J. mar. biol. Ass. India, 1993, 35 (1 & 2) : 131-134

WATER-INSOLUBLE PROTEINS OF THE PERIOSTRACUM OF *PERNA VIRIDIS* AND *MERETRIX CASTA*

D. YAMUNA AND SOWMINI BALAKRISHNAN

Dept. of Zoology, Presidency College, Madras-600 005

ABSTRACT

A study of water—insoluble fraction of proteins of periostracum of *Meretrix casta and Perna viridis* was made. The water—insoluble fractions (alkali soluble and alkali insoluble) were subjected to amino-acid analysis for an understanding of the nature of the proteins. A heterogeneous nature of the proteins was found out from the chromatographic analysis.

INTRODUCTION

THE STRUCTURE and chemical composition of the periostracum of several bivalves were studied by previous workers (Beedham, 1958 ; Hillman, 1964 ; Dunachie, 1963) ; Beedham and Owen, 1964 ; Sowmini, 1970). It is known that the protein of the periostracum has undergone Phenolic-tanning (Hillman, 1964 ; Beedham, 1958) as well as hardening by sulphur-bonding (Sowmini, 1970). From the previous work it is known that the periostracum is formed of a heterogeneous type of protein matrix (Degens *et al.*, 1967). An attempt is made in the present study to find out the nature of proteins that are involved in the hardening as well as tanning processes by chromatographic analysis.

MATERIAL AND METHODS

Material taken for the present study are the shells of *Perna viridis* and *Meretrix casta*. Decalcification of shell was carried out in 8% EDTA (Ethylene diamine tetra acetic acid). From the decalcified shell the periostracum that was separated mechanically were dried, ground to powder and extracted with light petroleum to remove lipid material and then with borate buffer solution of pH 9.2 to remove water soluble proteins (Hackman, 1953). A second protein fraction was obtained by extracting the residue from these purification operations with N-NaOH at 50°C for 5 hours. The insoluble material was then separated by centrifugation and washed with water until the washings were no longer alkaline. The filtrate and washings were brought to pH 3 to 4 with H_2SO_4 ; the solution half saturated with Ammonium sulphate ($(NH_4)_2$ SO_4) and the precipitate separated was collected by centrifugation.

The protein precipitate was resuspended in distilled water, dialysed against distilled water for 2 days, evaporated to dryness in Vacuum extracted with ethanol and ether, to remove lipid.

The residual periostracal material (insoluble in water and dilute alkali) was analysed chromatographically for amino acids, by following the method of Giri and Rao (1952). The standards were also run simultaneously along with the sample. The amino-acids detected in the hydrolysates from both the

fraction (soluble in alkali and the insoluble fraction) are presented in Tables 1 and 2.

TABLE 1. *Amino-acids present in protein fractions in the periostracum of* Perna viridis

Amino-acids	Insoluble in water soluble in Alkali	Insoluble in water and Alkali
Leucine, Isoleucine (1) ..	+	++
Phenyl alanine (2) ..	+	++
Valine (3) ..	+	+
Methionine (4) ..	+	+
Tyrosine (5) ..	+	++
Proline (6) ..	+	+
Hydroxy proline (7) ..	+	+
Glutamic acid (8) ..	—	+
Alanine (9) ..	+	+
Glycine (10) ..	+	+++
Serine (11) ..	+	++
Aspartic acid (12) ..	+	++
Lysine (13) ..	+	++
Arginine (14) ..	+	++
Histidine (15) ..	—	++
Cystine (16) ..	—	++
Cystein (17) ..	+	++
Cysteic acid (18) ..	+	+

Number in parentheses are the code numbers in Fig. 1

\+ Positive (Feeble band)
++ Intense Band
+++ Highly Intense Band
— Negative (absence of band)

TABLE 2. *Amino-acids present in protein fractions in the periostracum of* Meretrix casta

Amino-acids	Insoluble in water soluble in Alkali	Insoluble in water and alkali
Leucine, Isoleucine ..	++	++
Phenyl alanine ..	++	++
Valine ..	++	+
Methionine ..	+	+
Tyrosine ..	+	++
Proline ..	+	+
Hydroxy proline ..	+	+
Glutamic acid ..	+	++
Alanine ..	+	++
Glycine ..	+	+++
Serine ..	+	++
Aspartic acid ..	+	++
Lysine ..	+	+
Argenine ..	+	+
Histidine ..	+	+
Cystine ..	—	+
Cystein ..	+	+
Cysteic acid ..	—	—

\+ Positive
++ Intense Band
+++ Highly Intense Band
— Negative or absence of Band

RESULTS AND DISCUSSION

Although the amino-acid analysis of periostracum of bivalves has been reported by Sowmini (1970), the results reported here are not indicative of the nature of protein fractions present in the periostracum and their role.

A comparison of the protein fractions analysed in *Perna viridis* and *Meretrix casta* in the present study with the results of the amino-acids analysis of periostracum of *Mytilus viridis* by Degens and Spencer (1966) is of interest. These authors used the entire periostracum for the quantitative estimation of the different amino-acid present in it and reported a very high percentage of glycine as well as comparatively sizeable quantities of Tyrosine and Phenylalanine. The occurrence of Glycine and proline in large proportion may be suggestive of the presence in the periostracum of the collagenous protein (Gross and Piez, 1960). The occurrence of Tyrosine may be suggestive of protein containing aromatic amino-acids involved in the tanning of the periostracum. Similarly sulphur-containing amino-acids may be indicative of a protein fraction with organic sulphur. Very

similar results have been reported by Hare (1963) from the amino-acid analysis of *Mytilus edulis* and *Mytilus californianus*. He noted a large percentage of glycine, comparatively less quantity of Tyrosine, Proline and Cystene, containing and the third collagenous in *Perna viridis* and *Meretrix casta* (Fig. 1).

In the periostracum of *Perna viridis* and *Meretrix casta* there is evidence from the

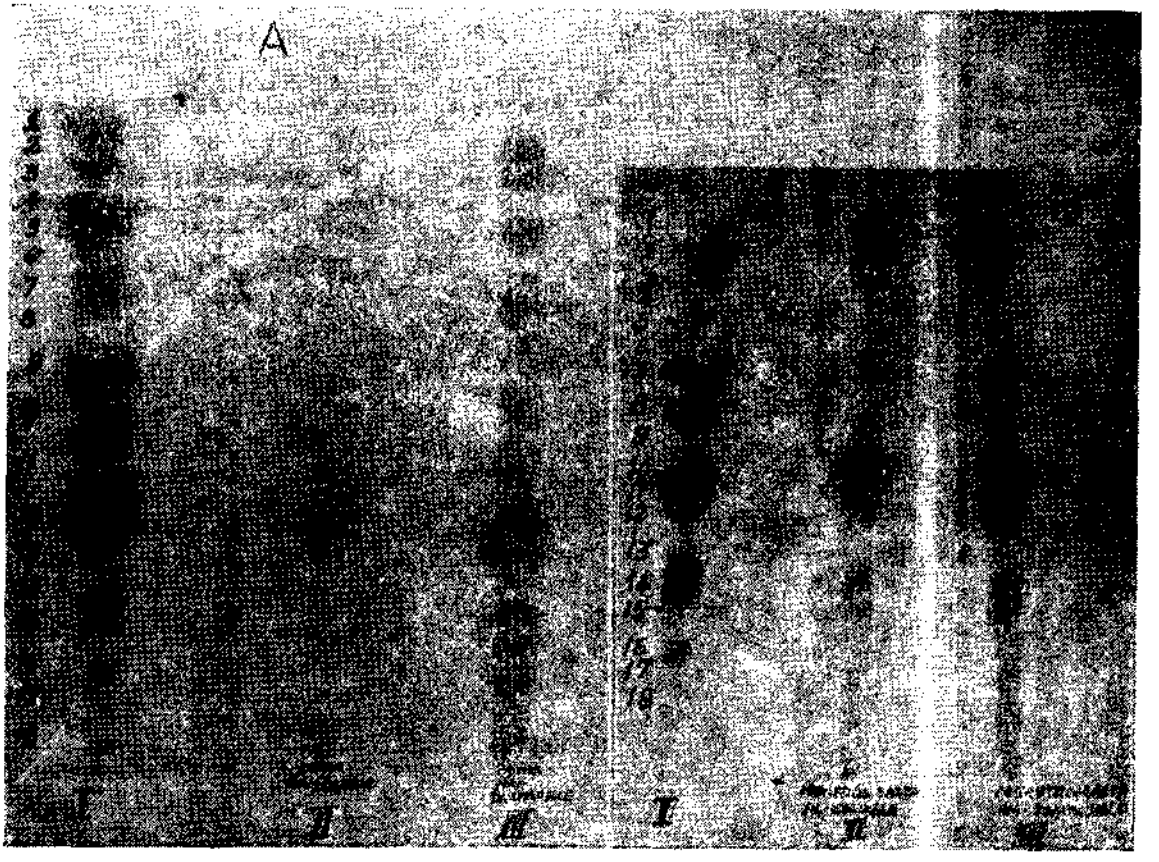

Fig. 1. Protein fractions in the periostracum of: A. *Perna viridis* and B. *Meretrix casta*. I. Mixed standard solution, II. Alkali soluble fraction and III. Alkali insoluble fraction.

It is suggestive that the amino acids reported in the periostracum of *Mytilus edulis* and *Mytilus californianus* support the suggestion of the presence of three types of proteins, a protein rich in aromatic amino acids, another sulphur results of histochemical tests of the presence of more than one protein fraction (Sowmini, 1970). Such occurrence may be correlated with amino-acid analysis.

REFERENCES

BEEDHAM, G. E. 1958 Observations on the non-calcareous of the shell of lamellibranch. *Q. Jl. Microsc. Sci.*, **99** : 341-357.

———— AND K. OWEN 1964. The mantle and the shell of *Solemya parkinsoni* (Protobranchs : Bivalvia). *Proc. Zool. Soc. Lond.*, **145** : 405-430.

DEGENS, E. T. AND D. W. SPENCER 1966. Data file on amino-acid distribution in calcified and uncalcified tissues of shell forming organisms. *Tech. Rep. Woods Hole Oceanographic Inst.*, **66** : 27-32.

——— 1967. Palaeobiochemistry of molluscan shell protein. *Comp. Biochem. Physiol.*, **20** : 553-579.

DUNACHIE, J. F. 1963. The periostracum of *Mytilus edulis*. *Trans. Roy. Soc., Edinburgh*, **65** : 383-411.

GIRI, K. V. AND N. A. N. RAO 1952. Circular paper chromatography I. A. Technique for separation and identification of amino-acids. *J. Indian Inst. Sci.*, **34** : 95-105.

GROSS, J. K. AND K. A. PIEZ 1960. Calcification in biological systems. *Publ. Amer. Assoc., Advancement Sci., Washington*, D.C.

HACKMANN, R. H. 1953. Chemistry of insect cuticle. I. The water soluble proteins. *Biochem. J.*, **54** : 362-367.

HARE, P. E. 1963. Amino-acids in the protein from aragonite and calcite in the shells of *Mytilus californianus*. *Science*, **139** : 216-217.

HILLMAN, R. E. 1964. The functional morphology of the fourth-fold of the mantle of the northern Quanog, *Mercenaria mercenaria*. *Ibid.*, **134** : 1754-1755.

SOWMINI, P. 1970. *Studies on skeletal structures in molluscs (with special reference to periostracum)*. Ph. D. Thesis, University of Madras.