Spherules and nanotubules involved in elaboration of crustacean cuticle during the molt cycle: A correlative TEM-AFM study

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Frequent biphasic molting is a unique feature of terrestrial isopod crustaceans. In intramolt animals the anterior part of the body is still covered with both cuticles, while the old cuticle of the posterior part is shed and the new cuticle calcifies. The intramolt stage is convenient for structural analysis of pre- and postecdysal cuticles of the same animal. Resorption of minerals from the cuticle and storage of calcium in ventral parts of the body during premolt is an important adaptation of isopod crustaceans to terrestrial lifestyle. In Porcellio scaber the sternal deposits are composed of amorphous calcium carbonate and calcium phosphate. Calcium spherules are formed by aggregations of nanogranules in a specialized aggregation zone [1]. In our previous work we presented evidence of calcium transport in the integument of intramolt Ligia exotica [2]. In this study we describe the structure and distribution of spherules and nanotubules in premolt Ligia italica and intramolt Ligia exotica by correlative TEM and AFM microscopy.

Small pieces of cuticle were fixed in 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1M cacodylate buffer, postfixed in 1% OsO4, dehydrated and embedded in a mixture of Araldite and Epon. Ultrathin sections were imaged with CM 100 electron microscope and the block face of the same sample was analysed by AFM.

In premolt animals numerous spherules surrounded by electron-dense ecdysal matrix are formed by the resorption of material from disintegrating lamellae of the old endocuticle (Fig.1 a). The size of the spherules composed of concentric layers with electron dense deposits is about 0.5 μm. Fine electron-dense deposits are also present in the pore canals of the new exocuticule, at the microvillar projections of epithelial cells and in the intercellular spaces (Fig.1 b). The AFM micrographs of the same sample show that the spherules are loaded with harder material from the ecdysal matrix, as progressing from the basal lamellae of the old cuticle through ecdysal space towards the surface of the new cuticle (Figs.1c, d). The ecdysal matrix close to the old cuticle exhibits harder texture compared to softer texture of the rest of the matrix. Spherules attached to the new epicuticular layer exhibit a hard granular texture. The granular material is concentrated in the intercellular spaces and in the exocuticular pore canals and it is finely dispersed in the cytoplasm and nuclei of epithelial cells. Spherules are loaded with calcium resorbed from the old cuticle and transported to the new cuticle where it enters the epithelium through paracellular and transcellular mechanisms. X-ray spectra of anterior tergites show high concentrations of calcium and phosphorous in the old endocuticle and ecdysal space of premolt animals and in the new exocuticle of intramolt animals. Nanotubules with a diameter of 20-25μm which connect the spherules and new epicuticule are abundant in the ecdysal space of intramolt animals (Fig.2 a, b). Immunocytochemical localization of tubulin was confirmed in the epithelial cells but not in the ecdysal space. The origin and role of the nanotubules and the structure of ecdysal matrix involved in the transport and nucleation of calcium remains to be investigated.

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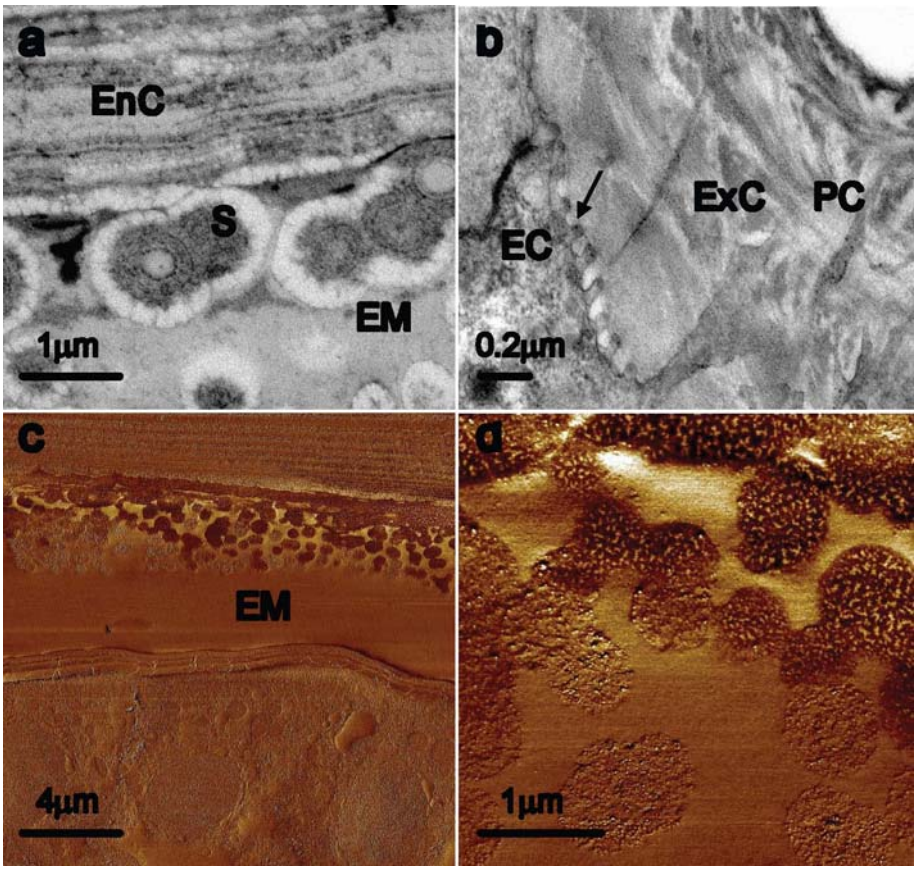

Figure 1: Integument of anterior tergites in premolt Ligia italica. a) Disintegration endocuticle (EnC) and formation of spherules (S) surrounded by ecdysal matrix (EM). Epithelial cells (EC) with microvillar projections (arrow) secrete new exocuticle (ExC) wit network of pore canals (PC). c) Distribution and structure of spherules surrounded by ecdy matrix (EM) of different texture. d) Structure of spherules with softer and harder textures.

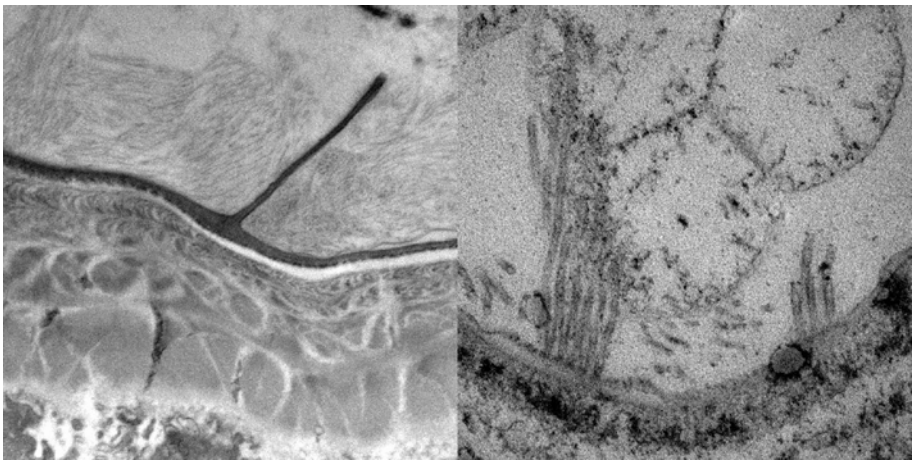

Figure 2: Integument of anterior tergites in intramolt Ligia exotica. a) Nanotubules (NT) in the ecdysal space (ES) close to the new cuticle (C). b) Nanotubules (NT) connect the spherules (S) and the new epicuticle (EpC).

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