BBA 61164

Purification of β-xylosidase and its action on O-β-xylosyl L-serine and stem bromelain glycopeptide

For the structural analysis of glycoproteins, purified enzymes which specifically hydrolyze the sugar portion of glycoproteins are required Several such enzymes have been discovered and purified, however no attempt to purify β-xylosidase (β-D-xyloside xylohydrolase, EC 3 2 1 37) has been carried out Recently, xylose was found in several glycoproteins as an integral constituent. Purification of β-xylosidase is of special interest, because O-β-xylopyranosyl L-serine is a type of linkage between carbohydrate and protein found in certain acid mucopolysaccharide–protein complexes[1,2]

This paper describes the purification of β-xylosidase from the liver of a marine gastropod, *Charonia lampas*, and its action on O-β-xylosyl L-serine and stem bromelain glycopeptide

As already reported, crude enzyme solution[3] from liver released xylose from stem bromelain glycopeptide[4] in addition to fucose and mannose.

The assay of β-xylosidase was carried out as follows. The incubation mixture was composed of 0.1 ml of enzyme solution and 0 2 ml of 0 2 M acetate buffer (pH 4.0), containing 0 2 mg of *p*-nitrophenyl β-D-xylopyranoside and 0 1 mmole of NaCl After incubation for 15 min at 37°, 2 ml of 1 M Na_2CO_3 solution were added and liberated *p*-nitrophenol was measured with the absorbance being at 420 mμ. Protein content of the enzyme preparation was measured by the method of LOWRY *et al* [5] with bovine serum albumin as a standard The entire procedure of purification was carried out between 0° and 5°. 250 g of the liver of *C lampas* were homogenized with 1000 ml of 1 M NaCl solution for 5 min. After centrifugation, the supernatant was brought to 37% saturation with $(NH_4)_2SO_4$ and centrifuged The precipitate was dissolved in 50 ml of distilled water, dialyzed against distilled water overnight, adjusted to pH 4 by the addition of 1 M acetic acid and centrifuged. About 500 ml of the supernatant solution were dialyzed against 0 01 M acetate buffer (pH 4 0) overnight, heated at 55° for 3 min and centrifuged The supernatant was applied to a column of CM-cellulose (5 cm × 60 cm) buffered with 0 01 M acetate buffer (pH 4 0) and washed with the same buffer containing 0.1 M NaCl solution It was then eluted with the same buffer containing 0 4 M NaCl The major active fractions of the eluate (about 340 ml) were pooled, concentrated to 13.5 ml by dialysis against polyethyleneglycol, applied to a column of Sephadex G-200 (3 cm × 100 cm) equilibrated with 0 1 M NaCl and eluted with the same solution The major active fractions of the eluate (90 ml) were pooled and applied to a column of hydroxylapatite (2 cm × 2 cm). After the column was washed with 2% Na_2SO_4 solution, β-xylosidase was eluted with 4% Na_2SO_4 solution. The most active fractions of the eluate (15 ml) were pooled and stored at −20° as purified β-xylosidase

With this procedure 88-fold purification was attained with a recovery of 5% The purified β-xylosidase hydrolyzed 1 83 μmoles of *p*-nitrophenyl β-D-xyloside per min per mg of protein.

This purified β-xylosidase preparation was practically free from protease activity and from all other glycosidase activities tested (α-glucosidase, α- or β-galactosidase, α- or β-mannosidase, α-L-fucosidase, α- or β-*N*-acetylglucosaminidase and β-*N*-acetyl-

galactosaminidase), except a weak β-glucosidase activity, which may be regarded as the secondary action of the β-xylosidase (M FUKUDA AND F EGAMI, unpublished data)

The purified β-xylosidase hydrolyzed *O*-β-xylosyl L-serine The reaction mixture consisted of 148 μg of enzyme, 100 μg of *O*-β-xylosyl L-serine[6], 10 μl of 1 M acetate buffer (pH 4 0) and 100 μmoles of NaCl. Its total volume was 110 μl and a few drops of toluene were added During incubation for 18 h at 37°, 19 8 μg of xylose were released as measured by the method of PARK AND JOHNSON[7] Thin-layer chromatography of the reaction products was carried out on plates coated with silica gel using the solvent system *n*-propanol–ethyl acetate–water (7 3.1, v/v/v). Only one spot, corresponding to xylose, could be detected by anisaldehyde–H_2SO_4 reagent[8] In control experiments, no trace amounts of xylose or of other sugars could be detected This enzyme also hydrolyzed stem bromelain glycopeptide The reaction mixture consisted of 0 478 μmole of stem bromelain glycopeptide[9], 100 μl of 0 1 M citrate–phosphate buffer (pH 4 0), 6 mg of NaCl and 550 μg of enzyme Its final volume was 300 μl and 3 μl of toluene were added After incubation for 65 h at 37°, the reaction mixture was desalted and subjected to paper chromatography using ethylacetate–acetate–water (3·1 3, v/v/v) On the chromatogram only xylose was detected by alkaline silver nitrate reagent[10] In the control experiment, no trace amounts of xylose or other sugars could be detected 0 42 μmole of xylose was released as measured by quantitative paper chromatography These results clearly indicate that stem bromelain glycopeptide has a xylose at the non-reducing terminus Further details will be reported elsewhere

The authors are greatly indebted to Dr. F. TOMINAGA for the gift of *O*-β-xylosyl L-serine They also wish to thank the Seikagaku Kogyo Co for the supply of *C lampas*

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Received February 22nd, 1968