

Biosensors Based on Protein Adsorption on Nanoporous TiO₂ Films

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Strategies for the immobilisation of biomolecules is a key issue in the development of bioanalytical devices such as optical and electrochemical biosensors. In this paper, we consider a novel approach to protein immobilisation: immobilisation upon preformed nanoporous TiO₂ films.

Nanoporous TiO₂ films have a high surface area, are optically transparent, exhibit good electrical conductivity and can be prepared by low cost screen printing technologies. Therefore they provide a novel and versatile immobilisation surface for the development of optical and electrochemical biosensors based on the reactivity of the immobilised proteins.

It is found that cytochrome c (Cyt-c), hemoglobin (Hb), myoglobin and maltose binding protein immobilisation on such films may be readily achieved from aqueous solutions at 4 °C with a high binding stability and no detectable protein denaturation. The nanoporous structure of the film greatly enhances the active surface area available for protein binding (by a factor of up to 800 for an 8- μ m-thick film). It was found that the binding was mainly electrostatic and among the factors having an important role in controlling the binding we have identified pH, protein charge, solution ionic strength and length of preparation time. The range of proteins immobilized can be further extend to low IEP proteins such as catalase and green fluorescence protein by pretreating the TiO₂ films in poly-L-lysine solution.

Redox proteins immobilised on nanocrystalline TiO₂ films retain their redox activity and exhibit direct and reversible electrochemistry, characterised both by cyclic voltammetry and UV/Vis spectroelectrochemistry. The conductivity range of the metal oxide can be further extended by the use of alternative metal oxides. In particular we demonstrate that nanocrystalline ZnO films can be used to immobilise successfully and electrochemically reduce Cyt-c, and that such films exhibit conductivity at potentials positive of those observed for TiO₂.

In this paper we will focus on bioanalytical applications of haemoglobin immobilised on nanocrystalline TiO₂ films. We demonstrate both optical and electrochemical sensing function. These films are used for the optical detection of dissolved carbon monoxide and nitric oxide in aqueous solutions (see figures 1 & 2).

In this case the redox state of Hb is cycled electrochemically to allow repeat measurements, whilst the binding of CO or NO is measured optically.

Secondly, the electrical conductivity of the films in combination with the catalytic properties of the immobilised Hb have been used for the development of an amperometric NO biosensor.

We conclude that proteins adsorb on the TiO₂ films in a stable and functional way and the protein/TiO₂ films can be used for the development of optical and electrochemical sensors.

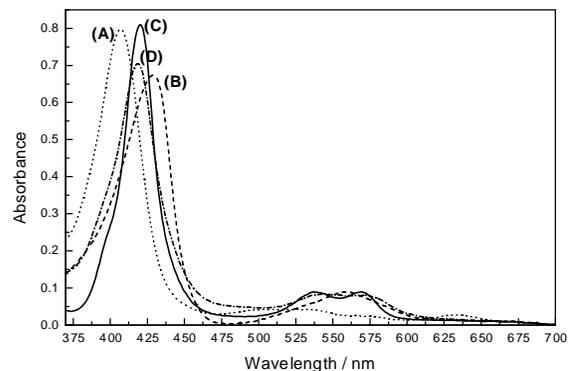


Figure 1: Absorption spectra of (A): met Fe(III)Hb (B): deoxy Fe(II)Hb, (C) carboxy Fe(II)Hb and (D) nitrosyl Fe(II)Hb immobilised on a nanoporous TiO₂ film.

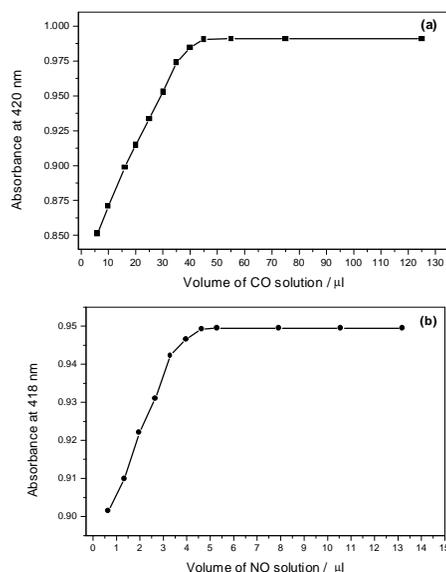


Figure 2: Binding curves of immobilised Hb on a TiO₂ film (a) for CO and (b) for NO. Deoxy Fe(II) Hb titrated against additions of saturated CO and NO solution and the increasing absorption intensity was monitored at 420 and 418 nm respectively.

References:

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