Electrochemistry of Hemin Self-Assembled from Aqueous Hexadecyltrimethylammonium Bromide (CTAB) Solution on Single-Wall-Carbon-Nanotube-Modified Glassy Carbon Electrodes

by Jiwei Liu, Jingxia Qiu, Kai Sun, Jin Chen, and Yuqing Miao*

Zhejiang Key Laboratory for Reactive Chemistry on Solid Surfaces, Institute of Physical Chemistry, Zhejiang Normal University, Jinhua 321004, P. R. China (phone/fax: +86-579-82283109; e-mail: biosensors@zjnu.cn)

Hemin could be dissolved directly in H_2O solution in the presence of the cationic surfactant hexadecyltrimethylammonium bromide (CTAB). The hemin is adsorbed on the surface of single-wall-carbon-nanotube-modified glassy carbon (GC) electrodes by self-assembly. The modified electrodes exhibit a well-defined, reversible redox peak with the reduction potential at -0.30 V and a potential separation of 61 mV at pH 7.0. The peak current is found to increase linearly with the scan rate in the range $25-200$ mV, and the peak potential is pH-dependent. The modified electrodes exhibit obvious signal enhancement for the electrochemical reduction of O_2 and H_2O_2, and oxidation of L-cysteine, which is useful to develop their application in electroanalysis and biosensors.

1. Introduction. – The application of electrochemical methods to study electron-transfer reactions in biomolecules, *e.g.*, enzymes, directly with electrodes are becoming a mature field. Such investigations can provide a model for the mechanistic study of electron exchange between biomolecules in biological system. However, natural enzymes and proteins are expensive and unstable, and electron transfer of the electrically active center is hindered due to the large molecular mass and matrix. Therefore, studies on small biomolecules as catalysts have become an active area of research.

Hemin, a natural porphyrinatoiron complex, is a preferred target due to its stability in solution, low molecular mass, and low cost. The electrochemical properties of hemin have been investigated on different substrates [1–4]. Many studies indicated that hemin and similar porphyrindatoiron complexes have similar electrocatalytic properties for O_2, H_2O_2, and nitrite [5–7]. The catalytic response of hemin and similar compounds mostly depends on the efficient modification of materials and methods. Great efforts have been made in search of materials suitable to provide a convenient biocompatibility and favorable microenvironment, for biomolecules. Many researches into encapsulation or fixation of biomolecules by means of surfactant films, polyions, conducting polymers and nanomaterials have been reported [8–13]. The existence of these modified materials may provide favorable microenvironments and orientations for these biomolecules, and can also improve the catalytic activities toward various substrates.

In general, hemin is insoluble in H_2O and only dissolved in basic solution or EtOH solution. The most common method to cast hemin on an electrode is by absorption

from a basic aqueous solution or an EtOH/H$_2$O solution [14–18]. We now found that hemin can be dissolved directly in H$_2$O in the presence of the cationic surfactant hexadecyltrimethylammonium bromide (CTAB).

Carbon nanotube (CNT) is one of the most promising candidates for nano-electronics and biosensors due to its excellent properties, such as a good electrical conductivity, high chemical stability, and notable mechanical strength [19]. In the present study, we used single-wall CNTs as electrode materials for the immobilization of hemin. The hemin in aqueous CTAB solution could easily be adsorbed on the surface of CNT-modified glassy carbon (GC) electrodes. CNTs act as a 'bridge' for the electrons transfer between hemin and the underlying electrode. The 'bridge' can also improve the electrocatalytic activities and provide favorable microenvironments for the hemin.

2. Experimental. – 2.1. *Materials.* Hemin chloride from *SCRC* (Shanghai, P. R. China) was used without further purification. Cationic surfactant CTAB was from *Shanghai Sanpu Chemical Co. Ltd.* and L-cysteine (Cys) from *Sigma*; they were used as received. The single-wall CNTs (COCC, CAS, China) employed in this work were subjected to a treatment *via* sonication in conc. H$_2$SO$_4$/HNO$_3$ soln. according to [20] (see below). All other chemicals were of anal. grade and used without further purification. All solns. were prepared with ultra-pure H$_2$O from *Milli-Q* H$_2$O.

2.2. *Apparatus.* Voltammetric measurements were performed with an *Autolab-PGSTA30* electro-chemical instrument in a three-electrode system. Glassy carbon (GC) electrodes with or without modification were used as working electrodes. Pt-Wire and Ag/AgCl (3M KCl) were used as counter electrode and reference electrode, resp. The experiments were performed at r.t.

2.3. *Hemin – CNT-Film-Modified Electrode.* The CNTs were washed by sonicating in a 3 : 1 mixture (*v/v*) of conc. H$_2$SO$_4$ and conc. HNO$_3$ soln. for 3 h, followed by a thorough washing with ultra-pure H$_2$O until the filtrate became neutral. After drying at r.t., the CNTs were dispersed in ultra-pure H$_2$O to form a 1 mg/ml CNT suspension. Hemin chloride was dissolved in 10 mM aq. CTAB soln.; its concentration was 0.3 mM. Prior to modification, the GC electrodes were mechanically polished, first with 0.1 and then with 0.05 μm alumina slurries, to result in a mirror finish. After polishing, the electrodes were sonicated in ultra-pure H$_2$O for 1 min, followed by a thorough rinse with ultra-pure H$_2$O. Then, 5 μl of the aq. CNT suspension was cast on the GC electrode and exposed to for drying. The CNT-modified GC electrodes (=CNTs/GC electrodes) were used as the working electrodes and for further modification. For further modification, 2 μl of aq. hemin/CTAB soln. was dropped on the modified electrode surface and left for 6 h at 4°. The resulting electrodes are denoted as hemin/CNTs/GC electrodes. The procedure is illustrated in the *Scheme*.

Scheme. *Procedure of the Stepwise Modification of a Glassy Carbon* (GC) *Electrode Resulting in a Hemin/CNTs/GC Electrode* (CNTs = carbon nanotubes)
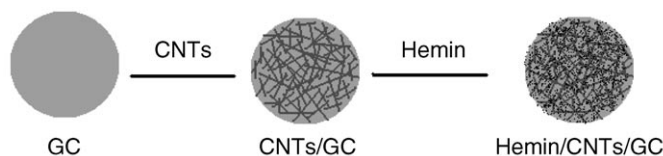
3. Results and Discussion. – The most common method to cast hemin molecules on an electrode is by absorption from a basic aqueous solution or EtOH/H$_2$O solution (see *Sect. 1*). As described in *Sect. 2.3*, we used instead an aqueous hemin solution prepared in the presence of CTAB.

Fig. 1 shows a typical cyclic voltammogram (CV) recorded in N_2-saturated phosphate-buffered saline (PBS; pH 7.0) at a hemin/CNTs/GC electrode. Compared with the current response of the bare GC electrode, a high current response is observed with the CNTs/GC electrode due to the existence of the CNT film. When hemin solution is attached to the CNTs/GC-electrode surface, a couple of well-defined redox waves is observed with a peak-to-peak separation of *ca.* 61 mV at 100 mV·s^{-1}. Obviously, the response of the hemin/CNTs/GC electrode is attributed to the redox of the electroactive centers in the assembled hemin. No characteristic redox peaks are observed when the electrode was prepared by dropping a hemin/CTAB solution onto the GC electrode without CNTs. Hence, it is impossible for hemin to be assembled directly on a bare GC electrode.
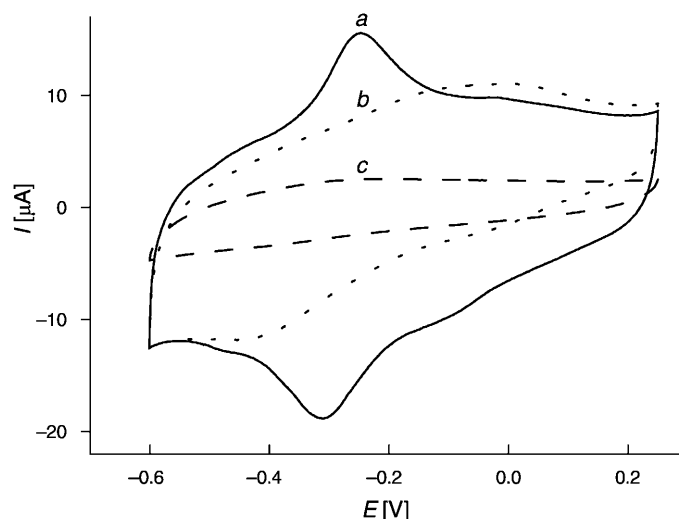

Fig. 1. *CVs at 0.1 V·s^{-1} in N$_2$-saturated PBS* (pH 7.0) *containing 0.1M KCl for the* a) *hemin/CNTs/GC electrode,* b) *CNTs/GC electrode, and* c) *GC electrode*

The relationship between peak currents and scan rates is given in *Fig. 2*. Both the anodic and cathodic peak currents increase linearly with scan rates between 25 and 200 mV·s^{-1}, indicating a surface-controlled electrochemical process. As shown in *Fig. 2*, the peak potentials expand slightly with the increase of the scan rate, also indicating that the electron-transfer rate is very fast.

The stability of the hemin/CNTs/GC electrode was also studied in N_2-saturated 0.01M PBS (pH 7.0) containing 0.1M KCl between -0.6 and 0.25 V at a scan rate of 100 mV·s^{-1} for 100 cycles. The potentials and peak currents of the modified electrode remain almost unchanged after 100 cycles, indicating that the resulting hemin-modified CNT film was stable.

The peak potential of hemin and similar compounds adsorbed at the electrode surface has been shown to depend on the pH value [21]. To assess the properties of hemin adsorbed at the surface of CNTs, the influence of pH on the peak potential of the modified electrode was examined. *Fig. 3* shows a series of the CVs of the hemin/CNTs/
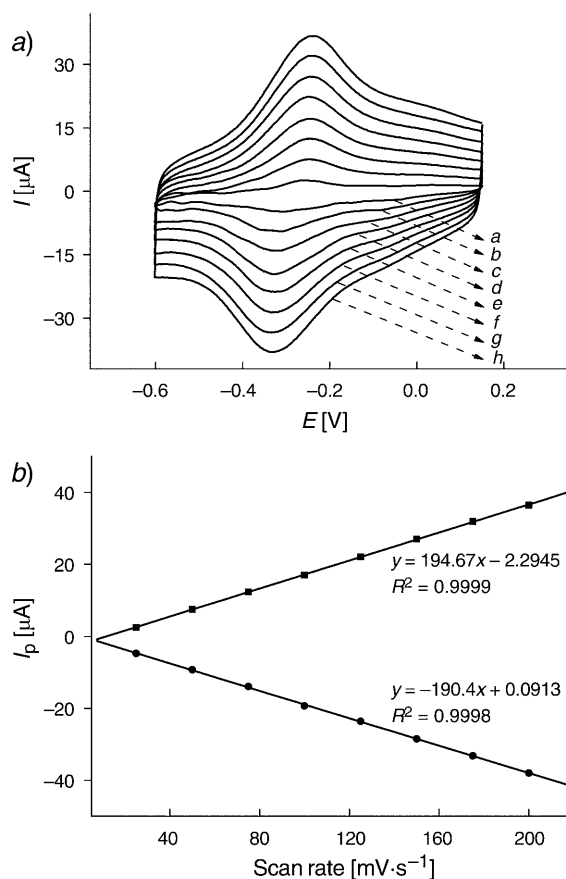
Fig. 2. a) *CVs of the hemin/CNTs/GC electrode in N_2-saturated PBS* (pH 7.0) *containing 0.1M KCl at different potential scan rates* (*a* 25, *b* 50, *c* 75, *d* 100, *e* 125, *f* 150, *g* 175, *and h* 200 mV · s^{-1}). b) *Relationship between the peak currents and the scan rates.*

GC electrode at different pH values. Both the reduction and oxidation peaks of the modified electrode are shifted to more negative values with increasing pH. The formal potentials ($E^{\circ\prime}$) have a linear relationship in the pH range 5 – 9 (*Fig. 3*, inset).

The reduction of O_2 is an important analytical target due to its important role in electrochemical energy conversion in fuel cells and metal – air batteries [22 – 24]. Recently, hemin and similar compounds have been used to investigate electrocatalytic O_2 reduction. *Fig. 4* compares the CVs of GC, CNTs/GC, and hemin/CNTs/GC electrodes in the presence (O_2-saturated) and absence (N_2-saturated) of O_2. Obviously, the hemin/CNTs/GC electrodes greatly increase the reduction overpotential and current, indicating that the immobilized hemin exhibits excellent electrocatalytic activity toward electrochemical reduction of O_2. The new reduction peak at − 0.1 V is probably due to the dimer of hemin, which was reported also by other groups [25][26].
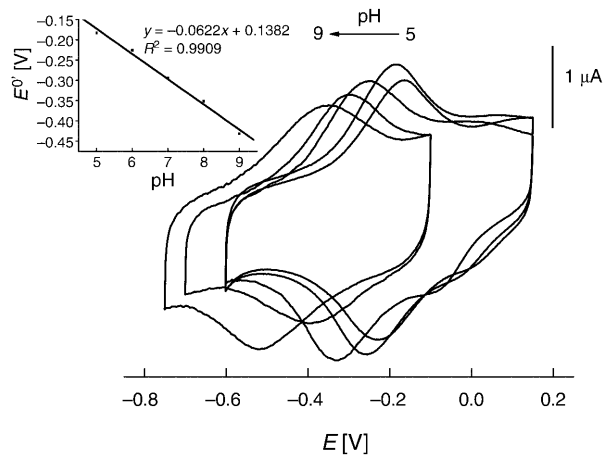
Fig. 3. *CVs of the hemin/CNTs/GC electrode in N$_2$-saturated PBS of pH values from 5 to 9*. Inset: plot of $E^{\circ\prime}$ *vs.* solution pH.
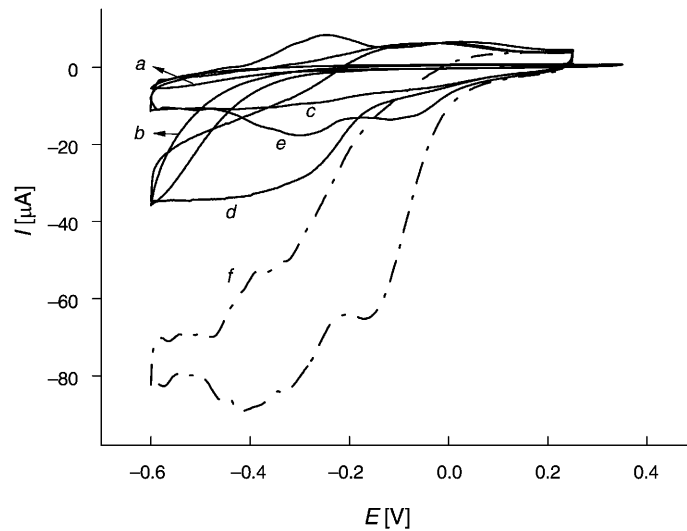

Fig. 4. *CVs of the GC (a and b), CNTs/GC (c and d), and hemin/CNTs/GC (e and f) electrode in 0.01M PBS (pH 7.0) containing 0.1M KCl, N$_2$-saturated (a, c, and e) or O$_2$-saturated (b, d, and f)*. Scan rate: 100 mV · s^{-1}.

H$_2$O$_2$ is the product of the reactions catalyzed by a large number of oxidases. It has been also shown that hemin has a catalytic activity for the reduction of H$_2$O$_2$. *Fig. 5* shows CVs of CNTs/GC and hemin/CNTs/GC electrodes in PBS containing 0.1M KCl (pH 7.0) in the presence or absence of H$_2$O$_2$. When H$_2$O$_2$ was added to the PBS solution containing KCl (pH 7.0), an increase of the cathodic current and a decrease of anodic current could be observed. The hemin/CNTs/GC electrode shows a higher

reduced response to H_2O_2 than the CNTs/GC electrode. Therefore, the hemin/CNTs/GC electrode could be used to develop H_2O_2 or oxidase-based sensors.
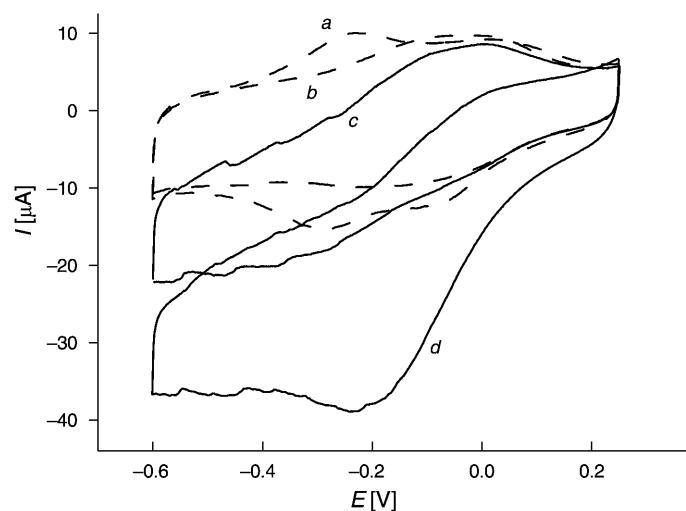
Fig. 5. *CVs of the Hemin/CNTs/GC (a and d) and CNTs/GC (b and c) electrode in 0.01M PBS containing 0.1M KCl* (pH 7.0) *in the presence of H_2O_2 (a and b) or in the absence of H_2O_2 (c and d). Scan rate:* 0.1 V·s⁻¹.

Thiols are a class of compounds characterized by the presence of −SH groups. An accurate determination of thiols is extremely important because thiols are prominent maker molecules, and the changes in thiols can provide a critical insight into their physiological functions or in the diagnosis of disease states. However, most of the analytical methods suffer from difficulties due to high costs, complex sample preparation, and low sensitivity, all of which limit their utility. In contrast, electro-analysis is a more attractive method because it has the advantages of simplicity and high sensitivity. The direct oxidation of thiols at solid electrodes is slow and requires a high potential. Recently, several studies have used modified electrodes to detect thiols. The approach here is to employ the redox cofactor hemin as a biocatalyst for the detection of thiols. *Fig. 6* compares the CVs of GC, CNTs/GC, and hemin/CNTs/GC electrodes in the presence and absence of L-cystein (Cys). Slight voltammetric responses of Cys with a single irreversible oxidation wave are produced at the bare GC electrode at high potential. However, greatly decreased overpotentials and enhanced oxidation currents of Cys were observed at the hemin/CNTs/GC and CNTs/GC electrode, respectively. The oxidation currents at the hemin/CNTs/GC electrode are larger than at the CNTs/GC electrode, indicating that the immobilized hemin exhibits good electrocatalytic activity toward electrooxidation of Cys.

4. Conclusions. – The hemin can be dissolved in an aqueous CTAB solution instead of a basic solution or other organic solutions. The obtained colloid of hemin is directly added onto the CNTs/GC-electrode surface, thus anchoring hemin on the modified electrode. Here, the CNTs serve as a 'bridge' to immobilize hemin onto the electrode
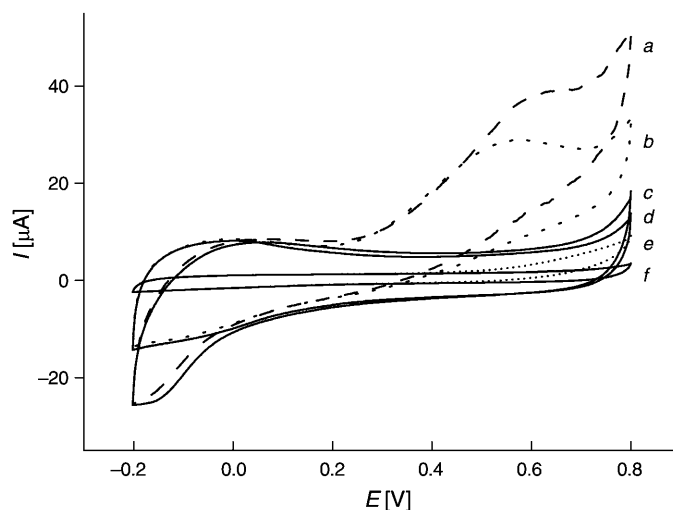
Fig. 6. *CVs showing the catalytic oxidation of Cys with the hemin-modified GC electrode: comparison of the GC (e and f), the CNTs/GC (b and d), and hemin/CNTs/GC (a and c) electrodes in PBS* (pH 7.0) *containing 0.1*M *KCl in the presence of Cys (a and b) or in the absence of Cys (c and d)*

surface. The presence of the CNTs causes an improved immobilization effect of hemin and an enhanced electron transfer between hemin and the GC electrode. The hemin/CNTs/GC electrode exhibits good electrocatalytical responses toward O_2, H_2O_2, and Cys. It is useful to assemble hemin from a neutral solution and to develop the application of the hemin/CNTs/GC electrode in electroanalysis and biosensors.

REFERENCES

[1] Q. Shi, W.-B. Cai, D. A. Scherson, *J. Phys. Chem., B* **2004**, *108*, 17281.
[2] C. G. Nan, Z. Z. Feng, W. X. Li, D. J. Ping, C. H. Qin, *Anal. Chim. Acta* **2002**, *452*, 245.
[3] T. Lötzbeyer, W. Schuhmann, H.-L. Schmidt, *J. Electroanal. Chem.* **1995**, *395*, 341.
[4] F. Patolsky, T. Gabriel, I. Willner, *J. Electroanal. Chem.* **1999**, *479*, 69.
[5] N. Zheng, Y. Zeng, P. G. Osborne, Y. Li, W. Chang, Z. Wang, *J. Appl. Electrochem.* **2002**, *32*, 129.
[6] W. Zheng, J. Li, Y. F. Zheng, *Biosens. Bioelectron.* **2008**, *23*, 1562.
[7] B. Wang, X. Du, M. Wang, W. Gong, J. Anzai, *Electroanalysis* **2008**, *20*, 1028.
[8] N. Sultana, J. B. Schenkman, J. F. Rusling, *Electroanalysis* **2007**, *19*, 2499.
[9] Y. Hu, H. Sun, N. Hu, *J. Colloid Interface Sci.* **2007**, *314*, 131.
[10] M. K. Beissenhirtz, J. Kafka, D. Schäfer, M. Wolny, F. Lisdat, *Electroanalysis* **2005**, *17*, 1931.
[11] Y. Li, X. Lin, C. Jiang, *Electroanalysis* **2006**, *18*, 2085.
[12] D. Shan, E. Han, H. Xue, S. Cosnier, *Biomacromolecules* **2007**, *8*, 3041.
[13] I. Willner, B. Willner, E. Katz, *Bioelectrochemistry* **2007**, *70*, 2.
[14] J.-S. Ye, Y. Wen, W.-D. Zhang, H.-F. Cui, L. M. Gan, G. Q. Xu, F.-S. Shen, *J. Electroanal. Chem.* **2004**, *562*, 241.
[15] G. L. Turdean, I. C. Popescu, A. Curulli, G. Palleschi, *Electrochim. Acta* **2006**, *51*, 6435.
[16] M. T. de Groot, M. Merkx, A. H. Wonders, M. T. M. Koper, *J. Am. Chem. Soc.* **2005**, *127*, 7579.
[17] A. García de la Rosa, E. Castro-Ouezada, S. Gutiérrez-Granados, F. Bedioui, A. Alatorre-Ordaz, *Electrochem. Commun.* **2005**, *7*, 853.
[18] P. Kara, D. Ozkan, K. Kerman, B. Meric, A. Erdem, M. Ozsoz, *Anal. Bioanal. Chem.* **2002**, *373*, 710.

[19] Z.-G. Wang, B.-B. Ke, Z.-K. Xu, *Biotechnol. Bioeng.* **2007**, *97*, 708.

[20] L. Xu, Y. Zhu, L. Tang, X. Yang, C. Li, *Electroanalysis* **2007**, *19*, 717.

[21] Y.-X. Sun, S.-F. Wang, *Bioelectrochemistry* **2007**, *71*, 172.

[22] J. Zhang, Y. Mo, M. B. Vukmirovic, R. Klie, K. Sasaki, R. R. Adzic, *J. Phys. Chem., B* **2004**, *108*, 10955.

[23] A. N. Golikand, L. Irannejad, *Electroanalysis* **2008**, *20*, 1121.

[24] M. H. Shao, T. Huang, P. Liu, J. Zhang, K. Sasaki, M. B. Vukmirovic, R. R. Adzic, *Langmuir* **2006**, *22*, 10409.

[25] T. M. Bednarski, J. Jordan, *J. Am. Chem. Soc.* **1967**, *89*, 1552.

[26] F. Qu, Z. Zhu, N.-Q. Li, *Electroanalysis* **2000**, *12*, 831.