TWO FORMS OF α-L-FUCOSIDASE FROM PIG KIDNEY AND THEIR ACTION ON NATURAL OLIGOSACCHARIDES

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SUMMARY

Two forms of α-L-fucosidase from pig kidney, designated by us as α-L-fucosidase I and α-L-fucosidase II, were separated by means of gel filtration on Sephadex G-200. Both forms differed in thermostability, dependence of activity on pH, stability on storage at various pH and action on natural oligosaccharides with different structure.

In our previous communications (1,2) we reported the purification and some properties of α-L-fucosidase from pig kidney. The detailed specificity studies of α-L-fucosidase using various synthetic substrates indicated that the enzyme was highly specific; it hydrolyzed only those glycosidic linkages which involved L-fucose nonreducing residue. It was also shown that α-L-fucosidase did not release fucose from blood group (A+H) substance and glycoprotein of Tamm and Horsfall. More recently we have shown that fucose-containing oligosaccharides and glycopeptides fragments, obtained by alkaline degradation of native (A+H) blood group substance, were also substrates for α-L-fucosidase (2,3). However, the nature of linkages attacked by α-L-fucosidase, as well as the possible influence of the sugar residue, adjacent to the fucosyl unit, was not clarified in these experiments.

In the present paper we report separation of two forms of α-L-fucosidase and describe their action on various natural oligosaccharides.

MATERIALS AND METHODS

Substrates. p-Nitrophenyl-α-L-fucoside (1) was used as synthetic substrate. The oligosaccharides: 2'-fucosyllactose, lactodifucotetraose, lacto-N-fucopentaose I, lacto-N-fucopentaose II and lacto-N-difucohexaose I were kindly given by Dr. A.Gauhe. The preparation of lacto-N-fucopentaose III was kindly given by Dr. V.Ginsburg.

Preparation of the enzyme. Partially purified preparation of α-L-fucosidase was obtained from pig kidney as described earlier (1) with slight modifications: the fraction with the highest activity was obtained between 0.45 and 0.52 $(NH_4)_2SO_4$ saturation, heated at 55° (15 min), desalted by means of Sephadex G-25, freeze-dried and used for gel filtration on Sephadex G-200.

Enzyme assay. The activity of the enzyme was assayed by measuring the quantity of nitrophenol liberated from p-nitrophenyl-α-L-fucoside; 0.4 M glycine-NaOH buffer (pH 10.5) was added to the samples.

One unit of the enzyme was defined as the amount which would increase the optical density by 0.01 per min. at 400 nm under standard conditions.

In experiments with oligosaccharides as substrates the reaction mixtures (final volume 0.4 ml) contained 0.3 ml of the enzyme (60 and 40 units for α-L-fucosidase I and II, respectively) and 0.1 ml of 0.05 M sodium acetate buffer (pH 5.0), containing from 166 to 332 mg of the oligosaccharide. The time of incubation at 37° was 7-12 hours. The activities of α-L-fucosidase I and α-L-fucosidase II were assayed by measuring the quantity of the liberated fucose by the method of Bhattacharyya and Aminoff (4), slightly modified (5).

The content of fucose in oligosaccharides was determined by the method of Dische and Shettles (6).

RESULTS AND DISCUSSION

Fractionation of α-L-fucosidase preparation on a column with Sephadex G-200 gave two distinct peaks (Fig.1), which were designated as α-L-fucosidase I and α-L-fucosidase II. The activity of α-L-fucosidase I, which was eluted with a smaller volume of the buffer and presumably had higher molecular weight exceeding that of α-L-fucosidase II about two-fold.

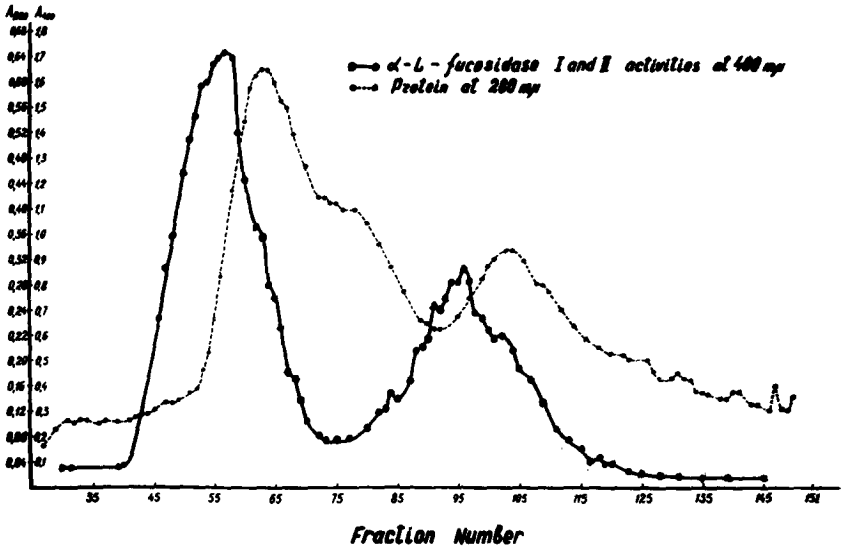

Fig.1. Elution profile of two forms of α-L-fucosidases from Sephadex G-200 column. 2.5 ml enzyme solution (200 mg of freeze-dried preparation) was placed on the first of the 3 consecutively - linked columnes (each 1.8x100 cm) of Sephadex G-200. A solution of 0.05 M acetic buffer (pH 5.0), containing 1 mM EDTA was passed through the columns; 2.4 ml fractions were collected. Flow rate was 7 ml/hr, operating pressure - 11 cm. Fractions were assayed for α-L-fucosidase activity with p-nitrophenyl-α-L-fucoside as substrate (final concentration 1 mM). The reaction mixture contained: 0.15 ml of the fractions and 0.1 ml of the substrate; time of incubation (37°) - 7 min, after which 0.25 ml of 0.4 M glycine-NaOH buffer (pH 10.5) were added and the optical density was read in 1 cm cells at 400 nm.

Comparative study of the properties of both fucosidases was undertaken. For that purpose fractions NN 35-55 of the first peak and fractions NN 95-120 of the second peak were pooled and concentrated with the aid of Ficoll.

Effect of pH on activity and stability of α-L-fucosidase I and II. Both fucosidases had the pH optimum of 5.2. Residual activity of fucosidase I at pH 3.5, 4.0 and 4.5 was 38, 65 and 85%, respectively, of that displayed by the enzyme at pH optimum. At the same pH values, the residual activity for fucosidase II was only 23, 40 and 65% (Fig.2). Fucosidase I was more stable on storage in the pH region 4.5-5.5 (residual acti-

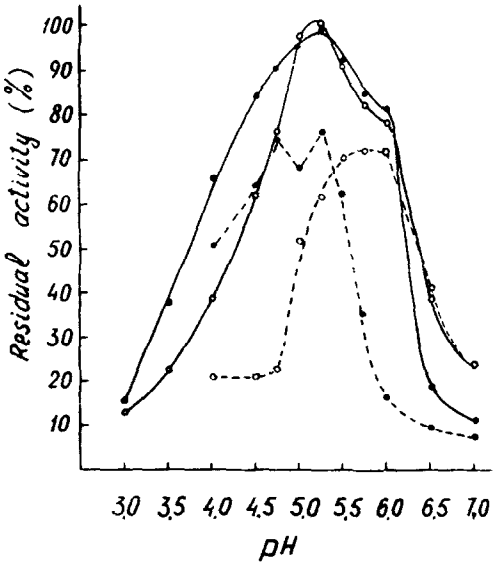

Fig.2. Effect of pH on activity (——) and stability (----) of α-L-fucosidase I (●) and α-L-fucosidase II (○). The enzymatic activity was assayed with p-nitrophenyl-α--L-fucoside (1 mM) as substrate in 0.1-0.2 M citrate--phosphate buffers of pH 3 to 7 at 37°. Samples were kept at various pH values at +2-4° for 5 days. Activity of samples of both fucosidases, kept at pH-optimum (5.2) at 2-4° for 5 days, were used as controls. The composition of incubation mixtures was the same as in Fig.1, except that different amounts of enzymes (α-L-fucosidese I - 4 units, α-L-fucosidase II - 0.75 units) were added. The mixtures were incubated at 37° for 15 min (α-L-fucosidase I) or 60 min (α-L-fucosidase II).

vity of fucosidase I under these conditions was 65%). Fucosidase II was more stable on storage in the pH region 5.5-6.0 and lost 80% of its activity at pH 4.0-4.7 (Fig.2).

Thermostability of α-L-fucosidases I and II. Controlled heating of the fucosidase I preparation had no effect on its activity. Under the same conditions the initial activity of fucosidase II was reduced by 35-55% (Fig.3).

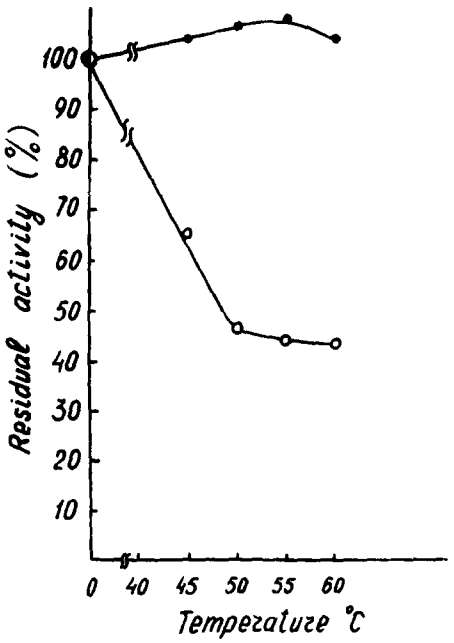

Fig.3. Effect of controlled heating on α-L-fucosidase I (•) and α-L-fucosidase II (o). Samples were placed in a bath preheated to the specified temperature. After 10 min the tubes were placed in ice and residual activity was assayed as in Fig.1, except that different amount of enzymes (α-L-fucosidase I - 3.6 units, α-L-fucosidase II - 1.7 units) were added. The mixtures were incubated at 37° for 15 min (α-L-fucosidase I) or 30 min (α-L-fucosidase II).

Hydrolysis by fucosidases I and II of various fucose-containing oligosaccharides. Both fucosidase preparations hydrolysed to completion p-nitrophenyl- α-L-fucoside and more or less effectively splitted off fucose from 2-fucosyllactose,

Table 1. SUBSTRATE SPECIFICITY OF α-L-FUCOSIDASE I AND II.

Substrate	Structure	Liberation of fucose (%)	
		α-L-Fucosidase I	α-L-Fucosidase II
1. p-Nitrophenyl-α-L-fucopyranoside		100	100
2. 2'-Fucosyllactose	Gal(β1→4)Gluc; Gal(α1→2)Fuc	65	73
3. Lactodifucotetraose	Gal(β1→4)Gluc; Gal(α1→2)Fuc; Gluc(α1→3)Fuc	10	5
4. Lacto-N-fucopentaose I	Gal(β1→3)GNAc(β1→3)Gal(β1→4)Gluc; Gal(α1→2)Fuc	0	0
5. Lacto-N-fucopentaose II	Gal(β1→3)GNAc(β1→3)Gal(β1→4)Gluc; GNAc(α1→4)Fuc	33	14
6. Lacto-N-fucopentaose III	Gal(β1→4)GNAc(β1→3)Gal(β1→4)Gluc; GNAc(α1→3)Fuc	36	0
7. Lacto-N-difucohexaose I	Gal(β1→3)GNAc(β1→3)Gal(β1→4)Gluc; Gal(α1→2)Fuc; GNAc(α1→4)Fuc	0	0

Abbreviations: Gal, D-galactose; Gluc, D-glucose; Fuc, L-Fucose; GNAc, N-acetyl-D-glucosamine.

lactodifucotetraose and lacto-N-fucopentaose II (Table 1). Both enzymes did not split off fucose from lacto-N-fucopentaose I and lacto-N-difucohexaose I. Fucose was split off from lacto-N-fucopentaose III only by α-L-fucosidase I but not by α-L-fucosidase II.

The data obtained suggest that in the enzyme preparations from pig kidney there are two forms of α-L-fucosidase which significantly differ in dependence of activity on pH values, stability on storage, thermostability and substrate specificity.

It is interesting to note that oligosaccharides from human milk are very similar in structure to carbohydrate chains of blood group substances (7). As it was shown by us previously, splitting of fucose from oligosaccharide fragments of blood group substances did not proceed to completion even at prolonged incubations (2,3). On the basis of the data reported here this finding can be explained by some pecularities in the specificity of α-L-fucosidases. The character of splitting of oligosaccharides studied indicates that it is not only the type of the bond or the nature of the sugar to which fucose is bound that are important for fucosidases action, but also the nature of the carbohydrates adjacent to the fucosyl residue.

REFERENCES

1. Wiederschain, G. and Rosenfeld, E.L., Biokhymiya, 32, 173 (1967).
2. Wiederschain, G. and Rosenfeld, E.L., Bull. Soc. Chim. Biol., 51, 1075 (1969).
3. Wiederschain, G., Likhosherstov, L.M. and Senchenkova, S.N., Biokhymiya, 35, 831 (1970).
4. Bhattacharyy, A.K. and Aminoff, D., Analyt. Biochem., 14, 278 (1966).
5. Wiederschain, G. and Kolibaba, L., Vopr. Med. Chim., 15, 637 (1969).
6. Dische, Z. and Shettles, L.B., J. Biol. Chem., 175, 595 (1948).
7. Watkins, W., Sciences, 152, 172 (1966).