THE PRESENCE OF O-SERYL-N-ACETYLGALACTOSAMINIDE GLYCOSIDASE IN HELIX POMATIA

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The carbohydrate moiety of ovine submaxillary glycoprotein is known to consist of about 800 disaccharide residues of the structure N-acetylneuraminyl(2→6)N-acetylgalactosamine (Graham and Gottschalk, 1960). Most of these disaccharides are linked O-glycosidically to peptide-bonded serine and threonine residues (Bhavanandan et al., 1964; Harbon et al., 1964; Carubelli et al., 1965).

Two years ago it was shown that a crude enzyme preparation from bovine spleen is able to split in glycopeptides the O-glycosidic linkage between unsubstituted N-acetylgalactosamine and serine and threonine residues respectively. The crude enzyme preparation was obtained from ox spleen homogenate by fractional precipitation with ammonium sulfate (30%, w/v). The glycopeptides were prepared from OSM[1] by treatment first with Pronase and then with neuraminidase; their isolation was effected by column chromatography and their average mol. weight was 660 (Bhavanandan et al., 1964). In a subsequent paper (Bharga-

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[1] The abbreviations used are: OSM, ovine submaxillary glycoprotein; NANA, N-acetylneuraminic acid.

va et al., in press) it was reported that the crude enzyme preparation acted on the above O-glycosidic linkage at a much higher rate when this linkage was present in large glycopeptides (as in NANA-free OSM and in glycopeptides derived therefrom of average mol. weight 7000) than when it was located in short glycopeptides (average mol. weight 800). The enzyme catalysing the release of free N-acetylgalactosamine from its linkage to serine and threonine residues was found to be different from the long-established N-acetyl-ß-D-hexosaminidase (EC 3.2.1.30), also present in the crude enzyme preparation and assayed with phenyl N-acetyl-ß-D-glucosaminide as substrate. The glycosidase has been separated from the hexosaminidase and purified to some degree. The new enzyme was tentatively termed O-seryl-N-acetylgalactosaminide glycosidase.

Recently we found that the digestive juice of Helix pomatia, supplied by L'Industrie Biologique Française, Gennevilliers (Seine), as a source of ß-D-glucuronidase and sulfatase, liberated N-acetylgalactosamine from NANA-free OSM and nitrophenol from p-nitrophenyl N-acetyl-ß-D-glucosaminide. In an effort to separate the two enzymic activities and to purify the O-seryl-N-acetylgalactosaminide glycosidase, 5.75 ml of the digestive juice containing 983 mg protein (determined by the absorbance at 280 mμ with crystalline bovine serum albumin as standard) was applied at 4° to a Sephadex G-150 column (174 x 2.4 cm), equilibrated with 0.25 M NaCl. The proteins were eluted with 0.25 M NaCl in three peaks. The glycosidase activity was recovered in the first 32 fractions (4 ml each) of the first peak. The hexosaminidase activity started to elute in the first peak and continued until the end of the second peak. The 32 fractions were pooled, dialysed and lyophilized; they contained

495 mg protein. The lyophilized material, dissolved in 3 ml of 0.25 M NaCl, was applied to a Sephadex G-200 column (126 x 2.5 cm) under the conditions described above; 2 ml fractions were collected. Of the 127 protein-positive fractions tubes 11 - 55 exhibited glycosidase activity. Hexosaminidase activity was found in all protein fractions, with the bulk of the enzyme contained in tubes 54 - 114. The contents of tubes 11 - 55 were pooled, dialysed and lyophilized; the protein content was 150 mg.

The lyophilized material was rechromatographed on Sephadex G-200 under similar conditions. Each third protein-positive fraction (1.5 ml each) was analysed for protein and for glycosidase and hexosaminidase activities. The glycosidase activity was assayed with 2.9 mM NANA-free OSM (the concentration referring to bound hexosamine) as substrate in 0.025 M citrate buffer, pH 4.8, at 37° for 16 hr. The hexosaminidase activity was assayed with 4.5 mM p-nitrophenyl N-acetyl-ß-D-glucopyranoside as substrate in 0.05 M citrate buffer, pH 4.4, at 37° for 30 min. The released N-acetylgalactosamine was estimated according to Reissig et al. (1955). The liberated p-nitrophenol was determined according to Findlay et al. (1958). The elution pattern is shown in Figure 1.

The specific activities of the hexosaminidase and glycosidase in the original digestive juice at the beginning of the purification procedure were 56.4 and 0.081 milliunits/mg protein respectively. The pooled material of tubes 85 - 127 of the last eluate (see Fig. 1), assayed under same conditions for the specific activities of hexosaminidase and glycosidase, gave values of 87.4 and 1.83 milliunits/mg protein respectively. This means that the ratio of the specific activities of the two en-

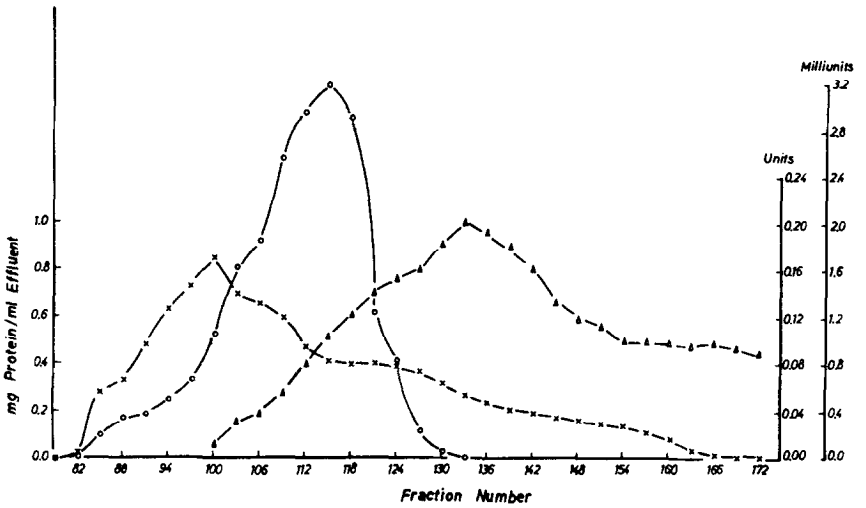

Fig. 1. Elution pattern of the enzymes O-seryl-N-acetylgalactosaminide glycosidase and N-acetyl-ß-D-hexosaminidase from a Sephadex G-200 column after two previous gelfiltrations. o—o, specific activity of O-seryl-N-acetylgalactosaminide glycosidase in milliunits/mg protein; △—△, specific activity of N-acetyl-ß-D-hexosaminidase in units/mg protein; x—x, protein in mg/ml effluent.

zymes decreased from 696.3:1 in the original juice to 47.8:1 in the purified material and that the glycosidase was purified 22.6 fold, uncorrected for the loss of activity of the glycosidase during the isolation procedure (see below). The protein content of the pooled material was 25 mg.

The pH optima of the two enzymes were determined with the pooled material (tubes 85 - 127 of the last eluate) and the result is shown in Figure 2.

In a separate experiment it was found that the specific activity of the glycosidase (stored in an opened ampoule at 4° in the presence of toluene) decreased over a period of 21 days by 72.8%, while the corresponding loss of hexosaminidase activity was only 37.6%.

It would thus appear that the enzyme O-seryl-N-acetylgalactosaminide glycosidase is present in Helix pomatia. The

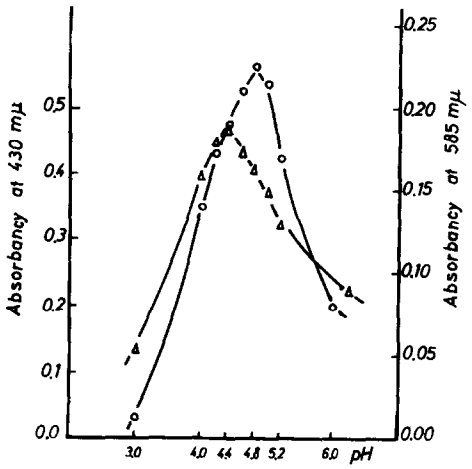

Fig. 2. Activities of O-seryl-N-acetylgalactosaminide glycosidase (o—o) and N-acetyl-ß-D-hexosaminidase (△—△) towards NANA-free OSM and p-nitrophenyl N-acetyl-ß-D-glucosaminide respectively at different pH values. The assays were carried out as described earlier in the text. The activities of the glycosidase and hexosaminidase are shown as the absorbancies at 585 and 430 mμ respectively of the liberated N-acetylgalactosamine and p-nitrophenol in their colour tests.

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occurrence of N-acetyl-ß-D-hexosaminidase in this snail was first described by Neuberger and Pitt-Rivers (1939). Both in mammals and in Helix pomatia these two enzymes seem to be closely associated.

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