A NEW DESTRUCTION OF BLOOD GROUP SUBSTANCES. ISOLATION OF GLYCOPEPTIDE CONTAINING O-GLYCOSIDIC CARBOHYDRATE-PEPTIDE LINKAGE

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SUMMARY: Degradation of blood group substances using chemical and enzymatic methods has been carried out. A glycopeptide with an O-glycosidic carbohydrate-peptide linkage has been isolated for the first time from biopolymers of such a type. It's structure has been determined as O-(N-acetyl-galactosaminyl)-Thr--Ala.

Blood group substances (BGS) are known to be resistant to proteolytic enzymes (1) that makes practically impossible to accomplish their degradation into small fragments required for structural studies of the peptide chain and particularly the regions of carbohydrate-peptide linkages. In view of the fact that carbohydrate chains prevent proteolytic hydrolysis we managed to split about 50% of the carbohydrates from BGS by means of a highly active glycosidase preparation from Clostridium perfringens (2). But attempts to hydrolyse the modified BGS by proteolitic enzymes failed. Recently, we succeeded in splitting off about 95% of the carbohydrates from BGS using a new approach based on a combination of chemical and enzymatic methods whereas the peptide backbone of BGS was practically unaffected. The obtained polypeptide fragment has been easily splitted by proteolytic enzymes (3). These results enabled us for the first time to develop a practical procedure of BGS de-

gradation resulted in splitting of the high molecular weight glycoprotein into a mixture of aminoacids, peptides, and low molecular weight glycopeptides containing, in average, one monosaccharide and several aminoacid residues. From this mixture we succeeded in isolating of an individual glycopeptide with the O-glycosidic linkage between N-acetylgalactosamine and threonine residues. This provided conclusive experimental evidence for the occurence of the O-glycosidic carbohydrate-peptide linkage in BGS.

6.1 g of BGS (A+H) isolated from hog stomach linings according to (4) was oxidized by aqueous sodium periodate (0.04M $NaJO_4$, 1.55 l, 5°, 24 h), reduced with KBH_4 (7.5 g, 5°, 3 days) and hydrolyzed at pH 1.5 (3 h, 100°). The resulting polymer was isolated on Bio-Gel P-10 and subjected to the action of a glycosidase preparation from Cl.perfringens as reported in (2). The polymer was isolated on Bio-Gel P-10 and incubated with pronase (20:1) in 0.2M tris-HCl buffer, pH 7.5, for 7 days at 37°. The glycopeptide fraction as followed from hexosamines and aminoacids determination was isolated on Bio-Gel P-2, and again treated with a glycosidase preparation from Cl.perfringens and then the glycopeptide fraction isolated by Bio-Gel P-2 chromatography treated with pronase. Gel-filtration on Bio-Gel P-2 resulted in two glycopeptide fractions. Fraction I (about 400 mg) eluted with the void volume of a column was not further investigated, fraction II (about 200 mg) with a lower mobility contained along with aminoacids practically only N-acetylgalactosamine (no galactose, galactosamine:glucosamine ratio ca.10:1). Fraction II was subjected to chromatography on cation-exchange resin Aminex AG 50W-X2 (200-325 mesh, pyridinium form, column 1.8 x 80 cm equilibrated with 0.05M

pyridine-formiate buffer pH 2.6) using linear gradient elution with 0.05M ⟶ 0.2M, pH 2.6 buffer (750 ml + 750 ml). Then the stepwise elution was performed using 0.2M pH 3.6 (300 ml), 0.2M pH 4.3 (300 ml) and 0.3M pH 6.3 (300 ml) pyridine-formiate buffers, fraction volume 5 ml. The elution curve was made up by means of ninhydrin reaction and revealed 19 peaks. The fractions corresponding to each peak were combined and the ratio of aminoacids and hexosamines was determined after hydrolysis (4N HCl, 24 h, 100°) using the aminoacid analyzer (Biotronic LC 4010). Fraction eluted between 1325-1450 ml consisting by galactosamine 1.0, Thr 1.2, Ser 0.1, Pro 0.5, Ala 0.4, Val 0.2, Leu 0.2 (molar ratios) was subjected to preparative paper chromatography (Whatman 3MM, solvent system butanol-pyridine-acetic acid-water 45:30:9:36). The zone with galactosamine:Thr:Ser:Ala = 1.0:1.2:0.3:1.4 ratio was purified by preparative high-voltage paper electrophoresis (Whatman 3MM, 60 V/cm, 1 h) in formic acid-acetic acid buffer pH 1.9. The glycopeptide thus obtained gave upon hydrolysis alanine 1.02, threonine 0.97 and N-acetylgalactosamine 1.0 (relative molar ratio). The homogeneity of this glycopeptide was proved by paper chromatography and electrophoresis (80 V/cm, 1 h in buffers with pH 1.9, 3.6 and 6.5). The structure of the glycopeptide was determined by application of two successive Edman degradations according to (5) with estimation of aminoacids after hydrolysis (4N HCl, 20 h, 100°) using aminoacid analyzer. The results obtained show threonine to be on the N-terminus of glycopeptide and consequently the glycopeptide has the structure O-(N-acetylgalactosaminyl)-Thr-Ala.

Thus, the new procedure led to isolation of first short glycopeptide from BGS. Further study of other glycopeptide

fractions obtained by this method seems to be promising for investigating the detailes of the structure of BGS peptide chain in the regions of carbohydrate-peptide linkage. We believe that this approach to BGS degradation using other proteolytic enzymes (papain and trypsin) would give rise to larger glycopeptides suitable for a more thorough study of the peptide chain of BGS. It should be noted that the structure of the peptide chain of BGS has been subjected so far only to speculative discussion.

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