THE ISOLATION AND THE STRUCTURE OF NEW GLYCOPEPTIDE FROM BLOOD GROUP SUBSTANCES

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Summary: Three new glycopeptides with O-glycosidic and one glycopeptide with N-glycosylaminic carbohydrate-peptide linkages have been isolated after degradation of blood group substances (BGS). Their structure have been determined as O-(α-GalNAc)-Ser(I), O-(GalNAc)-(Pro)-Ser(II), O-(GalNAc1→3 GalNAc)-(Thr-Ala)-Ser(III),N-(β-GlcNAc)-Asn(IV). The isolation of glycopeptide I confirmed α-configuration of O-glycosidic carbohydrate-peptide bonds. The structure of glycopeptide III with two galactosamine residues is in accordance with the data on the presence of hexosamine core of BGS carbohydrate chains.

Recently we reported /1/ on the isolation of glycopeptide O-(N-acetylgalactosaminyl)-threonyl-alanine, from degradation products of blood group substances (A+H) (BGS). Now we have succeeded in isolation of other glycopeptides with O-glycosidic and N-glycosylaminic carbohydrate-peptide linkages and elucidation of their structure.

Degradation of BGS isolated from pig stomach lining has been carried out according to the procedure /1/. The glycopeptide fraction II isolated after gel-filtration (fig.1) was subjected to chromatography on Aminex AG 50W-X2 resin /1/. The elution curve was made up by means of ninhydrin reaction and revealed 19 peaks (fig.2). The fractions corresponding to each peak were combined and the ratio of amino acids and hexosamines was determined after hydrolysis (4N HCl, 100°, 24 h) using

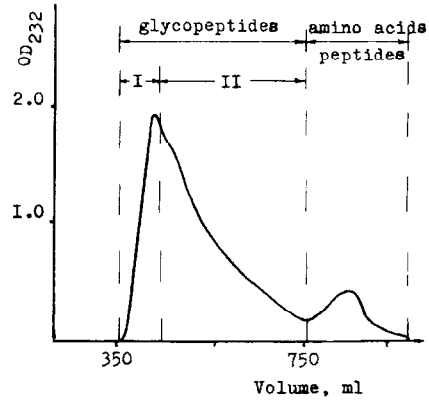

Fig.1. Fractionation of BGS degradation products on Bio-Gel P-2 (column 3 x 200 cm, elution with 0.1M acetic acid).

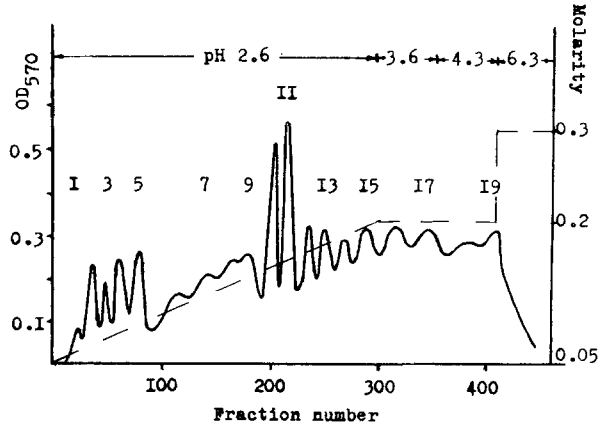

Fig.2. Chromatography of glycopeptide fraction II (see fig.1) on Aminex AG 50W-X2 column (1.8 x 80 cm), elution with gradient of pyridine-formate buffer, fraction volume 5 ml. Fractions were assayed by absorbance at 570 nm after reaction with ninhydrin.

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the amino acid analyzer (Biotronic LC 4010). Fractions 1-9 and 18-19 contained amino acids, galactosamine and small amount of glucosamine; fractions 10-17 contained amino acids and galactosamine only. Fraction 2,5 and 14 (fig.2) had the simplest amino acid composition and were used for the isola-

tion of glycopeptides. For this purpose the fractions were subjected to preparative high-voltage paper electrophoresis and paper chromatography /1/. In several cases two electrophoretic separations and paper chromatography were used for obtaining homogeneous glycopeptides.

The structure of glycopeptides was determined by application of Edman degradation according to /2/ and periodate oxidation (0.015M $NaIO_4$, 20°, 2 h); oxidation products were treated with $NaBH_4$ (1.5% solution of $NaBH_4$, 20°, 2 h) then hydrolyzed (4N HCl, 100°, 20 h) and analyzed using amino acid analyzer. The following glycopeptides were isolated from degradation products of BGS:

I. Ser (5.3)
|
O-α-GalNAc

II. Pro-Ser (5.1.2.1)
|
O-α-GalNAc

III. Thr-Ala-Ser (14.4.2)
|
O-α-GalNAc3 ← I GalNAc

IV. Asn (2.3)
|
N-β-GlcNAc

O-(α-N-acetylgalactosaminyl)-serine (I). Glycopeptide 5.3*) with GalN:Ser = 1.0:0.98 molar ratio was isolated by electrophoresis at pH 1.9 (zone 3). Periodate oxidation of I results in destruction of N-acetylgalactosamine only. The structure of this glycopeptide was confirmed by Edman degradation which completely excluded the structure with two residues of serine and N-acetylgalactosamine. α-Configuration of O-glycosidic carbohydratepeptide linkage was deduced from the high positive optical rotation value $[\alpha]_D^{20}$ + 117° (c 0.015; H_2O; calculated from the determination of serine using amino

*) When numbering glycopeptides the first figure is the number of a fraction after ion-exchange chromatography (fig.2) and next figures are the numbers of the zone (from the less mobile) after successive paper electrophoresis and chromatography.

acid analyzer). This result is in accordance with the data obtained by Donald et al. /3/ who have established α-configuration of carbohydrate-peptide linkages in BGS from the other source.

O-(α-N-acetylgalactosaminyl)-(prolyl)-serine (II). Glycopeptide 5.1.2.1 with GalN:Ser:Pro = 1.0:0.85:1.0 was isolated by electrophoresis at pH 1.9 (zone 1), and at pH 3.6 (zone 2) and subsequently by paper chromatography (zone 1). α-Configuration of O-glycosidic bond for this and other glycopeptides described has been accepted by analogy with glycopeptide I.

O-/3-O-(N-acetylgalactosaminyl)-α-N-acetylgalactosaminyl/-(threonyl-alanyl)-serine (III). Glycopeptide 14.4.2 with GalN:Thr:Ser:Ala = 2.0:1.08:1.0:1.2 was isolated by electrophoresis at pH 1.9 (zone 4) and at pH 3.6 (zone 2). No galactitol acetate could be detected when III was subjected to hydrolysis (N HCl, 4 h, 100°), $NaBH_4$ treatment and acetylation /4/ followed by GLC analysis using Pye series 104 model 64 gas chromatograph (3% OV-17 on Gas Chrom Q, 100-120 mesh, column size 0.4 x 150 cm, 215°), thus demonstrating the absence of galactose in III. Periodate oxidation of III results in the complete destruction threonine and only 50% of galactosamine. Consequently two N-acetylgalactosamine residues are linked with 1 → 3 or 1 → 4 bonds in a disaccharide, which is attached to serine. The presence of 1 → 3 linkage between N-acetylgalactosamine residues was established by comparison of data on alkaline degradation of methylamide of glycopeptide III and of model compound O-/4-O-(β-acetylglucosaminyl)-β-N-acetylglucosaminyl/-N-benzyloxycarbonyl-L-serine methylamide /5/. In the former case 70% of galactosamine was destroyed, while in the latter case (1 → 4 linkage) glucosamine was not

affected. Glycopeptide III had to be first converted into methylamide since free carboxyl group of serine inhibits β-elimination /6/. It was acetylated with acetic anhydride - pyridine mixture (1:1, 20 min, 100°) and then esterified by diazomethane and treated with 8% methylamine in abs. methanol (36 h, 0°) /5/; methylamine was removed and methylamide of III without isolation was treated with 0.05M aqueous Na_2CO_3 (4 h, 70°). O-(β-N-acetylglucosaminyl)-L-serine was used as a model compound for selection of amidation and degradation conditions.

N-(β-N'-acetylglucosaminyl)-asparagine (IV). Glycopeptide 2.3 with GlcN:Asp = 1.0:0.93 was isolated by electrophoresis at pH 1.9 (zone 3). Its elution time upon ion-exchange chromatography (47 min, column 0.9 x 55 cm packed with Aminex A-6 resin, elution with 0.2M sodium citrate-hydrochloric acid buffer, pH 2.2 at 60°, 80ml/h) and electrophoretic mobilities at pH 3.6 and 1.9 were identical to those of synthetic sample of N-(β-N'-acetylglucosaminyl)-L-asparagine. The structure of IV and configuration of N-glycosaminic bond were confirmed by enzymatic hydrolysis with β-aspartyl-glycosylamine-amidohydrolase /7/ with subsequent identification and quantitative determination of aspartic acid.

From the data presented here and in the previous paper /1/ some preliminary conclusions can be made concerning the structure of peptide and carbohydrate chains of BGS near the regions of carbohydrate-peptide linkages. 1) The isolation of glycosides of serine (I-III) and threonine /1/ is the first direct evidence of the presence of O-glycosidic carbohydrate-peptide bonds with both hydroxyamino acids. 2) The isolation of glycopeptide I confirmed α-configuration of these bonds.

3) The structure of glycopeptide III is in accordance with the data on the presence of the carbohydrate core in blood group substances built up of N-acetylhexosamine residues which is adjacent to the peptide chain /8/. 4) In the glycopeptides isolated hydrophobic acids - alanine and proline are bound either with NH_2- or COOH-group of hydroxyamino acid linked with carbohydrates. 5) The isolation of glycopeptide IV supports our recent suggestion /9/ that there are N-glycosylaminic carbohydrate-peptide bonds alongside with O-glycosidic bonds in BGS isolated from pig stomach lining according to /10/.

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