Calcification Model Overview:
Fundamental mechanisms on the shell formation in bivalve *Anodonta cygnea*

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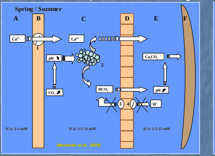
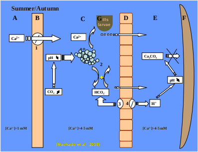
Introduction

The freshwater bivalves Anodonta cygnea live in a hypotonic environment with low calcium concentration (≈1 mM) and so an active transportation of the calcium ion into the body should occur followed by storage on transient calcium carbonate and calcium phosphate microspherules resulting from the balance of the internal pH and calcium ion contents in the body fluids. These internal calcareous reserves provide a continuous supply of calcium and carbonate ions to the shell. The calcification of the shell is a regulated and complex process, in which calcium carbonate gets embedded in an organic matrix consisting mainly of soluble and insoluble compounds. Both organic and inorganic components of the shell must be secreted or transported by the mantle into the extrapallial fluid, which contacts the shell surface in bivalves. The regulation of the extrapallial fluid composition and thus of shell formation is mainly due to the outer mantle epithelium (OME) activity. An early hypothesis for calcification of the shell required active transport of calcium from the haemolymph to the extrapallial compartment in the mantle. However, more recently, our studies (Coimbra et al. 1988, Machado et al. 1988, Lopes-Lima et al. 2008) showed that a high transepithelial permeability of A. cygnea OME cells to calcium ions combined with a calcium gradient towards the shell and other parameters favor a high calcification rate in the spring-summer period.



(Machado *et al.*, 2008)



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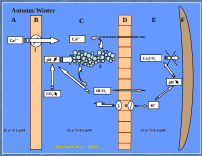

(Machado *et al.*, 2008)

Discussion

The CO_2 is the major component of the pH-buffering system in bivalve fluids showed relevant seasonal pCO_2 changes in the haemolymph of A. cygnea. These results associated with other from our previous work are summarized in a physiological model, about the shell biomineralization, with an emphasis on the acid-base influence on the mantle compartment. The haemolymphs CO_2 content increase during spring/summer while they decrease during autumn/winter inducing a respiratory acidosis or a respiratory alkalosis situation, respectively. The respiratory acidosis can be a determinant factor inducing calcium and HCO_3^- releases from the transitory deposits of calcareous spherules in the internal organs (mantle and gills) of A. cygnea, This acidosis mechanism associated with increases on the electrochemical gradient and permeability of the outer mantle epithelium (OME), favors the HCO_3^- and Ca^{2+} movements towards the shell compartment. On the contrary, in the extrapalial compartment, the deposition, under $CaCO_3$, is facilitated by a higher pH tendency due to the decrease of the proton pump activity in the (OME) cells.

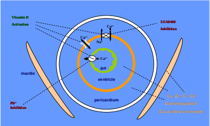

Diagram of calcium mechanism in *Anodonta cygnea*

(Machado *et al.*, 2008)

At beginning of autumn, reversal behavior occurs during autumn with reduction of mother shell $CaCO_3$ precipitation by a high proton pump activity and low OME permeability, although still with an internal respiratory acidosis. This will redirect the calcium and bicarbonate towards the larvae shell in the gills. On the winter period, the residual HCO_3- secretion from the OME, added now by the respiratory alkalosis and eventual external phosphate absorption, should increase the internal CaCO3/CaPO3 precipitated under calcareous spherules (Coimbra et al. 1988; Machado et al. 1988; Lopes-Lima et al. 2008).

The continuous calcium absorption from the external environmental is due to a probable calcium active transporter (channel, exchanger or pump), which may be located on other tissue (intestine, foot, gill or inner mantle epithelia). This active mechanism is necessary due to the hypotonicity of the environment and contributes to the enrichment on the total calcium supply of the bivalve organism.

Conclusions

As a final conclusion, it is possible to propose a general scheme for seasonal pCO_2, HCO_3^- and Ca^{2+} and pH variations. In fact, the CO_2 increase occurs as primary and determinant cause to the acid pH tendency which works as a physiological trigger promoting the subsequent dissolution of calcareous concretions during spring-autumn. On the contrary, the HCO_3^- occurs earlier as a main factor for an alkalosis situation inducing the calcium microspherules deposition in the mantle during the winter-spring period.

So, a simple process based on a high calcium permeability of basal membranes of OME cells, on a calcium-gradient towards the shell and a PMCA-like protein with a function in cytoplasmic calcium regulation, which, as a whole, drives calcium across the OME cells without a toxic effect.

References

1. Coimbra J, Machado J, Fernandes PL, Ferreira HG, Ferreira KG (1988) Electrophysiology of the mantle of *Anodonta cygnea*. J Exp Biol 140:65–88

2. Machado J, Coimbra J, Sá C, Cardoso I (1988a) Shell thickening in *Anodonta cygnea* by induced acidosis. Comp Biochem Physiol A Physiol 91A(4):645–651

3. Lopes-Lima M, Lopes A, Casaca P, Checa A, Machado J (2007) Seasonal Correlation of pH, *p*CO2, *p*O2, HCO3i and Ca2+ with Acidosis/ CalciWcation in *Anodonta cygnea*. Comp Biochem Physiol B 178(1): 17-25

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