# COMPOSITION OF THE GLYCOPEPTIDES ISOLATED FROM THE STRUCTURAL GLYCOPROTEINS OF AORTAS OF DIFFERENT SPECIES.

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The structural glycoprotein component (SGP) of different connective tissues amounts from 10 to 25 % of the total proteins(Robert and al.,1967).Aorta is particularly rich in such components, its insoluble stroma, obtained after exhaustive extraction with a 1M $CaCl_2$ solution still contains firmly bound glycoprotein releasing about 1,0mg sialic acid/g of dry stroma. This glycoprotein is antigenic and seems to be involved in autosenzitisation phenomena related to the degenerative changes of the arterial wall. It cross reacts with SGP-s extracted from other organs,as cornea,tendon and heart valve. (Goldstein and al., 1967).

In this communication we present evidence for a similar structure of the glycopeptides obtained by pronase digestion of porcine, horse and sheep aorta-SGP-s.

The urea soluble glycoproteins were prepared according to Robert and al.,(1965) from porcine, horse and sheep aorta, and stored in the lyophilised state. The crude urea extract was purified in the following way : 1g of lyophilised glycoprotein was suspended in 20 ml of a mixture of chloroform and methanol (2:1) and kept at boiling temperature for 1 minute cooled, and filtered. This procedure was repeated 3 times. 1g filtered delipidated residue was stirred in 25 ml water at 37°C for 1 hour and the suspension was centrifuged. The washings of the residue were pooled with the supernatant and evaporated in vacuo below 40°C. (Water soluble fraction). The

water-insoluble residue was dryed and analyzed separately. Cellogel electrophoresis at 13 volts/cm in veronal buffer at pH 8,2 demonstrated a peak with a shoulder in the delipidated extract (Figure 1 A) and a single peak in the water soluble fraction (Figure 1 B).

The pronase digestion of the purified residue was carried out as follows : the digestion mixture in a 0,05 M Tris-0,01M $CaCl_2$ buffer solution adjusted to pH 8,0 with HCl contained 50mg/ml of the glycoprotein and 1 mg of pronase (w/w). This mixture was kept at 47°C for 70 hours, then passed through a Sephadex G25 column. High voltage electrophoresis of the hexose peak on paper and on silicagel thin-layer at 1500 and 800 volts respectively in pyridine acetic acid buffer solution at pH 3,8 indicate, that the bulk of the sugar containing fraction had a slight cathodic migration identical to that of the neutral amino acids. Estimation of the molecular weight of the glycopeptide on Sephadex G 75 according to Roberts (1966) and using the glycopeptide of the fibrinogen (Mester and al., 1963) as a standard gave an approximative value of 4-5000.

Mineral acid hydrolysis of the glycopeptide yielded galactose, glucose, mannose and hexosamines (Table I and II). The hexose and hexosamine ratios were determined by thin-layer chromatography on Eastman Kodak 511 V sheets according to Moczar and al., (1967). The partial hydrolysis with ion exchange resin (Amberlite IR 120) according to Montreuil and al., (1965) yielded galactose, mannose and oligosides, (Table III and IV). Two dimensional thin-layer chromatography according to E. Moczar and M. Moczar (1967) indicated the presence of a 1-6 dimannoside.

Table I

Proportion of hexoses and hexosamines in the glycopeptides from the delipidated extract (SGP) of aortas.

| Species | Glucose | Galactose | Mannose | Glucosamine | Galactosamine |
|---|---|---|---|---|---|
| pig | 1,0 | 4,0 | 6,5 | 7,8 | traces |
| horse | 1,0 | 3,3 | 5,3 | 7,0 | traces |
| sheep | 1,0 | 3,3 | 5,3 | 8,1 | traces |

Table II

Proportion of hexoses and hexosamines of the glycopeptides isolated from the water extract and the residue of the delipidated fraction of aortas.

| Species | | Glucose | Galactose | Mannose | Glucosamine | Galactosamine |
|---|---|---|---|---|---|---|
| pig | water sol.fr. | 1,0 | 3,0 | 4,0 | 3,0 | 1,0 |
| | residue | 1,0 | 5,7 | 3,7 | 6,6 | 0,6 |
| horse | water sol.fr. | 1,0 | 3,6 | 4,0 | 4,6 | 1,1 |
| | residue | 1,0 | 4,4 | 7,0 | 15,3 | 0 |

Table III

Proportion of the hexoses in the partial hydrolysate of the delipidated glycopeptides from the urea extract.

| Species | Glucose | Galactose | Mannose |
|---|---|---|---|
| pig | 1,0 | 3,3 | 2,0 |
| horse | 1,0 | 2,5 | 1,2 |
| sheep | 1,0 | 3,0 | 1,2 |

Table IV

Proportion of the hexoses in the water extracts and in the residues of the delipidated glycopetides from the urea extracts.

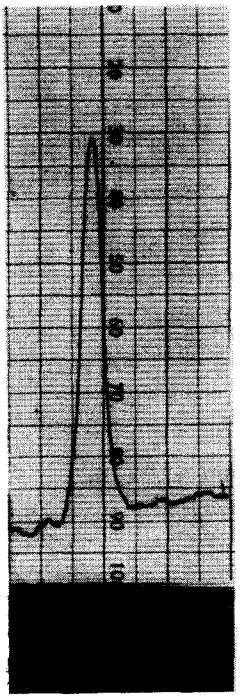
| Species | | Glucose | Galactose | Mannose |
|---|---|---|---|---|
| pig | water soluble fraction | 1,0 | 1,2 | 0,9 |
| | residue | 1,0 | 3,5 | 3,5 |
| horse | water soluble fraction | 1,0 | 2,4 | 1,9 |
| | residue | 1,0 | 3,3 | 3,3 |

The relative selective predominance of galactose and glucose in the partial hydrolysates compared to the total hydrolysate suggests, that galactose and glucose are situated mostly at the non reducing terminal position.

The amino-acid composition of the glycopeptides in the water soluble fraction as that of the water insoluble resi-

due from the SGP-s of pig and horse aortas gives similar patterns on twodimensional thin-layer chromatography(Schleicher & Schüll 1440S plates). The following amino acids could be detected : alanine, threonine, aspartic acid, glutamic acid, glycine, cystine, serine and hydroxylysine.

These results indicate the presence of galactose and glucose endgroups on SGP-s of aorta. Similar results were obtained on a SGP-fraction of heart valves (Moczar and al., 1967). Though part of these hexose end groups may be covered by fucose and sialic acid, split during the TCA-treatment part of them may be available on the surface of the molecule and may thus explain the immunochemical cross-reactions, between SGP-s and with the streptococcus A polysaccharide (Goldstein, Halpern and Robert 1967).



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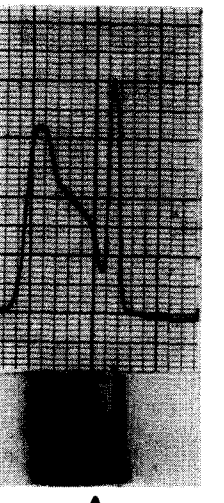

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Figure 1

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