PRIMARY STRUCTURE OF *N*-TERMINAL PART OF MOLECULE OF DOLPHIN MYOGLOBIN

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1. Introduction

The partial primary structure of Black Sea dolphin (*Dolphinus delphis*) myoglobin has been determined by Karadžova and coworkers [1] by classical methods. The sequences of amino acids at positions 17–31, 60, and 84–85 have not been established. The difficulties encountered in the sequential investigation of the *N*-terminal part of the molecule of myoglobins can be ascribed to the low solubility of tryptic fragments arising from this part of the molecule.

Another approach to the solution of this problem – the stepwise degradation of amino acid residues from the native molecule of the globin by the phenylisothiocyanate technique – has been proposed by Edman [2]. The automation of the procedure in the amino acid sequenator improved the yield to 98% and thus enabled the amino acid sequence of the 60-residue *N*-terminal fragment of the molecule of humpback whale myoglobin to be determined.

This paper has followed the same line of approach to fill the existing gap in the amino acid sequence at position 17–31 in the molecule of Black Sea dolphin myoglobin and at the same time served as a practical test of the amino acid sequenator built in this laboratory according to Edman's model [2].

2. Material

The main component of dolphin myoglobin was prepared by the procedure of Karadžova and coworkers [3]. The globin of the main component MbI was obtained by precipitation with acidified acetone according to Rossi-Fanelli [4].

Standard samples of phenylthiohydantoins of amino acids were products of Mann Research Laboratories, Quadrol was from Carlo Erba, Italy, heptafluorobutyric acid and silica gel D 5F from Fluka, A.G. The remaining chemicals and solvents were of practical grade purity. All chemicals and solvents were purified according to Edman [2]. Efforts to prepare Quadrol and *n*-propanol free of aldehyde impurities were unsuccessful.

3. Methods

The sequenator was built according to Edman's apparatus [2]. Alterations in the construction are without effect on its individual functions and will be reported elsewhere. The sequence of the program except for the changes mentioned below, the reaction times, and the volumes of the extracting agents were kept within narrow limits as prescribed [2].

The following alterations were made. In the reaction mixture, trifluoroacetic acid was replaced by propionic acid and the extractibility of the buffer was

thus improved. The extraction of the reaction medium by ethyl acetate was repeated twice and thus the sequence of extractions in the first part of the program was: reaction with phenylisothiocyanate, evaporation, extraction with benzene, extraction with ethyl acetate, evaporation, extraction with ethyl acetate and evaporation. The subsequent addition of heptafluorobutyric acid is in accordance with the original program. The repetition of the reaction with heptaflurobutyric acid and the second extraction with butyl chloride, i.e. operations No. 22–28 in the original program [2], were omitted in certain experiments since the interfering effect of the background was minimal due to the low number of degradation steps.

The cyclization of thiazolinones was effected in 30% ethanol, adjusted to pH 1 by hydrochloric acid, for 60 min at 80°. In repeated experiments, the cyclization of the thiazolinone of serine, degradation step No. 3, was carried out in 1 N HCl, for 10 min at 80°, and the yield of the hydantoin of serine was improved.

The identification of the phenylthiohydantoins by thin-layer chromatography on silica gel D 5F in systems D, E, and H has been described by Edman [5]. The phenylthiohydantoins of leucine and isoleucine, degradation steps No. 2, 7, 28, 29, and 30, were distinguished in system D and in the system benzene: heptane, proposed by Schroeder [6]. In order that the maximum resolving power of the latter system be achieved, the ratio of benzene to heptane must be determined experimentally so that $R_{F(\text{PTH-Leu})} = 0.8$. The length of the path of the solvent between the origin and the front was in all systems 10 cm. The phenylthiohydantoin of arginine was identified by thin-layer electrophoresis [2] and by the Sakaguchi reaction.

The average repetitive yield of the degradation was calculated from the yields of PTH-glycine isolated in degradation steps No. 1, 5, 15, and 23.

4. Results

The globin (8 mg) was dissolved in 250 µl of water and applied to the walls of the spinning cup. The protein was lyophilized in 15–20 min. For the subsequent procedure see the 3rd paragraph of Methods.

The degradation of 31 amino acid residues was repeated four times. Individual amino acid residues were degraded in an average yield of 96.5%. Determined amino acid sequence:

Gly-Leu-Ser-Asp-Gly-
1 2 3 4 5

-Glu-Trp-Gln-Leu-Val-Leu-Asn-Val-Trp-Gly-Lys-Val-
6 7 8 9 10 11 12 13 14 15 16 17

-Glu-Ala-Asp-Val-Ala-Gly-His-Gly-Glu-Asp-Ile-Leu-
18 19 20 21 22 23 24 25 26 27 28 29

-Ile-Arg
30 31

5. Discussion

The result of this paper provides additional evidence in favor of the similarity in the primary structures of myoglobins from different aquatic mammals. The structure determined by us here is in agreement with the partial structure reported earlier [1] save for two exceptions. The presence of glutamic acid instead of glutamine at position 26 can be explained by deamination of the material during the degradation, since the globin had been exposed altogether for 13 hr to the effect of alkaline medium before glutamic acid at position 26 was liberated. This explanation is acceptable only on condition that the rates at which individual glutamine residues are deaminated differ by one or several orders. In another experiment the same material was kept 26 hr in the same buffer as that in which the reaction with phenylisothiocyanate takes place and the degradation was begun after this period. The concentration of PTH-glutamic acid derived from glutamine at position 8 was not higher than usual, i.e. 10–15%.

The finding of glycine at position 15 is in disagreement with the earlier report [1] which ascribed alanine to this position. We believe that the structure determined by us is correct because it has resulted from straightforward degradation of the intact globin molecule. A survey of the *N*-terminal parts of the molecules of myoglobins from related aquatic mamals horse and man is shown in table 1. In our opinion, the table deserves interest with respect to the replacement of alanine for glycine at position 15 in different species.

The structure reported in this paper completes the structure of dolphin myoglobin proposed by Karadžova and coworkers [1], as shown in table 2.

Table 1

Position	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Sperm Whale	Val	Leu	Ser	Glu	Gly	Glu	Trp	Gln	Leu	Val	Leu	His	Val	Trp	Ala	Lys	Val	Glu	Ala	Asp	Val	Ala	Gly	His	Gly	Gln	Asp	Ile	Leu	Ile	Arg
Humpback Whale				Asp	Ala							Asn	Ile																		
Harbor Seal	Gly			Asp				His				Asn			Gly				Thr		Leu							Glu	Val		
Porpoise	Gly			Asp					Gln			Asn			Gly						Leu								Val		
Horse	Gly											Asn			Gly						Ile							Glu	Val		
Dolphin	Gly			Asp								Asn			Gly										Glu						
Human	Gly			Asx	(Gly, Glx)				–			Asx			Gly					Pro	(Asp,Ile,Ala,Gly,His,Gly,Glx,Glx)							Val			

A comparison showing the *N*-terminal amino acid frequences of the molecule of myoglobin from sperm whale (*Physeter catodon*) [1], harbor seal (*Phoca vitulina*) [7], humpback whale (*Megaptera nodosa*) [2], porpoise (*Phocaena phocaena*) [7], dolphin (*Dolphinus delphis*) (this paper), horse [8], and man [10]. The structure of sperm whale myoglobin is given in full, the structure of myoglobins from other species are presented only by amino acids in which they differ from the amino acid sequence of sperm whale myoglobin.

Table 2

Val	Leu	Ser	Glu	Gly	Glu	Trp	Gln	Leu	Val	Leu	His	Val	Trp	Ala	Lys	Val	Glu	Ala	Asp	Val	Ala	Gly	His	Gly
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Gln	Asp	Ile	Leu	Ile	Arg	Leu	Phe	Lys	Gly	His	Pro	Glu	Thr	Leu	Glu	Lys	Phe	Asp	Lys	Phe	Lys	His	Leu	Lys
26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50
Thr	Glu	Ala	Asp	Met	Lys	Ala	Ser	Glu	Asx	Leu	Lys	Lys	His	Gly	Asp	Thr	Val	Leu	Thr	Ala	Leu	Gly	Ala	Ile
51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75
Leu	Lys	Lys	Lys	Gly	His	His	Asp	(Ala,	Glx)	Leu	Lys	Pro	Leu	Ala	Gln	Ser	His	Ala	Thr	Lys	His	Lys	Ile	Pro
76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
Ile	Lys	Tyr	Leu	Glu	Phe	Ile	Ser	Glu	Ala	Ile	Ile	His	Val	Leu	His	Ser	Arg	His	Pro	Ala	Gln	Phe	Gly	Ala
101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125
Asp	Ala	Gln	Gly	Ala	Met	Asx	Lys	Ala	Leu	Glu	Leu	Phe	Arg	Lys	Asp	Ile	Ala	Ala	Lys	Tyr	Lys	Glu	Leu	Gly
126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150
Phe	His	Gly																						
151	152	153																						

The present state of knowledge of the primary structure of dolphin (*Dolphinus delphis*) myoglobin. Amino acid sequences 1–31 are from this paper, sequences 31–153 are those reported by Karadžova and co-workers [1].

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