THE PARTIAL AMINO ACID SEQUENCE OF SHEEP HEART MYOGLOBIN

K. HAN, D. TETAERT, M. DAUTREVAUX, Y. MOSCHETTO and G. BISERTE
Laboratoire de Chimie Biologique, Faculté de Médicine, Place de Verun 59-Lille, France

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1. Introduction

Several years ago a comparative study of the covalent structure of horse, beef, sheep and hog myoglobin was undertaken [1]. Since the differences observed by mapping the tryptic hydrolysates of these myoglobins appeared significant, it seemed interesting to further investigate the covalent structure of myoglobin molecules. The covalent structure of horse and beef heart myoglobins was first determined [2, 3]. The purpose of such a comparative study was discussed in a previous paper [3]. In order to extend this structural study, we investigated the covalent structure of sheep heart myoglobin. This paper presents a partial sequence of sheep myoglobin, in which 118 of the 153 residues of the protein chain can be positioned uniquely on the basis of the isolation and characterization of tryptic peptides.

2. Experimental

The heme moiety was removed from myoglobin molecule and globin denatured by guanidine hydrochloride before tryptic digestion. Because of a close analogy between sheep, horse and beef myoglobin sequences [2, 3], it was possible to make a tentative alignment of the isolated sheep myoglobin peptides with the beef myoglobin sequence as a model. The complete sequence determination appeared unnecessary for some peptides whose amino acid composition, *N*-terminal amino acid, paper electrochromatographic migration and peptide elution pattern on resin chromatography proved identical with those of corresponding tryptic peptides of beef myoglobin. When amino acid composition of a sheep myoglobin peptide was different from the homologous bovine tryptic peptide, its sequence was determined by dansyl-Edman technique, hydrazinolysis and hydrolysis by carboxypeptidases and/or leucine aminopeptidase.

Tryptic digestion was performed by adding 4% (w/w) enzyme (Trypsin Seravac) to the substrate solution and allowing the enzymic digestion to proceed at 38° for 150 min at pH 8.75. Separation of the peptides thus obtained was achieved by column chromatography on resin Chromobeads P (Technicon). Some peptides were extensively purified by paper electrophoresis at pH 3.9 or by paper chromatography in a butanol–acetic acid–water system (4:1:5). The purity of the peptides was controlled by end-group analysis and fingerprint technique. The quantitative amino acid analyses were performed with an automatic amino acid analyzer. The *N*-terminal groups of the peptides were determined by the dansyl-technique [4]. The *C*-terminal residues were determined after liberation by carboxypeptidases B and A. The amino acid sequences of the peptides were determined by the combined dansyl-Edman technique [5]. All these procedures were detailed in previous papers [2, 3].

3. Results and discussion

The sequence of the tryptic peptides of sheep myoglobin, set side by side with the known primary structure of beef heart myoglobin, is presented in fig. 1. Our results provide evidence for the existence of 5 sites in which the two myoglobins differ. In 4 cases, the differences could proceed from the change of a single nucleotide in the codon. At position 142,

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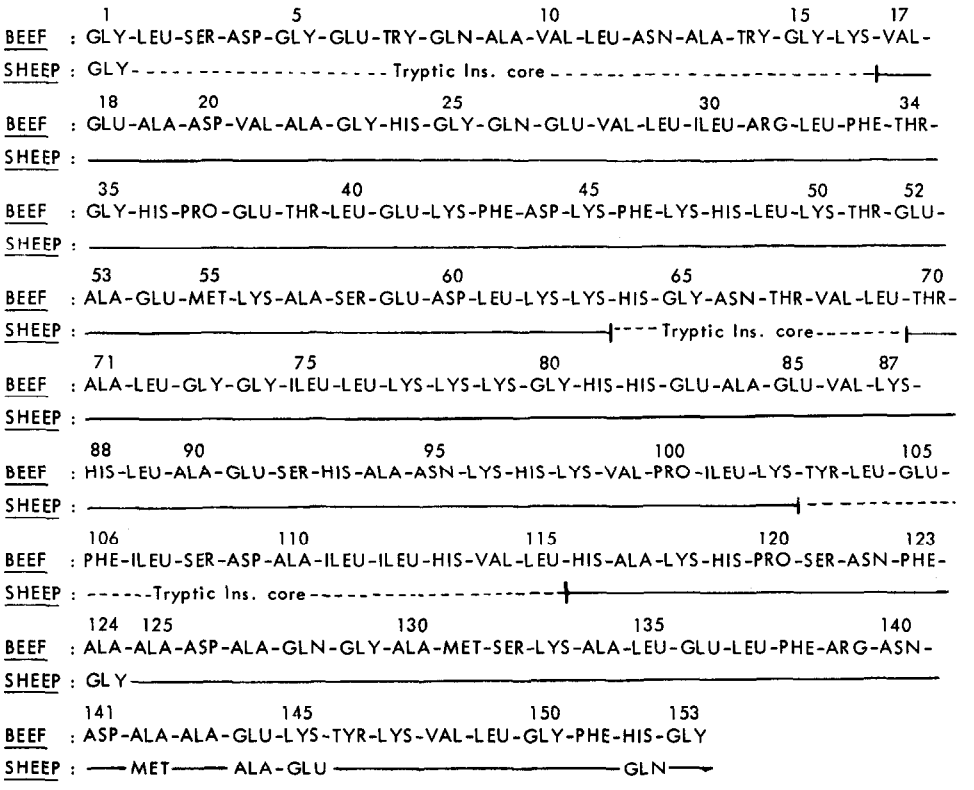

Fig. 1. Comparison of structure of beef myoglobin (top line) with tryptic hydrolysate of sheep myoglobin (bottom line). Wherever the residues are identical in both sequences the entry is omitted.

the replacement of alanine in bovine myoglobin by a methionine residue in ovine myoglobin should imply a double mutation in the coding bases [6].

We have reported here the sequence of 23 tryptic peptides. A tryptic insoluble core, representing approximately one quarter of the whole molecule and containing 35 residues remains undetermined at the present time. The cyanogen bromide cleavage of the three methionyl bonds present in the sheep globin molecule is now being conducted in order to obtain large soluble peptides corresponding to the tryptic insoluble core and overlapping the rest of the molecule.

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