CHARACTERIZATION OF O-SERYL-N-ACETYLGALACTOSAMINIDE GLYCOHYDROLASE AS AN α-N-ACETYLGALACTOSAMINIDASE

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Summary

Evidence is presented strongly suggesting that O-seryl-N-acetylgalactosaminide glycohydrolase of Lumbricus terrestris is an α-glycosidase acting on N-acetyl-α-D-galactosaminides but not on the corresponding glucosaminides.

We have reported previously that an enzyme, O-seryl-N-acetylgalactosaminide glycosidase*, isolated from ox spleen (1,2), Helix pomatia (3) and Lumbricus terrestris (4) and purified by a factor of 190 (L. terrestris) with respect to specific activity (units/mg protein), splits the O-glycosidic linkage joining N-acetylgalactosamine to peptide-bonded serine and threonine residues. This type of linkage is present in OSM (mol. wt. about 1 x 10^6), the carbohydrate moiety of which consists of approximately 800 disaccharide units of the structure N-acetylneuraminosyl (2→6)N-acetylgalactosamine. At

* The abbreviations used are: OSM, ovine submaxillary glycoprotein; NANA, N-acetylneuraminic acid; GlcNAc, N-acetyl-D-glucosamine; GalNAc, N-acetyl-D-galactosamine; ser, L-serine; $serNH_2$, L-serine amide; SAGG, O-seryl-N-acetylgalactosaminide glycohydrolase.

least 98 % of these disaccharides are linked O-glycosidically to serine and threonine residues of the polypeptide chain (5).

Substrates for SAGG are NANA-free OSM or glycopeptides prepared thereof, one specificity requirement of the enzyme being a non-reducing terminal position of the N-acetylgalactosamine residue. The enzyme was shown to be different from the established ß-N-acetylglucosaminidase (EC 3.2.1.30) (2). The action mechanism of SAGG is that of hydrolysis (6).

For the further characterization of the enzyme it seemed to be essential to know (a) the anomeric configuration of the susceptible N-acetylgalactosaminidic linkage and (b) the degree of its specificity with respect to the steric configuration at carbon atom 4 of the amino sugar. In order to obtain information on these two points a number of model compounds were tested for their susceptibility to SAGG.

Material and Methods

The following glycosides were synthesized: phenyl-ß-GlcNAc (7); phenyl-α-GlcNAc (8); phenyl-α-GalNAc (8); GlcNAc(ß,1→3) N-benzyloxycarbonyl-serNH_2; GalNAc(ß,1→3)N-benzyloxycarbonyl-serNH_2 and GlcNAc(ß,1→3)serNH_2. The serylglycosides and their derivatives were obtained by Koenigs-Knorr condensation of 1-chloro-3,4,6-tri-O-acetyl-2-acetamido-2-deoxy-glucose and galactose respectively with N-benzyloxycarbonyl-L-serine benzyl ester in the presence of silver carbonate in anhydrous chloroform. The condensation product, after purification, was de-O-acetylated by anhydrous methanol half-saturated with NH_3 and used as such or after removal of the benzyloxycarbonyl group. Details of the preparation and properties of the crystalline compounds will be described elsewhere.

The determination of the enzymatically released phenol and of the N-acetylhexosamines liberated from the synthetic or natural O-seryl-glycosides was carried out as previously (2). OSM, NANA-free OSM and the purified enzymes SAGG and ß-N-acetylglucosaminidase were prepared as described previously (2,7).

Results

Of all substances tested only NANA-free OSM and phenyl-α-GalNAc were substrates for SAGG, the Michaelis constants for

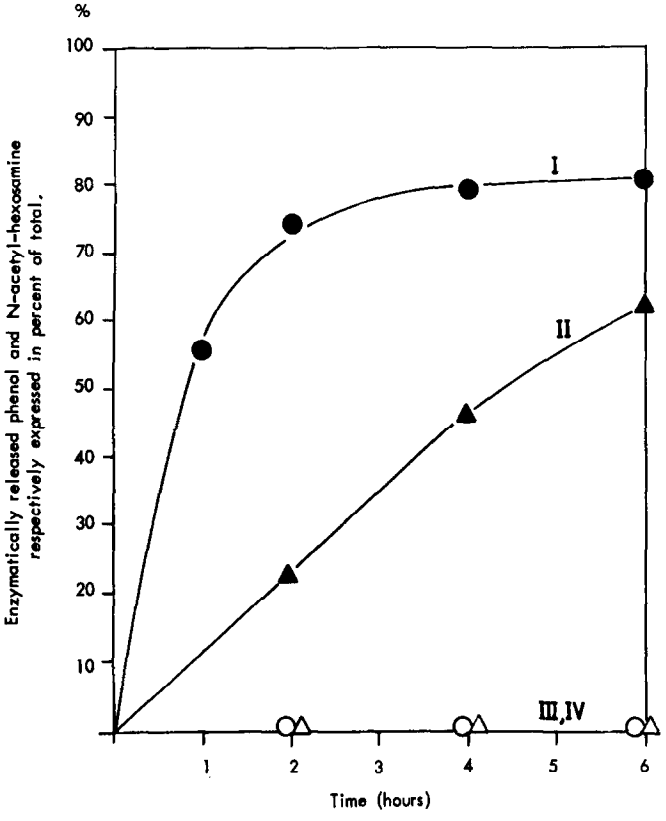

Fig. 1. Some kinetic data on the activity of SAGG

I, phenyl-α-GalNAc; II, NANA-free OSM; III, phenyl-α-GlcNAc; IV, GalNAc(ß,1→3)N-benzyloxycarbonyl-SerNH$_2$. Final concentration of I, III and IV was 4.7 mM, that of II 0.87 mM in the assay, the molarities referring to bound N-acetylhexosamine. The activity of the enzyme added was 2.0 mU/ml assay, using an enzyme preparation of 102 mU/mg protein.

the two compounds being K_m = 1.54 x 10^{-4} M (referred to bound GalNAc) and 1.25 x 10^{-2} M respectively. Some kinetic data are shown in Fig. 1. Phenyl-α-GlcNAc, phenyl-ß-GlcNAc, N-acetyl-ß-D-glucosaminide and galactosaminide of N-benzyloxycarbonyl-serine amide and GlcNAc(ß,1→3)serNH_2 were not susceptible to the enzyme. However, phenyl-α-GlcNAc was cleaved by an α-N-acetylglucosaminidase, also present in L. terrestris but separable from SAGG. The O-seryl-N-acetylhexosaminides in their ß-configuration were readily split by ß-N-acetylglucosaminidase (EC 3.2.1.30).

Discussion

The results would indicate that most probably SAGG is an α-glycosidase. In contrast to ß-N-acetylglucosaminidase which splits ß-glycosides of GlcNAc and GalNAc SAGG acts only on α-glycosides of GalNAc. The nature of the aglycone has a marked influence on the Michaelis constant.

It is of some interest that GalNAc linked O-glycosidically to serine and threonine residues in OSM is in α-configuration, whereas the linkages of GlcNAc to peptide-bonded asparagine, for instance in ovalbumin, of D-galactose to peptide-bonded hydroxylysine in collagen and of D-xylose to peptide-bonded serine in chondroitin 4-sulfate-protein are of ß-configuration. The wide distribution of SAGG in vertebrates and invertebrates (9) might suggest a more common occurrence of α-D-N-acetylgalactosaminidic linkages in biological structures than is known at present.

When these experiments were in progress, Dr. B. Weissmann, Chicago, Ill.,reported at a Meeting of the American Glycosaminoglycan - Glycoprotein Group at Bethesda, Md., on October 8, 1968 on the "Action of purified mammalian α-ace-

tylgalactosaminidase preparations on linkages in some glycoproteins".

Acknowledgement

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