GUANIDINATION OF HORSE HEART MYOGLOBIN

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1. Introduction

In a previous communication [1], we have reported an almost complete amino acid sequence of the "median" large peptide (76 amino acids) released from globin of horse myoglobin by cleavage with cyanogen bromide. However, the overlaps between Lys 77 and Lys 78 (see fig. 1) were not determined by classical tryptic and chymotryptic digestions. Because we have found in the tryptic hydrolysates free lysine and the dipeptide Lys-Lys, and in chymotryptic hydrolysates Lys-Lys-Gly-His-His-Glu-Ala-Glu-Leu (peptide : S2E3S1P9) [2], we have deduced the probable sequence – Leu (76) – Lys (77) – Lys (78) – Lys (79) – Gly (80). Commercial chymotrypsin contains residual tryptic activity. Even if the commercial chymotrypsin is previously treated by TLCK *, the residual "trypsin-like activity" splits the lysyl bond between the 77th and 78th residues [1], and makes difficult the characterization of the overlaps. It is known that trypsin (and "trypsin-like activity" of chymotrypsin) does not split peptide bonds involving homoarginine [3,4]. Therefore we have used guanidination to modify the globin. The chemically modified globin is then digested by chymotrypsin (TLCK-treated). The resulting fragments are separated by high voltage electrophoresis and descending chromatography. In this paper, the characterization of a decapeptide (from residues 77 to 86), containing three residues of homoarginine, is described.

* Or 1-chloro-3-tosylamido-7-amino-2-heptanone.

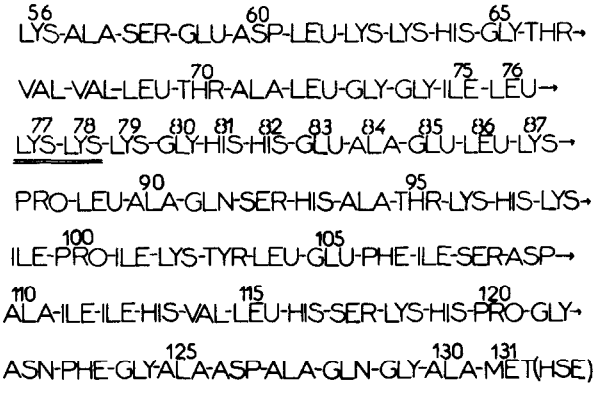


Fig. 1.

2. Experimental

2.1. *Guanidination*

The globine (2 g) was added to a 1 M solution of *O*-methyl-iso-urea of pH 8.0–8.5 and the pH adjusted to 11.0 with 6 M sodium hydroxide. The final concentration of globin was 1% and that of *O*-methyl-isourea 0.5 M. The reaction was carried out at 4°C for 192 hours (8 days) with daily control of pH (11.0). The solution was dialyzed against 0.05 M phosphate buffer, pH 7, for 24 h, then against water (24 h), filtered to remove small amounts of insoluble material and lyophilized. The dried material was treated again with *O*-methyl-isourea for an additional 192 hours (8 days) and dialyzed against buffer (24 h) and lyophilized.

2.2. *Extent of guanidination*

A sample of 5 mg guanidinated globin was hydrolized with constant boiling hydrochloric acid in an

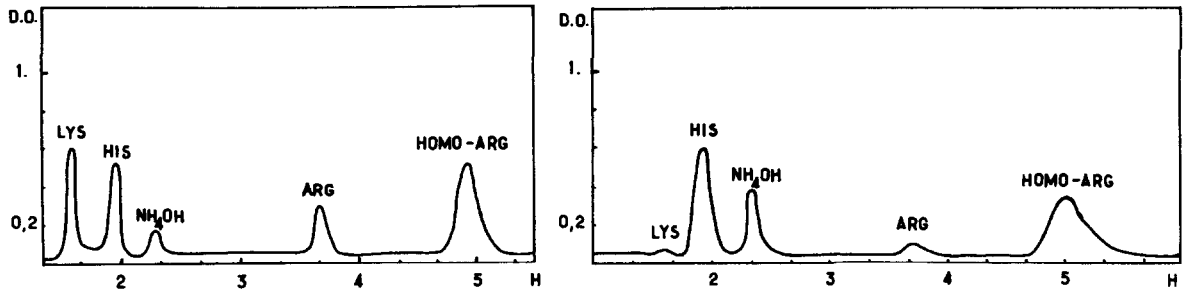


Fig. 2. (a) Standard sample of basic aminoacids. (b) Acidic hydrolysate of guanidinated globin.

evacuated sealed tube at 105°C for 24 hours. Quantitative analysis of basic amino acids were performed on a "Technicon Auto-analyzer" with the short column procedure described by Moore, Spackman and Stein [5]. Reaction of the globin with *O*-methyl-isourea led to a quantitative disappearance of lysine and a corresponding increase of the homoarginine content of the protein (fig. 2).

2.3. *Isolation of chymotryptic peptides*

Experimental conditions of chymotryptic cleavage of guanidinated globin are identical to those for chymotryptic digestion of unmodified protein [1, 6]. Chymotrypsin was previously treated by TLCK [7] (E/S : 1/50 w/w, 38°C during 6 h). The dialysate was concentrated by rotative evaporation and then lyophilized. The lyophilized products were fractionated by high voltage electrophoresis [8] : pH 1.9 (formic acid 8%), 40 volts-cm during 2 h. Each electrophoresis fraction was fractionated by descending chromatography in butanol-acetic acid-water (4 : 1 : 5) (fig. 3).

2.4. *Structural studies*

a) The peptide isolated by electrophoresis and chromatography were systematically hydrolyzed by 5.6 N HCl at 110°C for 24 h. Quantitative amino acid analyses were performed in a "Technicon amino acid Analyser".

b) *N*-terminal groups were determined by the Dansyl technique [8–10].

c) Sequential degradation of peptides : the method of Edman modified by Dopheide et al. [11] was used.

d) Partial amino-acid sequences were determined by a combination of "Dansyl-Edman" techniques [12].

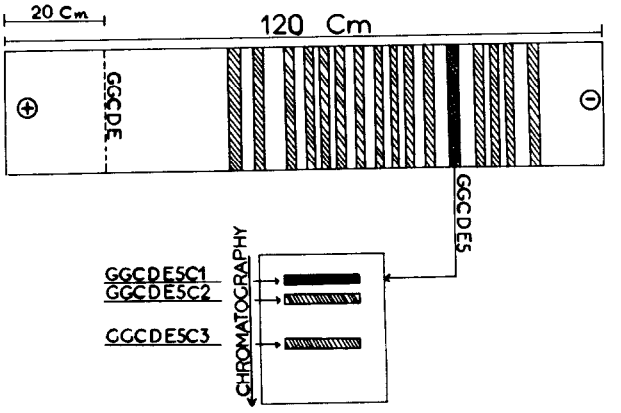
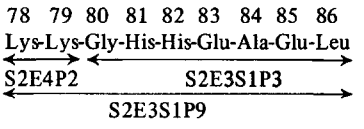


Fig. 3. High voltage electrophoresis.

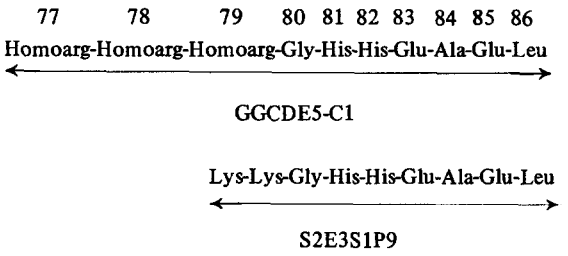
3. Results and discussion

Amino acid composition of the decapeptide GGCDE5-C1 has been determined: Glu(1.88) [2]; Gly(0.94) [1]; Ala(1.54) [1]; Leu(0.93) [1]; His(2.00) [2]; Homoarg(2.68) [3].

This decapeptide contains some impurities as shown by the presence of 0.54 mole of alanine in excess. However the sequence of chymotryptic peptides of unmodified globin can be established without ambiguity:



The guanidinated chymotryptic peptide (GGCDE5-C1) differs from the chymotryptic peptide (S2E3S1P9) only by an additional homoarginine residue. If we set side by side GGCDE5-C1 and S2E3S1P9, we can ascertain amino acid sequence of both peptides.



The sequence of the four amino acids at the *N*-terminal position of peptide GGCDE5-C1 was determined by "Dansyl-Edman" technique. The formation of the peptide GGCDE-C1 could be expected from the specificity of chymotrypsin, namely the cleavage at the carboxyl end of leucine residues; the peptide GGCDE5-C1 is located between Leu(76) and Lys(87) (fig. 1) : therefore, by chymotryptic digestion of guanidinated globin, we have obtained the decapeptide required to establish the overlapping sequence at Lys 77 and 78.

The homoarginine residues in chemically modified proteins are very stable and guanidination of proteins yields soluble derivatives which are very suitable for subsequent enzymatic hydrolysis. This technique may be extensively used for the determination of amino acid sequences of the type -X-$(Lys)_n$-Y-.

References

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