HYDROLYSIS OF FUCOIDAN BY ABALONE LIVER α-L-FUCOSIDASE

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1. Introduction

α-L-Fucosidase from abalone liver (*Haliotis gigantea* Gmelin) was reported to hydrolyze methyl α-L-fucoside and fucosidic linkages of fucoidan and blood-group substances [1]. However, this enzyme has been shown to be composed of two enzyme components [2]. One component acts on *p*-nitrophenyl α-L-fucoside as well as fucosidic linkages of porcine submaxillary mucin, and shows optimal activity at around pH 2 (pH 2-enzyme). The other component acts on the synthetic substrate, but not on the mucin, and shows highest activity at around pH 5 (pH 5-enzyme). On the other hand, a fucoidanase which shows hydrolytic activity toward fucoidan, but no activity toward *p*-nitrophenyl α-L-fucoside and blood-group A substance was reported from the livers of related species (*H. rufescens* and *H. corrugata*) [3]. These previous studies suggest that α-L-fucosidase of this genus is differentiated in the substrate specificity. The present paper reports on an exofucoidanase action of the pH 5-enzyme of *H. gigantea* Gmelin.

2. Materials and methods

Fucoidan was prepared from the brown algae, *Ecklonia cava* Kjellman, by the procedure of Percival and Ross [4]. The preparation contained 31.9% L-fucose [5], 1.2% protein (as serum albumin) [6], 1.5% uronic acid (as glucuronic acid) [7], 23.2% sulfate [8], and 22.7% ash. Paper chromatographic examination of its acid hydrolyzate, which was produced after hydrolysis with 1 N HCl at 100° for 4 hr, indicated the presence of two sugars besides L-fucose, neither of which was identified. This fucoidan preparation was converted into the sodium salt by treatment with Dowex 50 (Na^+ form), and used as substrate. α-L-Fucosidase was prepared from livers of abalones (*H. gigantea* Gmelin) as previously reported [2], and the preparation which corresponded to the previously described S4 preparation was used. Fucoidanase assay was carried out with the reaction mixture composed of 2.0 ml of 0.158% fucoidan, 0.5 ml of 0.1 M citrate-0.2 M phosphate buffer at pH 4.5, and 0.5 ml of 0.6% aqueous enzyme solution, at 37°. A reaction mixture in which the substrate solution was replaced by the same volume of water was used as control. Aliquots (0.5 ml) were withdrawn from the reaction mixture, and reducing sugar produced was estimated by the Somogyi-Nelson method [9]. The degree of hydrolysis of fucoidan was calculated on the basis of the concentration of L-fucose residues as determined by the cysteine-sulfuric acid method [5]. α-L-Fucosidase activity was assayed in the same way as reported [2], using *p*-nitrophenyl α-L-fucoside as substrate. Paper chromatographic examination of reaction products was performed by ascent on Toyo No. 51A filter paper in butanol-pyridine-water (6:4:3, v/v) [10] with alkaline silver nitrate [11] as the spray reagent.

3. Results and discussion

Fucoidanase activity was estimated by determination of reducing sugar produced in the reaction mixture. Fig. 1 shows the activity-pH relation curve of the fucoidanase action of the S4 preparation, as compared with that of the α-L-fucosidase. Optimal pH of the fucoidanase activity was observed at around pH 4.5, a slightly lower pH than the optimal pH of the "pH 5-enzyme". However, comparison of both curves sug-

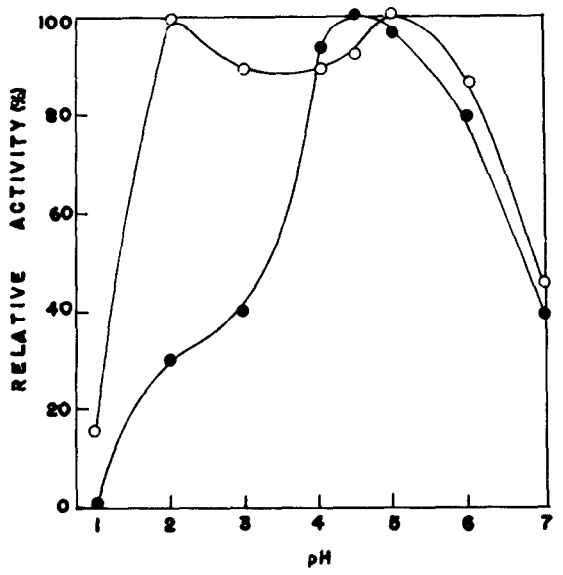

Fig. 1. Activity-pH relation curve of fucoidanase in comparison with that of α-L-fucosidase. pH 1.0 to 2.0, 0.017 M citrate-HCl buffer; pH 3.0 to 7.0, 0.017 M citrate-0.034 M phosphate buffer. •——•, Fucoidanase; ○——○, α-L-fucosidase.

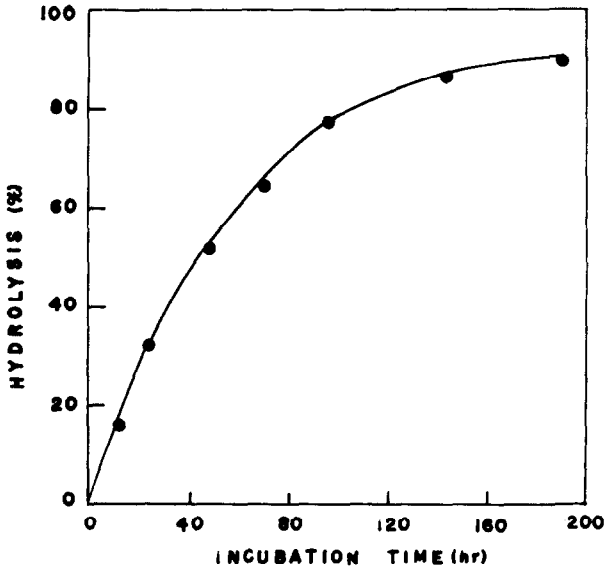

Fig. 2. Hydrolysis of fucoidan by fucoidanase.

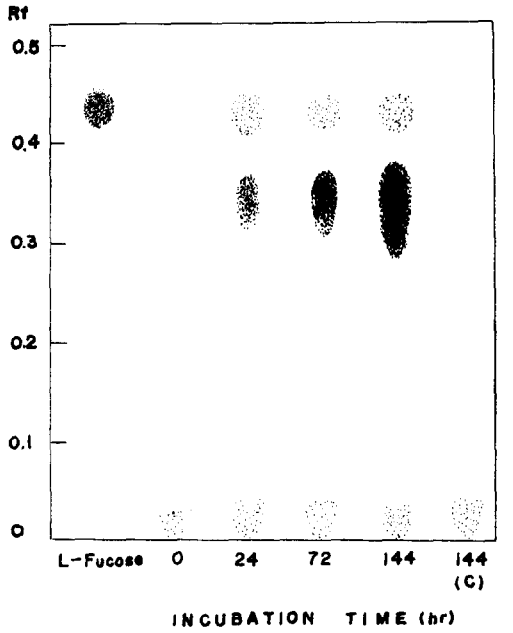

Fig. 3. Diagrammatic illustration of an ascending paper chromatogram showing the formation of reaction products from fucoidan by the action of fucoidanase. (C), Fucoidan was ommitted.

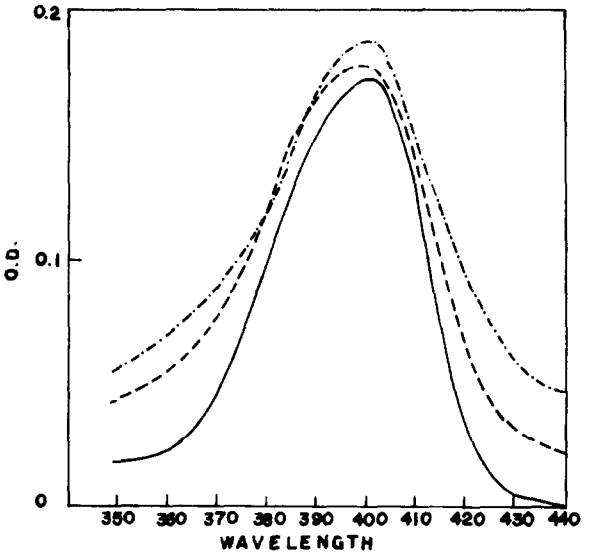

Fig. 4. Comparison of absorption spectra of chromogens produced by reaction with cysteine-sulfuric acid (5).
- - - - -, The sugar product showing R_f 0.43;
-·-·-·-, the sugar product showing R_f 0.34;
——, L-fucose (see fig. 3).

gests that the "pH 5-enzyme" probably relates to the fucoidanase action. In fucoidanase action, the hydrolytic cleavage of fucosidic linkages of fucoidan must take place without release of sulfuric acid residues [3]. In addition, as mentioned before, the fucoidan sample used contained sugar other than L-fucose. Therefore, the formation of reducing sugar in the reaction mixture does not directly measure the fucoidanase action. In order to show the fucoidanase action, in parallel with experiments to estimate the limit of enzymic hydrolysis of fucoidan, paper chromatographic studies on the formation of reaction products were carried out. For the latter experiments, an aliquot (0.8 ml) was withdrawn from the reaction mixture, heated at 100° for 5 min, and cooled in cold water. The precipitate formed was removed by centrifugation, and the solution was taken to dryness under reduced pressure in a desiccator over silica gel. The residue was dissolved in 0.02 ml of water, and an aliquot (0.005 ml) was chromatographed, using L-fucose as the reference sugar. The time course of the enzymic hydrolysis of the fucoidan is presented in fig. 2. The degree of hydrolysis is calculated on the basis of the concentration of L-fucose residues in the substrate, determined by the cysteine-sulfuric acid method [5]. It indicates that the fucosidic linkages of the fucoidan were almost completely hydrolyzed. On the chromatogram, two spots were detected by the alkaline silver nitrate reagent [11], as shown in fig. 3. The minor spot, which corresponds in R_f to L-fucose (0.43) was detected after 24 hr of incubation, but the spot was consistently faint over the entire course of the reaction. On the other hand, the major spot of lower R_f (0.34) was gradually enhanced as the time of incubation was prolonged. In order to identify both products, a 30 ml-volume of the reaction mixture was prepared, and was incubated for 6 days at 37°. The solution was treated in the same way as described above, and was submitted to the paper chromatographic separation. After the papers had been dried, the zone containing each product was cut from the papers. The products were extracted from the strips with warm water, and their solutions concentrated to a syrup under reduced pressure at 60°. Both samples were examined as to absorption spectrum of the chromogen produced by their reaction with cysteine-sulfuric acid [5], in comparison with that of L-fucose. Fig. 4 shows that both products formed chromogens yielding spectra similar to each other and to L-fucose. The characteristic absorption maximum at around 396 mμ was present in all cases. The product showing the lower R_f was analyzed for L-fucose [5] and for bound sulfate [8]. The molar ratio, L-fucose:sulfate, was 1:0.8.

These experimental results indicate that the minor sugar product corresponds to L-fucose and the major sugar product probably to an L-fucose monosulfate. As already metioned, the intensity of the L-fucose spot remained constant during the entire incubation. This fact strongly suggests that the free L-fucose was not produced from the major product indirectly, but was formed directly from the fucoidan, and that the enzymic hydrolysis of fucosidic linkages of the fucoidan occurred with little release of sulfuric acid residues. This also suggests the presence of monosulfated L-fucose residues in the fucoidan.

The fucoidanases of *H. rufescens* and *H. corrugata* have been reported to hydrolyze fucoidan incompletely and to produce predominantly oligosaccharides [3]. By contrast, the present enzyme preparation brought about almost complete hydrolysis of fucoidan. At the same time, the paper chromatogram revealed only the two spots of end products, and no other spot suggesting the formation of oligosaccharides was detected. This difference in the reaction pattern leads to the conclusion that the former enzymes can be regarded as endofucoidanases and the latter enzyme as an exofucoidanase. We presume, that the "pH 5-enzyme" probably acts as this exofucoidanase.

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References

[1] Y.Fukushima and N.Hiyama, Hirosaki University Med. (Hirosaki Igaku), 10 (1959) 149.
[2] K.Tanaka, T.Nakano, S.Noguchi and W.Pigman, Arch. Biochem. Biophys. 126 (1968) 624.
[3] N.M.Thanassi and H.I.Nakada, Arch. Biochem. Biophys. 118 (1967) 172.
[4] E.G.V.Percival and A.G.Ross, J. Chem. Soc. 1950 (1950) 717.
[5] Z.Dische and L.B.Shettles, J. Biol. Chem. 175 (1948) 595.
[6] O.M.Lowry, N.J.Rosebrough, A.Farr and R.J.Randall, J. Biol. Chem. 193 (1951) 265.
[7] Z.Dische, J. Biol. Chem. 167 (1947) 189.
[8] F.Egami and N.Takahashi, Bull. Chem. Soc. Japan 30 (1957) 442.
[9] N.Nelson, J. Biol. Chem. 153 (1944) 375.
[10] S.Inoue, Biochim. Biophys. Acta 101 (1965) 16.
[11] W.E.Trevelyan, D.P.Procter and J.B.Harrison, Nature 166 (1950) 444.