyl glycoside.

(*F*) *Glycoconjugate-substrate specificity.* The aglycon specificity of the α-L-fucosidase preparation purified from cherrystone clams was examined by use of naturally occurring glycoconjugates having (1→2)-, (1→3)-, and (1→4)-α-L-fucopyranosyl linkages (Table II).

Each [^{14}C]fucose-containing glycosphingolipids (0.3–1.0 nmol) was incubated with taurodeoxycholate (0.05 mg), bovine serum albumin, (0.05 mg), NaCPA buffer (pH 4.5) (0.01 mmol), and α-L-fucosidase enzyme fraction (2–4 μg of protein) in a final total volume of 50 μL. After 48 h at 37°, the mixture was analyzed by high-voltage borate electrophoresis[26]. The radioactivity was quantitatively determined by liquid-scintillation techniques.

When L-fucose-containing glycopeptides (40 μg) or oligosaccharides were used as substrates, detergent and bovine serum albumin were not included in the incubation mixtures. The amount of L-fucose liberated was determined by the L-fucose dehydrogenase method.

The extent of NAD^+ reduction (L-fucose hydrolyzed) for some of the glycoconjugates tested is shown in Figs. 9 and 10, and the results are summarized in

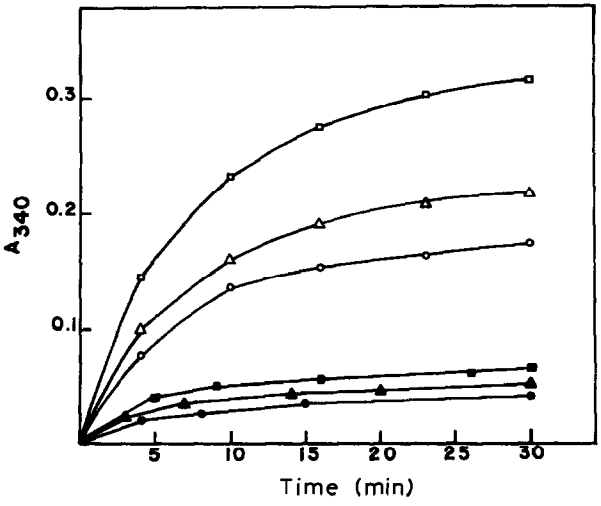

Fig. 9. Extent of NAD^+ reduction by oligosaccharides after incubation with α-L-fucosidase. Incubation conditions were the same as described in the text. Lacto-*N*-fucopentaose I: (●—●) 0.09, (▲—▲) 0.17, and (■—■) 0.27 mg; lacto-*N*-fucopentaose II: (○—○) 0.09, (△—△) 0.15, and (□—□) 0.30 mg.

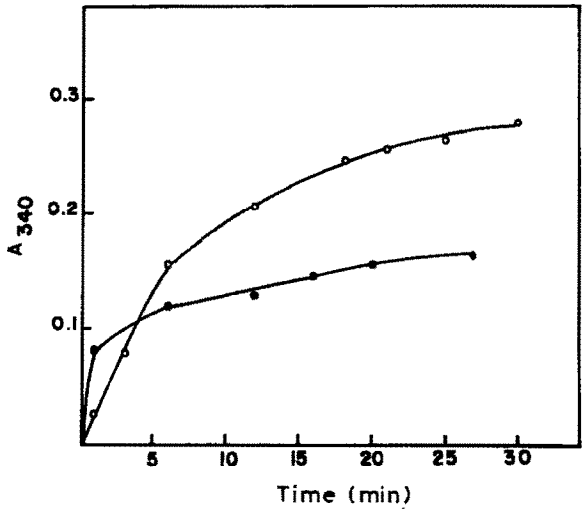

Fig. 10. Extent of NAD^+ reduction by glycopeptides after incubation with α-L-fucosidase. See the text for incubation conditions: (●—●) bovine thyroglobulin glycopeptides (2.14 mg), (○—○) human salivary glycoproteins (H-type; 2.24 mg).

Table II. The relative rate of hydrolysis was greater for substrates having a (1→2)- or (1→3)-α-L-fucopyranosyl linkages than for substrates having a (1→4)-α-L-fucopyranosyl linkage.

DISCUSSION

Differences in the specificity of mammalian and nonmammalian α-L-fucosidase (Table III) have been observed, but until recently a good source of

TABLE III

SUBSTRATE SPECIFICITY OF SPECIFIC L-FUCOSIDASES

Source	*Substrate*		*α-L-Linkage cleaved*
	Synthetic[a]	*Natural*[b]	
Microorganisms			
Clostridium perfringens/*Aspergillus niger*	−	+	(1→2)
Trichomonas foetus	−	+	(1→2), (1→3), and (1→4)
Molluscs			
Turbo cornutus, Charonia lampas, and Venus mercenaria	+	+	(1→2), (1→3), and (1→4)
Animal tissues			
Epididymis, kidney, and liver	+	+	(1→2)

[a]*p*-Nitrophenyl α-L-fucopyranoside. [b]Glycolipids, glycoproteins, or oligosaccharides.

(1→3)-bond-cleaving α-L-fucosidase was not available. α-L-Fucosidase purified (300-fold) from cherrystone clams by affinity chromatography on agarose–*N*-(ε-caproyl)-α-L-fucopyranosylamine has an extremely broad aglycon specificity, characteristic of α-L-fucosidases isolated from murine gastropods. The enzyme hydrolyzes a synthetic substrate (*p*-nitrophenyl α-L-fucopyranoside) and acts on (1→2)-, (1→3)-, and (1→4)-α-L-fucopyranosyl linkages in oligosaccharides, glycolipids[27–31], and complex glycoproteins (Table II). The K_m value of 0.26mM for the *p*-nitrophenyl α-L-fucopyranoside substrate is similar to the values reported for other mollusc fucosidases[32–34]. The enzyme has a broad pH optimum range between 4.5 and 5.5.

Isoelectric focusing in polyacrylamide gels revealed the presence of several isoenzymes. As many as ten α-L-fucosidase components have previously been isolated from tissues and body fluids by analytical starch-gel electrophoresis or isoelectric focusing[6,11–21]. The structural relationships of the different forms remain unclear, although several investigators have suggested that the isoenzymes differ in the proportion of sialic acid residues.

Treatment of the purified α-L-fucosidase with 1% sodium dodecyl sulfate and 5% 2-mercaptoethanol, followed by electrophoresis, indicated the presence of one major subunit having M_r 50 000. The enzyme is a glycoprotein, as evidenced by its positive periodic acid–Schiff stain. Recently, a human liver α-L-fucosidase gene has been cloned[35] and this enzyme has a subunit M_r of 50 000 also.

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