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TiO_2 phytate films as hosts and conduits for cytochrome *c* electrochemistry

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Abstract

Cytochrome *c* is accumulated into a film of TiO_2 nanoparticles and phytate by adsorption from an aqueous solution into the mesoporous structure. Stable voltammetric responses and high concentrations of redox protein within the TiO_2 phytate layer can be achieved. Two types of electrode systems are reported with (i) the modified TiO_2 phytate film between electrode and aqueous solution phase and (ii) the modified TiO_2 phytate film buried under a porous gold electrode ('porotrode').

The electrical conductivity of TiO_2 phytate films is measured and compared in the dry and in the wet state. Although in the dry state essentially insulating, the TiO_2 phytate film turns into an electrical conductor (with approximately 4 Ω cm specific resistivity assuming ohmic behaviour) when immersed in aqueous 0.1 M phosphate buffer solution at pH 7. The redox protein cytochrome *c* is therefore directly connected to the electrode via diffusion and migration of electrons in the three dimensional mesoporous TiO_2 phytate host structure. Electron transfer from cytochrome *c* to TiO_2 is proposed to be the rate-determining step for this conduction mechanism.

Keywords: Cytochrome *c*; Process in Biochemistry; Bioelectrochemistry; Titania nanoparticles; Electron hopping; Sensors

1. Introduction

Mesoporous oxides in the form of thin films are relatively stable in aqueous environments and are versatile for a wide range of applications in sensors [1], as electrocatalysts [2], and for modified electrodes [3]. Considerable interest in TiO_2 films exists predominantly due to their application in hydrophilic coatings [4], in photovoltaic cells [5], as photocatalysts [6], and as pigments [7]. There have also been several previous reports on the immobilisation of biological redox systems such as oligonucleotides and DNA [8], heme proteins [9] or redox enzymes [10] in titanium dioxide [11–13] and other metal oxide hosts such as manganese dioxide [14] and tin dioxide [15]. By combining a rigid host structure, prepared for example from metal oxide nanoparticles of appropriate size, and a more fragile biological system, novel functional composite materials can be obtained [16]. In this report TiO_2 is combined with cytochrome *c* and it is shown that in addition to providing a rigid host structure, metal oxides can also contribute to the efficient transport of electrons.

The heme protein cytochrome *c* has been coupled successfully to electrode surfaces by Hill et al. [17]. Over the past two decades, this redox protein of 12 kD mass and approximately 3.7 nm size [18] has often been considered as a model system for biological electron transfer [19] and for bioelectrocatalysis [20]. The most prominent feature of the almost spherical cytochrome *c* structure is a strongly positively charged lysine rich region on the outer shell of the protein, which is responsible for docking to electron donor and acceptor sites with complementary negative surface charge [21]. A wide range of films with negative surface functionalities, e.g. carboxylate functionalised surfaces [22], polysulfonates [23], polyphosphate (DNA) modified surfaces [24], and dialysis membranes [25] have

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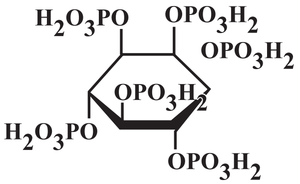

Scheme 1.

been proposed for reversibly docking cytochrome *c* and related heme proteins for applications in bioelectrochemistry and in biosensing. A recent monograph summarises developments in this field [26].

We have shown that cytochrome *c* is strongly adsorbed from aqueous solution into thin films of TiO_2 nanoparticles assembled with the help of phytic acid (see Scheme 1) 'binder' molecules [27]. After immersion of the TiO_2 phytate film coated onto tin-doped indium oxide (ITO) electrode surfaces, high concentrations of the redox protein in a stable and active state are immobilised. Increasing the film thickness in a layer-by-layer manner increases the amount of adsorbed redox active protein [27] and therefore improves the electrochemical response. This three-dimensional binding of the redox protein to the electrode surface was rationalised by a transport process similar to diffusion with either protein diffusion, electron hopping from site to site within the mesoporous film, or both occurring simultaneously [27].

In this report it is shown that TiO_2 phytate films, although electrically insulating in the dry state, conduct electrons when immersed in aqueous buffer solution. The resulting conductivity within the film is sufficient to explain the three-dimensional coupling of the redox protein cytochrome *c* to the electrode surface without the need of protein diffusion. It is also shown that the TiO_2 phytate films may be deposited onto plain non-conducting glass surfaces and then sputter-coated with a porous gold layer. The resulting 'buried' modified electrode (or 'porotrode') is cheap, readily prepared on a variety of substrates, electrochemically active similar to conventional modified ITO electrodes, and allows cytochrome *c* electrochemistry to be observed.

2. Experimental

2.1. Instrumentation

Electrochemical measurements were performed with a Eco Chemie PGSTAT20 Autolab bipotentiostat system. Conventional voltammetric experiments were conducted in staircase voltammetry mode and with a platinum gauze counter and saturated calomel reference electrode (SCE, Radiometer). The working electrode was either a modified glass microscope slide (13×75 mm, Fisher Scientific, UK) or tin-doped indium oxide (ITO) coated glass (9m×60 mm, resistivity 20 $\Omega\ \square^{-1}$) obtained from Image Optics Components (Basildon, Essex). Prior to conducting experiments, all solutions were purged with argon (BOC, UK). All experiments were carried out at a temperature of 22±2 °C.

Film conductivity measurements in dry and wet state were performed in a two- or four-electrode arrangement, respectively, with a second working electrode controlled in bipotentiostatic mode (vide infra). Scanning electron microscopy images were obtained with a Leo 1530 Field Emission Gun Scanning Electron Microscope system. Prior to SEM imaging, the sample surface was scratched with a scalpel blade.

2.2. Chemical reagents

Titania sol (anatase, ca. 6 nm diameter, 30–35% in aqueous HNO_3, pH 0–3) was obtained from Tayca, Osaka, Japan and diluted 100-fold with deionised water. $[Ru(NH_3)_6]Cl_3$ (Johnson Matthey), Horse heart cytochrome *c* (type VI, Sigma, molecular weight 12384 g mol^{-1}), $HClO_4$ (60%), KCl, KH_2PO_4, K_2HPO_4, phytic acid dodecasodium salt hydrate [*myo*-inositol hexakis (dihydrogen phosphate) dodecasodium salt], and 3-mercaptopropionic acid (Aldrich) were obtained commercially. Demineralised and filtered water was taken from an Elga water purification system (Elga, High Wycombe, Bucks, UK) with a resistivity of not less than 18 MΩ cm.

2.3. Electrode design

Films of TiO_2 phytate were deposited following a layer-by-layer dip coating strategy [28]. A clean glass or ITO surface (washed with ethanol and water, dried, and 30 min heat treated at 500 °C in air) is dipped into a colloidal solution of TiO_2 nanoparticles followed by rinsing. The surface charge of the resulting nanoparticle deposit is reversed by dipping into a solution of phytic acid (40 mM in pH 3 aqueous solution) followed by rinsing. The process, which is carried out by a robotic Nima dip coating carousel (DSG-Carousel, Nima Technology, Coventry, UK), can be repeated and results in a thickness increase of the TiO_2 phytate deposit of approximately 30 nm per layer [27]. Oxide films formed in this way are stable in air and may be stored under ambient conditions.

Gold coatings with 20 or 40 nm thickness were applied by sputter-coating (Polaron E5150 SEM gold sputter coating unit). A typical electrode cross section is shown in Fig. 1A. The electrode surface was scratched with a scalpel blade and the exposed oxide film between ITO substrate and gold coating can clearly be seen. The gold coating appeared porous (see Fig. 1B) even when 40 nm thick. The porous nature of the gold coating is probably due to the topography of the underlying oxide film.

2.4. Conductivity measurements

Conductivity measurements of thin TiO_2 phytate films were carried in dry and in wet state. Earlier measurements