Catalytic Reduction of Oxygen and Trichloroacetic Acid on Myoglobin/Polyethyleneimine Modified Electrodes

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The cytochromes P450 family of enzymes refer to hemeproteins that catalyze oxidation reactions including hydroxylations, epoxidations and heteroatom oxidations [1]. The great diversity of P450s and their specificity for the chemical substrate they oxidize provide an opportunity to develop promising catalysts for speciality chemical syntheses. In this framework, Myoglobin (Mb) can be used as model enzyme in the first stages of development of such catalytic processes, because this hemeprotein is more stable than cytP450s, commercially available, and catalyzes some of the same reactions as cytP450s.

Myoglobin has generally been immobilized on electrode surfaces either with insoluble surfactants forming cast films of protein [2], or with charged polyions and proteins deposited in alternate layers [3]. This so-called layer-by-layer procedure is known to provide good stability because of strong electrostatic interactions between protein and polyion. Myoglobin, with an isoelectric point of 6.8, has generally been used at slightly acidic pH, as a cationic protein. Layer-by-layer immobilization has consequently been carried out with negatively charged polyions such as poly(styrenesulfonate) (PSS) [4].

The purpose of this work was to study the immobilization of anionic Mb, with the positively charged polyion polyethleimine (PEI). All experiments were performed in slightly alkaline phosphate buffer pH 7.5, with a 0.28cm^2 surface area pyrolytic graphite electrode.

The classic layer-by-layer procedure consists in adsorbing alternate monolayers of protein and polyion, with electrode rinsing between each step. A new two-step procedure was attempted here, which only involved adsorption of an excess PEI, followed by adsorption of an excess protein, without intermediate rinsing. The purpose was to define a simpler method, which might be able to immobilize the greater amount of protein as possible. Cyclic voltammograms performed at 0.2V/s revealed clear oxidation and reduction peaks at –0.35 and –0.45 V/SCE respectively, characteristic of the Mb heme Fe^{III}/Fe^{II} redox couple. The two-step procedure gave poorly reproducible peak currents from 7 to 45μA. On the contrary the electrode constructed following the classic layer-by-layer procedure gave reproducible current peaks, which logically increased as a function of the number of superimposed layers up to 47μA with $\{PEI/Mb\}_6$ modified electrodes. It was concluded that the two-step procedure could easily provide very efficient electrodes, but still requires further work to give reproducible results.

Cyclic voltammograms performed at 0.01V/s in oxygen-saturated solution with $\{PEI/Mb\}_3$ modified electrodes showed a clear catalysis of oxygen reduction in the presence of myoglobin (Figure 1). The electrochemically driven reduction of trichloroacetic acid (TCA) was investigated in deoxygenated buffer in the same conditions (Figure 2). High catalytic current values were obtained, around 50μAmM^{-1}cm^{-2} at –0.2V/ECS, while previous studies indicated values around 0.4 μAmM^{-1}cm^{-2} with PSS/Mb modified electrodes. Anionic Mb seems to give highly efficient modified electrodes when immobilized with PEI. Nevertheless, addition of TCA shifted the solution to acidic pH, below the Mb isoelectric value, which logically induced the destruction of the film.

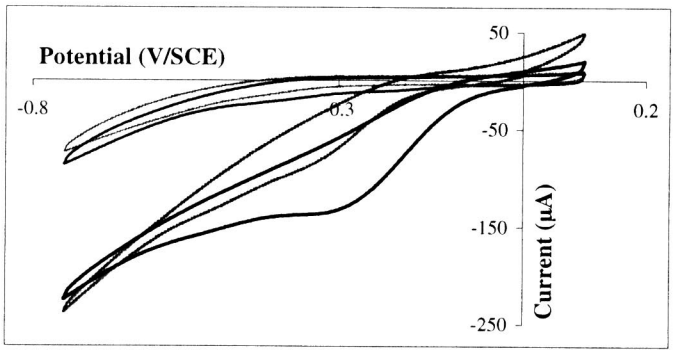

Figure 1: Cyclic voltammograms for $\{PEI/Mb\}_3$ films on PG electrode in the absence and in the presence of oxygen, pH 7.5, scan rate 0.01V/s.

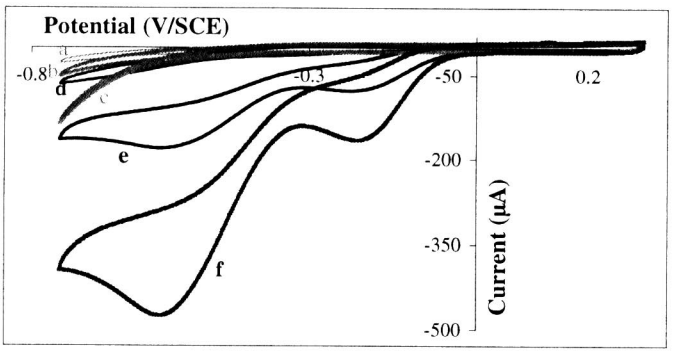

Figure 2: Cyclic voltammograms on PG electrode, pH 7.5, scan rate 0.01V/s, in the absence of oxygen for (a) PEI monolayer in buffer, (b) PEI monolayer in buffer containing 5mM TCA, (c) PEI monolayer in buffer containing 10mM TCA, (d) $\{PEI/Mb\}_4$ films in buffer, (e) $\{PEI/Mb\}_4$ films in buffer containing 5mM TCA, (f) $\{PEI/Mb\}_4$ films in buffer containing 10mM TCA.

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