THE AMINOTERMINAL SEQUENCE OF *DENDROSTOMUM PYROIDES* HEMERYTHRIN

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1. Introduction

The non-heme iron respiratory pigment hemerythrin is found in certain members of the Sipunculoidea, Priapulida, Brachiopoda and Annelida. The pigment has a molecular weight of 107,000 and is composed of 8 subunits. The only sequence information available is for the subunit of the hemerythrin from the sipunculid worm *Golfingia gouldii* [1–4]. We have described earlier the properties of the hemerythrin from the sipunculid *Dendrostomum pyroides* [5] and now report the sequence of the first 35 aminoterminal residues of this protein.

2. Materials and methods

D.pyroides were obtained from Pacific Biomarine Supply Company, Venice, California. *G. gouldii* were from the Marine Biological Laboratory, Woods Hole, Massachusetts. Hemerythrin was prepared as described previously [5] and converted to the apoprotein by the method of Groskopf et al. [1]. Samples of the apohemerythrin (225 nmoles) were sequenced by Edman degradation using a Beckman-Spinco Protein/Peptide Sequencer Model 890. The thiazolinone derivative of each amino acid was converted manually to the corresponding phenylthiohydantoin (PTH) derivative with 1 N HCl for 10 min at 80° [6]. The PTH derivatives were analyzed by gas-liquid chromatography according to the procedures of Pisano and Bronzert [7]. Those residues which could not be analyzed as the PTH derivative were silylated and analyzed by gas-liquid chromatography of the more volatile trimethylsilyl derivatives [7]. All the PTH derivatives were also examined by thin-layer chromatography using two solvent systems. Solvent system A contained chloroform–isopropanol–xylene–propionic acid (30:5:2:1) and system B contained chloroform–formic acid (100:5, v/v) [8].

3. Results

The automated sequence analysis of *Dendrostomum pyroides* hemerythrin gave high yields of the PTH derivatives and allowed unambiguous assignment of the first 35 aminoterminal residues. Fig. 1 shows a typical identification, by gas chromatography, of the PTH derivatives of residues 2 and 29, by their elution time in relation to that of a standard PTH amino acid.

The sequence determined for the aminoterminal region of *D. pyroides* hemerythrin is given in table 1 together with the sequence determined by Klotz and coworkers [1–4] for this portion of the pigment from *Golfingia gouldii*. These sequences are identical except for residues 9, 10 and 11. In the *D. pyroides* hemerythrin the sequence of these residues was determined to be Gly–Trp–Asp compared with the sequence of Val–Asp–Trp reported by Klippenstein et al. [4] for these residues in *Golfingia* hemerythrins. We have previously shown these two sipunculid proteins to have very similar amino acid compositions and peptide maps [5] and it was thought highly unlikely that a change in three consecutive positions in the sequence would occur in proteins which otherwise show so much resemblance. In order to clarify this point, the hemerythrin of *Golfingia gouldii* was subjected to analysis using the automated sequencer in the same manner as described for the *D. pyroides* protein, and carried through 12 cycles of Edman degradation (table 1). The sequence of residues 9–11 in *Golfingia*

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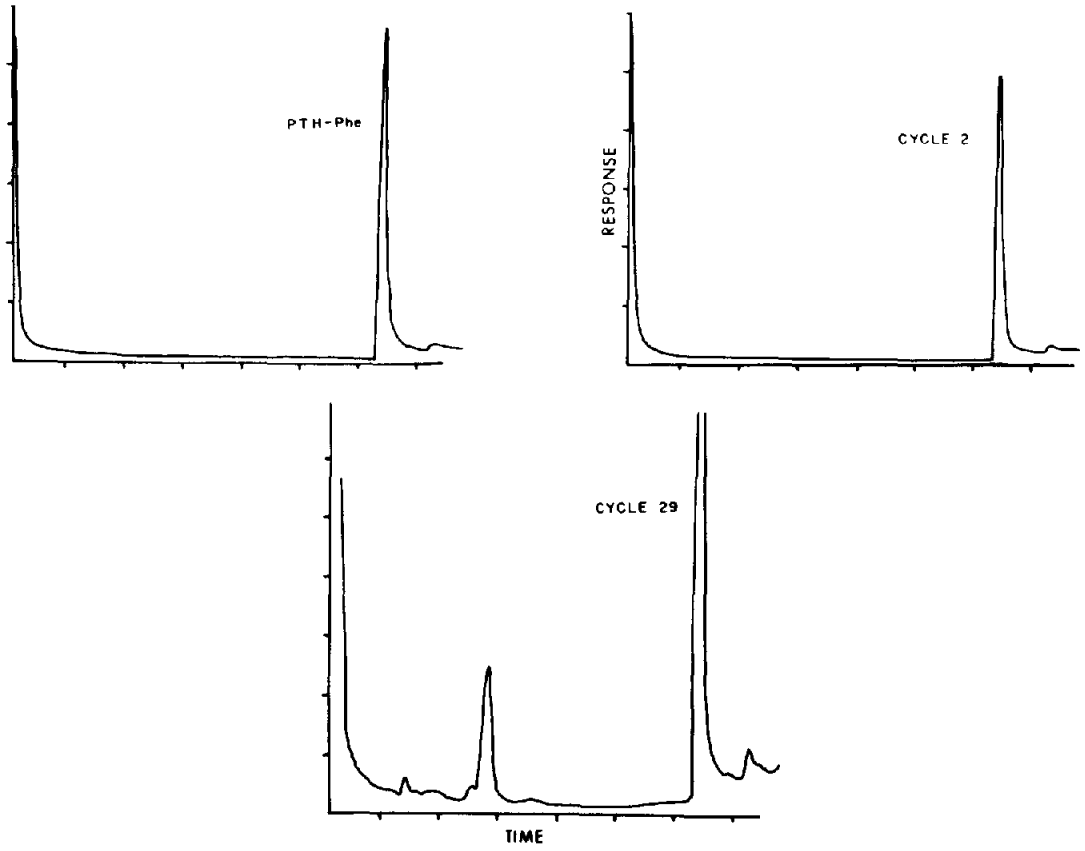

Fig. 1. Identification of PTH amino acid derivative, by gas-ligand chromatography, of residues 2 and 29 by their elution time in relation to that of a standard PTH amino acid.

Table 1
The aminoterminal sequence of *Dendrostomum pyroides* hemerythrin.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Dendrostomum	Gly–	Phe–	Pro–	Ile–	Pro–	Asp–	Pro–	Tyr–	Gly–	Trp–	Asp–	Pro–	Ser–	Phe–	Arg–
Golfingia[a]	Gly–	Phe–	Pro–	Ile–	Pro–	Asp–	Pro–	Tyr–	Val–	Asp–	Trp–	Pro–	Ser–	Phe–	Arg–
Golfingia[b]	Gly–	Phe–	Pro–	Ile–	Pro–	Asp–	Pro–	Tyr–	Val–	Trp–	Asp–	Pro			

	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Dendrostomum	Thr–	Phe–	Tyr–	Ser–	Ile–	Ile–	Asp–	Asp–	Glu–	His–	Lys–	Thr–	Leu–	Phe–	Asn–
Golfingia[a]	Thr–	Phe–	Tyr–	Ser–	Ile–	Ile–	Asp–	Asp–	Glu–	His–	Lys–	Thr–	Leu–	Phe–	Asn–

	31	32	33	34	35
Dendrostomum	Gly–	Ile–	Phe–	His–	Leu
Golfingia[a]	Gly–	Ile–	Phe–	His–	Leu

[a] Data of Klippenstein et al. [4].
[b] Present study.

hemerythrin was found to be Val–Trp–Asp, with conclusive identification of each of these residues by both gas and thin-layer chromatography. It therefore appears that residue 9, which is valine in the *Golfingia* protein, is occupied by glycine in the *Dendrostomum* hemerythrin, a change which is consistent with a single mutation. The present work indicates that residues 10 and 11 are tryptophan and aspartic acid respectively in both *Golfingia* and *Dendrostomum* hemerythrins and suggests that these residues were inverted in the earlier report of the *Golfingia* sequence [2, 4].

Our earlier comparison of peptide maps of tryptic digests of *Dendrostomum* and *Golfingia* hemerythrins showed two peptides in the *Dendrostomum* map which were not present in the *Golfingia* digest. One prominent *Golfingia* peptide was absent from the map of *Dendrostomum* hemerythrin. All other peptides of both hemerythrins appeared to occupy equivalent positions on the peptide maps. The present studies indicate that in the first 35 aminoterminal residues, the *Golfingia* and *Dendrostomum* hemerythrins differ only at residue 9 which is valine in the *Golfingia* pigment and glycine in *Dendrostomum* hemerythrin. Such a change in sequence would not, however, be expected to result in a marked change in the peptide map and suggests that the sequence differences responsible for the peptide map dissimilarities must lie elsewhere than in the aminoterminal regions of the protein. This is borne out by studies on the tryptic peptides of *Dendrostomum* hemerythrin which will be reported elsewhere.

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