GIANT EXTRACELLULAR HEMOGLOBIN *Amyntas gracilis* IMMOBILIZED ON SOLID SURFACES AND ITS POTENTIAL USE AS BIOSENSORS FOR ENVIRONMENTAL CONTAMINATION

Linhares, A. P. D.¹; Oliveira, J. B. S¹; Santiago, P. S¹.

¹Campus Experimental of Registro, Agronomy, UNESP / Registro.

Introduction

Giant extracellular hemoglobins, also known as erythrocruurins, have been investigated as a model of extreme complexity in oxygen-binding heme proteins. They are characterized by very high molecular mass and high redox stability.

Purpose

It has been studied as blood substitute and potential use in biotechnological applications such as biosensor for environmental contamination.

Material and method

In previous work were observed an effect of heavy metal (copper) at oxidation state (met-hemoglobin formation). The immobilization of molecules of biological interest on solid surfaces is essential for a biosensor biotechnology application. In this work studies were performed related to the immobilization of hemoglobin *Amyntas gracilis* (HbAg) on a solid surface (quartz) using the layer by layer (LBL) method, aiming to maintain the structure of the protein in its native form. Solid substrates with a negatively charged planar surface (quartz) were immersed in the solution containing the cationic polyethylene amine polyelectrolytes (PEI); and layers of polyelectrolyte were adsorbed. After rinsing in pure water the substrates were immersed in the solution containing 0,1 mg/mL HbAg at phosphate buffer 20mM pH 7.0. After each layer of the immobilization on the solid surface were made spectra optical absorption of the HbAg-PEI. Film growth was achieved up to at least ten (10).

Results

The alternating polyelectrolyte/protein layers were constructed in order to increase the binding layer capacity (i.e. sensitivity) of the thin film with respect to heavy metal detection. Above deposition of the tenth layer of the optical absorption spectrum showed the Soret band centered at 415 nm and two Q bands (535 and 575) characteristic of HbAg in its native form.
Conclusion

Above deposited HbAg were observed by fluorescence and optical absorption that structure protein was preserved.

Acknowledgments: Brazilian agencies CNPq for partial financial support.

Key Words: extracellular hemoglobin; optical absorption; Layer by layer