Direct electrochemistry and electrocatalysis with hemoglobin in water-soluble quantum dots film on glassy carbon electrode†

Qing Lu,[ab] Shengshui Hu,*[ab] Daiwen Pang[a] and Zhike He[a]

Received (in Cambridge, UK) 28th January 2005, Accepted 16th March 2005
First published as an Advance Article on the web 6th April 2005
DOI: 10.1039/b501401h

The direct electrochemistry of hemoglobin can be performed by immobilizing hemoglobin in a water-soluble quantum dots (CdSe–ZnS) film on glassy carbon electrode.

Quantum dots (QDs), which can also be called nanocrystals (NCs), are generally applied to semiconductor particles whose sizes are of the order of a few to hundreds of angstroms. They often have an inorganic core and a capping shell, such as CdSe–ZnS core–shell QDs. The optical and electronic properties are dominated by carrier confinement (electron/hole), which results in the size dependence of its optical properties, including light absorption and photoluminescence.[1–4] In 1998, Chan and Nie[5] and Bruchez *et al.*[6] reported first that QDs can be covalently coupled to biomolecules and used as fluorescent probes in biological detection, which shows the great potential of QDs in application to biological labelling. However, the report relating to bioelectrochemical properties of QDs is unusual. The problem that QDs are in general water-insoluble, which will limit their uses in biological applications, can be solved by capping QDs with an organic layer such as mercaptoacetic acid.[5] Considering the good biocompatibility of QDs, we used them to immobilize hemoglobin (Hb) on a glassy carbon (GC) electrode using Nafion as binder, and direct electrochemistry of Hb was performed. Hb immobilized in QDs film retained its biological activity and gave sensitive electrochemical reduction signals involving reactions with NO and H_2O_2.

The Nafion/QDs–Hb/GC electrode was prepared according to the steps thereinafter. The bare glassy carbon electrode was first polished with 0.05 μm alumina slurry and then sonicated in nitric acid (1 : 1), ethanol and twice-distilled water in turn. Hemoglobin was dissolved in a pH 6.0 phosphate buffer solution (3 mg mL^{-1}), then was mixed with the QDs (mercapto-coated CdSe–ZnS) solution in 1 : 1 (v/v) ratio. 5 μL of the mixture was cast onto the glassy carbon electrode and dried at ambient temperature over night. Before using, 1 μL 0.5% Nafion was used to cover the QDs–Hb film as a binder to hold the film on the electrode surface stably. The Nafion/QDs–Hb/GC electrode was stored at 4 °C in a phosphate buffer (0.1 mol L^{-1}, pH 7.4).

Fig. 1 shows cyclic voltammograms (CV)‡ of Nafion/QDs–Hb/GCE. A pair of well-defined and quasi-reversible redox peaks (curve a) with a formal potential ($E^{\circ\prime}$) of −0.367 V (*vs.* saturated calomel electrode) is observed, which reflect the direct electrochemistry of hemoglobin. There were no redox peaks at the bare GC electrode (curve b), the Nafion/GC electrode (curve d) and the Nafion/QDs/GC electrode (curve c). Curve e, which shows the cyclic voltammogram of Hb only immobilized on a bare GC electrode surface using Nafion, suggested that the electrochemical reaction of Hb at a bare GC electrode made little contribution to the observed redox peaks in curve a. The anodic peak potential (E_{pa}) and the cathodic peak potential (E_{pc}) are located at −0.338 V and −0.396 V, respectively. The separation of the peak potential (ΔE_p) is 0.058 V, indicating that Hb immobilized on the surface of the Nafion/QDs/GC electrode displayed a one-electron quasi-reversible electrochemical reaction.[7] The reduction and the oxidation peak currents exhibited a linear relationship with the scan rate in the range from 0.02 V s^{-1} to 0.5 V s^{-1}, and the charge consumed in coulombs, Q, obtained from integrating the anodic or cathodic peak area in cyclic voltammograms under the background correction was invariable in substance. Furthermore, the log I_{pc}–log V and log I_{pa}–log V plots were linear and the ratio of the slopes ($S_{I_{pc}}/S_{I_{pa}}$) was about one. These results indicate that the electrochemical reaction of Hb immobilized in Nafion/QDs film is surface-controlled behavior, not diffusion-controlled.[8,9] The formal potential of Nafion/QDs–Hb film is linearly dependent on the solution pH. Within the pH range 3.0–10.0, $E^{\circ\prime}$ shifts to the negative direction with the slope of −0.037 V pH^{-1}, which is smaller than the theoretical value of −0.059 V pH^{-1} for a single-proton coupled, reversible one-electron transfer. The reason for this is not clear now. However, the linear relationship between the formal potential and the solution pH suggests that the electron transfer in the protein is accompanied by proton transfer.[10]

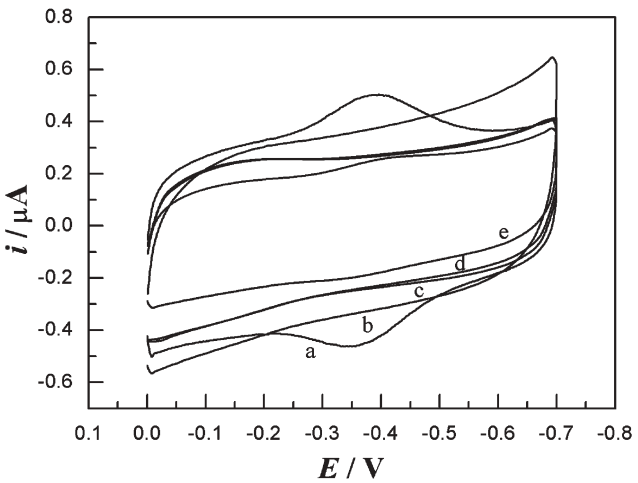

Fig. 1 Cyclic voltammograms at 0.06 V s^{-1} in pH 7.4 buffers for (a) Nafion/QDs–Hb film, (b) bare GC electrode, (c) Nafion/QDs film, (d) Nafion film, (e) Nafion/Hb film.

† Electronic supplementary information (ESI) available: Nyquist plots of EIS data. See http://www.rsc.org/suppdata/cc/b5/b501401h/

*sshu@whu.edu.cn

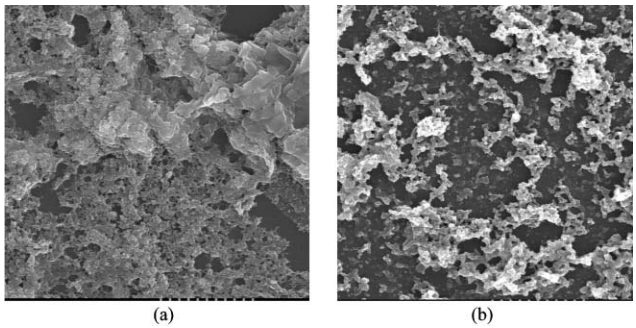

Fig. 2 SEM views with the same magnification: (a) Hb film; (b) QDs–Hb film.

Scanning electron microscopy (SEM) was used to characterize and compare the morphology of the Hb film, QDs film and QDs–Hb film (Fig. 2). The top view of Hb film on a GC block displays an agglomerate crystal structure (a) while the top view of QDs film revealed nothing because of the QDs' nano-dimensions. The top view of QDs–Hb film also exhibits a crystal structure (b) but every crystal unit is much smaller than that of Hb film, which suggests there exists interaction between Hb and the QDs. In order to validate the interaction between Hb and the QDs, electrochemical impedance spectroscopy (EIS) was carried out.§ For the diffusive species, the EIS data (refers to ferricyanide) include a semicircular part and a linear part, of which the semicircular part at high frequencies corresponds to the electron transfer limited process and the linear part at low frequencies corresponds to the diffusion process. The bare GC electrode EIS diagram exhibited an almost straight line that is characteristic of a diffusional limiting step of the electrochemical process. The Nafion/GC electrode EIS plan demonstrated a curve that is part of a semicircle with a large radius, which is characteristic of an electron transfer limited process. The EIS of electrodes modified with different films displayed a linear part and a semicircular part with different radii. The radius of the semicircular part becomes larger in turn for Nafion/Hb, Nafion/QDs–Hb and Nafion/QDs films, respectively, which indicates that the charge transfer resistance of Nafion/QDs–Hb film is in between that of Nafion/Hb film and Nafion/QDs film. The results of EIS may reflect the interaction between Hb and QDs. Hb in pH 6.0 phosphate buffer solution has positive charges, which will adsorb $Fe(CN)_6^{3-}$ and $Fe(CN)_6^{4-}$, while QDs modified with mercaptoacetic acid have negative charges that will block $Fe(CN)_6^{3-}$ and $Fe(CN)_6^{4-}$. When Hb was mixed with QDs, there were electrostatic attractions between them due to the different charges they had. The neutralization of charges would reduce the effect of adsorption or repulsion for $Fe(CN)_6^{3-}$ and $Fe(CN)_6^{4-}$.

The reflectance absorption infrared (RAIR) spectra of Nafion/QDs–Hb film had very similar shapes and positions of amide I and amide II bands to that of the Hb film alone, which suggested that Hb immobilized in Nafion/QDs film did not denature. The bioelectrocatalytic activity of Hb immobilized in Nafion/QDs film was confirmed through the experiments that catalyzed the reduction of NO and H_2O_2. The reduction peak potential of NO located at −0.80 V exhibited a linear relationship with the NO concentration in the range 0.18–4.32 μM (R = 0.998). The same result was obtained for H_2O_2, within the range 6.3–35.28 μM (R = 0.995), the catalytic reduction peak current was linearly related to the H_2O_2 concentration.

In summary, Hb can be immobilized in Nafion/QDs film cast on the GC electrode surface, and the direct electrochemistry of Hb can be achieved. The quasi-reversible electrochemical reaction is a surface-controlled reaction. EIS was used to validate the interaction of Hb and water-soluble QDs. The bioactivity of Hb immobilized in Nafion/QDs film is retained, and the catalytic reduction of NO and H_2O_2 was estimated.

Financial support from the National Nature Science Foundation of China (No.30370397 and No.20275028) is gratefully acknowledged.

Qing Lu,[ab] Shengshui Hu,*[ab] Daiwen Pang[a] and Zhike He[a]
[a]*Department of Chemistry, Wuhan University, Wuhan 430072, P R China. E-mail: sshu@whu.edu.cn*
[b]*State Key Laboratory of Transducer Technology, Chinese Academy of Sciences, Beijing 100080, P R China*

Notes and references

‡ Cyclic voltammetry was performed by using a CHI660B electrochemical workstation (CH Instruments, Shanghai, China) in a conventional three-electrode cell. A modified GC electrode was used as the working electrode, a saturated calomel electrode (SCE) and a platinum electrode were used as the reference electrode and the counter electrode, separately. Prior to each experiment, the buffer solutions were purged with high-purity nitrogen for at least 30 min and a nitrogen environment was then kept over the solution in the cell. All experiments were carried out in a 0.1 mol L^{-1} phosphate buffer (pH 7.4) solution, at room temperature. The scan rate was 0.06 V s^{-1}.

§ Electrochemical impedance spectroscopy was performed with a 263A Potemtiostat/Galvanostat (EG&G Instruments, USA). The experiments were carried out in the presence of a 5 mmol L^{-1} $K_3[Fe(CN)_6]$–$K_4[Fe(CN)_6]$ (1 : 1) mixture containing 0.1 mol L^{-1} KCl as a redox probe at the formal potential of the system, 0.226 V.

1 C. B. Murray, D. J. Norrid and M. G. Bawendi, *J. Am. Chem. Soc.*, 1993, **115**, 8706.
2 M. A. Hines and P. Guyot-Sionnest, *J. Phys. Chem.*, 1996, **100**, 468.
3 B. O. Dabbousi, J. Raddavanich Viejo and F. V. Mikulec, *J. Phys. Chem. B*, 1997, **101**, 9463.
4 X. G. Peng, M. C. Schlamp and A. V. Kadavanich, *J. Am. Chem. Soc.*, 1997, **119**, 7019.
5 W. C. Chan and S. Nie, *Science*, 1998, **281**, 2016.
6 M. Bruchez, Jr, M. Moronne and P. Gin, *Science*, 1998, **281**, 2013.
7 C. X. Cai and J. Chen, *Anal. Biochem.*, 2004, **325**, 285.
8 J. F. Rusling and A.-E. F. Nassar, *J. Am. Chem. Soc.*, 1993, **115**, 11891.
9 Y. Zhou, N. Hu, Y. Zeng and J. F. Rusling, *Langmuir*, 2002, **18**, 211.
10 H. Liu, Z. Tian and Z. Lu, *Biosens. Bioelectron.*, 2004, **20**, 294.