ORDERING OF TWO CYANOGEN BROMIDE FRAGMENTS OF HUMAN SERUM ALBUMIN

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1. Introduction

The cleavage of the six methionine residues of human serum albumin followed by disruption of the disulfide bonds produces seven peptide chains [1, 2]. A scheme of the albumin molecule at the level of cyanogen bromide chains has been proposed [2]. Two immunologically active fragments have been isolated in our laboratory. The first, called inhibitor, was obtained from human serum albumin degraded by rabbit spleen extract [3] and has a mol. wt. of 11 000 [4]. The second, called F1, was obtained from inhibitor degraded by trypsin and has a mol. wt. of 6600 [5]. A study of the structure of these fragments has been undertaken. In the course of this work, we have isolated a peptide chain from F1, the sequence of which has been partially determined. It overlaps two cyanogen bromide fragments which can be identified thus leading to a modification of the scheme proposed for the structure of the albumin molecule.

2. Materials and methods

Human serum albumin (Squibb fraction V prepared by ethanol fractionation) was kindly given by the American Red Cross. Inhibitor and F1 fragments were prepared as described previously [6, 5]. N terminal amino acids of the fragments were determined by the technique of Sanger and Thompson [7] following the conditions described by Fraenkel-Conrat et al. [8]. The peptides chains of F1 were isolated after reduction and alkylation by preparative electrophoresis in acrylamide–agarose gel plates (Indubiose plates, I.B.F., Paris) according to Uriel [9] and Bellon [10]. The sequential analysis of the N terminal region of this chain was performed upon 6 mg with a "Séquenceur de Protéines SOCOSI", using the degradation method of Edman and Begg [11].

3. Results and discussion

The seven peptide chains resulting from cleavage of the methionine residues and the disulfide bonds of human serum albumin have been named differently by MacMenamy et al. [1] and by Kusnir and Meloun [2]. Both groups have identified the chains corresponding to the N and C terminal end of the albumin molecule and Kusnir and Meloun have proposed an order for the remainder. Therefore, we shall use the nomenclature of the latter authors. Hydrolysis of albumin by cyanogen bromide produces three fragments called: N1, N2 and C. Disulfide bonds cleavage of N1 gives rise to two chains named I and II; N2 consists of one single chain named III. Disulfide bonds cleavage of C gives rise to four chains named IV, V, VI and VII. The Roman numerals of the chains indicate the order proposed, I corresponding to the N terminal and VII to the C terminal end of the albumin molecule.

Inhibitor contains only one methionine residue [4]. We have found two N terminal amino acids per mole: glutamic and threonine. It should therefore be made of two chains but they have not as yet been obtained independently. We have also found two N terminal amino acids per mole of F1, glutamic acid and alanine. The corresponding chains have been obtained separately by electrophoresis in acrylamide agarose gel

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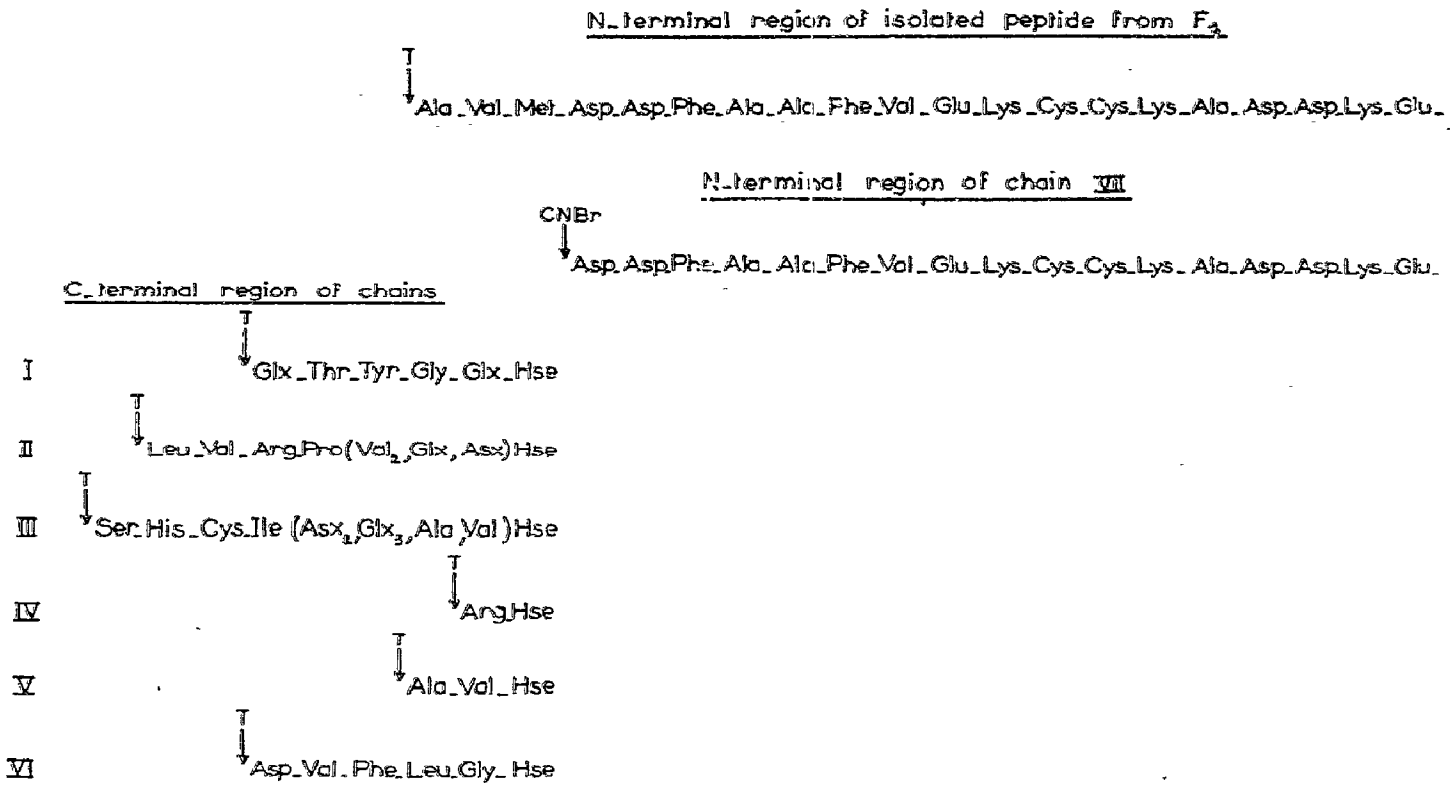

Fig. 1. Comparison of amino acids sequence of a peptide isolated from F1 and the N or C terminal sequences of cyanogen bromide fragments of human serum albumin. ↓ CNBr and ↓ T denote sites of cleavage by cyanogen bromide and trypsin.

plates. The alanine chain comes certainly from inhibitor after cleavage by trypsin of the peptide bond preceding the N terminal alanine of F1, since this amino acid is different from the two terminal amino acids of inhibitor. It has twenty seven residues, among them the methionine, and the first twenty have been sequenced.

Fig. 1 shows this sequence as well as the N terminal region of chain VII according to Meloun and Kusnir [12] and the C terminal region of other chains according to Kusnir and Meloun [2]. These last sequences have been established upon the peptides resulting from tryptic degradation of the chains and containing homoserine.

One sees that the third residue of the peptide isolated from F1 is the methionine. The sequence following it, is identical to the N terminal part of chain VII. The beginning of the sequence, Ala—Val—Met, has been compared to the C terminal part of the other chains. There are only two chains having a C terminal part which could fit with this sequence. One is the chain III; its C terminal region has not been completely sequenced but it contains alanine and valine. However, this peptide cannot correspond to the beginning of F1 peptide because it has no bond susceptible to cleavage by trypsin. Otherwise, chain V has the C terminal sequence Ala—Val and has, like F1, a peptide bond preceding alanine which has been cleaved by trypsin.

These results indicate therefore that it is chain V which is linked to the chain VII through a methionine residue.

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