XRD, TEM, HRTEM AND SAED investigation of the morphology and structure of the sea hare species *Aplysia punctata*

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Transmission electron microscopy (TEM), SAED and HRTEM were used in order to find and locate starting biominerals [1] in the early embryos of the sea hare species *Aplysia punctata.* The former results obtained by scanning electron microscopy (ESEM) with energy dispersive X-ray analysis (EDS) showed morphological changes during development of numerous (about 40 in each capsule) embryos incorporated in a jelly-like egg-string immediately after deposition on the substratum (in a form of a spaghetti-like tissue) until hatch of the veliger larvae [2]. X-ray diffraction patterns of *A. punctata* embryos confirmed that the very first aragonite crystals appeared 12 days after egg-strings deposition. Fully formed aragonite crystals [2] were recorded in a further stage of embryo developments even before hatching, which occurred 23 days after deposition.

The same series of powdered samples of egg-strings and embryos were also used for TEM, SAED and HRTEM investigation. A special attention was paid to the sample of 9 days old embryos (sample C4 in Figures 1 and 2) that appeared amorphous in XRD (Figure 1d). During observation of the amorphous area A by TEM, the appearance of the precipitation of first grains of a nanocrystalline phase was marked, showing a diffraction effect in SAED. This precipitation of the first nucleated phase was induced by the electron beam in the amorphous phase. Three separate regions were analyzed in order to give confidence of appearance of crystallographic phases. At higher magnification (area of 100x100 nm^2), after disappearance of the amorphous area, layers of monocrystalline dolomite in [010] orientation were noticed by SAED (Figure 2). In all regions faint nanocrystalline diffraction rings of calcite or aragonite were observed.

It appears from our observation and measurements by SAED that in *A. punctata* biomineralization process starts by formation of the dolomite crystals which serve as centers of crystallization for further aragonite deposition in the larval shell.

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3. The aid in ESEM observation of Prof. V. Bermanec is gratefully acknowledged.

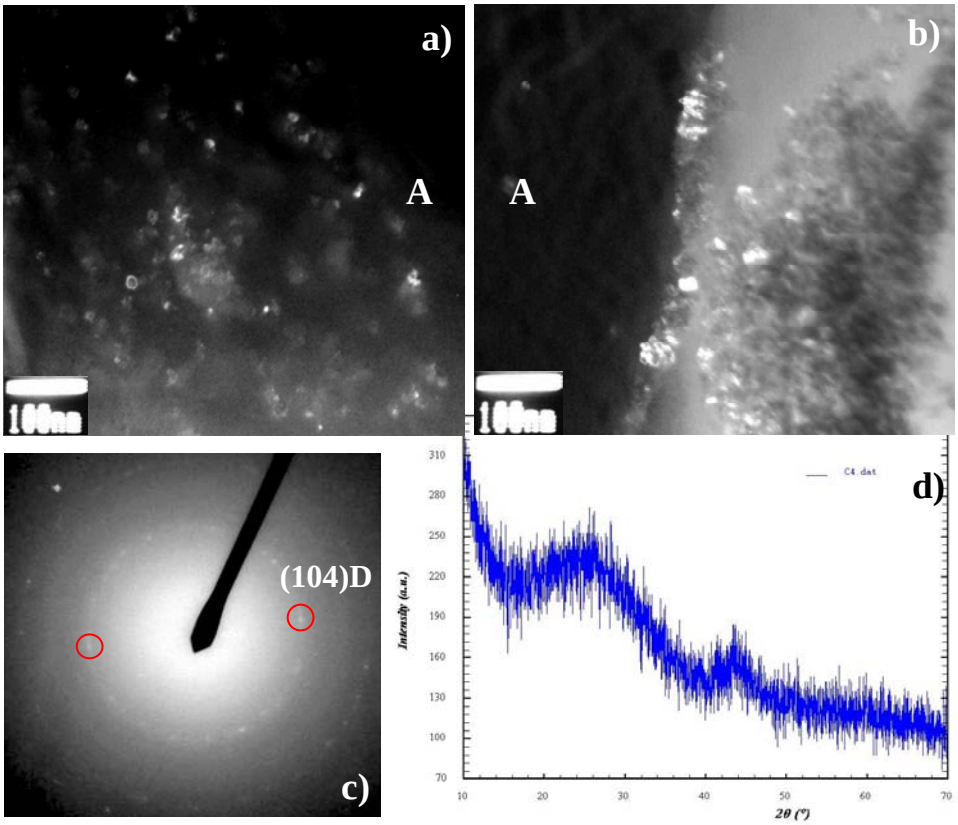

Figure 1. **a)** and **b)**: Dark field micrographs taken with (104) diffraction spot of dolomite, D, from the SAED (c). The appearance of the first crystalline phase with grain sizes smaller than 10 nm, from amorphous region A; (d) XRD pattern of sea hare *Aplysia punctata*, sample C4.

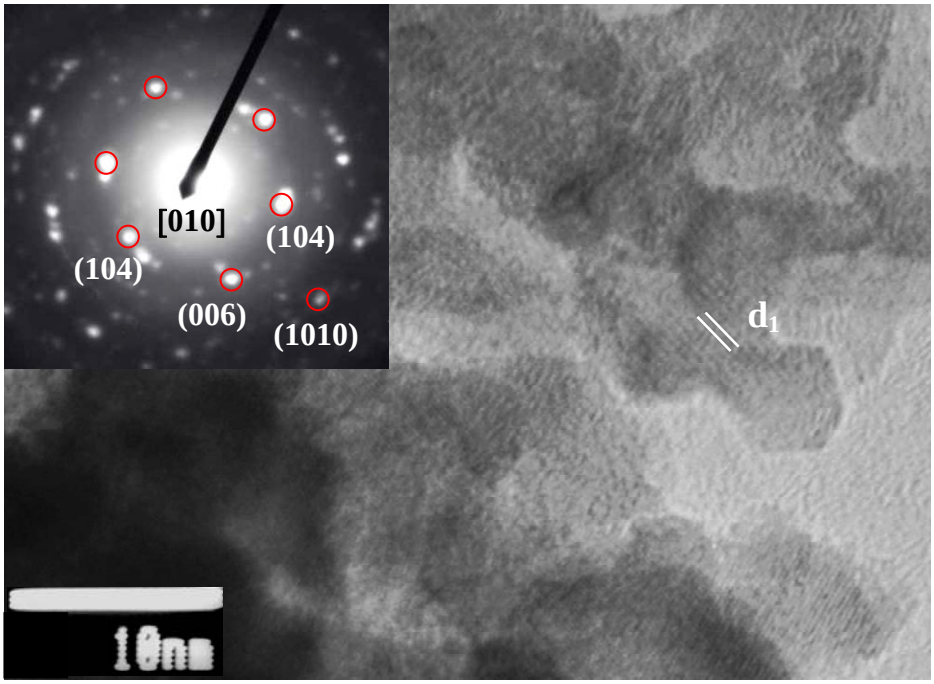

Figure 2. HRTEM image of Aplysia punctata, C4, with corresponding SAED pattern (inset) in [010] dolomite orientation. (104) dolomite lattice fringes with spacing 0.289 nm are marked d_1.

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