Using Electrochemical Approach to Evidence Close-Packing Arrangements in Urease-Lipid Langmuir-Blodgett films

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The Langmuir–Blodgett (LB) technique is a suitable method to obtain controlled architectures for amphiphilic materials and have been used to incorporate enzymes in a phospholipid matrix in to order to preserve their biocatalytic activity [1]. In order to understand the structural environmental of enzymes at molecular level, we incorporated the urease enzyme in LB films made from the phospholipid dipalmitoyl phosphatidyl glycerol (DPPG). Urease was injected in the aqueous subphase support for a DPPG monolayer at the air-water interface forming a mixed enzyme-phospholipids film, which was transferred to indium tin oxide (ITO) substrates (ITO/urease-DPPG) by using the LB technique. Also, urease adsorbed at a clear air-water interface was transferred to solid substrates (ITO/urease) for comparison. From cyclic voltammetry data, we observed a blocking effect to hexacyanoferrate (III) in electrolyte solution (0.5 mol L^{-1} phosphate buffer at pH 7.0) for both ITO/urease and ITO/urease-DPPG electrodes. However, the anodic and cathodic faradaic currents of $Fe^{II}(CN)_6^{4-}/Fe^{III}(CN)_6^{3-}$ redox couple were lower for the ITO/urease-DPPG electrode when compared to the ITO/urease. It is worth mentioning that the cyclic voltammogram obtained with ITO modified only with DPPG molecules showed a similar electrochemical response to that for an ITO/Enz electrode. Also, a slower kinetics of charge transport across the DPPG/Enz film was observed, with a difference of 100 mV between redox potential of 5 and 50 mV s^{-1} for ITO-DPPG/Enz. The apparent diffusion coefficients (D^{app}) for the hexacyanoferrate species were 2.9×10^{-6} cm^2 s^{-1} for ITO-DPPG/Enz and 4.9 × 10−6 cm^2 s^{-1} for ITO/Enz electrode, confirming that the DPPG/Enz layer affects significantly the charge transport of hexacyanoferrate species due to the close packing of the enzymes. Theses results was corroborated by nanogravimetry measurements through a quartz crystal microbalance, for which we observed a higher mass adsorbed of urease. A molecular area of 4500–5500 $Å^2$ was estimated, which points to a closely packed monolayer. The urease-DPPG surface packing favors a better orientation for the protein, for which the phospholipid matrix plays as protector environment. The latter was confirmed by biocatalytic studies using colorimetric measurements to detect the enzymatic conversion of urea in ammonium ions, when the bio-inspired interface (phospholipids) leads to a favorable conformation/orientation of the polypeptide structure which enhances the enzyme activity.

Keywords: Langmuir-Blodgett films, Proteins, Enzymes, Biosensing

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