Journal of Thermal Analysis, Vol. 32 (1987) 1265–1270

Short communication

GLYCOLIPOPROTEIN COMPLEX IN THE LOBSTER BRANCHIAL SKELETON

*D. Oviedo Vega, I. Garcia Alonso** and *M. Velasco Villanueva*

INSTITUTE OF CHEMISTRY, ACADEMY OF SCIENCES OF CUBA,
HAVANA, CUBA

(Received August 21, 1986)

A chitin-lipoprotein complex was detected in the pre-exuvial lobster shell. The aqueous extract of this skeleton contains a lipoprotein in its highest molecular weight fraction where the phospholipids constitute the main component. Changes in the lipoprotein crystalline structure with temperature were detected, but not the formation of typical liquid crystals.

The chitin-protein complexes constitute the matrix for the deposition of other substances, e.g. waxes and lipoproteins, which makes the whole structure impermeable [1]. Nevertheless, with the exception of the superficial waxes and carotenoids [2], neither the lipid components of the arthropod cuticle nor their interaction with the chitinous matrix have been extensively studied. The presence of cholesterol in the insect epithelium is known, and the tannizated cuticle is a consequence of the formation of an sterol-protein complex [1]. Some results concerning the changes of the lipid concentration in the integumental tissue of crabs have also been reported, in an attempt to find the connection between them and epicuticle formation [3]. The presence of lipoprotein in the lobster shell was suggested by Travis [4, 5]. Subsequently, a thermal study of the branchial lobster carapace in different moult stages was performed and the probable presence of a glycolipoprotein was found in the pre-exuvial stage [6]. In the chemical and structural characterization of the pre-exuvial lobster shell, lipoproteins were detected in the epicuticle and the exocuticle of their skeleton, but a glycolipoprotein complex was determined only in the last layer [7].

* To whom all correspondence should be addressed 19 971 Vadedó, Ciudad Habana, Cuba

John Wiley & Sons, Limited, Chichester
Akadémiai Kiadó, Budapest

Experimental

The starting materials were the lobster Panulirus argus branchial skeletons in the pre-exuvial and intermoult stages. After the shells had been cleaned, dried and ground, the protein was extracted. A simple extraction was made with water under room-temperature conditions, in order not to affect the general structure. The aqueous protein extracts were then freeze-dried. The delipidation of the pre-exuvial shell was performed as reported by Bihari–Varga [8].

For the thermal analysis, a derivatograph (MOM) was used at a heating rate of 10 deg/min. Calorimetric experiments were performed on a DuPont 910 DSC cell in 10 µl sealed Al pans at a heating-cooling rate of 5 deg/min.

Samples of the aqueous lipoprotein extract were examined with a Zeiss ML polarizing light microscope at heating-cooling rates of 1–3 deg/min.

Column chromatography was performed on Sephadex G–100, with ammonium bicarbonate (pH 8) as running buffer at a rate of 0.25 ml/min. Fractions of 3 ml were collected. The protein was detected via the absorption at 270 nm, while its concentration was determined by the Lowry method [9]. Protein electrophoresis was performed in polyacrylamide gel (acrylamide 10%, *N,N*-methylene-bis(acrylamide) 2.6%) with Tris-glycine buffer (pH 7.9). Lipoprotein electrophoresis was carried out in cellogel strips with Tris-barbital-EDTA buffer (pH 8.8). The strips were stained with Red oil (0.5%) [10].

The total lipids were determined by the method of Whatman and Price [11], the triglycerides by the method of Foster and Dunn [12], and the total cholesterol by the Pearson method [13]. For phospholipid determination, an extraction with Boor solution was performed. Sulphuric acid (4.5 *N*), perchloric acid (60%) and hydroquinone were added after evaporation. The absorbance measurements were made at 650 nm.

Results and discussion

Differences between the aqueous extracts isolated from the carapaces in the pre-exuvial and intermoult stages were detected in the results of protein electrophoresis (Table 1), column chromatography (Fig. 1) and thermal behaviour (Fig. 2). The

Table 1 Electrophoretic mobility in polyacrilamide gels

Extract from	Electrophoretic mobility	
Intermoult skelet.	0.90	0.96
Pre-exuvial skelet.	0.75	0.88

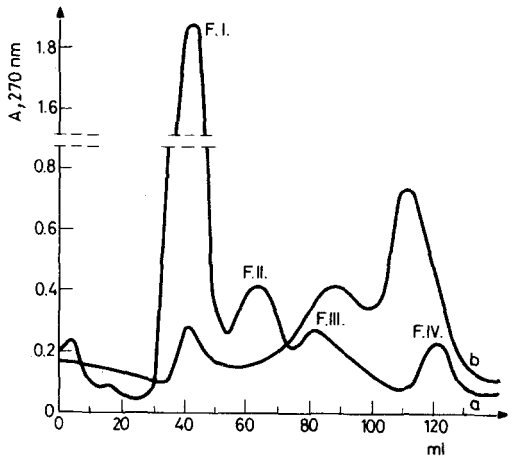

Fig. 1 Column chromatographic results. a) pre-exuvial aqueous extract; b) intermoult aqueous extract

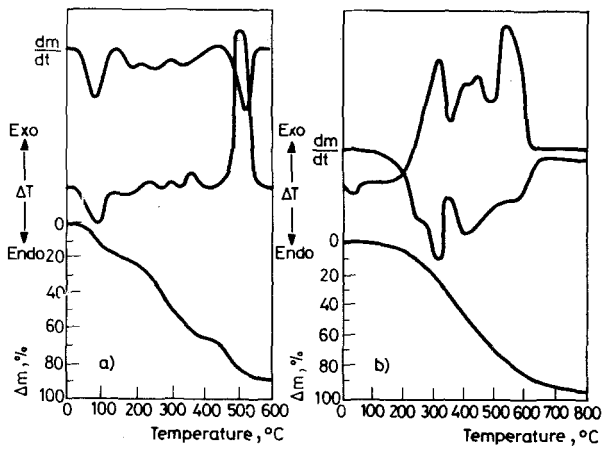

Fig. 2 DTA, DTG and TG curves of a) intermoult aqueous extract; b) pre-exuvial aqueous extract

Table 2 Lipidic composition of the pre-exuvial shell crude aqueous extract and its fractions

Sample	T. lipid, mg/ml	Phospholip.,		Cholesterol,		Triglycerid,		Lipop., test
		mg/ml	rel%	mg/ml	rel%	mg/ml	rel%	
Crude ext.	8.50	3.82	45	1.23	14	1.44	17	+
Fract. I	3.50	1.63	46	0.60	17	0.48	13	+
Fract. II, III, and IV	0	0	0	0	0	0	0	–

highest protein concentration in the pre-exuvial stage was found in the highest molecular weight fraction, and the lipoprotein electrophoresis was positive only for the extract relating to the pre-exuvial moult stage. Furthermore, the intermoult extract showed peaks at 260 and 520° in the DTG curve, while the pre-exuvial one was characterized by an inflection at 260°, followed by a peak at 320°.

Thermal transitions at 18.0, 33.5 and 37° were observed in the DSC curve for the crude water-soluble lipoprotein (Fig. 3). In addition, the sample showed birefringence and a crystalline structure dependent on temperature (Fig. 4). Nevertheless, the lobster shell lipoprotein does not seem to form liquid crystals, as a difference from serum lipoprotein [14].

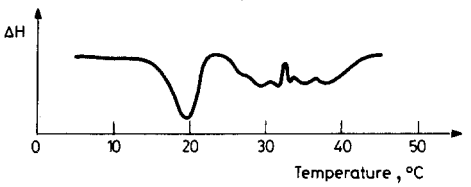

Fig. 3 DSC curve of pre-exuvial lipoprotein extract

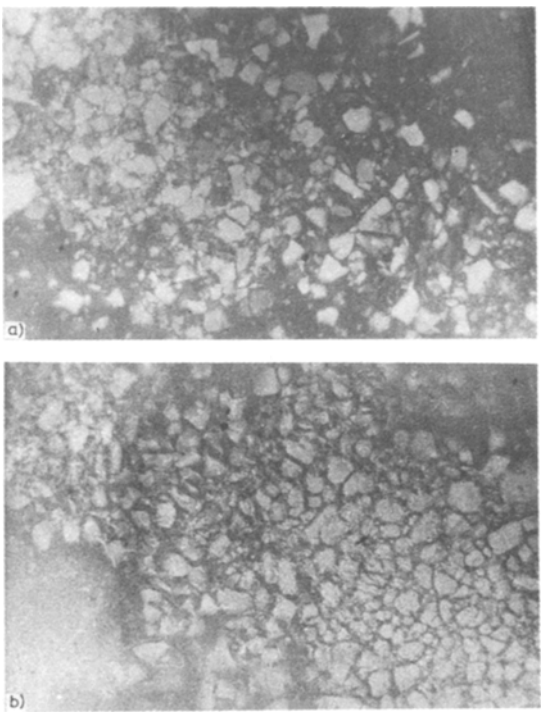

Fig. 4 Pre-exuvial lipoprotein extract. Polarizing light microscope. a) 22 °C; b) 80 °C

Since only cholesterol has been reported in the study of other crustaceans [3, 4], it is important to point out that the phospholipids constitute the main component in the chromatographic fraction and in the original extract.

The existence of glycolipoproteins in the original lobster cuticle reflects the form of the naturally existing lipoproteins.

Thermal analysis of the carapace showed that the DTG peak at 310° is again the most important one after lipid extraction with ether:ethanol (Fig. 5), while

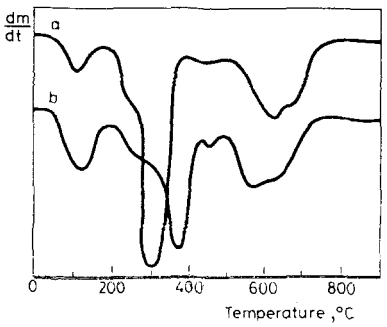

Fig. 5 DTG curves of pre-exuvial shell a) before delipidation; b) after delipidation

phospholipids and glycolipids were detected as the main components in the organic solvent phase [15]. The incorporation of lipoprotein into the polysaccharide matrix seems to confer a higher thermal stability on the system. Analogous results have been obtained by Bihari–Varga on atherosclerotic aortas [8].

The results support the role of chitin in lipid deposition during shell formation.

* * *

The authors express their gratitude to the Lipid Laboratory researchers of the Enrique Cabrera Hospital, and to Dr. M. Bihari–Varga and coworkers for their help in the lipoprotein characterization.

References

1 R. A. A. Muzzarelli, Enzymatic synthesis of chitin and chitosan. In: Chitin. Ed. Pergamon Press, 1977. p. 23.
2 D. L. Fox, Comp. Bioch. Physiol., B–44 (1973) 953.
3 R. Dennel, Integument and exoskeleton. The physiology of crustacea. Vol. 1, Ed. A. P. Waterman, 1960, Ch. 14.
4 D. Travis, Biol. Bull., 108 (1955) 88.
5 D. Travis, Biol. Bull., 113 (1957) 451.
6 A. I. García et al., Rev. Ciencias Químicas, Vol. 16, no. especial, 1985.
7 A. I. García, et al., Characterization of the lobster pre-exuvial branchial shell (not published).
8 M. Bihari-Varga, Acta Biochim. et Biophys. Acad. Sci. Hung., 3 (4) (1968) 375–383.

9 D. R. Lowry, Biol. Chem., 193 (1951) 265.
10 S. T. Norenberg, Electroforesis. Manual Práctico de Laboratorio. Barcelona 1968, p. 80.
11 D. D. Woodman et al., Clin. Chem. Acta, (1972) 38.
12 K. Foster, Clin. Chem., 19 (2) (1973) 338.
13 S. Pearson, Anal. Chem., 25 (1953) 813.
14 M. Bihari-Varga, Int. J. Biol. Macromol., 5, (1983) Vol. 5, 59.
15 V. D. Oviedo et al., Biol. Inf. Cient. IQBE N° 1 (1981) 43.