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Ultrastructural analysis of the integument during the moult cycle in *Ligia italica* (Crustacea, Isopoda)

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Abstract. The formation of the cuticle was investigated during moulting of the isopod crustacean *Ligia italica*. The intermoult cuticle is a four-layered lamellar structure composed of chitin-protein fibrils and mineralized in its upper half. The distribution of calcium carbonate in cuticle during moult cycle was determined by cytochemical methods and X-ray microanalysis. Epi-and exocuticle are secreted during premoult. Calcium is resorbed from the old cuticle and accumulates in the ecdysal gap as calcium granules. Endocuticle is secreted after moult when the mineralization of exocuticle starts. The shape and ultrastructure of epithelial cells change during cuticle secretion and mineralization. Mitochondria, bundles of filaments, calcium granules and large amounts of glycogen accumulate in the apical cytoplasm of cells in premoult animals.

Key words: Isopods–Moult cycle–Cuticle–Mineralization–X-ray microanalysis–Calcium granules

Introduction

Frequent moulting of isopod crustaceans results in permanent synthesis of the cuticle. The main cuticular components are chitin, proteins and amorphous calcium carbonate. The process of cuticle secretion and mineralization in moulting isopods was studied by several authors [1,2]. It has been shown that resorption of calcium in premoult, its storage in the sternal deposits and its mobilization in posmoult are important adaptations of isopods to the terrestrial way of life.

The ultrastructural analysis of the sternal calcium deposits in *Porcellio scaber* has shown that the deposits consist of an opaque region built by spherules and a translucent layer composed of an organic matrix and amorphous calcium carbonate [3,4]. Very little is known about the distribution and the dynamics of calcium fluxes between the cuticle and epithelium during different phases of moult cycle.

We have analyzed the sternal integument during the moult cycle in order to follow the secretion of cuticle and the pathways of calcium in the cuticle and epidermis. Ultrastructural features of epidermal cells related to cuticle secretion and calcium translocation during moulting were determined. The ultrastructural location and the pathways of calcium ions in the integument of premoult and intermoult animals were investigated by means of a X-ray microanalyser system attached to a scanning electron microscope.

Materials and methods

Experimental animals

The specimens of amphibious isopod *Ligia italica* Fab. from Adriatic coast of Slovenia were maintained in laboratory and inspected daily for the accurate determination of the phase of moult cycle.

Ultrastructural analysis

Scanning electron microscopy. Small pieces of the anterior sternal integument were taken by dissecting animals in different phases of the moult cycle. The samples were fixed in 3.5% glutaraldehyde in 0.2 M cacodylate buffer for 2 hours at 4°C, rinsed in buffer and postfixed in 1% osmium tetroxide. After dehydration through a graded series of ethanols the tissue was critical-point dried, sputter-coated with gold and examined with a Jeol 840A scanning electron microscope.

Cytochemical detection of calcium. Samples were fixed in 3.5% glutaraldehyde in 0.2 M cacodylate buffer for 45 minutes and rinsed in distilled water at pH=8. They were incubated in 5% lead acetate for 20 minutes at 37°C and postfixed in 1% osmium tetroxide in veronal acetate buffer at pH=7.2. Control samples were decalcified with 0.5 M EDTA for 30 minutes before incubation [5]. Some samples were fixed with the OsFeCN method and stained en bloc with uranyl acetate [6]. After dehydration tissue was embedded in Spurr. Non-contrasted ultrathin sections were examined with a Zeiss EM 9-S2 electron microscope.

X-ray microanalysis. Pieces of sternal integument were immersed in liquid nitrogen and dried by liophilization.They were mounted on aluminium stubs and examined by means of a Link eXL-10 microanalytical system attached to a Jeol 840A scanning electron microscope. Analyses were performed at 10 kV with a counting time of 100 sec.

Results and discussion

Ultrastructure of integument and location of non soluble calcium salts during moult cycle

The moult cycle of isopod crustaceans has been divided into four phases, pre-, intra-, post- and intermoult respectively. The intermoult integument in *Ligia italica* consists of simple epithelium covered by a four-layered cuticle as shown in Fig.1. The epicuticle is composed mainly of lipoprotein and is not mineralized, preecdysal exocuticle is a pigmented chitinous layer with fibers arranged helicoidally and calcium salts in the spaces between fibrils. The postecdysal lamellar endocuticle with pore canals is

calcified in its outer part. The inner part of the endocuticle and the membranous layer are not calcified. In early premoult the cuticle separates from the epithelium (apolysis), the calcium is resorbed from the old cuticle and accumulates at its base and in the ecdysal gap (Figs.2,3). In intramoult a new double-layered cuticle is secreted. Figs.4 and 5 show electron dense granular structures in epidermal cells and in the ecdysal gap. The size and the shape of granules corresponds to calcospherules described in *Porcellio scaber* [4].The epithelial cells contain large amounts of glycogen and bundles of filaments which presumably play an important role in the secretion of the new cuticle and the organic matrix of calcium deposits.

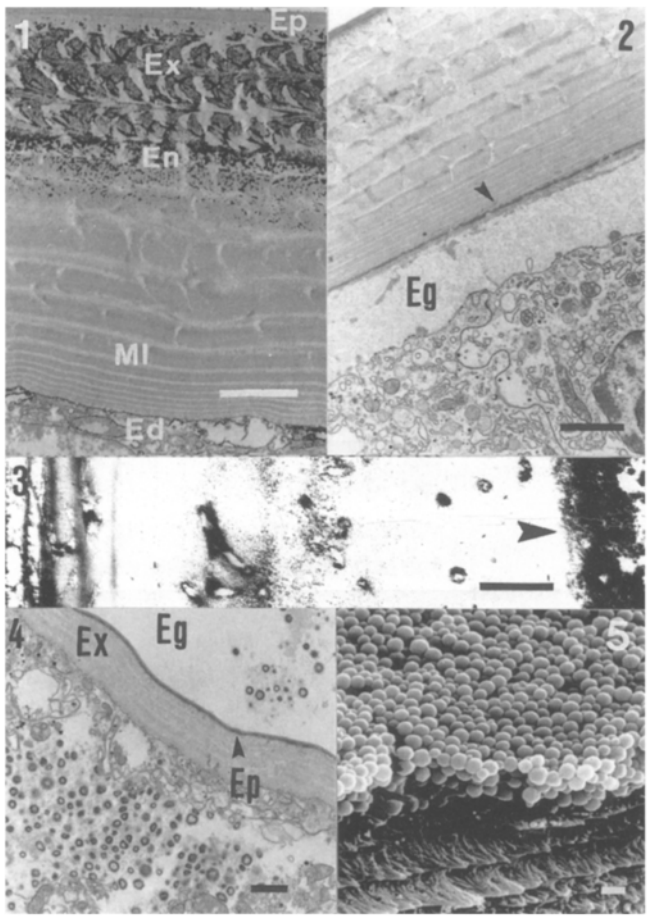

Fig.1 Intermoult sternal integument in *Ligia italica,* incubated in lead acetate. Electron dense precipitates show the distribution of calcium in exocuticle (Ex) and outer endocuticle (En). Scale bar 2μ. Ep-epicuticle, Ml-membranous layer, Ed-epidermis

Fig.2 Premoult sternal integument in *Ligia italica* stained with uranyl acetate *en bloc.* Accumulation of calcium at the base of the cuticle (arrowhead) facing the ecdysal gap (EG) after apolysis. Scale bar 2μ.

Fig.3 Intramoult cuticle in *Ligia italica* incubated in lead acetate. Electron dense deposits at the base of cuticle (arrowhead) mark the resorption of calcium and its accumulation in the ecdysal gap. Scale bar 1μ.

Fig.4 Intramoult sternal integument in *Ligia italica* stained with uranyl acetate *en bloc.* The new cuticle consists of electron dense epicuticle (Ep) and lamellar exocuticle (Ex). Electron dense granules accumulate in the cells and in ecdysal gap (EG). Scale bar 1μ.

Fig.5 SEM micrograph of the basal part of the cuticle facing the ecdysal gap in intramoult *Ligia italica.* Note granules (arrowhead) accumulated at the surface. Scale bar 1μ.

X-ray microanalysis of pre- and intermoult integument

X-ray spectra of anterior sternites in intermoult *Ligia italica* revealed that the exocuticle is mineralized with calcium carbonate whereas epithelial cells contain large amounts of Cl and small amounts of Ca ions (Figs. 6a,b). In premoult animals Ca and Cl ions are present at the base of the cuticle and in the ecdysal space (Fig.6c). X-ray spectra of granules which accumulate in the ecdysal gap revealed the presence of calcium as well (Fig.6d). These results support the ultrastructural observations (Figs.1,3,5) and help to follow the pathway of calcium through integument during the moulting of *Ligia italica*. Calcium is resorbed from the cuticle prior to exuviation and accumulates in the ecdysal gap as granules composed of amorphous calcium carbonate. In the intramoult phase which follows posterior exuviation, calcium is transported to epithelial cells and/or intercellular spaces and further to the haemolymph for the mineralization of the posterior exocuticle. These findings are consistent with the results on calcium transporting epithelia in the posterior caeca of amphipods [7].

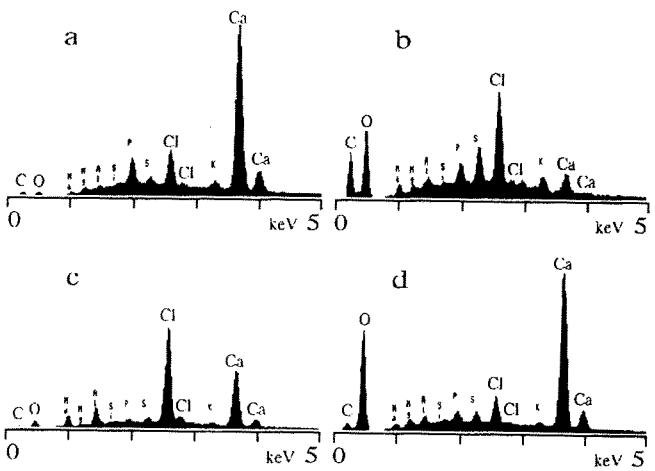

Fig.6 X-ray spectra from intermoult sternites a) exocuticle; b) epithelial cells c) the base of the premoult cuticle; d) granules in the ecdysal gap.

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