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Electrochemical study of photovoltaic effect of nano titanium dioxide on hemoglobin

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Abstract

Nano titanium dioxide (TiO_2) and hemoglobin (Hb) were co-modified on pyrolytic graphite (PG) electrode to study the photovoltaic effect of TiO_2 nanoparticles (NPs) on the electron transfer reactivity and catalytic activity of the protein. By means of cyclic voltammetry (CV) and FTIR measurements, the study was characterized in both aerobic and anaerobic environments. Experimental results revealed that the factors which mainly interacted with Hb were electron/hole pairs and reactive oxygen species (ROS) generated by the photovoltaic effect when TiO_2 NPs were irradiated under ultraviolet (UV) light. The electron/hole pairs generated on the surface of TiO_2 would influence the structure of Hb gently, so the electron transfer reactivity and catalytic ability of the protein slightly changed. In contrast, ROS interacted with Hb intensively, which brought in much conformational change to Hb and its active centers, and even cause some damage. Consequently, the electron transfer reactivity and catalytic activity of Hb changed with a process of increasing initially and decreasing afterwards.

Keywords: Titanium dioxide; Photovoltaic effect; Hemoglobin; Electrochemistry

1. Introduction

Titanium dioxide (TiO_2) is an n-type semiconductor material with a good biocompatibility, stability and environmental safety. It has been used in lots of areas such as paint industry, biomedicine and environmental engineering. Besides, the photovoltaic effect of TiO_2 derived from the special energy band-gap structure makes it a broader application in various fields such as electronic and chemical engineering, military and energy industry [1–3].

Recently, TiO_2 nanoparticles (NPs) have attracted great attention because of its novel characters in nanometer scale. It has a high specific surface area and abundant surface-active groups as well as the strong adsorptive ability. And the electron/hole pairs generated by the photovoltaic effect can quickly flee to the TiO_2 surface and react with adsorbed molecules, taking advantage of plenty of active sites and blank sites created on the particle surface [4]. Thus, due to its distinctive properties, TiO_2 NPs has been widely employed to manufacture solar cells [5,6] and electric color-change device [7], to sterilize and disinfect cloth, facilities in hospital and laboratory [8], as well as to decompose industry pollutants in water and pesticide remains [9–14].

Meanwhile, TiO_2 NPs have been used for immobilization of proteins and enzymes due to their high specific surface area, good biocompatibility and stability [15,16]. Li et al. has successfully assembled cytochrome c, myoglobin and hemoglobin (Hb) in TiO_2 NPs films, which can keep the natural conformation of the proteins and enhance their electron transfer reactivity [17]. Topoglidis and his co-workers have utilized TiO_2 NPs to immobilize heme proteins to study the adsorption mechanism of the proteins and their interaction with catalytic substrates, as well as to develop a series of biosensors to detect nitric oxide and carbon oxide [18–21]. Recently, Hu et al. have obtained the direct electron transfer of horseradish peroxidase in TiO_2 NPs films and have fabricated a hydrogen peroxide biosensor [22]. Moreover, the photovoltaic effect of TiO_2 NPs in UV wavelength has been applied to the photo-therapy of cancer cells and photoelectric dynamic remedy for some special diseases [23,24]. Topoglidis et al. have also used spectroscopic method to explore the photo-induced redox reaction of proteins and

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the enhanced electron transfer rate caused by photovoltaic effect of TiO_2 NPs [25]. Meanwhile, the degradation of DNA or proteins caused by photovoltaic effect of TiO_2 NPs has also been observed by several other groups [26–29]. The influence on the degradation of amino acid residues has been examined as well [30]. Besides, Scaiano et al. have found that a long-period exposure in air condition will cause the adsorbed horseradish peroxidase on TiO_2 NPs losing its activity [31].

In this paper, we report the photovoltaic effect of TiO_2 NPs on Hb, obtained by electrochemical technique. The finding here will be a help to study the photovoltaic effect of semiconductive NPs and its interaction with proteins, as well as to construct photoelectric biosensors.

2. Materials and methods

2.1. Reagents

Bovine hemoglobin and Polyethyleneimine (PEI) were purchased from Sigma, and the protein was used without further purification. TiO_2 was purchased from Haitai Nanometer Company of Nanjing (China). Other chemicals were all of analytical grade. All solutions were prepared with double-distilled water, which was purified with a Milli-Q purification system (Branstead, USA) to a specific resistance of >16 MΩ cm^{-1}, and stored in refrigerator at 4 °C.

2.2. Preparation of modified electrodes

PG electrode was prepared by putting a PG rod into a glass tube and fixing it by epoxy resin. Electrical contact was made by adhering a copper wire to the rod with the help of Wood alloy.

The PG electrode was firstly polished on rough and fine sand papers. Then its surface was polished to mirror smoothness with an alumina (particle size of about 0.05 mm)/water slurry on silk. Eventually, the electrode was thoroughly washed by ultrasonication in both double-distilled water and ethanol for about 5 min.

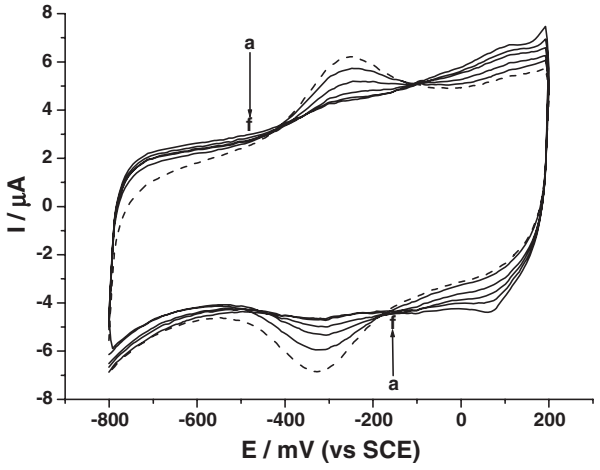

Fig. 1. Cyclic voltammograms of TiO_2 NPs–Hb co-modified PG electrode with the UV irradiation time of a) 0, b) 1, c) 2, d) 3, e) 4, f) 5 h in air conditions.

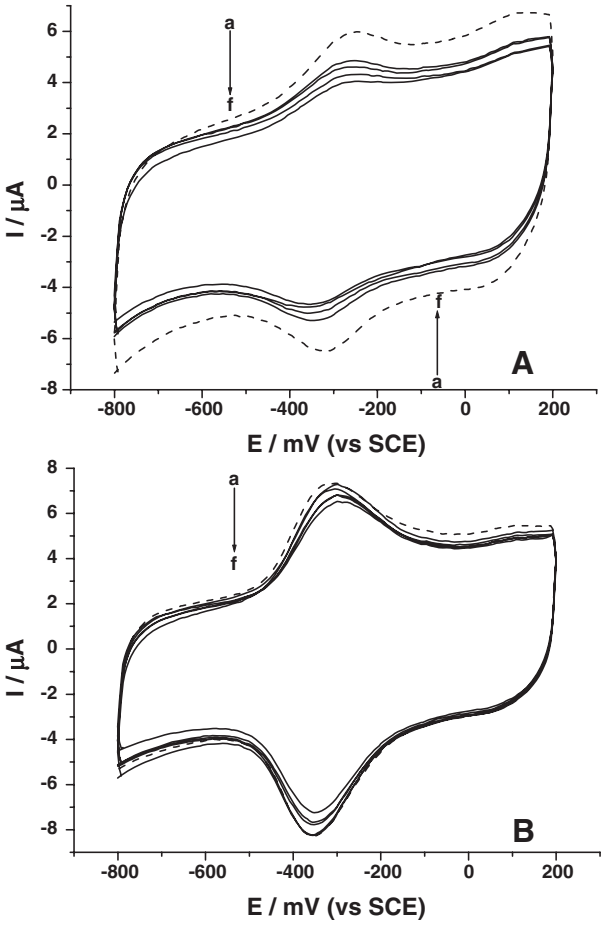

Fig. 2. Cyclic voltammograms of TiO_2 NPs–Hb co-modified PG electrode in nitrogen conditions (A) and PEI–Hb co-modified PG electrode in air conditions (B) with the UV irradiation time of a) 0, b) 1, c) 2, d) 3, e) 4, f) 5 h.

10 mg TiO_2 NPs was added into 10 mL double-distilled water and then ultrasonicated for 10~15 min to create a suspension with a concentration of 1 mg mL^{-1}. After being diluted by 10 times, the mixture of 10 μL TiO_2 NPs suspension and Hb solution (10 mg mL^{-1}, pH 8.0) was spread evenly onto the surface of the PG electrode which was afterwards covered with an Eppendorf tube and dried overnight to prepare uniform film. Finally, the modified electrode was thoroughly rinsed with double-distilled water. When not in use, the electrode was stored in aqueous solution

Table 1
The reducing rates of peak currents of Hb in different conditions

UV irradiation time t (h)	Experiment I TiO_2–O_2–UV Ratio (I_t/I_0)	Experiment II TiO_2–N_2–UV Ratio (I_t/I_0)	Experiment III PEI–O_2–UV Ratio (I_t/I_0)
0	1.0000	1.0000	1.0000
1	0.95232	0.915459	0.953303
2	0.726804	0.782005	0.946044
3	0.554553	0.693237	0.913138
4	0.387113	0.687802	0.878781
5	0.313574	0.611111	0.828938

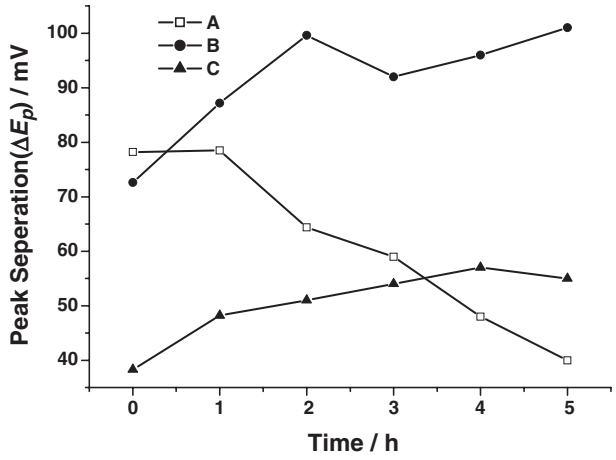

Fig. 3. Effect of UV irradiation time on the peak separation of TiO_2 NPs–Hb co-modified PG electrode in air (A) and nitrogen (B) conditions as well as the PEI–Hb co-modified PG electrode in air condition (C).

of Na_2HPO_4/NaH_2PO_4 (0.1 M) with pH 6.0 at 4 °C. The Hb–PEI co-modified electrode was prepared similarly by using PEI instead of the nano-material.

2.3. UV irradiation on modified electrodes

Electrodes were irradiated by a home-built UV lamp system as the UV light came from front-side (surface of TiO_2 film) with a distance of 10 cm. In anaerobic experiment, nitrogen was first blown into a glass tube for more than 10 min, which was used to cover the modified electrodes. Nitrogen was kept on blowing in the tube for 10 min until the tube was sealed to avoid nitrogen leaking.

2.4. Measurements

Electrochemical experiments were carried out with a VMP Potentiostat (PerkinElmer, USA) and a three-electrode system. A one-compartment glass cell with a modified PG working electrode, a saturated calomel reference electrode (SCE), and a platinum wire auxiliary electrode were used for the measurements, with a working volume of 10 mL. All the following potentials reported in this work are against the SCE.

Drops of Hb solution, TiO_2 NPs suspension or mixed solutions of a series of integral proportions of 10 mg mL^{-1} Hb and TiO_2 NPs solution were put on the chip, dried and irradiated by UV. The films were then taken off for IR measurements. Fourier transform infrared radiation (FTIR) spectroscopy was performed on a 170SX FTIR spectrometer (Nicolet, Madison, WI, USA).

2.5. Experiments

2.5.1. Experiment I

The Hb–TiO_2 NPs co-modified PG electrode was exposed under the irradiation of UV light in air condition for hours.

2.5.2. Experiment II

The Hb–TiO_2 NPs co-modified PG electrode was exposed under the UV light irradiation for hours in nitrogen condition.

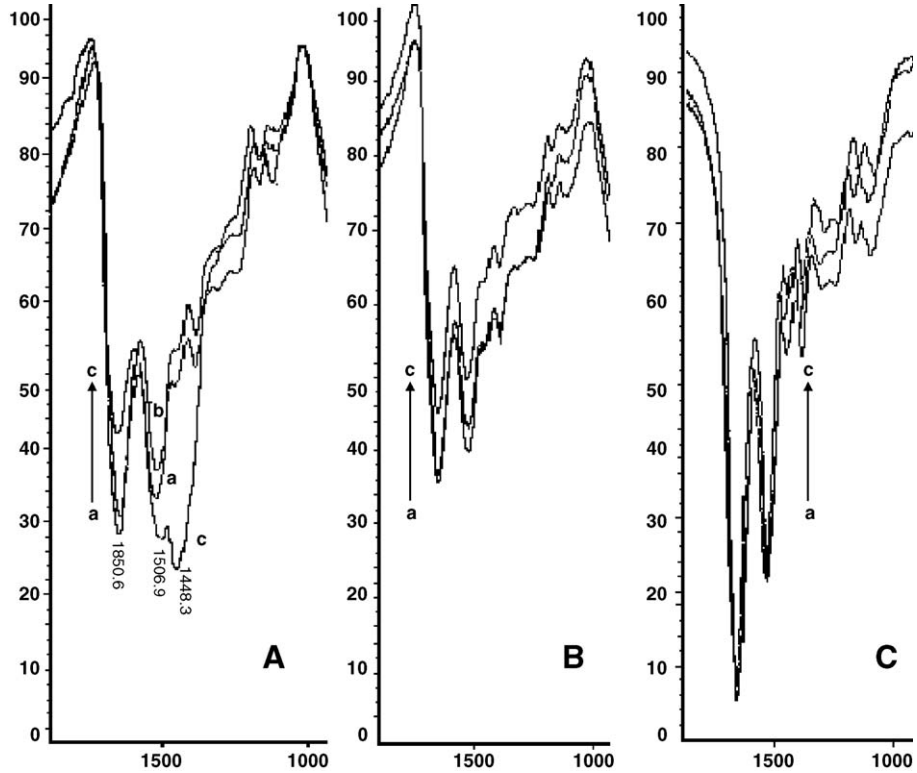

Fig. 4. FTIR spectra of mixture of Hb–TiO_2 NPs in air condition (A), nitrogen condition (B) and Hb single in air condition with the UV irradiation time of a) 1, b) 3, c) 5 h.

2.5.3. Experiment III

The Hb–PEI co-modified PG electrode was exposed under the UV light irradiation in air condition.

3. Results and discussion

3.1. Electrochemical study of photovoltaic effect of TiO_2 NPs on Hb

To investigate the influence of the photovoltaic effect of TiO_2 NPs on Hb, the Hb–TiO_2 NPs co-modified PG electrode is exposed under the irradiation of UV light first of all in air condition for hours (Experiment I). Results in Fig. 1 show that with the increasing irradiation time, the peak currents of Hb decrease gradually (from curve a to f). After irradiation for 5 h, the anodic and cathodic peak currents have fallen to 31.4% and 47.3% of their original, which indicates a loss of electroactive proteins after this photo-activating process.

When TiO_2 NPs undergo the UV light irradiation, electrons in the valence band of TiO_2 are promoted to the conduction band, generating electron/hole (e^-/h^+) pairs, which can quickly flee to the surface of NPs and produce reactive sites and blank sites. Accordingly, in air condition, reactive oxygen species (ROS) can be achieved by the interaction between electron/hole pairs and oxygen on the nanoparticles surfaces. After irradiation on TiO_2 NPs, the following reactions will take place [32]:

$$3O_2 \rightarrow 2O_3 \quad (1)$$

$$TiO_2 \rightarrow h^+ + e^- \quad (2)$$

$$O_2 + e^- \rightarrow O_2^{\cdot -} \quad (3)$$

Because of the existence of H_2O molecules in the air, although not so many, the following reactions may also occur on the surface of Hb–TiO_2 NPs modified PG electrodes:

$$h^+ + H_2O \rightarrow HO^{\cdot} + H^+ \quad (4)$$

$$O_2^{\cdot -} + H^+ \rightarrow HO_2^{\cdot} \quad (5)$$

$$2HO_2^{\cdot} \rightarrow H_2O_2 + O_2 \quad (6)$$

$$H_2O_2 + O_2^{\cdot -} \rightarrow HO^- + HO + O_2 \quad (7)$$

$$h^+ + HO^- \rightarrow HO^{\cdot} \quad (8)$$

$$H_2O_2 + e^- \rightarrow HO^- + HO^{\cdot} \quad (9)$$

Therefore, the above reactions will produce hydroxyl radicals, superoxide anion, ozone and electron pairs, which all can interact with Hb. Especially, both hydroxyl radicals and superoxide anions are highly reactive molecules as ROS. In order to evaluate the separate effect of these species on Hb, two other parallel experiments have been carried out with an Hb–TiO_2 NPs co-modified PG electrode under UV light irradiation in nitrogen condition (Experiment II) and an Hb–PEI co-modified electrode under UV light in air condition (Experiment III).

When the Experiment I is carried out, all the reactions mentioned above will affect Hb synthetically. However, only the reaction (2) will play effect in the Experiment II, in which the influence from ROS and ozone can be excluded, and the study can be focused on the effect from electron/hole pairs and UV light. On the other hand, since PEI does not demonstrate the photovoltaic effect, only UV light and ozone function as the effective factors in the Experiment III. Fig. 2 shows the decrease of the peak currents of Hb after irradiated by UV light for 1∼5 h in Experiment II and III. It can be observed that their reducing rates are not as intense as that in Experiment I. Table 1 gives the reducing rates of the anodic peak current of Hb in each parallel experiment after different irradiation time. It indicates that after irradiation for a period, the amount of electroactive proteins in each film will decrease to some extent gradually. But the reducing extents of Experiment III, II and I are with an increasing trend. So, it can be concluded that electron/hole pairs caused by the photovoltaic effect of TiO_2 NPs, $O_2^{\cdot -}$ and hydroxyl radical are the main factors to produce

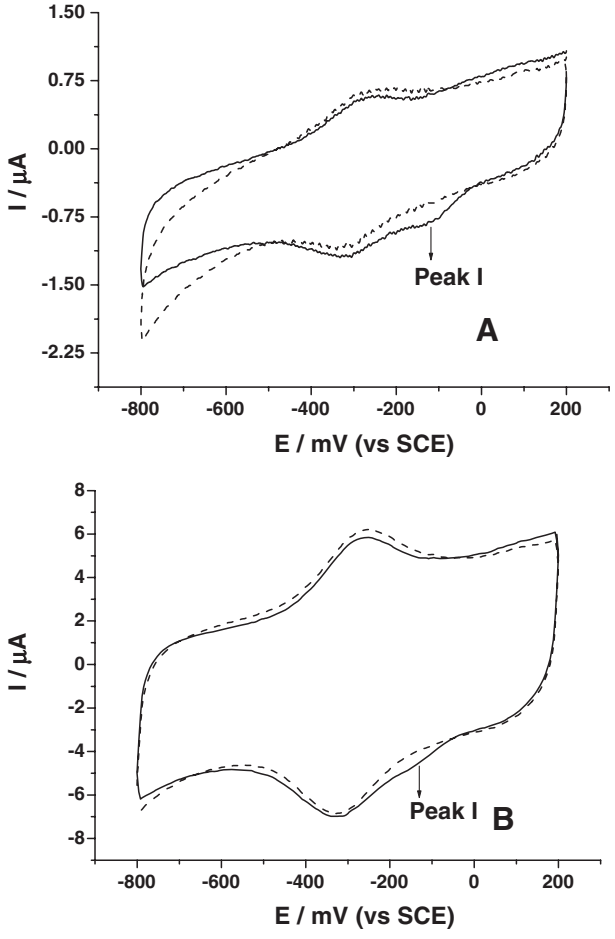

Fig. 5. Cyclic voltammograms of Hb–TiO_2 NPs co-modified PG electrode in 0.1 M PBS of pH 6.0 after injection of different volumes of air at 25 °C into the buffer with the scan rate of 20 (A) and 200 (B) mV/s. Dashed curve: 1 mL volume of air; solid curve: 5 mL volume of air.

the decrease of the peak current. UV light and ozone may also cause the decrease, however with less influence.

The peak separation ΔE of Hb, which indicates the electron transfer reactivity of the protein, displays a different changing trend in the three parallel experiments. Usually a larger ΔE presents a sluggish reactivity and vice versa. As is shown in Fig. 3, after UV irradiation the changing trends of ΔE in Experiment II and III show an enlargement with the increasing irradiation time. It implies that the reactivity of Hb is inhibited after UV irradiation. By contrary, ΔE of Experiment I becomes much smaller, which means that the electron transfer reactivity is increasing with the irradiation of UV light. Therefore, after the photovoltaic effect of TiO_2 NPs on Hb with the involvement of air which produces hydroxyl radicals and superoxide anion, the redox reactivity of the protein has been enhanced, although the amount of electroactive protein has decreased. These results also demonstrate that factors in each parallel experiment make different interactive modes on Hb.

3.2. FTIR measurement of conformational change

FTIR spectroscopic technique has been employed to study the conformational change of Hb after the UV irradiation, since FTIR is very sensitive to the conformational changes of the protein [33–36], and the conformational changes will affect the electron transfer reactivity. It has been known that the shapes of amide I and amide II infrared absorption band of Hb can provide detailed information about the secondary structure of the polypeptide chain [37,38]. The amide I band (1700~1600 cm^{-1}) is caused by C=O stretching vibrations of peptide linkages in the protein's backbone and the amide II band (1620~1500 cm^{-1}) is attributed to the combination of N–H bending and C–N stretching. In our measurement, the absorption bands of Hb are located at 1646 and 1515 cm^{-1}, which reflect the situation of á helix [39,40] and anti β sheet [41] in the protein, respectively.

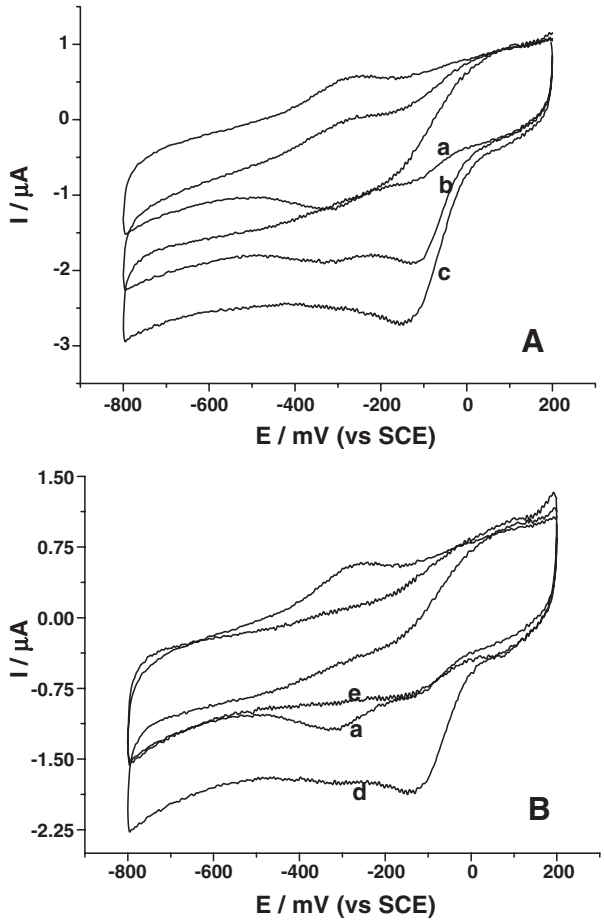

Fig. 6. Cyclic voltammograms of Hb–TiO_2 NPs co-modified PG electrode in 0.1 M PBS of pH 6.0 in the condition of oxygen free diffusion in 25 °C for 5 min with the UV irradiation of a) 0, b) 1, c) 2, d) 3, e) 5 h. Scan rate: 20 mV/s.

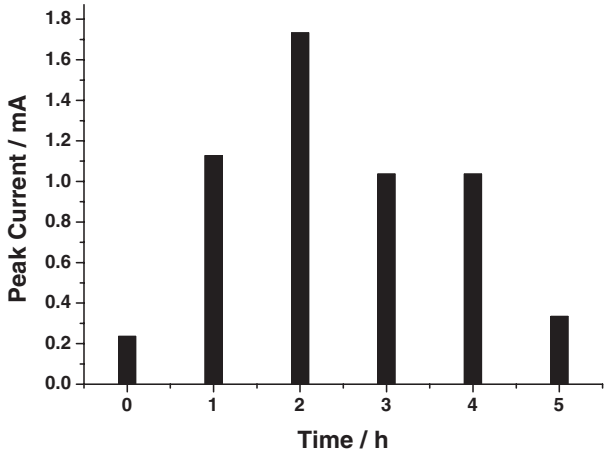

Fig. 7. The catalytic peak currents of Hb to O_2 on after the UV irradiation in air condition.

As shown in Fig. 4, after UV light irradiation, the three parallel experiments give different results. In the first 3 h irradiation, the absorbance of the two bands decreases similarly. However, in the experiment of Hb with TiO_2 NPs exposed in the air condition, after being irradiated for as long as 5 h, two new peaks with strong absorbance appear in 1498 and 1448 cm^{-1}, which can be attributed to the vibration of tyrosine (Tyr) residue, implying the possible exposure of Tyr residue to the out layer of Hb [42]. Since Tyr residue is generally buried in the hydrophobic area of the protein [43], its exposure indicates that the secondary structure of Hb has been badly damaged after 5 h UV irradiation. In the meantime, this conformational change can be beneficial to the electron transfer reactivity of the protein. It has been reported that Tyr residue is very sensitive to hydroxyl radicals and superoxide anion, and long time interaction between the ROS and the protein will

Table 2
The rate of catalytic peak currents of Hb to O_2 in different conditions

Irradiation time t (h)	Experiment I TiO_2–O_2–UV Ratio (I_t/I_0)	Experiment II TiO_2–N_2–UV Ratio (I_t/I_0)	Experiment III PEI–O_2–UV Ratio (I_t/I_0)
0	1.0000	1.0000	1.0000
1	4.75527	1.01825	0.65487
2	7.31224	0.92958	0.45133
3	4.37553	0.74721	0.67699
4	4.37131	0.34135	0.67257
5	1.4135	0.49592	0.50442

cause the degradation and breakage of the peptide chains [30], which will loose the secondary structure of the protein and further make the electroactive sites exposed. As a result, the electron transfer reactivity is enhanced. On the other hand, above experimental results reveal that UV irradiation and ozone that is derived from oxygen activated by UV light will only take little effect on the structure of protein. However, hydroxyl radicals and superoxide anion, which are generated by the photovoltaic effect of TiO_2 NPs in air condition, are highly reactive radicals [10,44–46], which may cause the degradation of Hb [47]. And, a longer irradiation period will bring in structural changes of the protein.

3.3. Investigation of catalytic ability

In vivo, Hb is a kind of oxygen-binding protein, working as an oxygen carrier in its ferrous state. And in vitro, it can catalyze substances such as oxygen, hydrogen peroxide and nitric oxide [48]. Thus we have also used such substrates to evaluate the catalytic ability of Hb under the special UV irradiation on TiO_2 NPs. Considering the possible structural damage of the protein from concentrated hydrogen peroxide and nitric oxide, oxygen is chosen to study the catalytic ability of Hb. As is shown in Fig. 5, a new reduction peak (peak I) at about −0.1 V appears after oxygen is added in the buffer solution. Since the reduction potential of oxygen at the bare PG electrode is around −0.6 V, the interaction between Hb and oxygen has reduced the reduction energy of oxygen, facilitating the deoxidization of oxygen.

Fig. 6 shows that after irradiation for less than 3 h, the Hb–TiO_2 NPs co-modified electrode exhibits a growth of catalytic current with the prolonging UV irradiation time. However, too much irradiation will decrease the catalytic current of Hb and make its catalytic ability undergo a process of increasing initially and decreasing afterwards, as shown in Fig. 7.

Table 2 has also shown the photovoltaic effect on the catalytic ability of the protein. It can be known that the catalytic activity of the protein cannot be enhanced if TiO_2 NPs are not involved, or if the photovoltaic effect is not excited.

Acknowledgements

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References

[1] M. Turkoglu, S. Yener, Design and in vivo evaluation of ultrafine inorganic-oxide-containing-sunscreen formulations, Int. J. Cosmet. Sci. 19 (1997) 193–201.
[2] G. Meacock, K.D.A. Taylor, M. Knowles, A. Himonides, The improved whitening of minced cod flesh using dispersed titanium dioxide, J. Sci. Food Agric. 73 (1997) 221–225.
[3] R. van der Molen, H. Hurksx, C. Out-Luiting, F. Spies, J. van't Noordende, H. Koerten, A. Mommaas, Efficacy of micronized titanium dioxide-containing compounds in protection against UVB-induced immunosuppression in humans in vivo, J. Photochem. Photobiol., B Biol. 44 (1998) 143–150.
[4] S.V. Tambwekar, M. Subrahmanyam, Photocatalytic generation of hydrogen from hydrogen sulfide: an energy bargain, Int. J. Hydrogen Energy 22 (1997) 959–965.
[5] B. O'Regan, M. Grätzel, A low-cost, high-efficiency solar-cell based on dye-sensitized colloidal TiO_2 films, Nature 353 (1991) 737–740.
[6] H. Renssmo, K. Keis, H. Linstrom, S. Sodergren, A. Solbrand, A. Hagfeldt, S.E. Lindquist, L.N.M. Muhammed, High light-to-energy conversion efficiencies for solar cells based on nanostructured ZnO electrodes, J. Phys. Chem., B 101 (1997) 2598–2601.
[7] J. Sotomayor, G. Will, D. Fitzmaurice, Photoelectrochromic heterosupramolecular assemblies, J. Mater. Chem. 10 (2000) 685–692.
[8] R. Cai, K. Hashimoto, K. Itoh, Y. Kubota, A. Fujishima, Photokilling of malignant cells with ultrafine TiO_2 powder, Bull. Chem. Soc. Jpn. 64 (1991) 1268–1273.
[9] E. Pelizzetti, V. Maurino, C. Minero, V. Carlin, E. Pramauro, O. Zerbinati, M. Tosato, Photocatalytic degradation of atrazine and other *s*-Triazine Herbicides, Environ. Sci. Technol. 24 (1990) 1559–1565.
[10] H. Hidaka, J. Zhao, E. Pelizzetti, N. Serpone, Photodegradation of surfactants, 8. Comparison of photocatalytic processes between anionic sodium dodecylbenzenesulfonate and cationic benzyldodecyldimethylammonium chloride on the TiO_2 surface, J. Phys. Chem. 96 (1992) 2226–2230.
[11] L. Maszkat, Photocatalytic degradation process, in: P.C. Kearney, T. Roberts (Eds.), Pesticide Remediation in Soil and Water, Wily, 1998, pp. 307–337.
[12] D. Bahnemann, Photocatalytic detoxification of polluted waters, in: O. Hutzinger (Ed.), The Handbook of Environment Chemistry, vol. II, 1999, pp. 285–351, Part L.
[13] A. Mills, S. Le Huntc, An overview of semiconductor photocatalysis, J. Photochem. Photobiol., A Chem. 108 (1997) 1–35.
[14] E. Moctezuma, E. Leyva, E. Monreal, N. Villegas, D. Infante, Photocatalytic degradation of the herbicide "paraquat", Chemosphere 39 (1999) 511–517.
[15] E. Topoglidis, B.M. Discher, C.C. Moser, P.L. Dutton, J.R. Durrant, Functionalizing nanocrystalline metal oxide electrodes with robust synthetic redox proteins, ChemBioChem 4 (2003) 1332–1339.
[16] K.J. McKenzie, F. Marken, M. Opallo, TiO_2 phytate films as hosts and conduits for cytochrome c electrochemistry, Bioelectrochemistry 66 (2005) 41–47.
[17] Q.W. Li, G.A. Luo, J. Feng, Direct electron transfer for heme proteins assembled on nanocrystalline TiO_2 film, Electroanalysis 13 (2001) 359–363.
[18] E. Topoglidis, A.E.G. Cass, G. Gilardi, S. Sadeghi, N. Beaumont, J.R. Durrant, Protein adsorption on nanocrystalline TiO_2 films: an immobilization strategy for bioanalytical devices, Anal. Chem. 70 (1998) 5111–5113.
[19] E. Topoglidis, C.J. Campbell, A.E.G. Cass, J.R. Durrant, Factors that affect protein adsoption on nanostructured titania films. A novel spectroelectrochemical application to sensing, Langmuir 17 (2001) 7899–7906.
[20] E. Topoglidis, A.E.G. Cass, B. O'Regan, J.R. Durrant, Immobilisation and bioelectrochemistry of proteins on nanoporous TiO_2 and ZnO films, J. Electroanal. Chem. 517 (2001) 20–27.
[21] E. Topoglidis, Y. Astuti, F. Duriaux, M. Gratzel, J.R. Durrant, Direct electrochemistry and nitric oxide interaction of heme proteins adsorbed on nanocrystalline tin oxide electrodes, Langmuir 19 (2003) 6894–6900.
[22] Y. Zhang, P.L. He, N.F. Hu, Horseradish peroxidase immobilized in TiO_2 nanoparticle films on pyrolytic graphite electrodes: direct electrochemistry and bioelectrocatalysis, Electrochim. Acta 49 (2004) 1981–1988.
[23] A. Fujishima, R. Cai, K. Hashimoto, H. Sakai, Y. Kubota, Biochemical application of TiO_2 photocatalysts, in: D.F. Ollis, H. Al-Ekabi (Eds.), Photocatalytic Purification and Treatment of Water, Elsevier, 1993, pp. 193–205.
[24] N. Huang, Z. Xiao, D. Huang, C. Yuan, Photochemical disinfection of *Escherichia coli* with a TiO_2 colloid solution and a self-assembled TiO_2 thin film, Supramol. Sci. 5 (1998) 559–564.

[25] E. Topoglidis, T. Lutz, R.L. Willis, C.J. Barnett, A.E.G. Cass, J.R. Durrant, Protein adsorption on nanoporous TiO_2 films: a novel approach to studying photoinduced protein/electrode transfer reactions, Faraday Discuss. 116 (2000) 35–46.
[26] W. Wamer, J. Yin, R. Wei, Oxidative damage to nucleic acids photosensitized by titanium dioxide, Free Radic. Biol. Med. 23 (1997) 851–858.
[27] H. Hidaka, S. Horikoshi, N. Serpone, J. Knowland, In vitro photochemical damage to DNA, RNA and their bases by an inorganic sunscreen agent on exposure to UVA and UVB radiation, J. Photochem. Photobiol., A Chem. 111 (1997) 205–213.
[28] S. Horikoshi, N. Serpone, S. Yoshikawa, J. Knowland, H. Hidaka, Photocatalyzed degradation of polymers in aqueous semiconductor suspensions. IV theoretical and experimental of constituent bases in nucleic acids at titania/water interfaces, J. Photochem. Photobiol., A Chem. 120 (1999) 63–74.
[29] S. Horikoshi, N. Serpone, J. Zhao, H. Hidaka, Towards a better understanding of the initial steps in the photocatalyzed mineralization of amino acids at the titania/water interface. An experimental and theoretical examination of L-alanine, L-serine and L-phenylalanine, J. Photochem. Photobiol., A Chem. 118 (1998) 123–129.
[30] L. Muszkat, L. Feigelson, L. Bir, K.A. Muszkat, Titanium dioxide photocatalyzed oxidation of proteins in biocontaminated water, J. Photochem. Photobiol., B Biol. 60 (2001) 32–36.
[31] T. Hancock-Chen, J.C. Scaiano, Enzyme inactivation by TiO_2 photosensitization, J. Photochem. Photobiol., B Biol. 57 (2000) 193–196.
[32] H. Al-Ekabl, N. Serpone, Kinetic studies in heterogeneous photocatalysis: 1. Photocatalytic degradation of chlorinated phenols in aerated aqueous solutions over TiO_2 supported on a glass matrix, J. Phys. Chem. 92 (1988) 5726–5731.
[33] D.M. Byler, H. Susi, Examination of the secondary structure of proteins by deconvolved FTIR spectra, Biopolymers 25 (1986) 469–487.
[34] R.W. Sarver Jr., W.C. Krueger, An infrared and circular dichroism combined approach to the analysis of protein secondary structure, Anal. Biochem. 199 (1991) 61–67.
[35] W.K. Surewicz, H.H. Mantsch, New insight into protein secondary structure from resolution-enhanced infrared spectra, Biochim. Biophys. Acta 952 (1988) 115–130.
[36] J.K. Koening, D.L. Tabb, in: J.R. During (Ed.), Analytical Applications of FTIR to Molecular and Biological Systems, Reidel, Boston, MA, 1980, p. 241.
[37] J.K. Kauppinen, D.J. Moffat, H.H. Mantsch, D.G. Cameron, Fourier self-doconvolution: a method for resolving intrinsically overlapped bands, Appl. Spectrosc. 35 (1981) 271–276.
[38] J.F. Rusling, T.F. Kumosinski, New advances in computer modeling of chemical and biochemical data, Intell. Instrum. Comput. 10 (1992) 139–145.
[39] A. Dong, P. Huang, W.S. Caughey, Protein secondary structures in water from second-derivative amide I infrared spectra, Biochemistry 29 (1990) 3303–3308.
[40] A. Dong, P. Huang, W.S. Caughey, Redox-dependent changes in β-extended chain and turn structure of cytochrome c in water solution determined by second derivative amide I infrared spectra, Biochemistry 31 (1992) 182–189.
[41] S. Krimm, J. Bandekar, Vibrational spectroscopy and conformation of peptides, polypeptides and proteins, Adv. Protein Chem. 38 (1986) 181–264.
[42] P. Hellwig, S. Grzybek, J. Behr, B. Luwig, H. Michel, W. Mantele, Electrochemical and ultraviolet/visible/infrared spectroscopic analysis of heme a and a_3 redox reactions in the cytochrome c oxidase from *Paracoccus denitrificans*: separation of heme a and a_3 contributions and assignment of vibrational modes, Biochemistry 38 (1999) 1685–1694.
[43] T. Brittain, Molecular aspects of embryonic hemoglobin function, Mol. Aspects Med. 23 (2002) 293–342.
[44] E. Pelizzetti, V. Maurino, C. Minero, V. Carlin, E. Pramauro, O. Zerbinati, M.L. Tosato, Photocatalytic degradation of atrazine and other *s*-Triazine herbicides, Environ. Sci. Technol. 24 (1990) 1559–1565.
[45] L. Muszkat, Photocatalytic degradation process, in: P.C. Kearney, T. Roberts (Eds.), Pesticide Remediation in Soil and Water, Willy, 1998, p. 307.
[46] A. Mills, S. Le Hunte, An overview of semiconductor photo-catalysis, J. Photochem. Photobiol., A Chem. 108 (1997) 1–35.
[47] K.J.A. Davies, A.L. Goldberg, Oxygen radicals stimulate intracellular proteolysis and lipid peroxidation by independent mechanisms in erythrocytes, J. Biol. Chem. 262 (1987) 8220–8226.
[48] L. Stryer, Biochemistry, 3rd edition, Freeman, New York, 1998.