Direct Electrochemistry of Myoglobin in MnO_2 Nanosheet Film

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A continuous myoglobin/MnO_2 nanosheet (derived via the delamination of layered manganese oxide) film was prepared by a simple method for the first time. The direct electrochemistry of myoglobin immobilized in the film was investigated using electrochemical methods.

The direct electron transfer of redox proteins, like myoglobin (Mb), can serve as a model for the study of metabolic processes in the biological systems and establish a foundation for fabricating a new generation (third generation) of biosensors, catalytic bioreactors, and biomedical devices.[1] But it is usually difficult for Mb to realize direct electron transfer on a conventional electrode. Many novel morphology nanomaterials, such as nanotubes[2] and nanoporous materials,[3] have been used to modify electrodes to facilitate the electron transfer of Mb. For example, Zhou et al.[2] found that Mb exhibited facile direct electrochemistry and higher catalytic reactivity in titanate nanotube (TNT) film than that in nanocrystalline TiO_2 film. It is believed that the introduction of novel nanomaterials with special morphology is a promising approach to greatly enhance electron transfer of Mb.

Recently, nanosheets have been produced as a new class of nanoscale materials by the exfoliation of related bulk layered compounds. Strikingly different from nanoparticles, the delaminated colloidal nanosheets have a morphological property of a thickness of molecular dimensions with lateral dimensions of submicro- to micrometers, so they received growing attention owing to their extremely high anisotropy, polyelectrolytic nature, etc.[4] Many functional nanosheet materials have been prepared and applied in photochemistry, electrochromic device, and so on. However, the application of functional nanosheet films in facilitating electron transfer of redox proteins has not been reported so far.

In this letter, we fabricated a novel Mb/MnO_2 nanosheet (derived via the delamination of layered manganese oxide) film. For comparison, Mb/nanosized precursor layered manganese oxide film was also prepared. Experimental results reveal that the immobilized Mb in the Mb/MnO_2 nanosheet film displays direct electrochemical behaviors, whereas no peaks are found at Mb/precursor layered manganese oxide modified electrode.

The precursor layered manganese oxide and MnO_2 nanosheet were prepared by the method reported in the literature.[5] Scanning electron micrograph (SEM) image shows that the thickness of the precursor is 10–30 nm (See Figure S1).[13] X-ray diffraction (XRD) patterns and transmission electron microscopy (TEM) image show that the precursor layered manganese oxide has been delaminated to MnO_2 nanosheet (See Figures S2 and S3).[13] Mb solution was prepared with pH 5.95 phosphate buffer solution (PBS). 10 µL of the mixture containing 0.026 mM Mb and 1.29 mg·mL^{-1} MnO_2 nanosheets was cast onto the surface of glassy carbon electrode (GCE). A small bottle was fit tightly over the electrode so that water was evaporated slowly and more uniform films were formed. The films were dried in air at 4 °C in a refrigerator. Then, the modified electrodes were dipped into ethanolic poly(vinyl butyral) solution (2% w/w) for 1 min to enhance the adhesive ability and the stability of the film. Mb/precursor layered manganese oxide film was made in the same way, and the concentrations were 0.026 mM Mb and 1.7 mg·mL^{-1} precursor layered manganese oxide, respectively.

Figure 1 shows the scanning electron images of Mb/precursor layered manganese oxide film and Mb/MnO_2 nanosheet film. As shown in Figure 1a, the platelets are aggregated together to platelike forms with a dimension of 5–15 µm on the electrode surface. However, in Figure 1b, edges of the nanosheets are faintly recognized and the film is smooth and continuous. The MnO_2 nanosheets favor not only face-to-face type interaction but also edge-to-edge type interaction between individual MnO_2 nanosheets, as observed for layered double hydroxides.[6] The two types of strong interactions between individual nanosheets produce continuous films. On the other hand, nanosheets generally adhere well to substrates because of their small dimensions and high charge density.[7]

Figure 2 displays the circular dichroism (CD) spectra of Mb in PBS and Mb in colloidal suspension of MnO_2 nanosheets. The double minima locate at 210 and 222 nm when Mb is in the native conformation in PBS. Similar minima in Mb/MnO_2 nanosheet CD spectrum are found at 211 and 222 nm, confirming that most of secondary structure is retained. MnO_2 nanosheets give no CD peaks in this region.

The electrochemical behavior of Mb/MnO_2 nanosheet film modified GCE was studied by cyclic voltammetry. Figure 3 shows the cyclic voltammograms of bare GCE, MnO_2 nanosheet/GCE, and Mb/MnO_2 nanosheet/GCE in 0.1 M PBS (pH 7.0). There are no redox peaks for bare GCE or MnO_2 nanosheet/GCE within the potential window. A pair of well-defined, quasi-reversible CV peaks located at −0.34 and −0.27 V (vs Ag/AgCl) is observed on the Mb/MnO_2 nanosheet/GCE (Figure 3c), which is the characteristic of the Mb heme Fe^{III}/

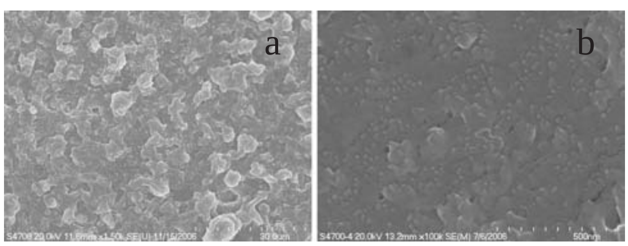

Figure 1. SEM images of Mb/precursor layered manganese oxide film (a) and Mb/MnO_2 nanosheet film (b).

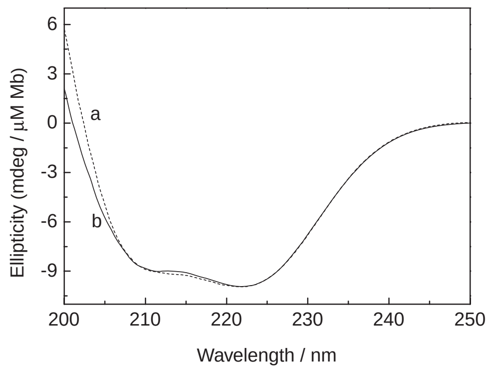

Figure 2. Circular dichroism spectra of Mb in pH 5.95 PBS (a) and Mb in colloidal suspension of MnO_2 nanosheets (b).

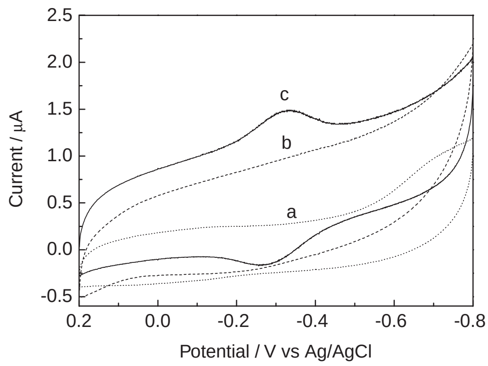

Figure 3. Cyclic voltammograms obtained with GCE (a), MnO_2 nanosheet/GCE (b), and Mb/MnO_2 nanosheet/GCE (c) in a 0.1 M PBS (pH 7.0). Scan rate: $0.1\,V{\cdot}s^{-1}$.

Fe^{II} redox couple.[2] This indicates that the direct electron transfer of Mb could be achieved through the incorporation into MnO_2 nanosheet film. The formal potential ($E^{0'}$) is calculated to be -0.305 V vs Ag/AgCl (-0.097 V vs NHE), which is similar to that obtained by the Mb-clay (-0.100 V vs NHE)[8] and Mb/sol–gel film (-0.099 V vs NHE)[9] on pyrolytic graphite electrode in a pH 7.0 PBS. The average surface concentration of electroactive Mb in MnO_2 nanosheet film is estimated to be $3.63 \times 10^{-11}\,mol{\cdot}cm^{-2}$.

In recent papers,[10] the possibility of heme release is thoroughly investigated. In the control experiment, $E^{0'}$ of hemin/MnO_2 nanosheet/GCE is -0.384 V vs Ag/AgCl, which is significantly different with the formal potential of Mb/MnO_2 nanosheet/GCE (See Figure S4).[13] In addition, the two films exhibit different electrocatalytic behaviors in O_2 reduction (See Figure S5).[13] We convince that heme release has not occurred in Mb/MnO_2 nanosheet film on GCE.

On the other hand, the Mb/precursor layered manganese oxide/GCE shows no peaks in this range (See Figure S6).[13] This may be attributed to unique properties of nanosheets, which are different from that of the precursor layered materials. Firstly, nanosheets can adsorb biomolecules strongly and interact particularly with biomolecules owing to their large specific surface area. It is reasonable that the electrostatic interaction could be favorable for protein adsorption in the interlayer of the MnO_2 nanosheets. Mb molecules are difficult to be intercalated in a precursor layered manganese oxide, so there is the only interaction of Mb with external surface of the precursor layered manganese oxide. Secondly, it is well known that the surface of MnO_2 nanosheets is negatively charged. The net charge of Mb is positive in this study (pH 5.95) (isoelectric point: 6.8),[11] so the electrostatic interaction between the protein and the matrix makes Mb in a favorable orientation to facilitate electron transfer. Thirdly, it is expected that continuous film can decrease the interfacial resistance to facilitate the electron transfer between electroactive center of Mb and the electrode.

The peak currents are linearly proportional to scan rates from 0.02 to $5\,V{\cdot}s^{-1}$ with a correlation coefficient of 0.999, indicating a characteristic of a thin-layer electrochemical process.[2] An average electron-transfer rate constant (k_s) is $13.2\,s^{-1}$, estimated by the method of Laviron.[12] The high electron-transfer rate suggests that MnO_2 nanosheet film provides a favorable microenvironment to shuttle electron transfer between the bioactive center of Mb and the GCE surface. Direct electron transfer for the Mb heme Fe^{III}/Fe^{II} redox couple is greatly enhanced.

In conclusion, a novel Mb/MnO_2 nanosheet film has been made on the surface of GCE for the first time. In the continuous film, direct electron transfer of Mb is realized and the electron transfer rate is fast. The Mb/MnO_2 nanosheet films may have a potential application in fabricating the third-generation electrochemical biosensors based on mediator-free electrochemistry of the proteins. The introduction of nanosheets can provide a promising platform for fabricating other protein (enzyme)-modified electrodes and immobilizing protein (enzyme) reactors.

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