INSTITUTIONEN FÖR BIOLOGI OCH MILJÖVETENSKAP

Calcium transport in the Pacific oyster, *Crassostrea gigas*

- in a changing environment

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Dissertation Abstract

Pacific oyster, *Crassostrea gigas,* is globally one of the most important farmed bivalve species. A prominent features of the *C. gigas* is the thick $CaCO_3$ shell covering the body of the animal and protecting it from the environment. To be able to produce the shell, the oysters need to take up calcium from the environment and transport it to the shell forming area. The mantle tissue, separating the rest of the body from the shell, is suggested to be of central importance for both uptake of calcium and its transfer to the shell. The final part in this route is the transfer of the ion across the outer mantle epithelium (OME). The Ca has been suggested to be transferred across the OME in one or more of the following forms: as ionic calcium (Ca^{2+}), as calcium bound to proteins or inorganic ligands, as $CaCO_3$ inside vesicles or cells in the hemolymph. The uptake of Ca and other ions for the shell formation, as well as the conditions affecting the calcification process, are dependent on external conditions such as salinity, temperature and pH. As climate change has predicted to change these conditions in the future, also the shell formation of oysters might be affected.

In this thesis, the uptake and transport of calcium from the environment to the shell forming area in *C. gigas* were investigated. Calcium uptake and transport in the hemolymph were analysed by exposing the oysters to water containing radioactive calcium after shell regeneration had been induced through an artificial cut, to accelerate shell formation. The uptake and transport of calcium in the different hemolymph fractions and mantle tissue were then followed. The transfer of calcium ions across the OME was investigated *in vitro* using live OME mounted in specialized Ussing chambers. The kinetics of the Ca^{2+} transport was assessed as were the effects of pharmacological tools inhibiting selected potential Ca^{2+} transporters and channels. Additionally, the mantle genome was searched for these potential ion transporters and channels. The expression of the proteins as well as their cellular localisation in the OME, was confirmed by immunohistochemistry and western blot. Finally, effects of a dilute environmental salinity on the OME ion transfer as well as on the mRNA expression of potential Ca^{2+} transporters and channels were examined

In *C. gigas* calcium was taken up from the environment and transported in the hemolymph mostly as Ca^{2+}. The transfer of Ca^{2+} across the OME consisted of a passive, paracellular component and a transcellular, active transport component. A combination of physiological and functional studies, transcriptome analysis and protein expression analyses through immunological methods made it possible to postulate a model for Ca transfer across the OME of *C. gigas*. The Ca was transferred following two pathway: 1) 60% was transcellularly transported and entered the OME cells through basally located voltage-gated Ca channels (VGCCs) and was then excreted across the apical membrane by Ca^{2+}-ATPases (PMCA) and Na^+/Ca^{2+}-exchangers (NCX), the latter using the Na^+ gradient created by a basal NKAs to function. 2) the remaining 40% was diffusing across the OME through the paracellular pathway. Ionic Ca^{2+} transfer, total active ion transport and paracellular permeability all decreased when *C. gigas* were exposed to diluted seawater (50%). The pattern of changes in mRNA expression of Ca transporters and channels in the OME cells suggest that the cells are trying to compensate for the decreased Ca levels in the diluted seawater. Expression of intracellular Ca-ATPases (SERCAs), transporting Ca^{2+} into intracellular stores decreases, while membrane bound Ca^{2+} channels and NCX mRNA expression increases. These changes suggest that the cells strive to maintain a high enough intracellular Ca^{2+} concentration to achieve a sufficient Ca^{2+} flow across the OME for shell growth. However, as the Ca^{2+} transfer across the OME decreased when exposed to 50 % seawater, these compensatory mechanisms were not sufficient. Overall, these results indicate that the oyster *C. gigas* may face problems with shell calcification in areas where the salinity of the seawater have been predicted to decreaseas a result of current climate changes.

Keywords: Climate change, Ussing chambers, calcium uptake, sodium/potassium ATPase, calcium ATPase, sodium/calcium exchanger, calcium channel, hemocytes, mantle epithelium