

STRUCTURE OF SOME PEPTIDES ISOLATED FROM CHYMOTRYPTIC DIGEST OF DOLPHIN MYOGLOBIN

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1. Introduction

The covalent structure of crystalline myoglobin prepared from muscle of Black Sea Dolphin (*Delphinus delphis*) [1] is being investigated through different experimental approaches. In earlier studies the heme moiety was removed from the myoglobin molecule and globin was cleaved by trypsin [2], cyanogen bromide [3] and chymotrypsin [4]. The resulting fragments were purified and sequenced. The characteristics of 14 peptides isolated from the chymotryptic hydrolysate have been reported elsewhere [4]. In this paper 17 additional peptides obtained from the same hydrolysate and accounting for 90 amino acid residues are described.

2. Experimental

The chymotryptic cleavage of dolphin globin was described in the previous paper [4]. Individual peptides were purified by paper chromatography in the system *n*-butanol–acetic acid–pyridine–water (30:6:20:24) and by descending paper electrophoresis [5] at 1500 V and pH 5.6. The quantitative amino acid analyses were performed in an Automatic Amino Acid Analyzer (Vývojové dílny ČSAV, Praha). The *N*-terminal groups of the peptides were determined by the Dansyl technique [6]. The *C*-terminal amino acids were liberated by hydrazinolysis and then identified as the corresponding Dansyl derivatives. Detailed information on this procedure has been presented elsewhere [7]. The amino acid sequences of the peptides were determined by a combination of the Dansyl and phenylthiohydantoin tech-

nique [8]. The latter has been modified in our laboratory for the application to short peptides [9]. The purity of the peptides was assayed by end-group analyses and the fingerprint technique.

3. Results and discussion

The quantitative amino acid composition and sequence of the peptides isolated in this study from the chymotryptic digest are given in table 1. In table 2 our results are set side by side with the known primary structure of sperm whale myoglobin.

The results of our study on the chymotryptic digest provide evidence for the existence of 12 sites in which the two myoglobins differ. These differences are identical with those observed in earlier work on the tryptic digest [2] and the cyanogen bromide hydrolysate [3] of dolphin myoglobin and can be accounted for by amino acid interchanges caused by the change of one base in the coding triplet [10].

References

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Table 1
Amino acid composition and structure of peptides isolated from chymotryptic digest of dolphin myoglobin. Tryptophan was detected only qualitatively.

Peptide	Lys	His	Arg	Trp	Asp	Glu	Thr	Ser	Pro	Gly	Ala	Met	Val	Leu	Ile	Tyr	Phe	Structures
1										0.60	1.00			1.10	1.00			Gly-Ala-Ile-Leu
2			+		1.00	1.03	0.97			1.93				1.07				Gly-Leu-Ser-(Asp,Gly,Glu,Trp)
3	1.05				1.00												1.00	Asp-Lys-Phe
4	0.98					1.00								1.07				Lys-Glu-Leu
5	1.90	0.90				2.00	1.00		1.10	0.90				1.00			0.90	Lys-Gly-His-Pro-Glu-Thr.
6	0.89	0.80												1.00				Leu-Glu-Lys-Phe
7	0.90	0.80	+		1.00	1.07	0.88		0.90	1.00			1.00					Lys-His-Leu
8	0.90	0.80												0.90				Asn-Val-Trp
9	0.90	0.80					1.10				1.20							Lys-Gly-His-Pro-Glu-Thr.
10	0.93									1.00								Leu
11					1.00	1.00				1.90	2.94	0.83						Ala-Thr-Lys-His.
12					1.94	1.14				1.92	3.04	0.70						His-Gly
13	2.00	0.91	0.89			1.12		1.14	1.00									Gly-(Ala,Asp,Ala,Glu,Gly,Ala,Met)
14							0.78			1.05						2.00	0.65	Gly-(Ala,Asp,Ala,Glu,Gly,Ala,Met,Asp)
15	2.30	0.90			1.00						1.71						0.89	Lys-(Ile,Pro,Ile,Lys,Tyr)
16	1.74	0.81			1.00		0.78			1.03			0.85	1.00				Ser-(Arg,His,Pro,Ala,Glu,Phe)
17	3.90	1.87			1.20	1.19			0.98	1.17	1.20							Arg-(Lys,Asp,Ile,Ala,Ala,Lys,Tyr)
																		Lys-(Lys,His,Gly,Asp,Thr,Val,Leu)
																		Lys-(Lys,Lys,Gly,His,His,Asp,Ala,Glu,Leu,Lys,Pro,Leu)

Table 2
Comparison of structures of sperm-whale myoglobin (top line) with peptides isolated from chymotryptic digest of dolphin myoglobin (bottom line).

1	10	20
Val. Leu. Ser. Glu. Gly. Glu. Trp. Gln. Leu. Val. Leu. His. Val. Trp. Ala. Lys. Val. Gly. Ala. Asp. Val. Ala. Gly. His. Gly. Gln. Asp.		
Gly. Leu. Ser. (Asp, Gly, Glu, Trp)	Asn. Val. Trp	
30	40	50
.Ile. Leu. Ile. Arg. Leu. Phe. Lys. Ser. His. Pro. Glu. Thr. Leu. Glu. Lys. Phe. Asp. Arg. Phe. Lys. His. Leu. Lys. Thr. Glu. Ala. Glu.		
	Lys. Gly. His. Pro. Glu. Thr. Leu. Glu. Lys. Phe. Asp. Lys. Phe. Lys. His. Leu. Lys. Thr. Glu. Ala. Asp.	
60	70	80
.Met. Lys. Ala. Ser. Glu. Asp. Leu. Lys. Lys. His. Gly. Val. Thr. Val. Leu. Thr. Ala. Leu. Gly. Ala. Ile. Leu. Lys. Lys. Gly. His.		
.Met	Lys(Lys, His, Gly, Asp, Thr, Val, Leu) Thr. Ala. Leu. Gly. Ala. Ile. Leu. Lys(Lys, Lys, Gly, His,	
90	100	
.His. Glu. Ala. Glu. Leu. Lys. Pro. Leu. Ala. Gln. Ser. His. Ala. Thr. Lys. His. Lys. Ile. Pro. Ile. Lys. Tyr. Leu. Glu. Pro. Ile. Ser.		
,His, Asp, Ala, Glu, Leu) Lys. Pro. Leu. Ala. Gln. Ser. His. Ala. Thr. Lys. His. Lys(Ile, Pro, Ile, Lys, Tyr) Leu. Glu. Pro. Ile. Ser.		
110	120	130
.Gln. Ala. Ile. Ile. His. Val. Leu. His. Ser. Arg. His. Pro. Gly. Asn. Phe. Gly. Ala. Asp. Ala. Gln. Gly. Ala. Met. Asn. Lys. Ala. Leu.		
.Glu. Ala. Ile. Ile. His	Ser(Arg, His, Pro, Ala, Glu, Phe) Gly(Ala, Asp, Ala, Glu, Gly, Ala, Met) Asp. Lys. Ala. Leu.	
140	150	
.Glu. Leu. Phe. Arg. Lys. Asp. Ile. Ala. Ala. Lys. Tyr. Lys. Glu. Leu. Gly. Tyr. Gln. Gly		
.Glu. Leu. Phe. Arg(Lys, Asp, Ile, Ala, Ala, Lys, Tyr) Lys. Glu. Leu. Gly. Phe. His. Gly		