THE PARTIAL AMINO ACID SEQUENCE OF DOG MYOGLOBIN

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1. Introduction

The covalent structure of horse [1], beef [2] and sheep myoglobin [3] has now been determined. The purpose of such a comparative study was discussed in a previous paper [2]; in order to extend this phylogenetical study, we investigated the covalent structure of dog myoglobin and we present in this paper a partial sequence in which 121 of the 153 residues are positioned exclusively on the basis of the isolation and characterization of tryptic peptides.

2. Experimental

Myoglobin was prepared from skeletal muscles of dog by the procedure previously described [4]. The heme moiety was removed from myoglobin molecule and globin denatured by guanidine hydrochloride and ethanol before tryptic digestion [5]; separation of the peptides was achieved by column elution chromatography (130 cm × 0.635 cm) on resin chromobeads P (Technicon) with an increasing gradient of pH and pyridine molarity as described in a previous paper [1]. Some peptides were extensively purified by paper electrophoresis at pH 3.9 or by paper chromatography in n-butanol–pyridine–acetic acid–water (150:100:30:120). Homogeneity of each peptidic fraction was controlled by finger-printing method with these systems. Quantitative amino acid analyses were performed with an automatic amino acid analyzer (JEOL 5 AH); the N-terminal groups of peptides were identified as their dansyl derivatives on polyamide sheets (5 × 5 cm) according to Gray and Hartley [6]. The amino acid sequence of each peptide was determined by the combined dansyl–Edman technique [7]. The amide residues, asparagine and glutamine, were determined by analysis of the amino acids released from peptides by leucine aminopeptidase.

For some peptides, whose paper electrochromatography behaviour, elution pattern on resin chromatography, amino acid composition and N-terminal amino acid proved to be identical with those of corresponding peptides of horse, beef, or sheep myoglobin, the complete sequence determination appeared unnecessary.

Because of a close analogy between all the myoglobin sequences, it was possible to make a tentative alignment of the isolated dog myoglobin tryptic peptides with the general myoglobin sequence as a model.

3. Results and discussion

Table 1 gives amino acid composition and N-terminal residue of each tryptic peptide. Identification and alignment of these peptides was made by analogy with amino acid sequence of other known myoglobins; their amino acid sequence is reported in table 2 by comparison with horse myoglobin. Sequence studies of peptide T4a, T5, T6, T7, T10a, T13, T19 and T20 were not performed because of their close analogy with the corresponding peptides of horse, beef or sheep myoglobin.

A tryptic insoluble core, representing approximatively one quarter of the whole molecule and containing 32 residues remains undetermined at the present time. The cyanogen bromide cleavage of the two methionyl bonds and the thermolysin hydrolysis are now being conducted in order to obtain soluble peptides corresponding to the tryptic insoluble core and overlapping the rest of the peptidic chain.

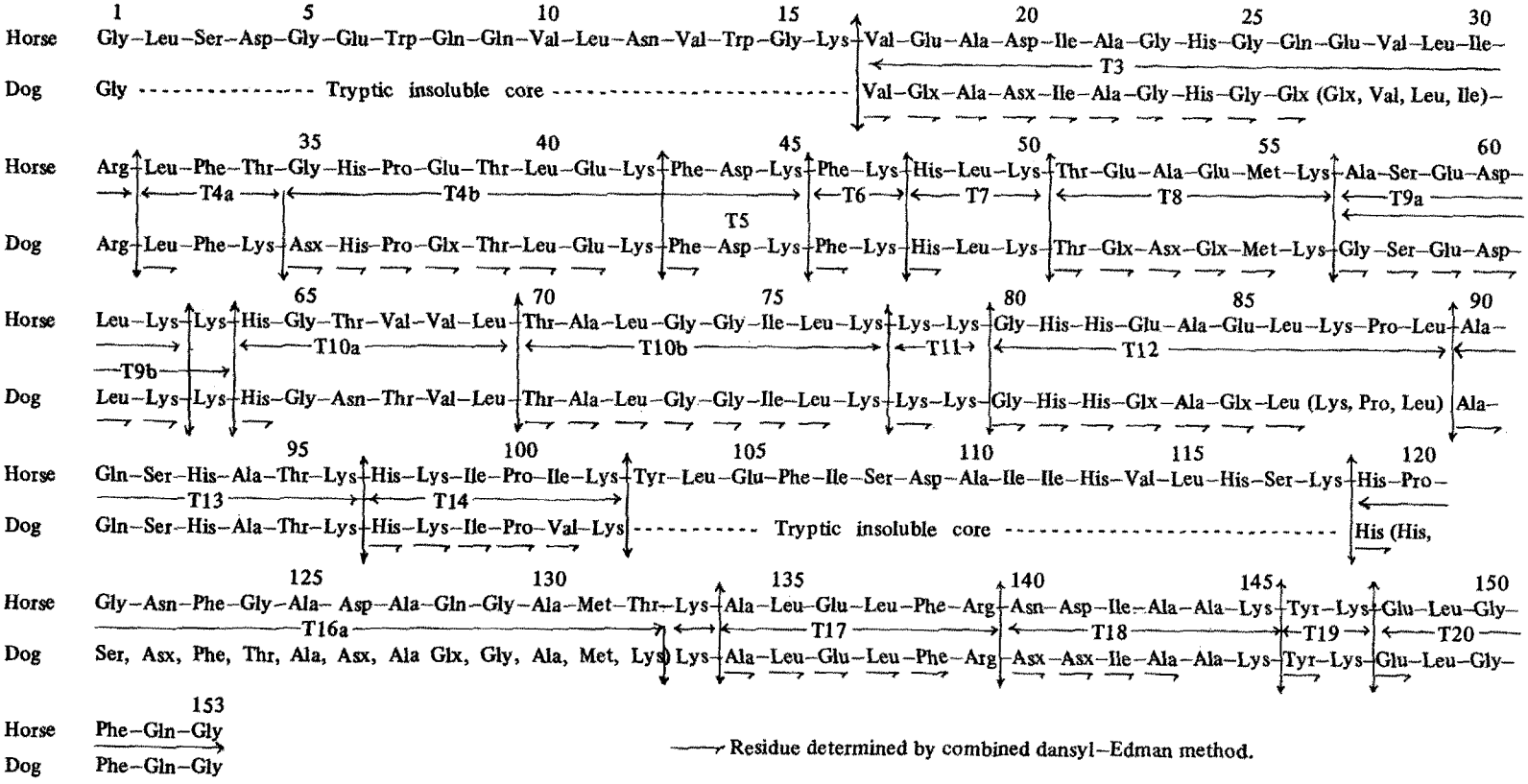
Table 1
Amino acid composition and N-terminal residue of tryptic peptides of dog myoglobin.

	T3	T4a	T4b	T5	T6	T7	T8	T9a	T9b	T10a	T10b	T11	T12	T13	T14	T16a	T17	T18	T19	T20
Lys	–	1.24	1.97	1.14	1.16	1.07	1.19	1.20	2.11	–	1.27	2.00	1.03	1.20	1.82	1.13	–	1.16	1.15	–
His	1.12	–	0.95	–	–	1.13	–	–	–	0.86	–	–	2.09	1.08	0.86	2.10	–	–	–	–
Arg	1.05	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1.08	–	–	–
Asp	1.07	–	2.95	1.01*	–	–	0.96	0.93	1.03*	1.02†	–	–	–	–	–	1.98	–	1.81☆	–	–
Thr	0.98	–	0.99	–	–	–	0.94	–	–	0.90	0.97	–	–	0.93	–	0.96	–	–	–	–
Ser	–	–	–	–	–	–	–	0.87	0.88	–	–	–	–	0.88	–	0.98	–	–	–	–
Glu	2.76	–	1.04	–	–	–	2.05	0.97	1.02*	–	–	–	1.86	1.00†	–	1.06	1.02*	–	–	1.93☆
Pro	–	–	0.90	–	–	–	–	–	–	–	–	–	0.89	–	1.25	–	–	–	–	–
Gly	2.15	–	–	–	–	–	–	1.00	0.95	1.16	2.00	–	1.02	–	–	1.10	–	–	–	2.15
Ala	1.01	–	–	–	–	–	–	–	–	–	1.18	–	1.00	1.90	–	2.94	0.95	1.99	–	–
Val	1.99	–	–	–	–	–	–	–	–	1.06	–	–	–	–	1.03	–	–	–	–	–
Met	–	–	–	–	–	–	0.85	–	–	–	–	–	–	–	–	0.83	–	–	–	–
Ile	0.87	–	–	–	–	–	–	–	–	–	0.80	–	–	–	1.04	–	–	1.00	–	–
Leu	2.02	0.86	1.09	–	–	0.80	–	1.03	0.98	0.99	1.78	–	1.94	–	–	–	1.98	–	–	0.99
Tyr	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.95	–
Phe	–	0.91	0.99	0.95	0.84	–	–	–	–	–	–	–	–	–	–	0.93	0.99	–	–	0.93
Total	14	3	11	3	2	3	6	6	7	6	8	2	10	7	6	14	6	6	2	6
N-terminal residue	Val	Leu	Asx	Phe	Phe	His	Thr	Gly	Gly	His	Thr	Lys	Gly	Ala	His	His	Ala	Asx	Tyr	Glu

* Asp or Glu: identified as the dicarboxylic acid.
† Asp or Glu: identified as the amide.
☆ Asp or Glu: one of which being identified as the amide.

Table 2

Tryptic peptides from dog myoglobin compared with peptidic chain of horse myoglobin.



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