Identification and Kinetic Characterization of Phosphatases Isozymes of *Lithobates catesbeianus* Tadpoles Tail During Metamorphosis

Gonçalves, A.M.[1,2]; Colosio, R.R.[1,2]; Santos, L.F.J.[2]; Santana, C.C.[1,2]; Pavarina, G.C.[2]; Neira, L.M.[2]; Oliveira, G.M.[2]; Vieira, V.I.[2]; Ferreira, G.C.[2]; Eto, S.F.[2]; Fernandes, D.C[1,2]; Pizauro, J.M.[2,1]

[1]Departamento de Bioquímica e Tecnologia, IQ/UNESP Araraquara, SP, Brasil;
[2]Departamento de Tecnologia, FCAV/UNESP Jaboticabal, SP, Brasil

Introduction: During metamorphosis, major post-embryonic changes occur in morphology, physiology, biochemistry, and behavior. An intriguing and little understood aspect of amphibians' metamorphosis is the anabolic and metabolic processes involved in tail regression, which is a large part of the tadpoles' body and releases nutrients necessary for the morphophysiological transformations of the aquatic larval stage into terrestrial adults. **Objectives**: In spite of the information accumulated to date, the mechanism of phosphorus metabolism during tail absorption remains unclear, thus, the aim of this study was to identify the phosphatases involved in this process, during metamorphosis. **Material and Methods**: The animals were kept in aquaria, at 27°C and separated by stages of development. Samples were taken from a pool of tails from animals of the stage 44. The tails were homogenized in 5mM Tris.HCl buffer, pH 7.5, containing 2 mM $MgCl_2$ and 1 µM $ZnCl_2$, centrifuged at 10,000 *g* for 10 minutes, at 4°C, then ultra-centrifuged at 100,000 *g* for two hours to separate the membrane-bound from soluble proteins, the pellet was resuspended in the same buffer and *p*-nitrophenylphosphate was used as substrate for the enzymatic assays. **Results and Discussion**: The ultracentrifugation separation showed the presence of two acid phosphatase isozymes, one soluble and another membrane-bound. Regarding the alkaline phosphatase, only a membrane-associated form was found. The study of the effect of substrate concentration on the enzymes activity revealed a Vmax of 104.51, 10.55 and 25.86 $U.mg^{-1}$ and $K_{0,5}$ values of 0.292, 0.198 and 0.329 for membrane-bound acid and alkaline phosphatases and soluble acid phosphatase, respectively. Both acid phosphatases presented negative cooperation, while alkaline phosphatase showed Michaelian behavior. **Conclusions**: The kinetic parameters support the hypothesis that there are three distinct phosphatases acting in the tail absorption during metamorphosis. More studies are being conducted in order to clarify their role in the studied process.

Key Words: Bullfrog, Enzymatic Kinetic, Phosphomonohydrolases.
Acknowledgements: CNPq and CAPES.