Nanoachitecture of the crustacean cuticle – visualization and analysis by a combined use of TEM, AFM and light microscopy

N. Žnidaršič[1], N. Matsko[2], M. TušekŽnidarič[1], W. Grogger[2], F. Hofer[2], J. Štrus[1]

1. Department of Biology, Biotechnical Faculty, University of Ljubljana, Večna pot 111, 1000 Ljubljana, Slovenia
2. Research Institute for Electron Microscopy (FELMI), Technical University of Graz, Steyrergasse 17, A8010, Austria

nada.znidarsic@bf.unilj.si
Keywords: cuticle, exoskeleton, TEM, AFM

The arthropod cuticle or exoskeleton is a complex extracellular matrix, secreted by a singlelayered epithelium. Cuticle performs numerous functions, including support, prevention of desiccation, protection against pathogens and predation and sensing. From the structural point of view crustacean cuticle is a very complex hierarchically structured biological composite material, consisting of chitinprotein fibers, minerals (mostly calcium) and lipids.

The results of recent studies addressing cuticle structure suggest that it is more complex as previously assumed and that classical interpretations need to be refined [1]. In addition, there is only limited data available about cuticle formation and differentiation, including mechanisms governing the organization of the chitinprotein network. One of the problems in resolving the complex and dynamic cuticle nanoarchitecture is accurate visualization and localization of all its constitutive parts – the carbohydrate chitin, different proteins and minerals in different phases of cuticle cycle – during formation and differentiation, in the 'steady state cuticle' and in degrading old cuticle of molting animals.

We propose a seashore amphibious crustacean Ligia italica (Crustacea: Isopoda) as an appropriate model to study cuticle formation, differentiation and resorption, due to the following reasons: animals are small and easily reared in the laboratory, they molt every two weeks and their cuticle is thin and soft enough to be prepared for different microscopic techniques without additional treatments, like decalcification. In this study we present a successful implementation of transmission electron microscopy (TEM), atomic force microscopy (AFM) and light microscopy to visualize and analyse the newly secreted cuticle, the ecdysal space between the new and old cuticle and the decomposing old cuticle. Samples of cuticle with underlying tissue were aldehyde fixed and embedded in Araldite/Epon resin. Ultrathin sections were imaged with TEM and the corresponding block face was analysed with AFM. Combination of TEM and AFM analyses enables us to get the information about the detailed ultrastructure, composition and mechanical properties of the sample in the nanometer range [2]. In addition, this approach is very convenient for deciphering dynamic processes like molting, where the structural and physicochemical properties change over short distances. By inspection of sequential semithin and ultrathin sections we can exactly determine the place of interest to be additionally analysed by AFM. Molting of dorsal body segments (tergites) involves gradual detaching of the old cuticle, accompanied by widening of the ecdysal space between the old and newly forming cuticle (Figure 1). Structural and chemical characteristics of both cuticles and ecdysal space differ distinctly according to the progress of molting process. Imaging of this area is of particular importance and interest, as ecdysal space is an unique extracellular compartment in which extremely dynamic processes of degradation, resorption and transport towards the forming cuticle occur during molting [3, 4]. In the same molting tergite different stages of cuticle detachment are observed: (i) closely

DOI: 10.3217/978-3-85125-062-6-325,

apposed old and new cuticles, (ii) slightly detached old cuticle and spherules in the ecdysal space (Figure 2) and (iii) wider ecdysal space separating the old degrading cuticle from the newly forming cuticle. Ultrastructure, composition and mechanical properties of all components involved can be successfully investigated at any required place by a combined TEM and AFM approach.

1. D. Raabe et al., Materials Science and Engineering A, **421** (2006) p143-153.
2. N.B. Matsko, Ultramicroscopy, **107** (2007) p95-105.
3. A. Ziegler, Micron, **36** (2005) p137-153.
4. J. Štrus et al., this conference

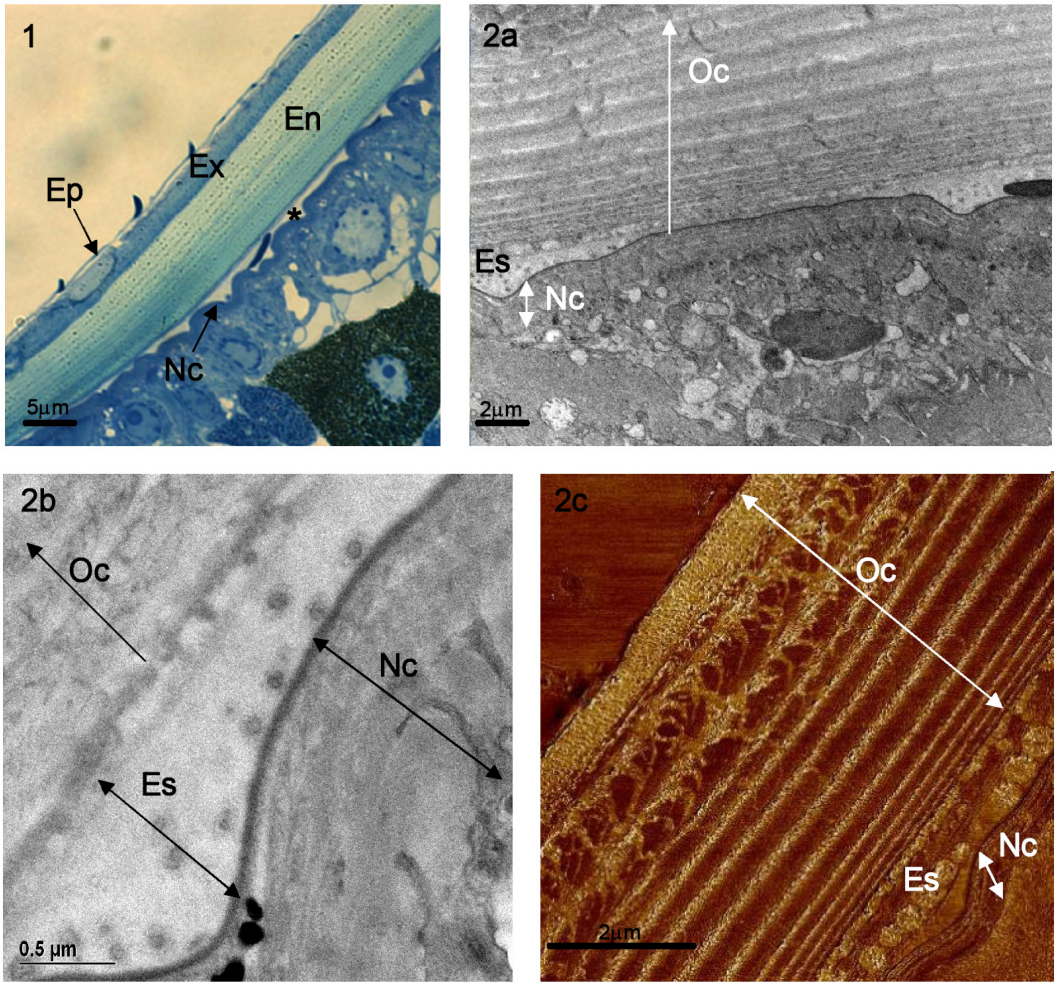

Figure 1. Sagittal section of the molting tergite showing the old detaching cuticle composed of epicuticle (Ep), exocuticle (Ex) and endocuticle (En); new cuticle (Nc) and ecdysal space (*).

Figure 2. TEM (2a, b) and AFM (2c) imaging of the place where the old cuticle (Oc) is slightly detached and the narrow ecdysal space (Es) contains spherules forming at the internal surface of the degrading cuticle, transferring constituents towards the newly secreted cuticle (Nc).

M.A. Pabst, G. Zellnig (Eds.): MC2009, Vol. 2: Life Sciences,
DOI: 10.3217/978-3-85125-062-6-325, © Verlag der TU Graz 2009