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SPECIFICITY PATTERNS OF DIFFERENT TYPES OF HUMAN FUCOSIDASE

RECOGNITION OF A CERTAIN REGION OF THE PYRANOSE RING IN SUGARS BY THE ENZYMES

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Summary

Data on the hydrolysis of fucosides, galactosides and arabinosides by different types of fucosidase are presented. The comparative study of the splitting of α-L-fucoside and β-D-arabinoside with a similar structure at C_1—C_4 of the pyranose ring and the preparation of the enzyme by affinity chromatography showed that both substrates were hydrolysed by the same enzyme, α-L-fucoside(β-D-arabinoside)hydrolase. The analogous investigation of the hydrolysis of β-D-fucoside, β-D-galactoside and α-L-arabinoside with a similar structure at C_1—C_4 of the pyranose ring demonstrated that these glycosides were split by the same enzyme, β-D-fucoside(β-D-galactoside, α-L-arabinoside)hydrolase, the activity of which is decreased dramatically in G_{M1}-gangliosidosis. The data obtained support the assumption that the specific action of different types of fucosidase is due to recognition by these enzymes of C_1—C_4 of the pyranose ring in the corresponding substrates. The problems of differential diagnosis of some glycosidoses (fucosidosis, G_{M1}-gangliosidosis and Fabry disease) are discussed on the basis of the data obtained.

Introduction

As was noted in our previous communications [1,2], fucosidases may be classified, according to the fucosyl residue split (L- or D-) and the glycosidic

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linkage (α- or β-) attacked, into four types: α-L-, β-L-, α-D- and β-D-fucosidases.

α-L-Fucosidase is widely distributed in human and mammalian organisms. The literature contains some information concerning properties, substrate specificity and molecular forms of this enzyme [3—9]. Recently the application of affinity chromatography made it possible to obtain homogeneous preparations of α-L-fucosidase from human liver [6,10], kidney [11], serum [12], brain [13], etc. It was found that the deficiency of α-L-fucosidase or the absence of some molecular forms of this enzyme led to the development of the severe neurovisceral storage disease, fucosidosis [14].

β-D-Fucosidase and α-D-fucosidase activities found by us previously in pig and human kidney appeared to be due to β-D-galactoside(fucoside)hydrolase and α-D-galactoside(fucoside)hydrolase, respectively [1,2].

The β-L-fucosidase activity in mammalian organisms was reported by Bosmann et al. [15—17].

Each type of α-D- and β-D-galactoside(fucoside)hydrolase requires a specific configuration of glycosidic linkage (α- or β-) and a specific position of OH groups at C_2—C_4 of the pyranose ring [2]. According to our hypothesis on the mammalian fucosidase specificity, these enzymes should hydrolyse not only the corresponding fucosides and galactosides, but also arabinosides (Fig. 1). It should be noted that it was previously stated that hydrolysis of β-D-fucoside, β-D-galactoside and α-L-arabinoside could be catalyzed either by the same enzyme or by different ones depending on their source [18]. For instance, in emulsin, the enzyme that hydrolyses β-glucosides, β-galactosides and β-D-fucosides also hydrolyses α-L-arabinosides. Rat epididymis resembles emulsin

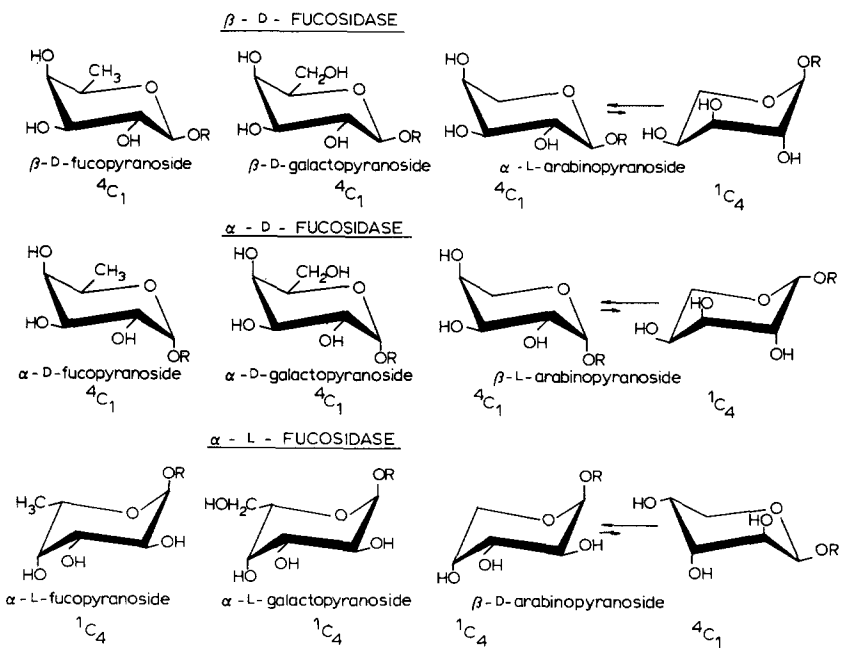

Fig. 1. The established and putative substrates for the different types of fucosidase.

except in that it lacks β-glucosidase activity. α-L-Arabinosidase activity of limpet is due to the enzyme which is capable of splitting both β-fucoside and β-glucoside and which is not capable of splitting β-galactoside. In barley preparations α-L-arabinosidase activity is more likely to be associated with β-galactosidase than with the enzyme that hydrolyses β-glycosides and β-D-fucosides.

The present paper reports further studies into the substrate specificity of different types of human fucosidase using corresponding arabinopyranosides. A preliminary report of these results has recently appeared [19].

Materials and Methods

Substrate and inhibitors. *p*-Nitrophenyl-α-L-fucoside and *p*-nitrophenyl-β-L-fucopyranoside were obtained from Serva. *p*-Nitrophenyl-α-D-fucoside and *p*-nitrophenyl-α-D-galactoside were prepared by the methods of Levvy and McAllan [20] and Westphal and Feier [21].

p-Nitrophenyl-α- and β-glycosides of L- and D-arabinose were obtained by the method of Westphal and Feier [21]. The Helferich reaction of tetraacetic derivatives of corresponding sugars was carried out in the presence of either $ZnCl_2$ or HgCN. The following compounds were obtained: *p*-nitrophenyl-2,3,4-triacetyl-β-L-arabinopyranoside (I) has m.p. 161°C, $[\alpha]_D^{20}$ + 238° (*c* = 1, CCl_3); *p*-nitrophenyl-2,3,4-triacetyl-α-L-arabinopyranoside had m.p. 105°C, $[\alpha]_D^{20}$—61° (*c* = 1, CCl_3); *p*-nitrophenyl-2,3,4-triacetyl-β-D-arabinopyranoside (II) had m.p. 155°C, $[\alpha]_D^{20}$—230° (*c* = 1, CCl_3); *p*-nitrophenyl-β-L-arabinopyranoside (III) had m.p. 182—184°C, $[\alpha]_D^{20}$ + 260° (*c* = 1, MeOH); *p*-nitrophenyl-β-D-arabinopyranoside (IV) had m.p. 184—186°C, $[\alpha]_D^{20}$—36° (*c* = 1, MeOH); *p*-nitrophenyl-α-L-arabinopyranoside had m.p. 204—206°C, $[\alpha]_D^{20}$—26.5° (*c* = 1, MeOH). *p*-Nitrophenyl-β-D-galactopyranoside (Chemapol, Czechoslovakia), 4-methylumbelliferyl-β-D-fucopyranoside (Koch-Light, U.K.), L-fucose (B.D.H. Laboratory Chemical Division, U.K.); L- and D-arabinose were obtained from the Institute of Chemistry, Slovak Academy of Sciences (Czechoslovakia).

Preparation of enzyme. The partially purified enzyme preparation with some glycosidase activity was obtained from human kidney (autopsy material) by the method described in Ref. 3: a 60% ammonium sulfate fraction was heated at 55°C (15 min) and desalted by means of Sephadex G-25 before isoelectric focusing (see below).

Enzyme assay. Enzyme activity was assayed by measuring the quantity of *p*-nitrophenol or 4-methylumbelliferone liberated from corresponding substrates. The reaction mixture (final volume 0.25 ml) contained 0.15 ml of the enzyme solution and 0.1 ml of the corresponding substrate (final concentration, 1 mM) in 0.05 M acetate buffer (pH 5.0) with 1 mM EDTA. The periods of incubation (temperature, 37°C) for various enzyme assays were as follows: 10 min for α-L-fucosidase, 2.5 h for β-D-galactosidase, 4.5 h for β-D-fucosidase, 13 h for β-D-arabinosidase and 144 h for α-L-arabinosidase. The released *p*-nitrophenol or 4-methylumbelliferone was determined after the addition of 0.4 M glycine/NaOH buffer (pH 10.5).

One unit of the glycosidase activity is defined as the amount of the enzyme which hydrolyses 1 nmol of the corresponding substrate per h.

The specific glycosidase activity is expressed as the number of units per

milligram of protein. Protein was determined by the method of Lowry et al. [22] with bovine serum albumin as the standard.

Isoelectric focusing. Isoelectric focusing was performed using a 110 ml column of LKB 8100 electrofocusing equipment with a pH range from 4 to 6 as described earlier [2]. Isoelectric focusing was performed on partially purified enzyme preparation (about 180 000 units of α-L-fucosidase activity).

The diluted enzyme solutions were concentrated by pressure dialysis using an Amicon ultrafiltration cell (Amicon Corp., Cambridge, MA). Diaflo XM-50 and PM-30 membranes were used, under a nitrogen pressure of 50 lb/inch^2.

Preparation of highly purified α-L-fucosidase. The enzyme was purified 2600-fold by an affinity chromatographic procedure utilizing Sepharose *N*-(ε-aminocaproyl)-β-L-fucosylamine as described in Ref. 11. A portion of the partially purified enzyme preparation (360 units/ml of the gel) was applied to a column (0.6 × 11 cm) of affinity adsorbent equilibrated in 10 mM NaH_2PO_4 (pH 5.5) and containing 0.02% NaN_3 (w/v). Then the column was washed with the same buffer until the absorbance at 280 nm was near to zero and eluted with the same buffer containing 1% L-fucose. The fractions with α-L-fucosidase activity were combined and concentrated using Diaflo PM-30 membranes.

Polyacrylamide gel electrophoresis. Electrophoresis of highly purified α-L-fucosidase preparations was performed in 7.5% polyacrylamide gel at pH 8.9 and 2 mA/tube for 20 min and 4 mA/tube subsequently according to the method of Davis [23]. Fucosidase activity was located by incubating the gels for 20 min at 37°C in 1 mM 4-methylumbelliferyl-α-L-fucoside in 0.05 M sodium acetate buffer, pH 5.0, and by the monitoring under ultraviolet light of the fluorescence of liberated aglycon after treatment of the gels with 0.4 M glycine/NaOH buffer, pH 10.4. Subsequently the gels were washed with water and stained for protein with 0.1% Coomassie brilliant blue in 30% trichloroacetic acid.

PMR-spectroscopy. PMR-spectra of compounds I and II were recorded on BS-497 'Tesla' apparatus. C^2HCl_3 and tetramethylsilane were used as the solvent and the internal standard, respectively.

Results

As was shown in our experiments, all of the three arabinopyranosides synthesized were split by the partially purified enzyme preparation from human kidney. However, β-D-, β-L- and α-L-arabinosidase activities were considerably lower than the corresponding fucosidase and galactosidase activities. Comparative activities of these glycosidases are presented in Table I.

As the original enzyme preparation contained all three arabinosidase activities, it was of interest to clarify the relationship between arabinosidases, fucosidases and galactosidases and to compare the properties of these enzymes.

We used isoelectric focusing as a method of revealing the relationship of arabinosidases to the corresponding fucosidases and galactosidases. The elution profiles of the corresponding glycosidases obtained after isoelectric focusing are presented in Fig. 2.

Fig. 2A shows the complete identity of elution profiles obtained with α-L-

TABLE I

SPECIFIC ACTIVITIES OF FUCOSIDASES, GALACTOSIDASES AND ARABINOSIDASES IN PARTIALLY PURIFIED ENZYME PREPARATIONS FROM HUMAN KIDNEY

Enzyme	Spec. Act. (units)
α-L-Fucosidase	1215.0
α-D-Fucosidase	3.8
β-D-Fucosidase	16.0
α-D-Galactosidase	32.0
β-D-Galactosidase	104.0
β-D-Arabinosidase	37.5
β-L-Arabinosidase	0.016
α-L-Arabinosidase	0.86

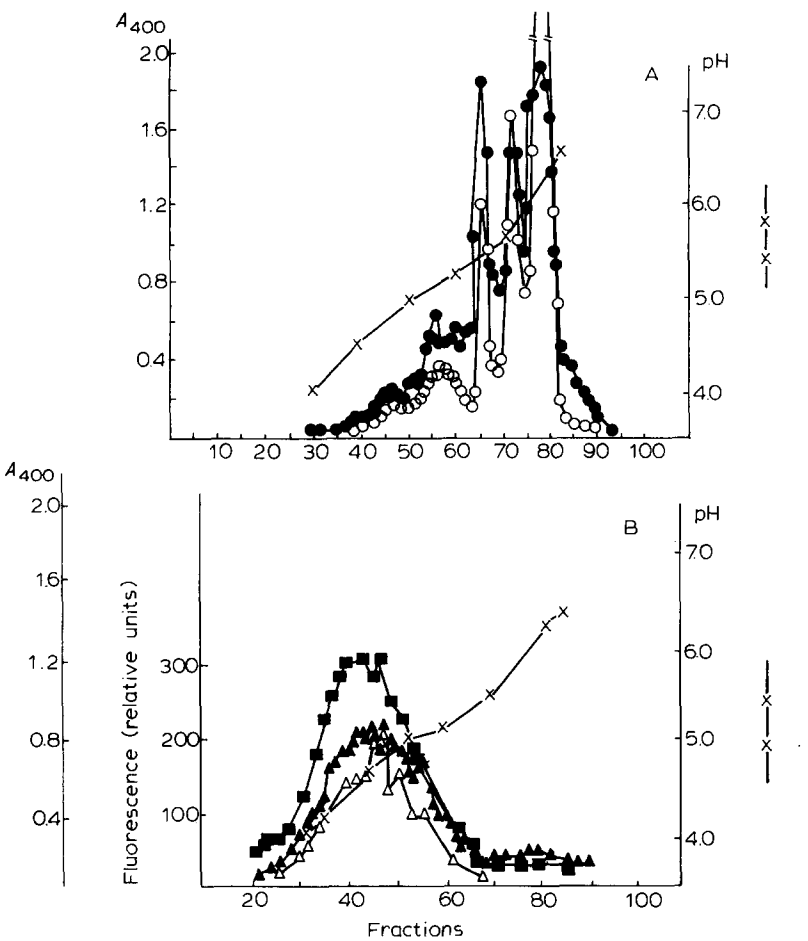

Fig. 2. Isoelectric focusing of (A) α-L-fucosidase (●), β-D-arabinosidase (○) and (B) β-D-fucosidase (■), β-D-galactosidase (▲) and α-L-arabinosidase (△). Procedures for isoelectric focusing and assaying glycosidase activities are described in the text.

fucoside and β-D-arabinoside as substrates. Isoelectric focusing of partially purified enzyme preparations revealed the similarity of elution profiles of β-D-fucosidase, β-D-galactosidase and α-L-arabinosidase (Fig. 2B).

A comparative study of the properties of α-L-fucosidase and β-D-arabinosidase

The effect of pH on the hydrolysis of α-L-fucoside and β-D-arabinoside by the partially purified enzyme preparations is shown in Fig. 3A. Both glycosidases exhibited maximal activities at pH 4.5.

The effect of heat treatment on α-L-fucosidase and β-D-arabinosidase is shown in Fig. 4A. It is evident that the changes in the two activities are absolutely identical. As shown in Fig. 4A, heating of the enzyme preparation at different temperatures for 10 min resulted in a decrease of both activities by only 10—15%.

The evidence for the identity of α-L-fucosidase and β-D-arabinosidase was obtained in the experiments on affinity chromatography of the initial enzyme preparation.

As shown in Fig. 5, the use of D-arabinose in the elution buffer instead of L-fucose led to the elution of α-L-fucosidase as well. However, elution with D-arabinose under the same conditions as in the case of L-fucose resulted in a very poor yield of α-L-fucosidase. The major part of α-L-fucosidase remained bound with the adsorbent and was eluted with 60 mM L-fucose. At the same time the increase in D-arabinose concentration up to 700 mM caused a complete elution of α-L-fucosidase, as was indicated by the absence of the peak of

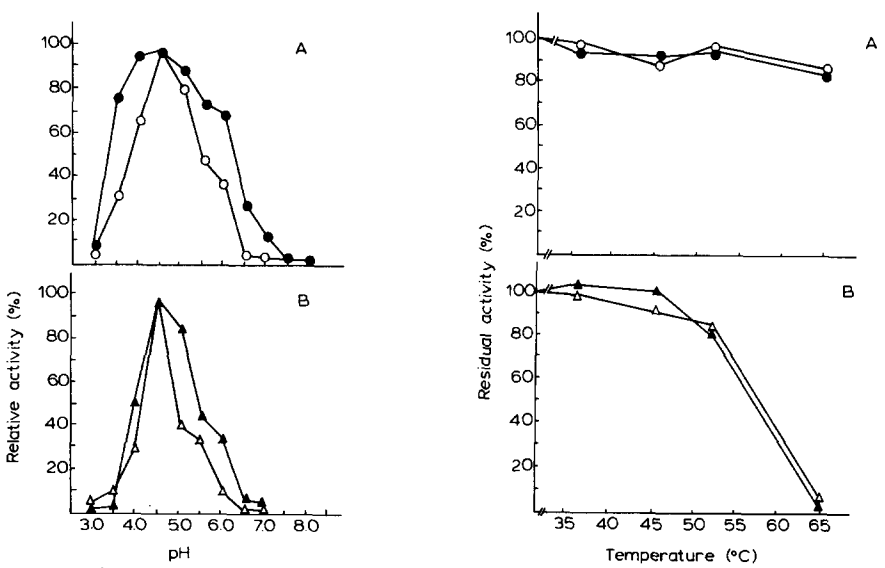

Fig. 3. Effect of pH on (A) α-L-fucosidase (●) and β-D-arabinosidase (○) and (B) β-D-galactosidase (▲) and α-L-arabinosidase (△). Samples were adjusted to the required pH at 0°C with 0.1—0.2 M citrate/phosphate buffer. Activities were expressed in percentages of the value determined at pH 4.5.

Fig. 4. Effect of heating on (A) α-L-fucosidase (●) and β-D-arabinosidase (○) and (B) β-D-galactosidase (▲) and α-L-arabinosidase (△) at pH 4.5. Results are expressed as percentages of residual activity as compared with the unheated controls.

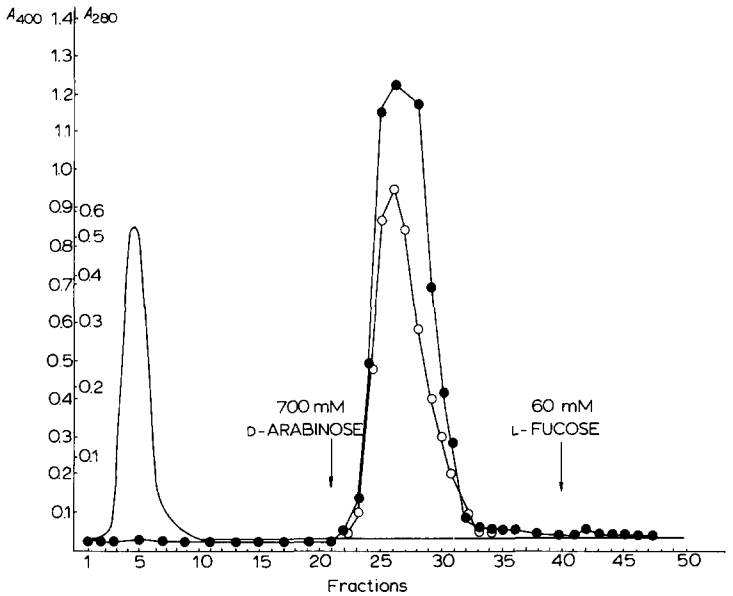

Fig. 5. Affinity chromatography of α-L-fucosidase on Sepharose-*N*-(ε-aminocaproyl)-β-L-fucosylamine. The column was eluted as described in Materials and Methods; α-L-fucosidase (●), β-D-arabinosidase (○); absorbance at 280 nm (———); arrows indicate when the corresponding inhibitors were added to the eluting buffer.

α-L-fucosidase activity during the elution with L-fucose. It will be noted that fractions obtained after the elution with D-arabinose and L-fucose split both substrates, α-L-fucoside and β-D-arabinoside (Fig. 5). The data on electrophoresis of these combined fractions indicated that the activities of protein bands found in gels corresponded to α-L-fucosidase.

The K_m values calculated for *p*-nitrophenyl-α-L-fucoside and *p*-nitrophenyl-β-D-arabinoside with the use of partially purified enzyme preparations were $(0.4—0.5) \cdot 10^{-3}$ M and $(13—15) \cdot 10^{-3}$ M, respectively.

A comparative study of the properties of β-D-galactosidase and α-L-arabinosidase

As shown in Fig. 3B, the maximal splitting of β-D-galactoside and α-L-arabinoside was observed at pH 4.5. At other pH values the pH vs. activity curves of β-D-galactosidase and α-L-arabinosidase were quite similar.

The data on thermostability of β-D-galactosidase and α-L-arabinosidase are presented in Fig. 4B. Both activities were changed to the same extent during heat treatment for 10 min at different temperatures and pH 4.5.

Heating of the enzyme preparation at 50° C resulted in a decrease of both the activities (by approx. 20%).

The data on α-L-arabinosidase activity in leucocyte extracts from a child with G_{M1}-gangliosidosis and his healthy parents are presented in Table II. A marked deficiency in both β-D-galactosidase and α-L-arabinosidase activities was observed in leucocytes of the child. The activity of β-L-arabinosidase was extremely low, causing difficulties in the comparative study of the properties

TABLE II

ACTIVITIES OF β-D-GALACTOSIDASE AND α-L-ARABINOSIDASE IN LEUKOCYTES OF CHILD Z WITH G_{M1}-GANGLIOSIDOSIS AND IN LEUCOCYTES OF HIS PARENTS (nmol OF *P*-NITROPHENOL/mg PROTEIN/h)

Examined	Activity			
	β-D-Galactosidase		α-L-Arabinosidase	
	units	%	units	%
Child Z.	15.75	8.6	0.0675	3.6
Mother Z.	45.25	25.0	0.325	18.0
Father Z.	120.0	65.0	0.725	40.0
Control	182.7	100	1.83	100
Range	100—320	—	0.57—4.32	—

of β-L-arabinosidase and the corresponding α-D-fucosidase and α-D-galactosidase. β-L-Fucosidase activity was not found in our experiments.

Discussion

The results obtained show that the enzyme preparation from human kidney possesses α-L-, β-D-, α-D-fucosidase activities, α-D-, β-D-galactosidase activities, as well as β-D-, β-L- and α-L-arabinosidase activities.

It was the purpose of our studies to ascertain whether these activities were due to independent enzymes or to the ability of certain fucosidases to hydrolyse the corresponding substrates with a similar structure at the C_1—C_4 region of the sugar pyranose ring. A study was undertaken with α-L-fucosidase, which besides α-L-fucoside and α-L-galactoside apparently uses β-D-arabinoside as a substrate (Fig. 1). As α-L-galactoside was not available, a comparative study was performed with α-L-fucoside and β-D-arabinoside as substrates. It was shown that splitting of both substrates occurs at similar pH and that heating of the enzyme preparation produces similar effects on both activities, which are manifested in practically homogeneous enzyme preparation, obtained by means of affinity chromatography using both L-fucose and D-arabinose solutions as eluents. The data obtained indicate that both substrates are hydrolysed by the same enzyme, α-L-fucoside(β-D-arabinoside) hydrolase.

The study of the splitting of β-D-fucoside, as compared with the splitting of β-D-galactoside and α-L-arabinoside, both of which are characterized by having the same position of OH group at C_1—C_4 as does β-D-fucoside, demonstrated that these glycosides were hydrolysed by the same type of fucosidase: β-D-fucoside(β-D-galactoside, α-L-arabinoside)hydrolase. This conclusion was confirmed by studies of α-L-arabinosidase activity in G_{M1}-gangliosidosis. This disease is characterized by a dramatic decrease in total β-D-galactosidase and β-D-fucosidase activities [24], which, as we have shown, are due to the same enzyme [1]. The decrease in the α-L-arabinosidase activity observed in our experiments supports the assumption that these three substrates are split by

the type of fucosidase indicated above.

β-L-Fucosidase activity was not found in our experiments and the activity of α-D-fucosidase was so low with β-L-arabinoside as substrate as to preclude comparative studies of pH-dependence, thermostability and isoelectric focusing profiles of β-L-arabinosidase and α-D-fucosidase and α-D-galactosidase.

However, the present data on the hydrolysis of β-L-arabinoside by the initial enzyme preparations, together with the previously reported data on α-D-fucoside(galactoside)hydrolase [2], provide indirect evidence that the splitting of β-L-arabinoside, α-D-fucoside and α-D-galactoside is catalyzed by the same enzyme.

Analysis of the data obtained shows that different types of fucosidase recognize the corresponding substrates by the structure of the C_1—C_4 regions of their glycons; the C_1—C_4 region is likely to be a site of hydrolytic action of these enzymes.

However, the data on specific activity of different fucosidases (Table I), as well as a comparison of the K_m values for α-L-fucoside (K_m = (0.4—0.5) · 10^{-3} M) and β-D-arabinoside (K_m = (13—15) · 10^{-3} M) hydrolysed by α-L-fucoside(β-D-arabinoside)hydrolase, show that the four types differ considerably in the nature of their substrates. These differences may be due to: (1) possible conformational peculiarities of the substrates, (2) the different nature of the substitutions at C_5 of pyranose rings in these substrates. For the verification of the first suggestion we performed PMR-spectroscopy of compounds I and II, the results of which we presented in Table III.

The similarity of key constants of the spin/spin interaction (CSSI), found by us for compounds I and II and given in [25] for tetraacetates of D-arabinopyranose, suggested that the ratio of conformations $^4C_1$: $^1C_4$ is 0.03 for β-D-arabinoside [25], when *p*-nitrophenol is the aglycon. So, according to the data obtained on PMR spectra, compounds I and II have preferential conformation $^4C_1$ and $^1C_4$, respectively.

In aqueous medium, as reported previously [25,26], the equilibrium is probably slightly shifted towards the increase in $^1C_4$ conformation for compound III and $^4C_1$ conformation for compound IV.

The difference between CSSI $^3J_{H4-H5}$ for arabinopyranosides in organic solvent (1.2 Hz) and for arabinopyranoses in ^2H_2O (3.4 Hz) is compelling evidence for this. However, a predominance of conformations $^4C_1$ for compound III and $^1C_4$ for compound IV remains essential in this case.

As for fuco- and galactopyranosides, it is generally accepted [27] that derivatives of D-galactopyranose and D-fucopyranose exist in $^4C_1$ conformation

TABLE III

ANALYSIS OF PMR-SPECTRA OF COMPOUNDS I AND II

5.88 m.d., H1, d., $J_{1,2}$ = 3.1 Hz, 1H; 5.56 m.d., H2, d.d., $J_{2,3}$ = 10.4 Hz, 1H; 5.35 md., H3, d.d., $J_{3,4}$ = 3.9 Hz, 1H; 5.41 m.d., H4 m., 1H; 3.79 m.d. and 3.9 m.d., H5a,c, AB-part of ABX-spectrum, $J_{4,5e}$ = 1.2 Hz; $J_{4,5a}$ = 1.8 Hz, $J_{5e,5a}$ = 13.0 Hz, 2H; 2.05 m.d.; 2.17 m.d., CH_3CO, s., 9H; 7.15 m.d. and 8.21 m.d., Ph, AA'BB'-spectrum, 4H.

independently of aglycon type, different type of substitution at OH groups and solvent.

Thus, the conformational analysis performed by us, as well as the available literature data, demonstrate conformational similarities of fuco-, galacto- and arabinopyranosides used as substrate by the corresponding types of fucosidase (Fig. 1).

The data obtained suggest that the variation observed in the hydrolysis of substrates by fucosidases is not due to the difference in substrate conformation, but rather to the nature of substitutions at C_5 (CH_2OH, CH_3 and H_2) of the pyranose sugar rings of substrates.

It is noteworthy that the substrate specificity of mammalian fucosidases differs from that of bacteria fucosidases, which are capable of splitting α-D-galactoside and α-D-fucoside but do not split β-L-arabinopyranoside [28].

The patterns of substrate specificity of mammalian fucosidases are also typical for other classes of enzymes participating in fucose metabolism, particularly fucose(arabinose)dehydrogenase from pig and rabbit liver, which uses D-arabinose and L-fucose as substrates [29,30].

The data on the specificity of different types of fucosidase may be helpful in the differential diagnosis of some glycosidoses [31]. According to our observations, the marked deficiency of α-L-fucosidase activity in fucosidosis must be accompanied by a dramatic decrease in β-D-arabinosidase activity. In the case of G_{M1}-gangliosidosis, as has been shown, the β-D-fucosidase and α-L-arabinosidase activities are decreased in addition to a decrease in β-D-galactosidase activity. Furthermore, in Fabry disease one may expect not only the decrease in α-D-galactosidase activity, but deficiency in α-D-fucosidase and β-L-arabinosidase activities.

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