Isolation of Cyanogen Bromide Cleavage Peptides from Myoglobins

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The chemical cleavage of apomyoglobins by the cyanogen bromide procedure [1] has provided fragments suitable for sequence analysis [2, 3] and for studies of physical [4–6] and immunochemical properties [7]. Offord [8] has used a peptide obtained from the cyanogen bromide cleavage of ribonuclease in studies aimed at the semi-synthesis of a protein.

The purpose of this report is to present conditions that reliably separate pure peptide fragments produced from the apomyoglobins of several species. A single gel filtration step obviates the need for additional purification [2, 3, 6]. The evidence reported here concerns specifically the apoprotein of sperm whale *(Physeter catodon)* and California sea lion *(Zalophus californianus)*. The procedure additionally has heen applied [9] without modification to the separation of the cyanogen bromide fragments of apomyoglobin from Amazon River dolphin *(Inia geoffrensis)*, Minke whale *(Balaenoptera acutorostrata)*, gray whale *(Eschrichtius gibbosus)*, Dall porpoise *(Phocoenoides dalli dalli)* and the bottlenosed dolphin *(Tursiops truncatus)*. Cyanogen bromide fragments from two of the variant myoglobins which occur to a minor extent in the sperm whale were also separated by this method [10].

Experimental

The fragmentations were done in a solution of apomyoglobin [11] (100 mg) in 70% formic acid (5 ml) with cyanogen bromide (40 mg) for 24 hours at room temperature under nitrogen. The material, obtained after lyphilization was dissolved in 10% acetic acid (2 ml) and applied to a column (2.45 × 182 cm) of Sephadex G-50 (20–44 microns) equilibrated to 10% acetic acid. Elution of the column with this solvent at a rate of 30 ml/h produced the pattern shown in Fig. 1. Comparison of the amino acid composition of the material isolated from peaks labelled B, C, and D with the composition of the expected peptides derived from the known sequence [12] of sperm whale myoglobin showed unequivocally that these peaks correspond respectively to 56–131 (76 residues), 1–55 (55 residues), and 132–153 (22 residues). Peak A was often highly asymmetric and probably represented aggregated peptides, unreacted peptides, and larger peptides resulting from partial cleavage, *i. e.* the 1–131 and the 56–153. Amino acid analysis of the material from any portion of peak A showed homoserine, homoserine lactone, and no methionine. The column could not cleanly separate the fragments

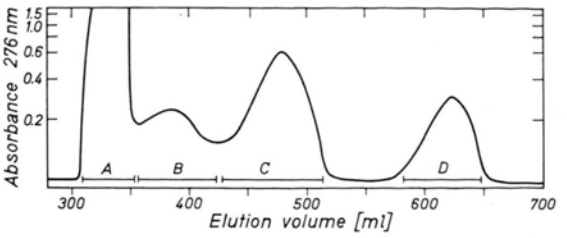

Fig. 1. Elution profile of cyanogen bromide fragments of sperm whale myoglobin.

Table I. Composition of the cyanogen bromide fragments of *Zalophus californianus* apomyyglobin.

Pool	B (56–131)		C (1–55)		D (132–153)	
Residues	Found	Predicted [a]	Found	Predicted [a]	Found	Predicted [a]
Lysine	11.87	12	5.88	6	3.06	3
Histidine	7.4	8	2.88	3		0
Arginine	1.14	1	1.00	1	1.99	2
Aspartic Acid	4.13	4	4.72	5	1.99	2
Threonine	3.52	4	0.96	1		0
Serine	3.52	4	1.70	2		0
Glutamic Acid	6.71 [d]	6	9.08 [d]	9	3.00	3
Proline	2.83	3	.98	1		0
Glycine	6.25	6	6.04	6	1.90	2
Alanine	7.70	8	1.31	1	2.84	3
Valine	1.99	2	3.78	4		0
Methionine [b]	0.3	1	0.3	1		0
Isoleucine	4.80	6	1.70	2	1.01	1
Leucine	8.24	8	8.14	8	3.02	3
Tyrosine	.88	1		0	0.93	1
Phenylalanine	1.92	2	2.62	3	2.05	2
Tryptophan	ND [c]	0	ND [c]	2	ND [c]	0
% Yield	63%		21%		43%	

[a] Composition predicted from the sequence [13, 14].
[b] Calculated as the homoserine lactone.
[c] Not determined.
[d] Homoserine may contribute to the glutamic acid peak.

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produced from samples of apomyoglobin larger than 250 mg. The degree of cleavage appeared to be improved by the inclusion of 0.5 to 1 mg of dithiothreitol in the initial reaction mixture [15].

Table I gives the amino acid composition of the cyanogen bromide fragments from the myoglobin of *Zalophus californianus*. The compositions of these fragments are consistent with the sequence of this protein recently established by Vigna, Gurd and Gurd [13, 14]. The identification of the peptides in other cases has been likewise confirmed by sequence determination [9, 10].

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