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Short communication

Reactivity of methemoglobin immobilized on TiO_2 nanoparticle films

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Abstract

Facile demetallation occurs upon contact of the methemoglobin with a mesoporous TiO_2 host in phosphate buffer media at pH 5.5 but not in acetate buffer media. As a result, voltammetric signals previously attributed to hemoglobin-based redox processes have to be re-interpreted.

Keywords: Hemoglobin; Titania; Cytochrome; Electrode; Voltammetry; Sensor

In our recent report [1] the absorption and the reactivity of methemoglobin in a porous TiO_2 electrode made from 40 nm TiO_2 nanoparticles were investigated. Voltammetric responses were interpreted based on the assumption that methemoglobin is binding to the porous host and that electron transfer between heme sites in the protein and the TiO_2 are possible. Although commonly observed in related reports [2], the true nature of the electrochemically active methemoglobin within the porous host is not well understood. For example, the potential problem of methemoglobin denaturation and the facile loss of the electrochemically active hemin sub-unit have recently been highlighted [3].

Further investigation of the recently reported [1] voltammetric responses for the reduction of methemoglobin on TiO_2 shows that within mesoporous hosts not only the loss of hemin but also *complete demetallation* of methemoglobin is possible. Perhaps surprisingly, voltammograms essentially identical to those reported recently for methemoglobin [1] can be obtained by direct immobilization of Fe^{3+} into the TiO_2 film. Fig. 1 shows voltammograms obtained after immobilization of Fe^{3+} into a 10 layer TiO_2 film electrode (essentially identical to those used in [1]). In the first potential cycle a characteristic irreversible reduction at ca. −0.6 V vs. SCE is observed and this is followed by the appearance of a new chemically reversible oxidation and reduction peak at ca. −0.08 and −0.32 V vs. SCE, respectively. These processes are readily explained based on the known redox chemistry of the $Fe^{3+/2+}$ system under these conditions [4]. Poorly water-soluble $FePO_4$ is formed initially immobilized on the TiO_2 surface. In the first reduction process (first cycle) electrons flow through TiO_2 at sufficiently negative potential and $Fe_3(PO_4)_2$ is formed. Redistribution of this more water-soluble redox system occurs during subsequent oxidation and $FePO_4$ is deposited directly onto the ITO electrode surface within the porous host. The chemically reversible process can be attributed to the $FePO_4$/$Fe_3(PO_4)_2$ redox system directly on ITO [4].

This interpretation applies not only to the voltammetric responses shown in Fig. 1 but also to all voltammetric data recently attributed to methemoglobin [1]. By carrying out the voltammetric experiment in the presence of 1 mM EDTA in 0.1 M phosphate buffer (pH 5.5), the $Fe^{3+/2+}$ redox system is removed by complexation after the first reduction (for both cases immobilized Fe^{3+} or immobilized methemoglobin, not shown). Methemoglobin immobilized in the presence of 1 mM EDTA does not lead to a voltammetric response (see Fig. 1iii) and in the presence of oxygen no catalytic effect is observed. In contrast, the same experiment conducted in acetate buffer pH 5.5 instead of phosphate buffer (see Fig. 1iv) does lead to an enhanced current of the oxygen reduction indicating the presence of hemin (or methemoglobin). Similar results were reported recently also for thin film electrodes made from TiO_2 and nano-cellulose [5].

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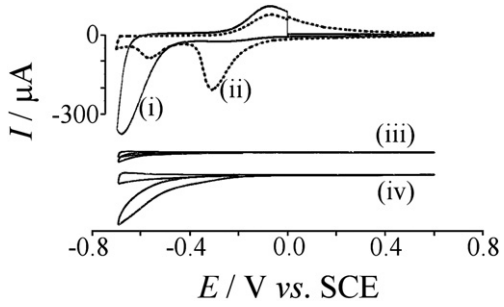

Fig. 1. (i) First potential cycle and (ii) third potential cycle for cyclic voltammograms (scan rate 0.1 V s^{-1}) obtained immersed in 0.1 M phosphate buffer pH 5.5 for the reduction of Fe^{3+} immobilized in a 10 layer TiO_2 film at ITO electrodes. Prior to voltammetric experiments, electrodes were immersed (1 h) in a solution of 60 μM Fe^{3+} in water followed by rinsing. (iii) Cyclic voltammograms (scan rate 0.1 V s^{-1}) obtained for the reduction of methemoglobin immobilized in a 10 layer TiO_2 film at ITO electrodes in the absence/presence of oxygen (air) in aqueous 0.1 M phosphate buffer (pH 5.5) containing 1 mM EDTA. Prior to voltammetric experiments, electrodes were immersed (1 h) in a solution of 1 mg mL^{-1} methemoglobin in 0.1 M phosphate buffer (pH 5.5) containing 1 mM EDTA. (iv) Cyclic voltammograms (scan rate 0.1 V s^{-1}) obtained for the reduction of methemoglobin immobilized in a 10 layer TiO_2 film at ITO electrodes in the absence/presence of oxygen (air) in aqueous 0.1 M acetate buffer (pH 5.5) containing 1 mM EDTA. Prior to voltammetric experiments, electrodes were immersed (1 h) in a solution of 1 mg mL^{-1} methemoglobin in 0.1 M acetate buffer (pH 5.5) containing 1 mM EDTA.

Based on these observations it seems likely that demetallation of methemoglobin occurs in the presence of phosphate (at pH 5.5) and in the presence of the porous host with negatively charged surface (phosphate bound to TiO_2 [6]). In contrast, acetate binding to the TiO_2 surface appears weaker thereby preventing the demetallation process. Hemin demetallation has been observed previously as a process occurring at pH 5.5 in the presence of phosphate and apoferritins [7].

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