THE PRESENCE OF TWO TYPES OF CARBOHYDRATE-AMINO ACID LINKAGE IN THE SAME GLYCOPROTEIN

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Proteins containing covalently-linked carbohydrate are extremely widespread in nature, occurring both extracellularly and as components of cell membranes. So far, two main types of linkage between carbohydrate and protein have been shown to occur in glycoproteins. The monosaccharide involved has invariably been hexosamine, which may be linked as an O-glycoside to the hydroxyamino acids, serine and threonine, as in submaxillary gland glycoproteins (Blix, 1963-Hashimoto, Tsuiki, Nisizawa and Pigman, 1963), or as an N-glycoside to the amide group of asparagine as in ovalbumin (Marshall and Neuberger, 1964) and IgG (Rosevear and Smith, 1961). Conclusive evidence for the existence of both types of linkage in the same glycoprotein has never been given, but recent studies in this laboratory have demonstrated the existence of O and N-glycosides in an A myeloma globulin, type K.

RESULTS AND DISCUSSION

An A myeloma globulin, type K, was prepared from serum by a combination of ammonium sulphate fractionation and chromatography on DEAE-cellulose (Bernier, Tominago, Easley and Putman, 1965) and after isolation appeared homogeneous by starch-gel and immuno-electrophoresis. Following digestion of the protein with Pronase (Clamp and Putnam, 1964) glycopeptide material was prepared by exclusion chromatography on a column (140 x 2.5 cm.) of Sephadex G-25 (bead form) and then subjected to further incubation with Pronase. Exclusion chromatography

of the second digest indicated the presence of three glycopeptide fractions. The first fraction, after purification by paper chromatography, was shown to contain a single glycopeptide by a modification of the two-dimensional technique (Clamp, Dawson and Hough, 1966) in which the duration of electrophoresis was extended to 3 hr. This glycopeptide corresponded to one provisionally reported to contain D-galactose and 2-acetamido-2-deoxy-D-glucose but with the isolation of additional material the aminohexose was found to be 2-acetamido-2-deoxy-D-galactose. Thus the main peak on gas-liquid chromatographic analysis had the same relative retention time (with respect to D-mannitol) as an authentic sample of methyl 2-acetamido-2-deoxy-α-D-galactose and the identification was confirmed by paper chromatography. Both the second and third fractions, after exclusion chromatography, contained a large number of glycopeptides with differing electrophoretic mobilities. After isolation, each of the glycopeptides was analysed for carbohydrate by gas-liquid chromatography. Amino acid analyses were carried out on certain of the glycopeptides by Dr. B. T. Pickering of the Department of Pharmacology, University of Bristol.

The results expressed in Table 1 show that aspartic acid was the only amino acid present in glycopeptide 2 and in the absence of an ester linkage, suggests the presence of an N-glycoside. Further, this glycopeptide gave a brown colour with ninhydrin as did an authentic sample of 2-acetamido-1-N-(4'-L-asparty1)-2-deoxy-β-D-glucopyranosylamine. A similar type of linkage is probably present in glycopeptide 4, which contains valine in addition to aspartic acid. Glycopeptide 10, however, contains mainly serine, threonine and proline and only traces of glutamic acid, aspartic acid, glycine and alanine, suggesting the presence of an O-glycosidic linkage involving serine and threonine. This type of linkage should undergo a β-elimination reaction under mild alkaline conditions (Anderson, Hoffman and Meyer, 1963) and treatment of the glycopeptide with dilute alkali and sodium borohydride (Carubelli, Bhavanandan and Gottschalk, 1965) resulted

Table 1 Ratio of the monosaccharide and amino acid residues in glycopeptides isolated from an A myeloma globulin, expressed to the nearest whole residue. The ratios, which are uncorrected are related to an L-aspartic acid content of 1 residue in glycopeptides 2 and 4 and to a D-galactose content of 3 residues in glycopeptides 7 and 10.

Glycopeptide No.	2	4	7	10
6-Deoxy-L-galactose	1	1	0	0
D-Mannose	4	3	0	0
D-Galactose	3	2	3	3
2-Acetamido-2-deoxy-D-galactose	0	0	3	3
2-Acetamido-2-deoxy-D-glucose	5	3	0	0
N-Acetylneuraminic acid	3	2	1	0
L-Aspartic acid	1	1	0	0
L-Proline	0	0	5	5
L-Serine	0	0	4	4
L-Threonine	0	0	3	4
L-Valine	0	1	0	0

in a significant increase in the content of alanine but no production of α-aminobutyric acid, indicating that the linkage was through serine. Gas-liquid chromatographic analysis of the reaction mixture showed a reduction in the 2-acetamido-2-deoxy-D-galactose and the appearance of 2-acetamido-2-deoxy-D-galactitol, whilst the D-galactose content remained unchanged. In addition, the unhydrolyzed glycopeptide gave a positive result with the Morgan-Elson procedure, presumably due to β-elimination during the alkaline-cyclization step and this corresponded to one third of the 2-acetamido-2-deoxy-D-galactose content. The Archibald procedure gave a molecular weight of approximately 2800 for this glycopeptide which was confirmed by freezing point osmometry. This suggests the presence of 3 residues of 2-acetamido-2-deoxy-D-galactose in each mole of glycopeptide, one of which was involved in the glycosidic linkage. Glycopeptide 7

contained one residue of sialic acid in addition to 3 each of D-galactose and 2-acetamido-2-deoxy-D-galactose and appeared to have similar amino acid composition.

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SUMMARY

Evidence is given for the presence of two distinct types of glycopeptide in a homogeneous A myeloma globulin preparation. The first type contains 6-deoxy-L-galactose, D-mannose, 2-acetamido-2-deoxy-D-glucose and N-acetylneuraminic acid and is linked to the protein through L-aspartic acid, whereas the second type contains only D-galactose and 2-acetamido-2-deoxy-D-galactose and is linked through either serine or threonine. 2-acetamido-2-deoxy-D-galactose has not previously been reported in human IgA globulins.

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