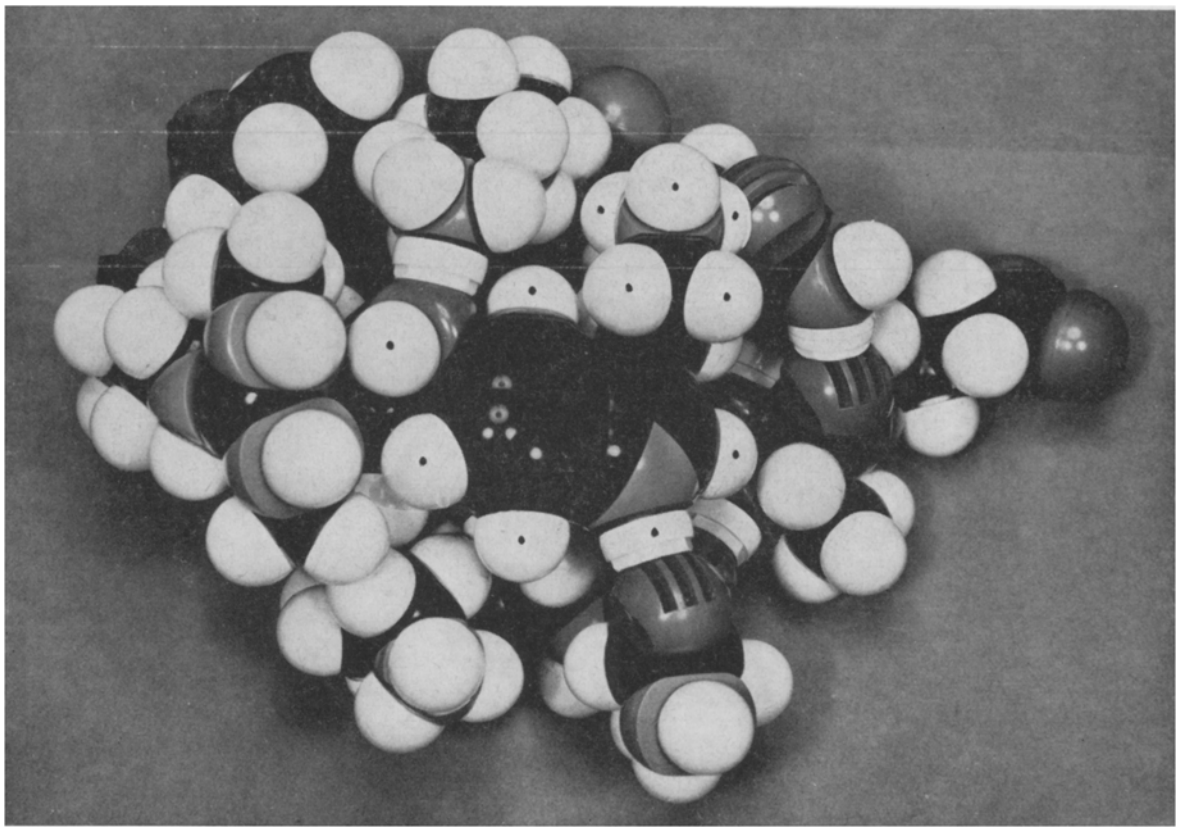

Fig. 2. CPK molecular model of the polypeptide forming a possible receptor site for 5HT shown bound. Aminoacids 107–112 are left out. They form a simple β-turn.

δ-hydrocarbon: ring hydrocarbons to Methyl-Arg, 2. *Polar*: $-NH_3+$ to Gln O: hydroxyl O from Arg $= NH_2^+$ and ring NH to Gln (102) O. Note that the Gln-Arg carbon-carbon bond (120–221) is twisted by a break in the β-conformation. The β CO = NH hydrogen bond is replaced by a hydrogen bond from Ser (114) OH to peptide O (Pro – 122) and possibly a second from Arg (112) to the next peptide O (123). This twist is necessary for the Arg-5HT interaction described. Gln (120) binds by a hydrogen bond to the carbonyl O of Ala (117).

The complex (Trp-Gln-Arg) somewhat resembles in size, form and charge distribution the molecule of strychnine. This may explain the convulsant activity of the basic protein when injected intraventricularly[1]. The function of 5HT in this position might be to maintain this segment of the protein molecule in its β-conformation or to disrupt it by a charge transfer operation (to Trp). This hypothesis can be tested by direct physical investigations using NMR, ORD and CD techniques of the conformation of these polypeptides[4].

Zusammenfassung. Es besteht die Annahme (CARNEGIE 1970), dass das basische Gehirnprotein als Rezeptor für Serotonin wirkt, und es wird der Vorschlag für molekularbiologische Reaktionen von Serotonin-Rezeptoren gemacht.

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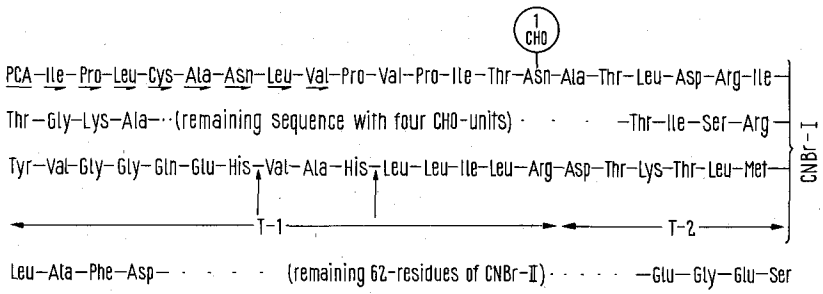
The Linear Amino Acid Sequence of-α_1Acid Glycoprotein

Although α_1-acid glycoprotein (orosomucoid), a human plasma globulin (for review see ref. [1]), is probably the most extensively studied protein with regard to the structure[2] and biosynthesis[3] of its carbohydrate moiety, it is only very recent that significant information concerning its amino acid sequence has been reported[4,5]. The complete elucidation of its primary structure has been hampered by the inability of investigators to establish the amino-terminal sequence, since digestion of α_1-acid glycoprotein by specific (chymotrypsin and trypsin) and nonspecific (pronase) proteases does not yield overlapping peptides, but affords the same pyrrolidone carboxylic acid, PCA-



The linear amino acid sequence of α_1-acid glycoprotein. PCA (—) was cleaved specifically with pyrrolidonecarboxylyl peptidase. The amino-terminal sequence of the enzymatically modified CNBr-I was then established by the direct Edman degradation technique (→). The sequence of the carboxyl-terminus of CNBr-I is indicated by the 2 peptides T-1 and T-2. The cleavage of T-1 effected by N-bromosuccinimide is signified by the 2 arrows (↑).

containing tetrapeptide, PCA-Ile-Pro-Leu. As will be shown in the present paper, this problem could be resolved by cleavage of PCA from the amino-terminal cyanogen bromide-fragment of this glycoprotein (CNBr-I)[4] by the highly specific enzyme, pyrrolidonecarboxylyl peptidase, and subsequent stepwise chemical degradation of the enzymatically modified CNBr-I. Moreover, the previously unknown peptide sequence that links CNBr-I to the carboxyl-terminal CNBr-fragment (CNBr-II)[5] of the protein, could also be elucidated.

For the present study α_1-acid glycoprotein was isolated from pooled normal human plasma[6] and demonstrated to be homogenous as judged by several criteria of purity[1,7]. This globulin possesses a single polypeptide chain[7] to which 5 heteropolysaccharide units[8], accounting for 40% of the protein by weight, are attached.

After cleavage of this glycoprotein with CNBr and subsequent reduction and alkylation, CNBr-I was isolated by Sephadex G-100 chromatography. For the removal of the amino-terminal pyrrolidone carboxylic acid residue, CNBr-I was treated with pyrrolidonecarboxylyl peptidase[9]. Another aliquot of CNBr-I was trifluoroacetylated[10] and digested with TPCK-trypsin. The resulting digest was passed through a Sephadex G-50 column in order to achieve an initial fractionation into a glycopeptide and a peptide fraction. The carbohydrate-free peptides, after removal of their trifluoroacetyl residues, were separated from each other and purified by conventional procedures[11]. One of the resulting peptides (T) that distinguished itself by its content of homoserine, indicating that it was derived from the carboxyl-terminus of CNBr-I, was selected for further investigation. Homogeneity of this peptide was established by high-voltage electrophoresis at pH 1.8 and 6.4, and by amino-terminal amino acid analysis. The amino acid sequence was established by the direct[12] and indirect[13] Edman procedures and by the analysis of carboxypeptidases A and B digests. Chemical cleavage at the histidine linkages was achieved with N-bromosuccinimide (NBS)[11].

The results of the present study reveal the following findings: 1. The specific enzymatic degradation of CNBr-I provides conclusive evidence that PCA represents the amino-terminus of this fragment, thus providing additional and corroborative evidence that PCA indeed is the amino-terminal residue of α_1-acid glycoprotein as reported earlier[5]. 2. After cleavage of PCA the enzymatically modified CNBr-I was directly accessible to Edman degradation. Eight steps (direct Edman procedure) afforded the sequence which overlapped by 5 residues with the previously established sequence of the 22-amino acid glycopeptide derived from CNBr-I[4] (Figure); and 3. One carbohydrate unit is in close proximity to the amino-terminus of the protein being attached to the 15 th residue (Figure).

For the elucidation of the carboxyl-terminal region of CNBr-I, the mentioned peptide T was digested with trypsin yielding a long (T-1) and a short (T-2) peptide (Figure). Because of the unusual sequence (-Leu-Leu-Ile-Leu-) located at the carboxyl-terminal sequence of T-1, this peptide was cleaved at its 2 histidine residues with N-bromosuccinimide. Utilizing the resulting pentapeptide, Leu-Leu-Ile-Leu-Arg, the structure of the carboxyl-terminal region of CNBr-I could be established unambiguously.

The present study essentially completes the linear amino acid sequence of α_1-acid glycoprotein. It should be noted that this is the first time that the amino acid sequence of a glycoprotein which possesses 5 carbohydrate units is described[14,15].

Zusammenfassung. Es gelang, die lineare Aminosäuresequenz von saurem α_1-Glykoprotein (Orosomucoid), einem Globulin des menschlichen Plasmas, aufzuklären.

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