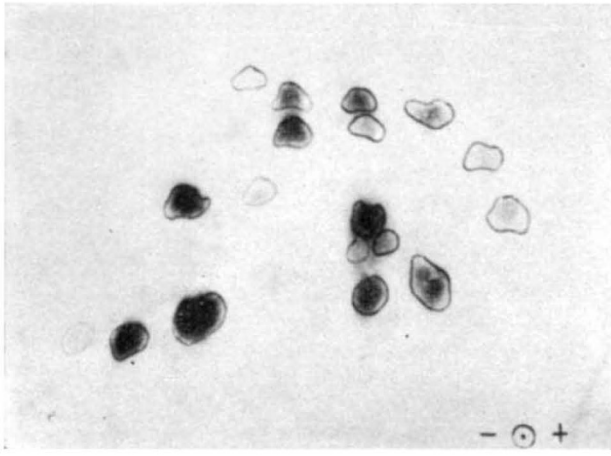
Amino-acid Composition of *Aplysia* Myoglobin

SEVERAL years ago we isolated from the buccal muscles of the common Mediterranean mollusc *Aplysia* a myoglobin which showed a number of interesting features when compared with mammalian myoglobins[1,2].

Preliminary amino-acid analysis indicated that the composition of *Aplysia* myoglobin is quite different from that of mammalian myoglobins, and in particular that there appears to be only one histidyl residue per molecule. This constitutes a particularly interesting feature in view of the role which is attributed to the iron-binding proximal and distal imidazoles in mammalian myoglobin and haemoglobin. In addition, *Aplysia* myoglobin was found to undergo a rapidly reversible thermal denaturation, which has been studied in some detail[3].



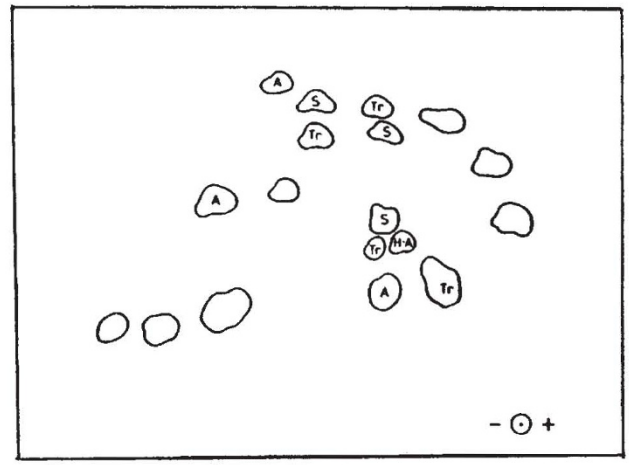


Fig. 1. Fingerprint and fingerprint tracing of tryptic digest of *Aplysia* myoglobin. Code for specific staining test: A = arginine; H = histidine; S = sulphur; Tr = tryptophan.

These peculiar properties, together with the detailed knowledge now available about the structure and physicochemical and functional properties of mammalian myoglobins, stimulated a more complete chemical analysis of *Aplysia* myoglobin. We report here the amino-acid composition and peptide pattern in *Aplysia* myoglobin; the new results complement the old observations and serve as a basis for sequence studies, which are in progress.

Aplysiae (*Limacina*) were obtained from the Zoological Station, Naples. The radulas, isolated from the animals just after capture, were frozen at −20° C and stored at this temperature until the extraction of myoglobin. Myoglobin was purified as described earlier[1]. Amino-acid analyses were carried out using the method of Spackman *et al.*[4]. The tryptophan content was determined by the *N*-bromosuccinimide method of Rao and Cama[5]. After tryptic digestion of the globin peptide patterns were obtained by the method of Ingram, as modified by Baglioni[6]. The sugar content of *Aplysia* myoglobin was analysed quantitatively by the method of Dubois[7] for the non-nitrogenous sugars, and by the method of Rondle and Morgan[8] for the 2-amino sugars.

As in previous studies, the purified *Aplysia* myoglobin was found to be homogeneous by starch gel electrophoresis and by ultracentrifugation. Analysis of the sugars in the purified protein has shown that *Aplysia* myoglobin does not contain non-nitrogenous or 2-amino sugars (sugar content less than 1 per cent).

The amino-acid composition of *Aplysia* myoglobin is reported in Table 1. The new data, which are the average of six separate analyses, are in general agreement with the preliminary ones[2] and confirm the large differences between *Aplysia* and mammalian myoglobins in respect of amino-acid composition. Particularly noteworthy in *Aplysia* myoglobin is the absence of tyrosyl residues and the presence of only one histidine per molecule.

Table 1. AMINO-ACID COMPOSITION OF *Aplysia* MYOGLOBIN

	Amino-acids (g/100 g of protein)	Residues (g/100 g of protein)	Nitrogen (g/100 g of total N)	No. of residues per molecule (molecular weight, 18,000)	No. of residues per molecule (nearest integer)
Lys	10·28	9·02	12·03	13·00	13
His	0·83	0·73	1·34	0·95	1
N-NH_2	1·08		5·74	(12·51)	(13)
Arg	3·81	3·41	7·51	4·06	4
Asp	13·78	11·92	8·85	19·11	19
Thr	1·34	1·13	0·98	1·96	2
Ser	8·47	7·02	6·90	13·58	14
Glu	6·44	5·65	3·72	8·03	8
Pro	4·18	3·53	3·11	6·30	6
Gly	4·90	3·73	5·56	12·02	12
Ala	15·72	12·54	15·07	32·54	33
Cys/2	—	—	—	—	—
Val	7·25	6·14	5·31	10·99	11
Met	2·50	2·20	1·40	3·09	3
I Leu	3·11	2·48	2·01	4·21	4
Leu	7·70	6·65	5·01	10·83	11
Tyr	—	—	—	—	—
Phe	15·27	13·61	7·87	17·04	17
Try	3·88	3·54	2·99	3·94	4
Sub-total	110·54	93·50	95·40		
Heme	2·93	2·93	1·65	1·00	(1)
Total	113·47	96·43	97·05		162

Partial specific volume = 0·734; calculated molecular weight = 17,953·85; nitrogen content (g/mole) = 2,941·68 = 16·38 per cent.

Peptide maps obtained from the tryptic digest are shown in Fig. 1. The number of peptides agrees with the arginine and lysine content of the protein. Only one peptide gives a specific reaction for histidine and this is consistent with the finding that there is only one histidine residue per molecule. Attempts to analyse the amino terminal groups by the D.F.B. method gave no answer. This suggests that the *N*-terminal group is acetylated in *Aplysia* myoglobin. Carboxypeptidases *A* and *B* failed to give any digestion products from either the whole protein or the apoprotein.

LEONARDO TENTORI
GEROLAMO VIVALDI
SALVATORE CARTA

Istituto Superiore di Sanità,
Rome.

ERALDO ANTONINI
MAURIZIO BRUNORI

Institutes of Biological Chemistry of Rome
and Camerino,
CNR Centre for Molecular Biology,
Rome.

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