THE AMINO ACID SEQUENCE OF THE CARBOXYL-TERMINAL CNBr-FRAGMENT 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 (orosomucoid), a normal human plasma protein, permitted isolation of a large fragment which was free of carbohydrate and was shown to represent the carboxyl-terminus of this protein. The amino acid sequence of this fragment which consisted of 70 residues and contained a remarkably high number of acidic and basic amino acids, was established.

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, its single polypeptide chain(1,4) consisting of approximately 210 amino acids has been investigated very little. Several investigators(1,5) have studied the amino acid sequences directly adjacent to the carbohydrate-protein linkages of this plasma globulin. The amino- and carboxyl-terminal amino acid residues have also been reported(1,4). In this paper we wish to describe briefly the sequence of the carboxyl-terminal CNBr- fragment of α_1-acid glycoprotein which consists of 70 amino acid residues.

MATERIAL AND METHODS

α_1-Acid glycoprotein was isolated from Cohn fraction VI of pooled normal human plasma(6) and appeared homogenous 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 in presence of 6 M guanidine hydrochloride was used(7). After removal of the reagents, the modified glycoprotein was reduced with mercaptoethanol and carboxymethylated(8). Subsequent chromatography on a Sephadex G-100 column permitted isolation of the expected three fragments (Fig.1). Rechromatography yielded fragment II in homogenous form as judged by several criteria

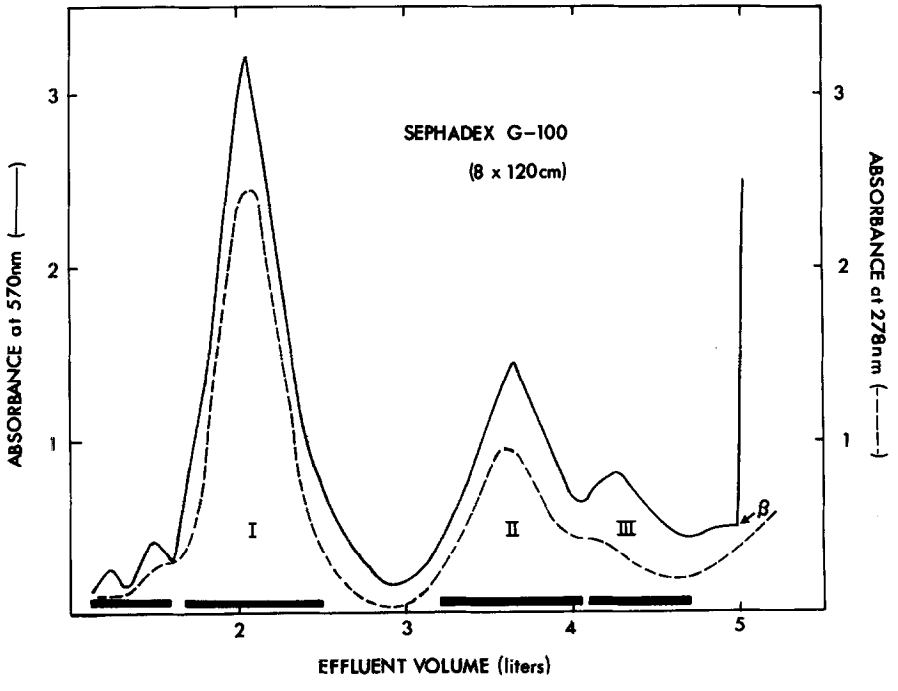


FIG. 1. Separation of the CNBr-fragments of α_1-acid glycoprotein by gel filtration. The effluent was monitored by the absorbancy at 280 nm and by the ninhydrin color at 570 nm formed after alkaline hydrolysis of appropriate aliquots. At the position (β), the reagents including β-mercaptoethanol were eluted.

of purity. This fragment which proved to be free of carbohydrate, did not contain any homoserine and, thus, represents the carboxyl-terminus of α_1-acid glycoprotein.

The chymotryptic and tryptic digestion of fragment II, the purification and the analysis for homogeneity of the resulting peptides were carried out by accepted procedures(8,9, 10). The amino acid sequences of these peptides were established by the direct(11) and subtractive(12) Edman degradation techniques and by digestion with carboxypeptidases A and B and aminopeptidase M.

RESULTS AND DISCUSSION

The partial amino acid sequence of the amino-terminal

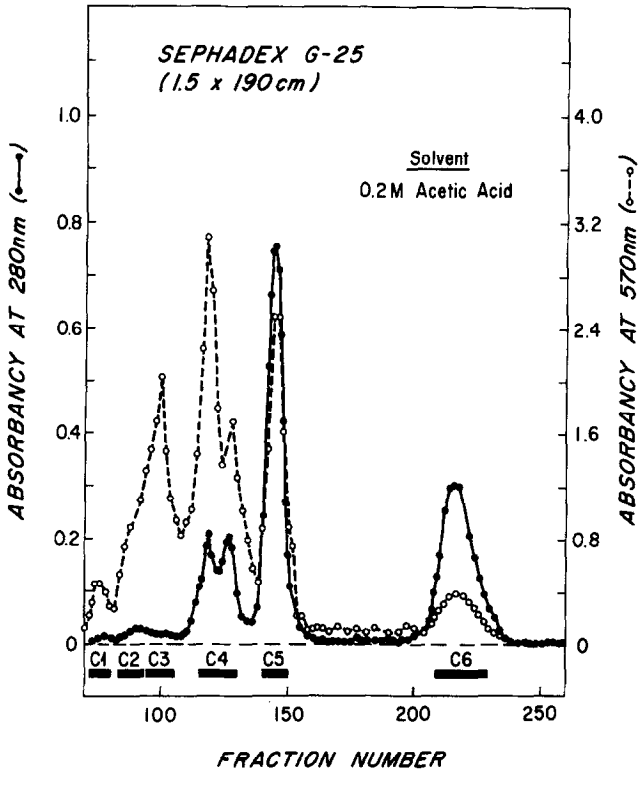


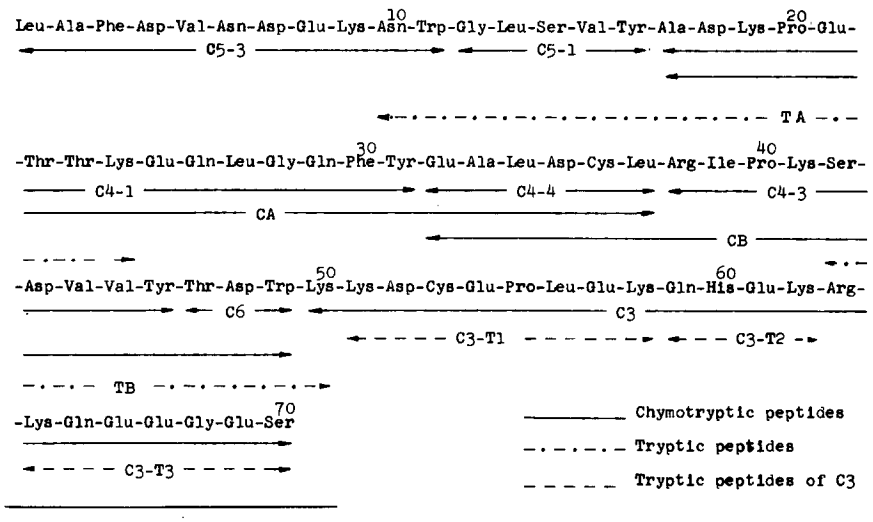
FIG. 2. Fractionation of a chymotryptic digest of the carboxyl-terminal CNBr-fragment of α_1-acid glycoprotein by gel filtration through a Sephadex G-25 column.

region of fragment II established by the direct Edman degradation was as follows: Leu-Ala-Phe-Asp-Val-Asn-Asp-Glu-Lys-Asn-Trp-Gly. Because of its large content of basic amino acids, fragment II was digested with chymotrysin for the first degradation. Separation of the resulting peptides on a Sephadex G-25 column (Fig.2) afforded two homogenous peptides (C_3 and C_6). From fraction C4, three homogenous peptides (C4-1, C4-3 and C4-4) were isolated. The peptides of fraction C5 were separated from each other yielding C5-1 and C5-3. The amino acid composition of these compounds confirmed further their homogeneity and accounted for all amino acids of fragment II.

Of the resulting amino acid sequences[1] required for establishing unequivocally the sequence of fragment II (Fig.3), the following is of particular interest. The sequence of C5-3 was found to be in agreement with that of the amino-terminus of fragment II. For the complete elucidation of the sequence of C4-1, it was necessary to study the tryptic peptides derived therefrom. To establish the sequence of C3, of which three residues were determined by direct Edman degradation, this peptide was digested with trypsin yielding three main peptides. Peptide C3-T1 was located near the amino-terminus of C3. The carboxyl-terminal amino acid of the heptapeptide (C3-T3) being serine was the same as that of C3 and, hence, of fragment II [as well as of the whole glycoprotein (1)]. Hence, peptide C3-T2 must be located between C3-T1 and C3-T3. Since the arginine residue

1 Only the sequences of those peptides, which afforded unambiguously the amino acid sequence of fragment II, are included in this report.

FIG.3. THE AMINO ACID SEQUENCE OF THE CARBOXYL-TERMINUS OF α_1-ACID GLYCOPROTEIN.



of C3 was not accounted for and because of the large number of lysine residues present in C3, fragment II was treated with citraconic acid anhydride (13) and then digested with trypsin. The resulting major peptides were elucidated and confirmed unambiguously the sequence of C3.

Short chymotryptic digestion of fragment II permitted the isolation of two large peptides (CA and CB). The amino acid composition and partial elucidation of the sequence of CA established the sequence and position of peptides C4-1 and C4-4. The partial sequence of CB confirmed the sequences and positions of peptides C4-3 and C4-4.

To ascertain further the sequence of the chymotryptic peptides, two large, overlapping peptides (T-A and T-B), isolated from a tryptic digest of fragment II, were investigated. The amino acid composition and partial sequence of peptide T-A determined the position of the peptides C5-3, C5-1 and C4-1. The amino acid composition of T-B established the sequence of C4-3, C6 and C3.

The complete amino acid sequence of the carboxyl-terminal CNBr- fragment of α_1-acid glycoprotein (Fig.3) accounts for approximately one third of the amino acids of the whole protein. It is of interest to note that this fragment contains a large number of basic and acidic residues which are particularly concentrated toward its carboxyl-terminus. Hence, the five carbohydrate units(4) of α_1-acid glycoprotein accounting for 40% of the weight of this protein, are located within the amino-terminal region(15). This hitherto unknown, unusually asymmetric distribution of the carbohydrate units with their sialyl residues and of the mentioned acidic and basic amino acids, appears to result in a relatively even distribution of the electrostatic charges along the polypeptide chain of this glycoprotein. This distribution of the electrostatic charges probably explains many of the physicochemical properties of α_1-acid glycoprotein(1). [Supported by Grants #GB-24813(NSF) and GM-10374(NIH)]

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