THE AMINO ACID SEQUENCES OF TWO LARGE GLYCOPEPTIDES DERIVED FROM THE CARBOHYDRATE-CARRYING REGION OF α_1-ACID GLYCOPROTEIN

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SUMMARY: Specific fragmentation with cyanogen bromide and subsequent reduction and carboxymethylation of α_1-acid glycoprotein, a normal human plasma globulin, permitted isolation of a large fragment which was shown to represent the amino-terminal half and to contain the total carbohydrate moiety of this protein. The amino acid sequences of two large glycopeptides derived from this fragment were established. One glycopeptide was composed of 22 amino acid residues and one carbohydrate unit, and the other consisted of 65 amino acid residues and carried four carbohydrate units.

INTRODUCTION

α_1-Acid glycoprotein (for review see Ref. 1) is probably the most extensively studied human plasma glycoprotein with regard to the structure (2) and biosynthesis (3) of its carbohydrate moiety. However, little work has been done concerning the elucidation of the sequence of its single polypeptide chain (1,4) which consists of approximately 210 amino acids. Several investigators (1,5) have studied the amino acid sequences directly adjacent to the carbohydrate-protein linkages of this plasma globulin. The amino (4)- and carboxyl-terminal amino acid residues of this conjugated protein have also been reported (1).

In this paper we wish to describe briefly the amino acid sequences of two large segments of the polypeptide backbone of α_1-acid glycoprotein which account for 40% of all amino acid residues and carry the five (6)

carbohydrate units of this protein. Specific cleavage by cyanogen bromide and subsequent reduction and carboxymethylation of this glycoprotein permitted isolation of the amino-terminal half of the total polypeptide chain to which these carbohydrate units are attached.

MATERIALS AND METHODS

α_1-Acid glycoprotein was isolated from Cohn Fraction VI of pooled normal human plasma (7) and appeared homogeneous as judged by several criteria of purity including amino- and carboxyl-terminal amino acid analyses (1,4).

For the initial fragmentation of this protein the highly specific cyanogen bromide reaction was used in presence of 6 M guanidine hydrochloride (8). After removal of the reagents by gel filtration through Sephadex G-25, the modified glycoprotein was reduced with mercaptoethanol and carboxymethy-

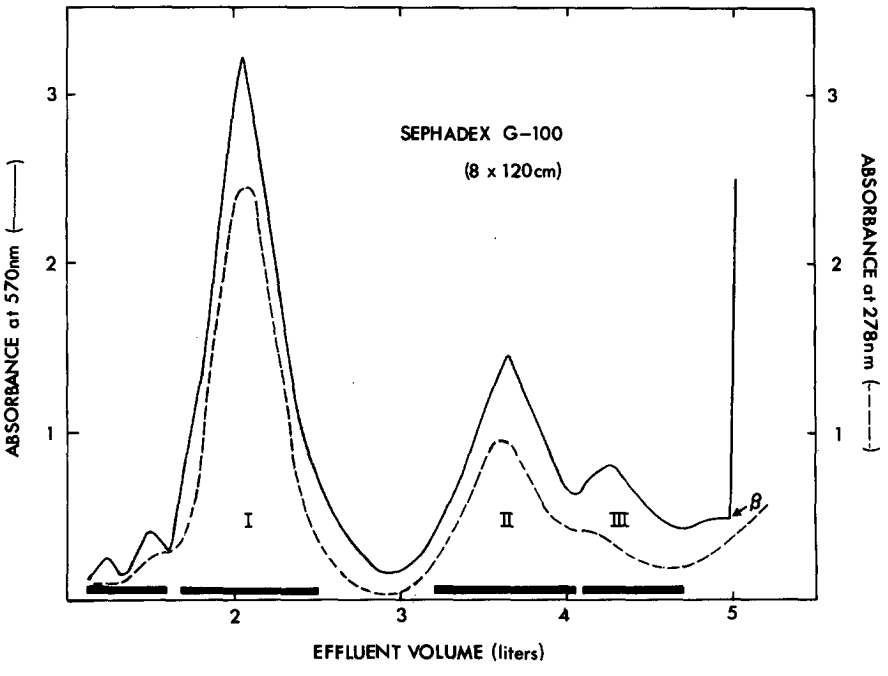


Figure 1: Separation of the CNBr-fragments of α_1-acid glycoprotein by gel filtration through Sephadex G-100.

lated (9). Subsequent chromatography on a Sephadex G-100 column permitted isolation of the expected three fragments (Figure 1). Fragment I when re-chromatographed appeared homogeneous as judged by amino-terminal amino acid analysis and proved to be free of α-amino groups. Since α_1-acid glycoprotein does not possess a free α-amino group, this fragment must have been derived from the amino-terminus of the glycoprotein (4). Fragment I was shown to carry the total carbohydrate moiety of the protein.

For the subsequent investigation of the amino acid sequence of fragment I, tryptic (TPCK-trypsin), chymotryptic and peptic digests were prepared from this portion of α_1-acid glycoprotein. Each digest was passed through a Sephadex G-50 column to obtain a gross separation into a glycopeptide and a peptide fraction. The glycopeptides of each digest were separated from each other and further purified by an appropriate combination of the following procedures: Chromatography on DEAE-, S-, and CM-celluloses, Dowex-1,X2 and BioRex-40, gel filtration through Sephadexes and high voltage electrophoresis (10). The carbohydrate-free peptides were prepared in pure form by chromatography on Dowex-1,X2, Dowex-50,X2, Sephadex G-25 and by high voltage electrophoresis at two of the following pH-values: 1.8, 3.4 and 6.4 and by amino acid and amino-terminal amino acid analyses.

Determination[1] of the amino acid sequences of the peptides and glycopeptides was carried out employing the direct (11) and subtractive (12) Edman degradation techniques and hydrolysis with carboxypeptidases A and B and aminopeptidase-M. In order to establish their complete sequences, some of the larger peptides were hydrolyzed further by chymotrypsin, trypsin or thermolysin.

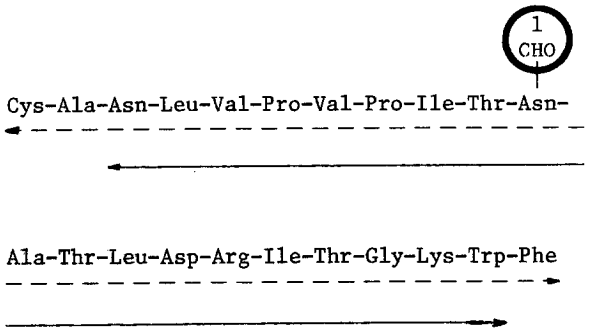
RESULTS AND DISCUSSION

The obtained results are summarized in Figures 2 and 3. Only the

[1]Further technical procedures necessary for the elucidation of amino acid sequences are described in recent publications (10,13,14).

Figure 2

THE AMINO ACID SEQUENCE OF A GLYCOPEPTIDE OF α_1-ACID GLYCOPROTEIN WHICH CONSISTS OF 22 AMINO ACID RESIDUES AND ONE CARBOHYDRATE UNIT



The solid line indicates the amino acid sequence of the main peptic glycopeptide and the broken line that of the major chymotryptic glycopeptide. The attachment of the carbohydrate unit is indicated by the sign $\overset{1}{CHO}$, and the number of the carbohydrate units is referred to in the text.

sequences of those peptides and glycopeptides which unequivocally yielded the amino acid sequences presented in the two figures are included in this report. The amino acid sequence of a glycopeptide which possesses 22 amino acid residues and one carbohydrate unit (Figure 2) and that of a very large glycopeptide consisting of 65 amino acid residues and carrying 4 carbohydrate units (Figure 3) were established. These five carbohydrate units account for the total carbohydrate moiety of α_1-acid glycoprotein (6). All carbohydrate units were found to be attached to asparagine residues with the following carboxyl-terminal sequences:

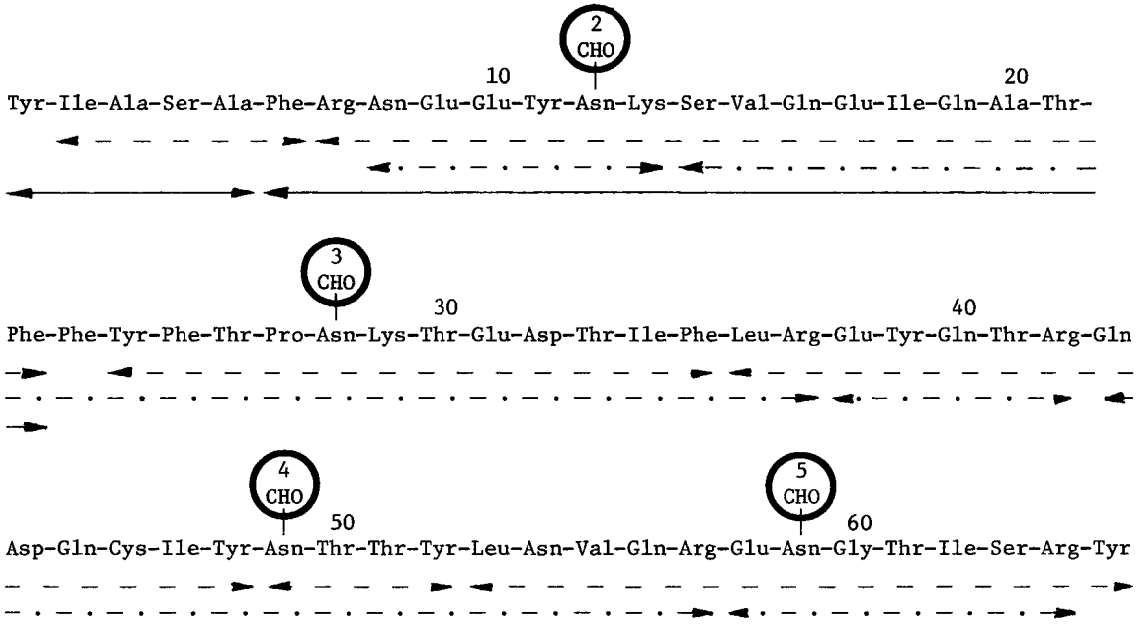
1. -Asn-Ala-Thr-
2. -Asn-Lys-Ser-
3. -Asn-Lys-Thr-
4. -Asn-Thr-Thr-
5. -Asn-Gly-Thr-

These five sequences support the theory of Eylar (15) and Neuberger and

Figure 3

THE SEQUENCE OF A 65-AMINO ACID RESIDUE-FRAGMENT

WITH FOUR CARBOHYDRATE UNITS OF α_1-ACID GLYCOPROTEIN



The sequence of the tryptic peptides and glycopeptides are indicated by (_ . _ . _).
For further information see Legend of Figure 2.

Marshall (16) according to which the carbohydrate units of globular glycoproteins are linked to asparaginyl residues that are included in the general tripeptide: -Asn-X-($\frac{Ser}{Thr}$)-. Of further interest is the finding that the distances between two adjacent carbohydrate units, as far as determined, vary considerably and amount to 16, 21 and 10 amino acid residues between carbohydrate units 2 and 3, 3 and 4 and 4 and 5, respectively. It can be seen that the distribution of the five carbohydrate units along the total polypeptide backbone of the glycoprotein is surprisingly asymmetric inasmuch as the carboxyl-terminal half of the polypeptide chain is free of sugar. However, both halves of this chain carry a large number of electrostatic charges: the amino-terminal half includes 16 sialyl residues (1) and the carboxyl-terminal half possesses a relatively high number of basic and acidic amino acid residues, as inferred from the composition of α_1-acid glycoprotein and the three CNBr-fragments derived from it.

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