Detection of calcium ions in the mantle epithelium of the abalone *Haliotis tuberculata*

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Abalone shells consist of 95wt% [1] $CaCO_3$ which is present as calcite respectively aragonite. Consequently for shell growth as well as for shell repair calcium ions are necessary. To reach the extrapallial space, where the shell is built, calcium ions are transported via the mantle epithelium.

In this work we investigate the path calcium ions take across the mantle epithelium in the abalone *Haliotis tuberculata*.

Small pieces of the mantle epithelium have been prepared using the tannic acid-antimonate method [2,3]. The antimonate binds to calcium ions and creates precipitates with large atomic number in the tissue. Ultrathin sections of the mantle epithelium have been investigated using transmission electron microscopy (TEM) and scanning transmission electron microscopy (STEM). In the STEM image in Figure 1 heavy roundish precipitates are visible. The larger precipitates (diameter ~300nm) are mainly located in the connective tissue (not visible in Figure 1), whereas the smaller ones (diameter <100nm) are located between the microvilli at the border of the epithelial cells.

To prove, that the precipitates really contain calcium ions, electron energy loss spectroscopy was performed in STEM mode. Figure 2 shows an EELS spectrum, which was taken at a larger precipitate (diameter ~300nm) located besides a nucleus of an epithelial cell. Additionally to the carbon K-edge at 284eV, the calcium L-edge occurs at an energy loss of 346eV. The signal of the smaller precipitates (diameter <100nm) located mainly between the microvilli is too low to create a distinct calcium signal.

For this reason the sections were treated with ethylenediaminetetraacetic acid (EDTA), which builds chelate complexes with calcium ions and can be used to dissolve calcium ions out of the embedded tissue. The comparison of an untreated and EDTA treated (100mM EDTA for 30min) section shows that most of the precipitates are dissolved (Figure 3). Therefore it is obvious, that the electron dense precipitates contain calcium ions.

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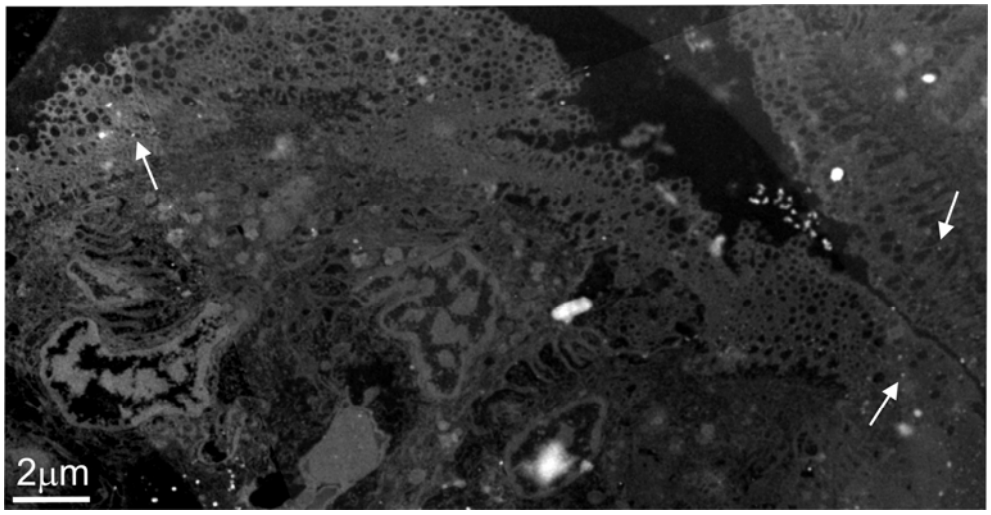

Figure 1. STEM image of epithelial cells of *Haliotis tuberculata*. The smaller precipitates (arrows) are mainly located between the microvilli.

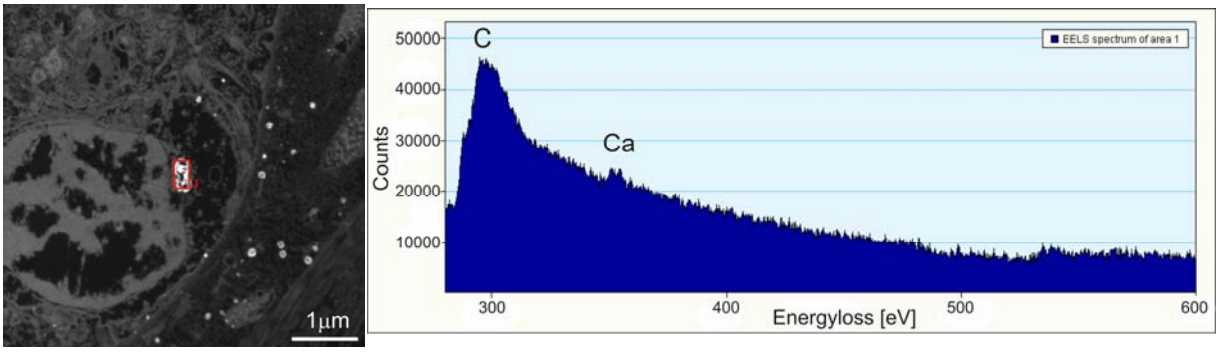

Figure 2. STEM image of an epithelial cell of *Haliotis tuberculata*. The EELS spectrum has been taken at the enframed precipitate. Additionally to the distinct carbon edge at 284eV, a calcium edge occurs at 346eV.

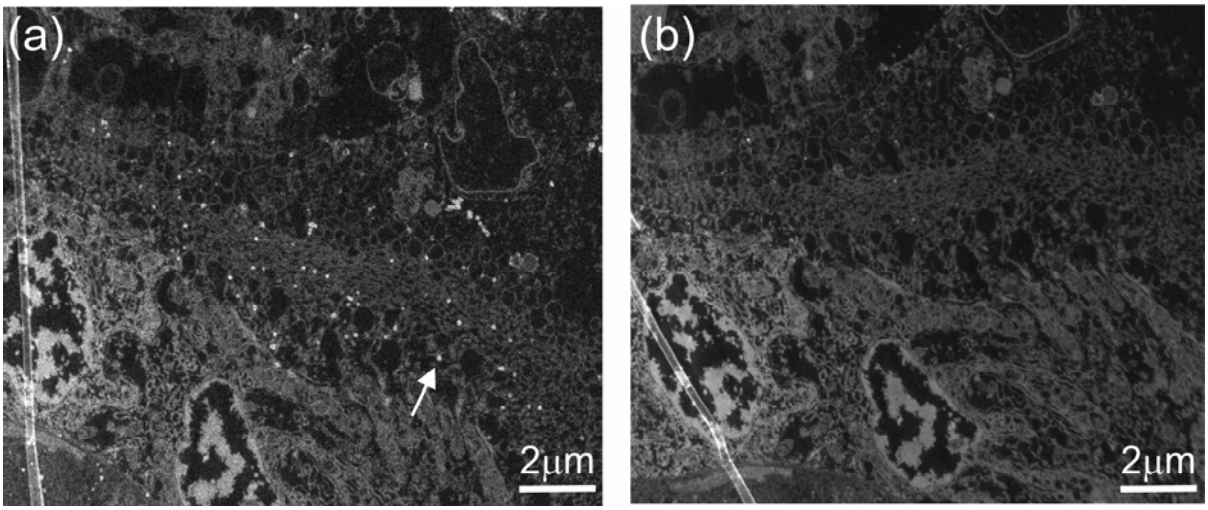

Figure 3. Comparison of an untreated (a) and an EDTA treated (b) section of epithelial cells of *Haliotis tuberculata*. The EDTA causes a dissolving of precipitates containing calcium ions. The arrow marks an antimonate-calcium-precipitate.

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