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The effects of conductivity and electrochemical doping on the reduction of methemoglobin immobilized in nanoparticulate TiO_2 films

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Abstract

Methemoglobin (bovine) is immobilized from aqueous phosphate buffer (pH 5.5) solution into thin porous TiO_2 (anatase) films at ITO electrode surfaces. Films of TiO_2 are produced in a deposition process employing 40 nm diameter TiO_2 nanoparticles suspended in dry methanol followed by calcination. The pore size in these films is sufficient for methemoglobin (ca. 6 nm diameter) to diffuse into the porous structure (over several hours) and to remain immobilized in electrochemically active form.

The electrochemical reduction of methemoglobin immobilized in TiO_2 and immersed in aqueous phosphate buffer at pH 5.5 is observed in two steps with (i) a small quasi-reversible voltammetric response at −0.16 V vs. SCE (Process 1) and (ii) an irreversible reduction peak at ca. −0.5 V vs. SCE (Process 2). The irreversible response is recovered only after slow chemical re-oxidation of hemoglobin to methemoglobin. At sufficiently negative applied potential "electrochemical doping" of the TiO_2 host is observed to lead to a considerably enhanced reduction Process 1. TiO_2 can be temporarily switched from a non-conducting (irreversible electron transfer) into a conducting (reversible electron transfer) state.

Keywords: Hemoglobin; Nanoparticle; Doping; Catalysis; Voltammetry; Sensor

1. Introduction

The incorporation of biological redox systems into mesoporous films has generated interest due to the possibility of direct electron exchange that can be achieved between the redox sites in the proteins and the mesoporous host electrode. Direct electrochemistry of redox proteins or enzymes can provide biomimetic models for fundamental studies as well as a basis for designing devices without the need for soluble electron transfer mediators [1]. There has been a wide range of approaches for the immobilisation of biological redox systems including adsorption [2], binding into 2D or 3D lipid structures [3], covalent binding [4], cast deposition of composites [5], trapping in porous matrices [6], or electrostatic binding into mesoporous films [7].

Hemoglobin or methemoglobin immobilized at electrode surfaces have often been employed as model redox systems and electrocatalysts. In studies by Rusling et al. [8,9] immobilization and electron transfer to hemoglobin was demonstrated. Siontorou and Nikolelis [10] have demonstrated the adsorption of hemoglobin into a self-assembled bilayer lipid membrane in order to produce a cyanide ion minisensor. The electrochemical properties of hemoglobin have also been investigated in clay films [11], in SiO_2 [12], SnO_2 [13], TiO_2 nanoparticle assemblies [14] and in DNA [15] or surfactant assemblies [16]. Previously the adsorption of hemoglobin [17] and cytochrome *c* [18,19] into mesoporous TiO_2 phytate thin films have been studied. The cytochrome *c* redox protein (diameter ca. 3.4 nm) adsorbed homogenously into the mesoporous TiO_2 film. However, the small pore sizes created from the 6 nm nanoparticle agglomerates of TiO_2 allowed the much larger hemoglobin protein (diameter ca. 6 nm) to adsorb only into the outer layer of the film [17]. It was observed that as the thickness of the film increased, a transition occurred from the hemoglobin being an electronically coupled protein to electronically insulated protein. Here the homogenous adsorption of methemoglobin is achieved by using TiO_2 films prepared with bigger 40 nm diameter particles. The larger particle size

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allows adsorption of the protein into larger pores and throughout the film and both the redox protein and the TiO_2 substrate contribute to the electrochemical characteristics of the film deposit. A temporary "doping effect" is observed after scanning the potential of the electrode into the Ti(III) formation. The quasi-reversible oxidation/reduction of hemoglobin immobilized in mesoporous TiO_2 can be strongly enhanced.

2. Experimental

2.1. Reagents

Chemical reagents such as perchloric acid (70% A.C.S reagent), KCl, KOH, phosphoric acid (85% wt.% solution in water, A.C.S reagent), were obtained commercially (Aldrich) and used without further purification. A suspension of 40 nm diameter TiO_2 (anatase) nanoparticles was prepared from a powdered form (AMT-600, Tayca Corp, Japan) 3% wt in dry methanol (Fisher Scientific, HPLC grade) by sonication (20 kHz Fisherbrand ultrasonic bath) for at least 30 min. Suspensions of TiO_2 in methanol were stable for several hours but underwent slow sedimentation (accelerated in the presence of water). Hemoglobin (Hb, bovine methemoglobin, H2625, from Sigma) was used without further purification. Unless stated otherwise, a 1 gL^{-1} Hb solution was prepared in aqueous 0.1 M phosphate buffer at pH 5.5. All solutions were prepared using deionised water with a resistivity of at least 15 MΩ cm (Elga, High Wycombe, UK).

2.2. Instrumentation

Electrochemical experiments were conducted with a PGSTAT 30 Autolab system (Eco Chemie, Netherlands) in a conical three-electrode cell. The counter electrode was platinum gauze and the reference electrode was a saturated calomel electrode (SCE, Radiometer). The working electrodes were prepared from tin-doped indium oxide (ITO) coated glass (1 cm × 6 cm, resistivity 15 Ω per square, Image Optics Components Ltd.). ITO electrodes were cleaned prior to film deposition by rinsing in ethanol followed by deionised water and furnace treated (Elite tube furnace) at 500 °C in air for 60 min. For Scanning Electron Microscopy (SEM), a JEOL JSM6310 system was used. UV/Vis spectra of solution and solid samples were obtained with a Helios γ spectrometer (Thermo Electron Corp.). The temperature during all measurements was maintained at 22 ± 2 °C.

2.3. Procedures and electrode design

A suspension of the nominal 40 nm TiO_2 particles was achieved by preparing a 3% wt solution in methanol and sonicating (see above). A clean ITO electrode was then dipped into the white suspension for 1 min, retracted, and the methanol was allowed to evaporate leaving behind a well-defined and uniform layer of TiO_2. The deposition process was repeated in order to build up several layers of TiO_2 particles. In this manner the required number of 'layers' of the 40 nm TiO_2 particles could be adjusted to allow variation in film thickness. The resulting film was then calcined in air at 500 °C for 1 h to improve adhesion and to remove impurities. Methemoglobin adsorption involved equilibrating the TiO_2 modified electrode in a solution of methemoglobin (1 gL^{-1}) prepared in aqueous 0.1 M phosphate buffer at pH 5.5, followed by rinsing in distilled water. Both methemoglobin in solution and after immobilization into TiO_2 films were investigated with UV/Vis spectroscopy and the presence of the characteristic Soret band at 406 nm [20] confirmed intact methemoglobin before and after immobilization. Electrodes were stored immersed in phosphate buffer solution pH 5.5 and remained stable over several weeks.

3. Results and discussion

3.1. Deposition and characterisation of nanoparticulate TiO_2 film electrodes

TiO_2 nanoparticles (anatase, ca. 40 nm diameter) were deposited from a suspension (3 wt.% TiO_2) in dry methanol. After immersion and solvent evaporation, tin-doped indium oxide (ITO) electrodes are coated with a thin and uniform layer of TiO_2 which increases in thickness during each new deposition step. Once deposited, the TiO_2 nanoparticles are not re-suspended in methanol and remain permanently immobilized at the electrode surface. After formation of the multi-layer TiO_2 film at the electrode surface, a calcination step (ca. 1 h at 500 °C in air) was employed to further improve adhesion. The characteristic electrochemical reduction response of the immobilized TiO_2 nanoparticles immersed in aqueous 0.1 M KCl is shown in Fig. 1.

The characteristic reduction process is predominantly capacitive in nature [21] and associated with the reversible formation of Ti(III) within the porous TiO_2 film [22]. From the increase in the charge under the reduction response it can be seen that the growth of the TiO_2 film is approximately proportional to the number of deposition cycles. In order to characterize the topography of the nanoparticle deposit, SEM images were obtained. Fig. 2 shows that rather than a thin homogeneous film, a patterned film deposit of aggregates of ca. 1 μm diameter is formed. Each of the aggregates consists of smaller TiO_2 particles with ca. 40 nm diameter.

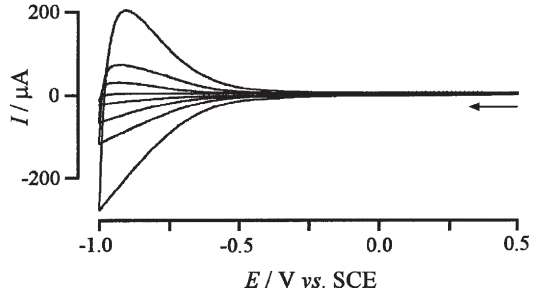

Fig. 1. Cyclic voltammograms (scan rate 0.1 Vs^{-1}) obtained for a 1 cm^2 ITO electrode coated with a 1, 3, 5, and 10 layer films of ca. 40 nm diameter TiO_2 particles (followed by calcination) immersed in aqueous 0.1 M KCl.

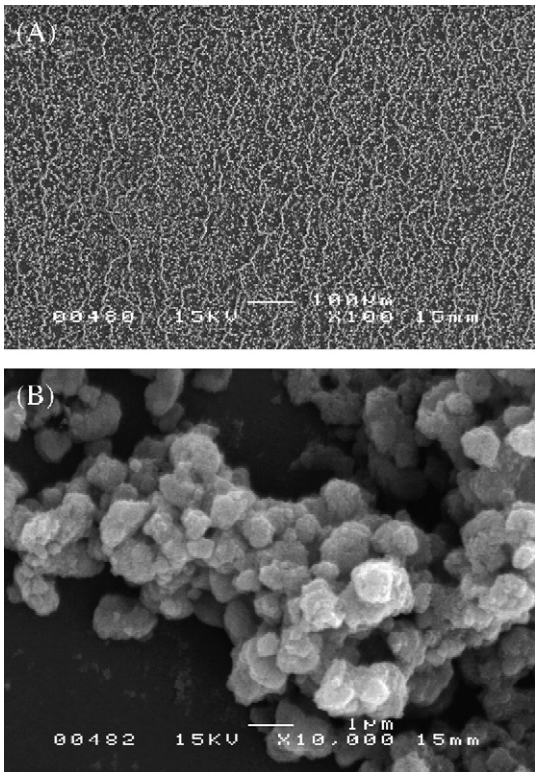

Fig. 2. Scanning electron microscopy image (SEM) of a 10 layer deposit of TiO_2 nanoparticles (calcined) at an ITO electrode surface. Due to the deposition conditions, the nature of the "layer" is highly non-uniform.

3.2. Immobilization and reactivity of methemoglobin in TiO_2 film electrodes

Next, TiO_2 thin film electrodes were exposed for 1 h to a solution of 1 gL^{-1} hemoglobin in aqueous 0.1 M phosphate buffer solution (pH 5.5). Under these conditions phosphate anions immediately adsorb onto the TiO_2 surface to give a negative surface charge [23]. The positively charged protein hemoglobin (the isoelectric point is approximately 7.4 [24,25]) is then attracted into the porous oxide and is thereby accumulated at the electrode surface. Fig. 3 shows typical cyclic voltammograms obtained with this kind of electrode. A bare ITO electrode (see Fig. 3i) shows only insignificant ability to adsorb the protein. A very weak and reversible response is observed at $E_{mid}=-0.16$ V vs. SCE. In contrast, a single layer of TiO_2 nanoparticles substantially increases this current response (see Fig. 3ii) and leads to a characteristic second reduction to be observed at a potential of ca. −0.7 V vs. SCE.

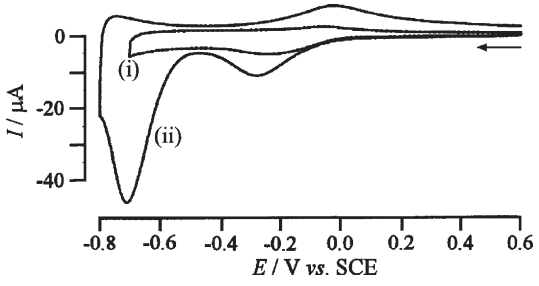

Fig. 3. Cyclic voltammograms (scan rate 0.1 Vs^{-1}) obtained for the reduction of adsorbed methemoglobin on (i) a bare ITO electrode and (ii) an ITO electrode covered with 1 layer of 40 nm diameter TiO_2 nanoparticles immersed in aqueous 0.1 M KCl. Prior to this experiment, electrodes were immersed in 1 mg mL^{-1} methemoglobin in 0.1 M phosphate buffer pH 5.5 for 1 h.

The quasi-reversible voltammetric signal at $E_{mid}=-0.16$ V vs. SCE is consistent with the Fe(III/II) one electron reduction of the hemin unit in methemoglobin [12,26]. Next, the reduction responses for methemoglobin are investigated in more detail. Fig. 4 shows cyclic voltammograms obtained in phosphate buffer solution (pH 5.5) for TiO_2 film electrodes with (A,B) one layer of TiO_2 and (C,D) 10 layers of TiO_2. The first reduction response is clearly present for both types of films. This process occurring at −0.16 V vs. SCE may be identified as the Fe(III/II) process probably involving all four hemin subunits of the methemoglobin molecule (Eq. (1)).

$$\text{Process1}: \mathrm{Fe(III)(Hb)} + \mathrm{e^-(ITO)} \rightleftarrows \mathrm{Fe(II)(Hb)} \quad (1)$$

It is interesting to note that the magnitude of the voltammetric response for process 1 during the first potential cycle is not increased upon increasing the thickness of the TiO_2 film (see Fig. 4A and C). Consecutively cycling the applied potential causes this voltammetric response to decrease in current.

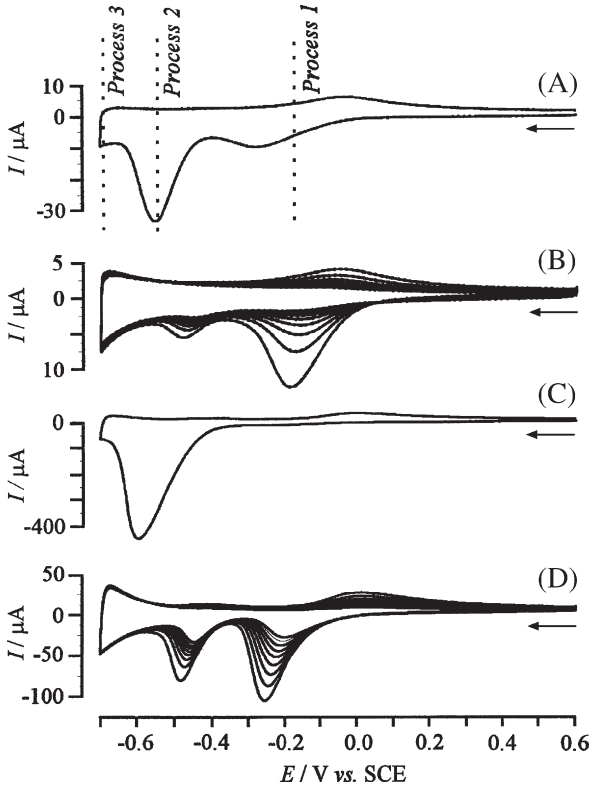

Fig. 4. Cyclic voltammograms (scan rate 0.1 V s^{-1}) obtained for the reduction of methemoglobin immobilized in a 1 layer (A,B) and in a 10 layer (C,D) TiO_2 film at ITO electrodes. Voltammograms were obtained in aqueous 0.1 M phosphate buffer (pH 5.5). (A) Scan 1 of 10 scans for a 1 layer TiO_2 film electrode. (B) Scans 2 to 10 for a 1 layer TiO_2 film electrode. (C) Scan 1 for a 10 layer TiO_2 film electrode. (D) Scans 2 to 10 for a 10 layer TiO_2 film electrode. Prior to voltammetric experiments, electrodes were immersed (1 h) in a solution of 1 mg mL^{-1} methemoglobin.

For the 10 layer TiO_2 film electrode, the voltammetric signal during the first reduction for Process 1 is very similar to that observed for the 1 layer TiO_2 film electrode. However, a very strong enhancement is observed in potential cycle 2 which is followed by a decay of the signal during subsequent cycles. This is a tell-tale sign for methemoglobin within the mesoporous TiO_2 film being accessed only after the first reduction process has occurred and attributed here to a conductivity effect (vide infra). Very similar conductivity effects have been described recently for a flavin adenine dinucleotide TiO_2 composite film [27].

A second reduction (Process 2) is observed at a potential of ca. −0.5 V vs. SCE. This reduction peak current is relatively small for 1 layer TiO_2 films but about 10-fold enhanced for the 10 layer TiO_2 film electrode. This reduction can therefore be identified as the Fe(III/II) process associated with electron conduction through the bulk TiO_2 film [28]. This reduction requires the transport of electrons through the porous oxide and therefore occurs at a more negative potential at which a sufficient concentration of electrons is available in the TiO_2 film (Eq. (2)).

$$\text{Process2} : \text{Fe(III)(Hb)} + e^-(TiO_2) \rightarrow \text{Fe(II)(Hb)} \quad (2)$$

Fig. 5 shows a schematic drawing explaining the different pathways for electrons during the methemoglobin reduction and the difference in the effect of TiO_2 film thickness on the voltammetric responses for Processes 1 and 2.

The description of Process 1 as based on electron transfer directly from ITO to methemoglobin (see Eq. (1)) is over-simplified and certainly involves a more complex "short range" conduction mechanism possibly via "hopping" or via conducting TiO_2 states.

For both, 1 layer and 10 layer TiO_2 film electrodes, the second reduction process is pronounced only during the first potential cycle and it gradually disappears during subsequent potential cycles. This loss of signal is not associated with the loss of methemoglobin from the porous structure but rather believed to be associated with the irreversibility of electron transfer in the system. An electrode used for several potential cycles shows essentially full recovery of the electrochemical activity when stored in aqueous 0.1 M phosphate buffer (pH 5.5) for 12 h. Therefore, methemoglobin is bound permanently and any loss of electrochemical activity is temporary and probably linked to conductivity effects in the TiO_2 host film. Fig. 6 shows typical voltammograms before and after recovery.

A third reduction response observed at potentials negative of −0.6 V vs. SCE (see Fig. 4) can be attributed to the reversible

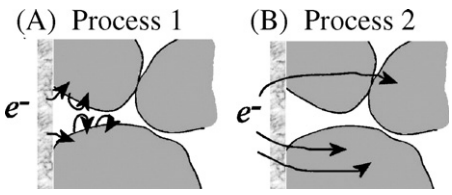

Fig. 5. Schematic drawing of the electron transport (A) via "short range" transfer from the ITO electrode (Process 1) and (B) via "long range" conduction through the bulk TiO_2 host material (Process 2).

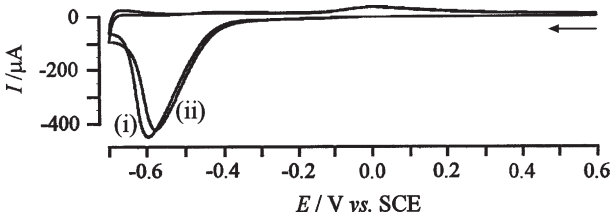

Fig. 6. Cyclic voltammograms (scan rate 0.1 Vs^{-1}) showing scan 1 for the reduction of methemoglobin in a 10 layer TiO_2 film immersed in 0.1 M phosphate buffer (pH 5.5) (i) with a freshly prepared electrode and (ii) with a used electrode after recovery in 0.1 M phosphate buffer (pH 5.5) for 12 h.

transfer of electrons into the TiO_2 host lattice (see Fig. 1). This process has been extensively studied [21,22] and linked to the Ti(IV/III) redox couple at the TiO_2 nanoparticle surface (Eq. (3)).

$$\text{Process3} : \text{Ti(IV)}O_2 + e^-(\text{ITO}) + H^+(aq) \rightleftarrows \text{Ti(III)}O_2H \quad (3)$$

All three types of redox processes observed for methemoglobin immobilised in a TiO_2 host film are surface based and dependent on the scan rate applied in the voltammetric experiment. Fig. 7 shows voltammograms obtained for the reduction of hemoglobin immobilized in a 10 layer TiO_2 film. Peak currents for Processes 1, 2, and 3 increase approximately linearly with scan rate.

From the initial potential cycle (see Fig. 7A) the amount of hemoglobin at the electrode surface can be estimated. The charge under the reduction peak is consistent with 390 μC or 1 nmol methemoglobin (assuming 4 electrons transferred per adsorbed hemoglobin molecule). In order to achieve this level of hemoglobin adsorption the roughness factor [28] required for the TiO_2 film electrode is $R_f = \frac{\text{area}}{\text{geometric area}} = 15$ (calculated by assuming a 6 nm diameter for each methemoglobin molecule) which is in good agreement with the SEM images shown in Fig. 2

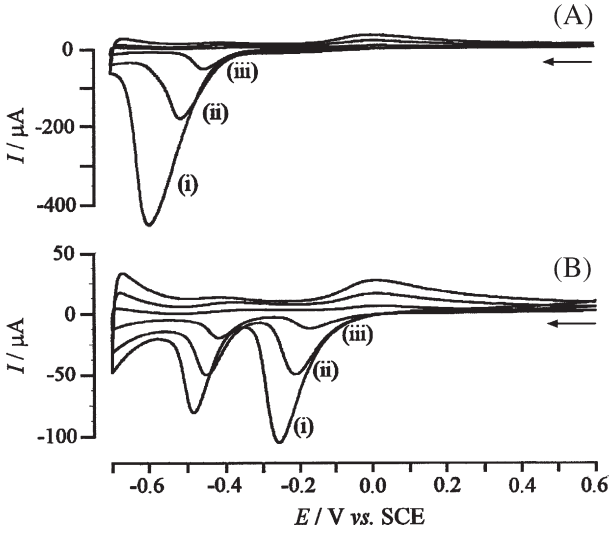

Fig. 7. Cyclic voltammograms (scan rates (i) 10 mVs^{-1}, (ii) 50 mVs^{-1}, (iii) 100 mVs^{-1}) for the reduction of hemoglobin immobilized in a 10 layer TiO_2 film electrode and immersed in 0.1 M phosphate buffer (pH 5.5) for (A) the first potential cycle and (B) the second potential cycle.

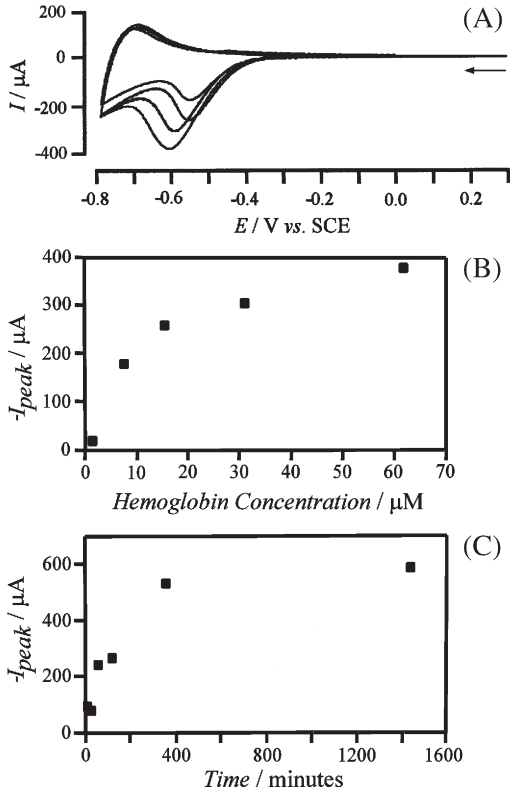

Fig. 8. (A) Cyclic voltammograms (scan rate 0.1 V s^{-1}) for the reduction of methemoglobin immobilized in a 10 layer TiO_2 film at a 1 cm^2 ITO electrode immersed in 0.1 M phosphate buffer at pH 5.5. Prior to voltammetric experiments the electrodes were soaked for 2 h in 60 M, 30 μM, 15 μM, and 7 μM hemoglobin in 0.1 M phosphate buffer at pH 5.5. (B) Plot of the cathodic peak current observed in the first potential cycle versus the concentration of methemoglobin during immobilization. (C) The effect of soaking time in 15 μM methemoglobin in 0.1 M phosphate buffer at pH 5.5 on the cathodic reduction peak during the first potential cycle.

(assuming that hemoglobin is adsorbed into the spaces between the 40 nm diameter TiO_2 particles).

3.3. Effects of concentration, deposition time, pH, and oxygen on the immobilisation and reactivity of methemoglobin in TiO_2 film electrodes

The immobilization of methemoglobin in 40 nm diameter TiO_2 nanoparticle films results in reproducible and stable electrodes with a characteristic Fe(III/II) reduction response. The immobilization process is believed to occur due to the electrostatic interaction between methemoglobin (which is positively charged at pH 5.5) and the phosphate coated (negatively charged) TiO_2 surface. In order to explore the ability of methemoglobin to bind, experiments were conducted with a set of electrodes and in a range of different concentrations of methemoglobin. Cyclic voltammograms shown in Fig. 8A demonstrate the effect of the methemoglobin concentration on the immobilization process. An increase in the amount of methemoglobin immobilized in TiO_2 is observed up to ca. 100 μM methemoglobin and approximately consistent with a Langmuir adsorption isotherm (see Fig. 8B). Next, the effect of time on the adsorption process is studied. For a 10 layer TiO_2 film electrode a considerable delay in adsorption is observed. The plot in Fig. 8C shows that only after 10 h the process approaches completion (for a 10 layer film deposit). This rather slow adsorption process is not surprising and due to the small pore size within the TiO_2 structure. It has been shown recently [17] that deposits with even smaller pore size (with 6 nm diameter TiO_2 nanoparticles) are essentially in-penetrable for the methemoglobin molecules. Here, 40 nm TiO_2 nanoparticles are employed and a pore size of typically 10–20 nm can be estimated. The penetration and transport of the protein within these pores are slow.

Next, the effect of the concentration of the aqueous phosphate buffer solution on the methemoglobin immobilization process is

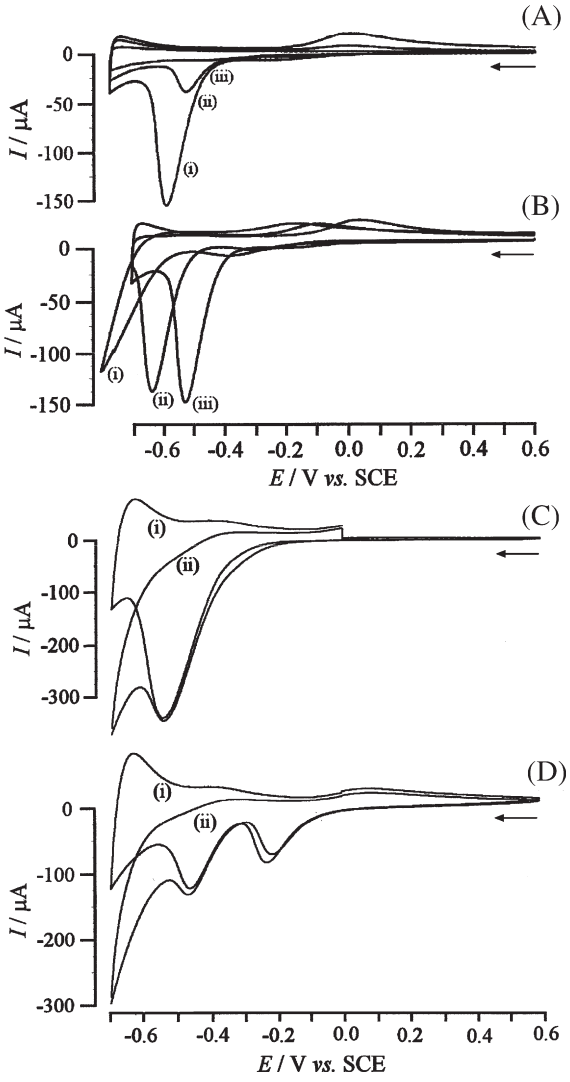

Fig. 9. Cyclic voltammogram (scan rate 0.1 Vs^{-1}) for the reduction of methemoglobin immobilized in a 10 layer TiO_2 film electrodes and immersed in 0.1 phophate buffer (pH 5.5). (A) Electrodes were soaked for 12 h in solutions containing (i) 0.1 M (ii) 0.5 M (iii) 0.01 M phosphate buffer (pH 5.5) and 0.1 mM methemoglobin prior to voltammetric experiments. (B) Electrodes were soaked for 12 h in a solution containing 0.1 mM methemoglobin in 0.1 M phosphate buffer at (i) pH 8.5, (ii) pH 7.0, and (iii) pH 5.5. (C) Potential cycle 1 and (D) potential cycle 2 for a 10 layer TiO_2 electrode (methemoglobin immobilized by soaking for 12 h 0.1 M phosphate buffer at pH 5.5) (i) in de-aerated and (ii) non-de-aerated 0.1 M phosphate buffer solution (pH 5.5).

studied. Voltammograms shown in Fig. 9A demonstrate the importance of this parameter. Both lower and higher concentrations change the behaviour of the protein and the highest voltammetric responses are observed after immobilization in 0.1 M phosphate buffer (pH 5.5). It is possible that at lower ionic strength the stronger interaction between host and guest significantly slows down the immobilization process whereas at high ionic strength a weaker interaction could stop the immobilization process entirely.

The effect of the pH of the buffer solution employed during cyclic voltammetric measurements is demonstrated in Fig. 9B. A clear shift of all three processes, the quasi-reversible Fe(III/II) reaction (Process 1), the irreversible Fe(III/II) reaction (Process 2), and the reduction of the Ti(IV) host lattice (Process 3), is observed with approximately 59 mV per pH unit. This observation is in agreement with literature reports for both TiO_2 [29] and immobilized methemoglobin [30].

Many studies have emerged in which methemoglobin is employed as an electrocatalyst. A range of processes from oxygen reduction [31], peroxide reduction [20], trichloroacetate reduction [32], and nitrite reduction [16] have been observed and attributed to the electrocatalytic effects of the porphyrinato-IX iron center. However, in particular the facile reduction of oxygen by hemoglobin is surprising because in nature this process has to be avoided at all costs. Hemoglobin is an oxygen transport protein and in intact hemoglobin the simple electron transfer is avoided by geometric constraints (distal and proximal histidines) in the vicinity of the binding site [33]. It is shown here that methemoglobin electrostatically immobilized into TiO_2 hosts is indeed essentially inert toward the oxygen reduction process. Voltammograms in Fig. 9C and D show the first and second potential cycle for electrodes in de-aerated phosphate buffer solution and in the presence of ca. 0.2 mM oxygen. Only process 3 (the formation of Ti(III)) is strongly affected by the presence of oxygen. For Processes 1 and 2 the direct electron transfer from reduced hemoglobin to oxygen in solution is too slow to be detected under the conditions employed here. In a recent report a similar observation of low reactivity towards oxygen was attributed to the presence of allosterically bound phytate ligands [17]. However, there is no phytate present in these experiments and the interaction of the protein with the negatively charged TiO_2 host may be regarded as another factor contributing to the effect. These results suggest that methemoglobin immobilized electrostatically into mesoporous TiO_2 is structurally intact and not an active electrocatalyst.

3.4. Electrochemical doping effects on the reduction of methemoglobin in TiO_2

The reactivity of redox systems immobilized into TiO_2 films is strongly affected by the conductivity of the host matrix. Only Process 1 (see Fig. 4) is associated with the "short range" transfer of electrons from the ITO electrode surface to the immobilized methemoglobin. Processes 2 and 3 require "long range" conduction of electrons within TiO_2. The concentration of mobile electrons within TiO_2 depends on the applied potential and the dramatic increase in this parameter at a potential negative of a potential of −0.5 V vs. SCE is demonstrated in Fig. 1. Due to the increase in Ti(III) sites the TiO_2 host becomes electrically conducting. The shape of the voltammograms shown in Fig. 1 suggests that the formation of Ti(III) is essentially reversible (very fast) and therefore capacitive in nature. However, there are different types of Ti(III) sites and a "memory" effect due to some less reversible Ti(III) sites can also be observed.

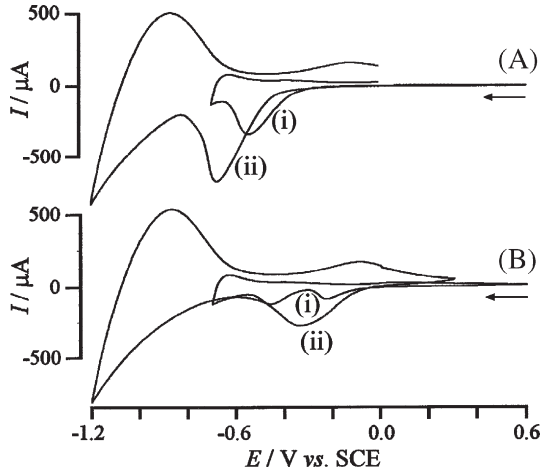

Fig. 10. Cyclic voltammograms (scan rate 0.1 Vs^{-1}) for the reduction of methemoglobin immobilized in a 10 layer TiO_2 film electrode immersed in 0.1 M phosphate buffer (pH 5.5). In (A) the first potential cycle and in (B) the second potential cycle are shown. The negative switching potential was chosen as (i) −0.7 V vs. SCE and (ii) −1.2 V vs. SCE. The unexpected difference in curves Ai and Aii is due to the use of two not fully compatible electrodes.

It is shown here that these less reversible Ti(III) sites are responsible for a temporary conductivity change. Data in Fig. 10i shows that initially Process 2 dominates (Fig. 10A) and then during the second potential cycle both Process 1 and 2 are observed (Fig. 10B). This effect is much more dramatic when the negative switching potential is changed from −0.7 to −1.2 V vs. SCE (see Fig. 10ii). Under these conditions Process 1 completely dominates during the second and following potential cycles indicating an increased electrical conductivity in the TiO_2 host. The reduction at −1.2 V vs. SCE is believed to lead to the formation of more long-lived Ti(III) sites (via "electrochemical doping") and therefore more facile "short range" electron exchange between ITO electrode and immobilized methemoglobin. A similar effect can be induced by UV light is known as "photo-doping" [34]. In a recent report by Li et al. [35] a composite of hemoglobin and TiO_2 nanoparticles immobilized at graphite electrodes was observed to become more reactive after exposure to UV radiation and this effect is likely to be also due to doping. The "electrochemical doping" effect is temporary and decays away over a period of several seconds. The observation of this "electrochemical doping" effect helps understanding the fundamental processes within the mesoporous TiO_2 host during reduction and oxidation processes and offer a novel "switching" mechanism for sensor developments.

4. Conclusions

It has been shown that methemoglobin is electrostatically bound and slowly accumulated into mesoporous TiO_2 hosts with

a sufficiently large pore space. The process is essentially irreversible but very mild and leaves the protein intact for electron transfer processes to occur. Two distinct reduction processes occur associated with (i) "short range" (quasi-reversible) reduction of methemoglobin in the vicinity of the ITO electrode surface and (ii) "long range" (irreversible) reduction via conduction through TiO_2. The electrical conductivity of TiO_2 is affected by the presence of Ti(III) sites and "electrochemical doping" is observed as a temporary conductivity enhancing process. Overall, the mechanism for the reduction of methemoglobin is complex and may involve different isomers of hemoglobin. Therefore more work (e.g. for similar redox systems or employing spectroelectrochemical experiments) will be required for further details of the mechanism to be resolved.

Acknowledgements

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