ELSEVIER

Journal of Photochemistry and Photobiology A: Chemistry 152 (2002) 53–60

Journal of Photochemistry and Photobiology A:Chemistry

www.elsevier.com/locate/jphotochem

Photoelectrochemistry of photosynthetic reaction centers embedded in Al_2O_3 gel

Jiequan Zhao [a], Ning Ma [a], Baohong Liu [a], Yonglong Zhou [b], Chunhe Xu [b], Jilie Kong [a,*]

[a] *Department of Chemistry, Fudan University, Shanghai 200433, PR China*
[b] *Shanghai Institute of Plant Physiology, Chinese Academy of Sciences, Shanghai 200433, PR China*

Received 1 January 2001; received in revised form 29 March 2002; accepted 10 May 2002

Abstract

Bacterial photosynthetic reaction center (RC) from *Rhodobacter sphaeroides* strain RS601 was immobilized on the glassy carbon electrode, and its photoelectrochemical responses were investigated. The effects of the H_2O/Al ratio, protein concentration and pH of the electrolyte on photocurrent were measured. The results showed that: (1) the H_2O/Al ratio affected the pore size of sol–gel matrix, resulting in the maximum of photocurrent and peak shape changing; (2) the photocurrent increased with the load amount of protein; (3) the optimal pH range for photocurrent was in neutral pH region. The absorption and circular dichroism (CD) spectra and photocurrent results showed that the structure and activity of protein were kept in Al_2O_3 gel films. This work supplied a promising approach to fabricate artificial biomimic solar cell.

Keywords: *Rhodobacter sphaeroides*; Reaction center; Al_2O_3 sol–gel; Photocurrent; Circular dichroism spectra

1. Introduction

The photosynthetic reaction center (RC) separated from purple bacteria, *Rhodobacter Sphaeroides*, is a transmembrane pigment–protein complex, contains four bacteriochlorophyll (BChl); a BChl, two bacteriopheophitin a (BPhe) and two quinones (Q_A and Q_B) [1]. The sequence of photoinduced electron transfer inside the RC has been widely studied, and a very high quantum efficiency (ca. 100%) of the photoinduced charge separation of the RCs has been evaluated [1,2]. Therefore, different photosynthetic materials have been immobilized on an electrode surface and studied with the aim of using the advantage of RCs as very high efficient natural light energy converter [3].

Low temperature sol–gel process was an attractive avenue for the immobilization of proteins [4–9]. This is due to a number of advantages, including tunable physical properties, mechanical rigidity, chemical inertness, high photochemical and thermal stability and negligible swelling both in aqueous and organic solvents [4,5]. Moreover, a sufficient amount of trapped interstitial water contained in gels plays an important role in the retention of the tertiary structure and active reactivity of encapsulated biomolecules [9]. It has been used widely to fabricate the biosensors, catalysts and even bioartificial organs [6–9].

As reported previously, the RCs and light harvesting complexes of purple bacteria were immobilized in polyacrylamide gel, and absorption and photoacoustic spectra were investigated [10,11]. However, the photoelectrochemical properties of RC immobilized in gel were rarely reported.

In our laboratory, the positively charged Al_2O_3 sol–gel has been found to be a suitable matrix for the immobilization of enzymes [8,9]. In this study, we try to immobilize the RC in the Al_2O_3 sol–gel matrix to fabricate photoelectrodes. The results showed that the photoelectric responses for RC-Al_2O_3 gel films were affected by various parameters, such as the H_2O/Al ratio, the protein concentration, pH of electrolyte, etc. In this paper, the typical photochemical responses in Tris–HCl buffer solution containing 4 mM sodium dithionite were presented. The influences of H_2O/Al ratio, protein concentration and pH of electrolyte on photoelectric responses were addressed for fresh photoelectrodes.

2. Materials and methods

2.1. Reagents

The preparation of a stock standard alumina sol–gel solution was the same as described in reference [9]. Sodium

* Corresponding author. Tel.: +86-21-65642405; fax: +86-21-65641740.
E-mail address: jlkong@fudan.ac.cn (J. Kong).

1010-6030/02/$ – see front matter © 2002 Published by Elsevier Science B.V.
PII: S1010-6030(02)00188-0

dithionite was obtained from Dong–Huan United Chemical Plants, Beijing. The sodium dithionite solution was prepared freshly before use. The supporting electrolyte was the Tris–HCl buffer solution for pH 8.0 consisted of 50 mM Tris (hydroxymethyl)– (Tris) aminomethane and 50 mM KCl. All other chemicals were of analytical grade and were used without further purification.

The RC was obtained from Shanghai institute of plant physiology, the Chinese Academy of Sciences. The RC from the photosynthetic bacterium RS601 (one of *Rhodobacter Sphaeroide* strain, containing carotenoid) was separated and purified as described in reference [12]. The purified RC was kept in pH 8.0 Tris–HCl buffer, containing 0.1% (w/w) lauryldimethylamine-*N*-oxide (TL buffer) and was stored at −2 °C.

2.2. Preparation of the photoelectrodes

A glassy carbon electrode (GCE, surface area: 0.1385 cm^2) was used as the base electrode for the sol–gel-modified photoelectrodes. Prior to coating, the GCE was polished first with fine emery paper, followed by 0.3 and 0.05 μm aluminum oxide power on a chamois leather, rinsed thoroughly with deionized water after each polishing step, then successively washed with 1:1 nitric acid, acetone and doubly distilled water in an ultrasonic bath.

The immobilization of RC protein in the alumina sol–gel matrix was accomplished by the addition of 10 mm^3 10 μM RC and 10 mm^3 of stock standard sol–gel solution (ratio of H_2O/Al was 100:1). With a micropipette, aliquots (15 mm^3) of such a colloid were deposited on the surface of a cleaned GCE. The electrode was then stored at 4 °C in the refrigerator for overnight drying and the resulting electrode was rinsed with water and stored at 4 °C when not in use.

2.3. Apparatus and measurements

The photocurrent was measured in three-electrode cell with an RC-modified glassy carbon electrode as working electrode, saturated calomel as reference electrode and a platinum flake as auxiliary electrode. The solution consisted of 5 cm^3 Tris–HCl buffer and 200 mm^3 0.1 M sodium dithionite. The working electrode was illuminated with a 60 W incandescent lamp through a filter ($\lambda = 600$ nm, 10^{-2} W cm^{-2}). All photocurrent tests were carried out in an electrochemical box. The photoelectric signals given in this paper were recorded by CHI-660A electrochemistry workstation (CHI Instrument Co., USA) with technology of amperometric *i–t* curve, and the IR compensation was 100% during testing. The electrode potential was set at its open-circuit voltage before each testing (∼−0.05 V), and the background dark current was less than 10 nA. All the potentials used in paper were versus the standard hydrogen electrode (SHE).

The RC gel film for UV–VIS absorption spectroscopy and CD measurements was prepared on a quartz slide by following method: 10 mm^3 of 10 μM RC solution and 10 mm^3 of stock standard sol–gel solution were deposited onto a quartz slide. The quartz slide was then stored at 4 °C in the refrigerator for overnight drying. The only RC film (or the sol–gel film) was made by depositing 10 mm^3 of 10 μM RC (or 10 mm^3 of stock standard sol–gel solution) and 10 mm^3 doubly distilled water onto the quartz slide, followed by overnight drying at 4 °C.

The UV–VIS absorption spectra were obtained using a SM-240 CCD spectrophotometer (CVI spectral instruments, Putnam, CT, USA) at room temperature. CD measurements were taken with a JASCO J-710 spectropolarimeter (JASCO spectropolarimeter power supply) purged with N_2 at a flow rate of 5 dm^3 min^{-1}. CD spectra were taken from 185 to 350 nm, using a scan speed of 100 nm min^{-1} with a response time of 0.25 s at 24 °C. Spectrum was recorded as the averages of three repeat scans. The spectrum of the Al_2O_3 gel on the quartz slide was subtracted as background.

3. Results and discussion

The hydrophilic Al_2O_3 sol–gel matrix was used to immobilize the photosynthetic RC protein. The positively charged Al_2O_3 sol–gel are expected to immobilize the negatively charged RC through electrostatic interaction, which may account for the observed good stability and reactivity of the protein.

3.1. The typical photoelectric response of the RC thin films

Fig. 1(a) showed the photoelectric responses of the RC thin films fabricated by sol–gel method. The results were very similar to the typical photoelectric responses of the RC thin films fabricated by other techniques, such as Langmuir–Blodgett (LB) [13] or self-assembled monolayers (SAM) technique [12]. The generated photocurrent magnitude was about 1 μA cm^{-2}. Comparing with the previously reported ones prepared by SAM technique [14], the magnitude of the photocurrent from the RC films by sol–gel method was greater two magnitude than that by SAM technique. This was easy to understand because there should be greater amount of RCs in sol–gel film than those in SAM film. The magnitude of the photocurrent from the RC LB film was uncertain, so it was difficult to compare their values.

For any RC-free films, no obvious photocurrent was observed, while for all the prepared electrodes coated with RC protein, obvious reproducible anodic photocurrents were observed immediately when the photoelectrode was illuminated. It was indicated that the generation of photocurrent was associated with the primary donor (the bacteriochlorophyll dimer or P) in the RC, rather than the other cofactor. After the P in RC was excited by light, the positive ion (P^+) produced by charge separation had strong oxidation (the midpoint potential of P^+/P, 0.48–0.52 V [15,16]), and

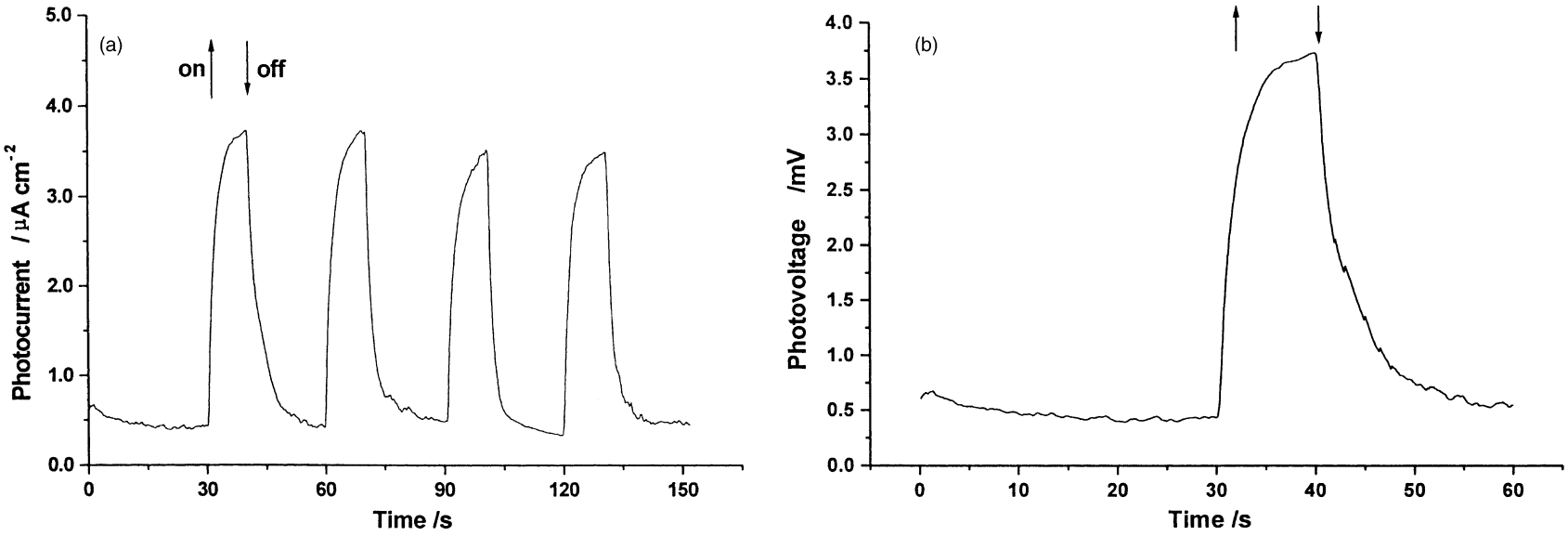

Fig. 1. The photoelectric responses of the RC-Al_2O_3 gel film induced by switching on (↑) and off (↓) the light in pH 8.0 Tris–HCl solution containing 4 mM sodium dithionite. (a) Photocurrent response; (b) photo-voltage response. The RC thin film fabricated with 1.5 mg dm^{-3} RC and H_2O/Al ratio of 200:1. The testing potential was about −0.05 V.

oxidized sodium dithionite in solution, resulting in generation of anodic photocurrent. The possible mechanism of photoelectric conversion should be as follows:

$$\text{cathode}: \quad P \xrightarrow{h\nu} P^+, \quad P^+ + e \rightarrow P$$

$$\text{anode}: \quad S_2O_4^{2-} + 4OH^- - 2e \rightarrow 2SO_3^{2-} + 2H_2O$$

The result also showed that the rise and decay of photocurrents were slow. This could be explained as follows: the RC protein was immobilized in sol–gel film, it needed some time that the sodium dithionite in solution diffused in the interior of the sol–gel film and reacted with P. So the slow rise and decay of photocurrents were observed.

In addition, the photovoltage response of the film was also observed, which was very similar to its photocurrent response (see Fig. 1(b)). The photo-voltage was about 3 mV, consistent with the previous result [17]. The reason of small photo-voltage for RC was as follows: the photosynthetic RC is a transmembrane protein [1]. For the RC isolated from the purple, the cell membrane was usually destroyed. The electric potential difference across the cell membrane didn't exist and the mediator (sodium dithionite) could short the primary donor (P) and the electron acceptor in RC. So small photovoltage was observed and the photoconversion efficiency was very low (<1%). As reported previously, if the electric potential difference across the membrane (in bilayer lipid membrane) and $FeCl_3$ existed, more than 350 mV of photovoltage was observed [18].

3.2. The effect of H_2O/Al ratio on photocurrent

The H_2O/Al (mol/mol) ratio of the sol–gel was an important parameter of electrode fabrication [9]. The effect of H_2O/Al ratio on photoelectric responses was shown in Fig. 2. In Tris–HCl buffer solution containing 4 mM sodium dithionite, the electrode responses obtained for different H_2O/Al ratios of the sol–gel were shown in Fig. 2(a). The results showed that the H_2O/Al ratio was an important factor for the photocurrent, and the maximal photocurrent was arrived at H_2O/Al ratio of 200:1. This result could be due to the influence of the H_2O/Al ratio on the pore size of the sol–gel matrix [9]. When the H_2O/Al ratio was small, the pore size of the sol–gel matrix was also small. The matrix was dense and the film was non-uniform. A too small pore size also slowed down the diffusion of mediator between the protein and the substrate surface leading to a small electrode response. A large H_2O/Al ratio accelerated the rate of alkoxide hydrolysis, increased the porosity and the specific surface area of the alumina gel. At a too high ratio, the pore size of the matrix was so large that the protein could leach out of the film easily [5,9].

For fresh-made photoelectrode, if H_2O/Al ratio was too low (e.g. 100), two oxidation peaks in photocurrent response curve were observed (see Fig. 2(b), curve 1). On the other hand, one oxidation peak and one reduction peak were observed, meanwhile the maximum of oxidation peak declined obviously when the H_2O/Al ratio was too high, e.g. 600 (see Fig. 2(b), curve 2). This could be explained easily according to the redox potential of species and the ET processes in RC as follows: the redox species in RC are P, BPhe, Q_A and Q_B. The redox potentials for the species were: about 0.48–0.52 V for P/P^+ [15,16], from −0.3 to −0.5 V for $Bphe^-$/BPhe [19], from 0 to −0.1 V for Q_A^-/Q_A [20,21], 0–0.08 V for Q_B^-/Q_B [21,22], respectively. When the RC was excited by light, the P was oxidized; meanwhile, the electron was transferred from the excited P to BPhe in about 3 ps, and arrived the Q_A about 200 ps, then to Q_B within 0.1 s. As usually, the electron could transfer from the excited P to Q_A and the electron transfer from Q_A to Q_B was blocked in pH 8.0 Tris–HCl buffer containing about 4 mM sodium dithionite (the open-circuit potential was about −0.05 V). So one oxidation peak was observed at this open-circuit potential. If the H_2O/Al ratio was small, it was difficult that the sodium dithionite in solution diffused in the interior of the sol–gel film, and the electron could transfer from Q_A to Q_B. The Q_B^-/Q_B was able to oxidize the sodium dithionite, so two oxidation peaks were observed. If the H_2O/Al ratio was large, it was easy that the sodium dithionite in solution diffused in the interior of the sol–gel film, the electron transfer from BPhe to Q_A was blocked. After illumination, there were a lot of the reduced $BPhe^-$ in RC, which returned to ground state and reduced the sodium dithionite, so one oxidation peak and one reduction peak were observed.

According to the above-mentioned experiments results and our previous experiences [8,9], the H_2O/Al (mol/mol) ratio of 200:1 was used in the experiments.

3.3. The effect of the RC concentration on photocurrent

In order to elucidate the effect of the immobilized amount of RC molecules on the photocurrent generation, the photoelectric responses of RC films were investigated with respect to the various concentrations. As shown in Fig. 3, the photocurrent increased with the increase of the RC concentration. It was easy to understand that the increase of the immobilized RC molecules amount resulted in the photocurrent increasing. On these glassy carbon electrodes, the average photocurrent increased about 28 nA when the concentration of RC increased 1 mg dm^{-3} or the mass of RC increased 1 μg.

3.4. The effect of pH on the photocurrent

The pH of the electrolyte could affect the surface potential and the biochemical activity of RC protein. In RC, the binding of proton was the first step in establishment of proton electrochemical potential, the driving force for ATP formation [23,24]. Fig. 4 depicted the relation between the maximal photocurrent and the pH of the electrolyte. The optimal pH range for photocurrent apparently existed in neutral pH region. The observed pH effect was consistent with the previous results that the activity of RC arrived to

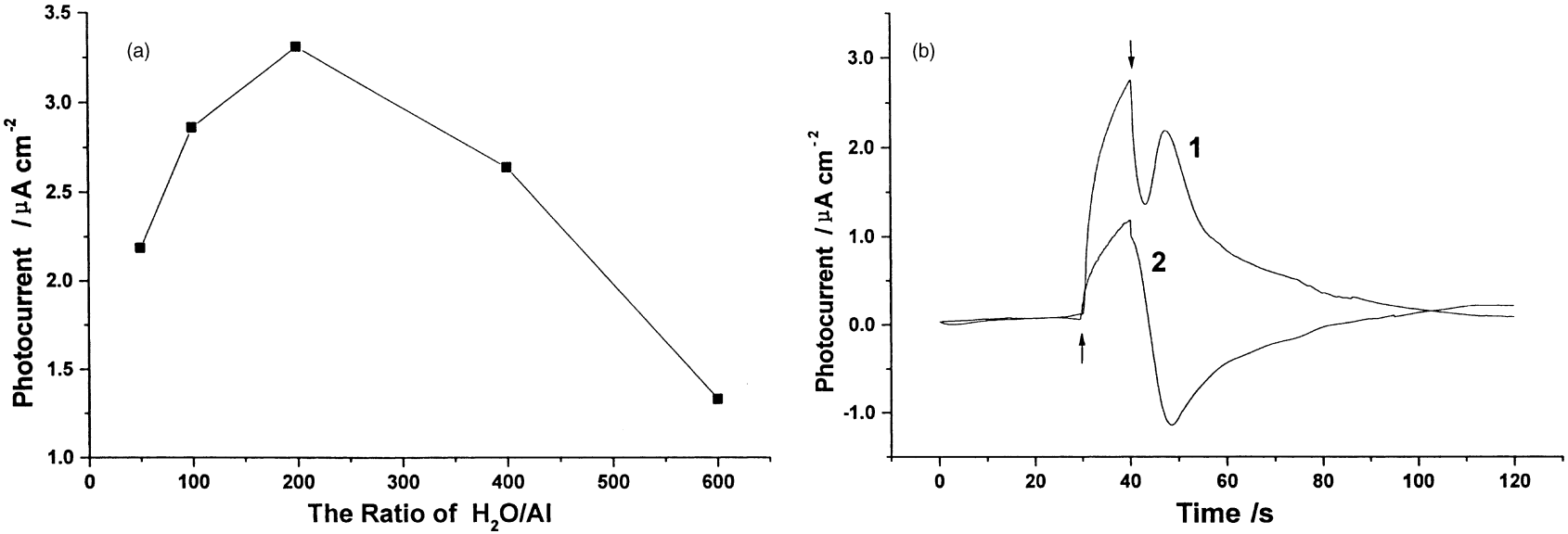

Fig. 2. the effect of H_2O/Al ratio on photocurrent. The testing potential was about −0.05 V. (a) The dependence of the photocurrent and the H_2O/Al ratio; (b) the photocurrent responses of electrode induced by switching on (↑) and off (↓) the light, the electrodes were prepared with the different H_2O/Al ratio, (1) 100/1; (2) 600/1.

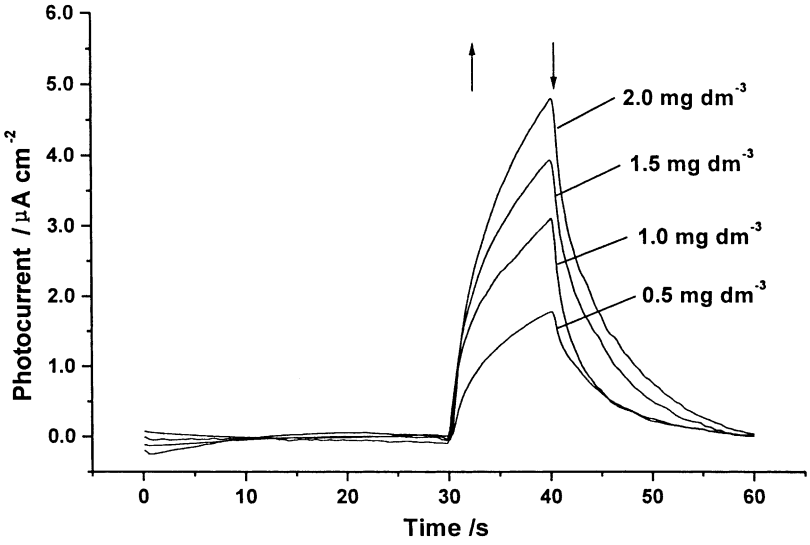

Fig. 3. The photocurrent responses of the electrode induced by switching on (↑) and off (↓) the light. The testing potential was about −0.05 V. The electrodes were prepared by a 200:1 H_2O/Al ratio and the 0.5, 1.0, 1.5 and 2.0 mg dm^{-3} RC, respectively.

the maximum at the pH region 6–9 and fell significantly at higher or lower pHs [25]. The similar phenomenon was also observed for purple membrane [26].

3.5. UV–VIS absorption spectra

RC absorption gives a very useful conformational probe for the study of the photosynthetic proteins. Fig. 5 presented the ground state absorption spectra of all investigated samples measured without additional illumination. RS601 RC showed major absorption at 760, 802 and 870 nm, which correspond predominantly to Q_Y transitions for BPhe, BChl and P in RC, respectively [27]. The absorption of RC in Al_2O_3 sol–gel matrix and immobilized on the quartz slides was similar to the one's of the RC in the Tris–HCl buffer, which showed that the native status for RC embedded in sol–gel matrix was kept.

3.6. Circular dichroism (CD) spectra

CD measurements provided an excellent means of studying the protein structure [28,29]. In order to study the structure of RC protein in Al_2O_3 gel film, the CD spectra of RC protein film and the RC-Al_2O_3 gel film on the quartz slides were measured (shown in Fig. 6). Change of base lines in the CD spectrum for RC film and RC-Al_2O_3 film may be because of the difference of concentration in film.

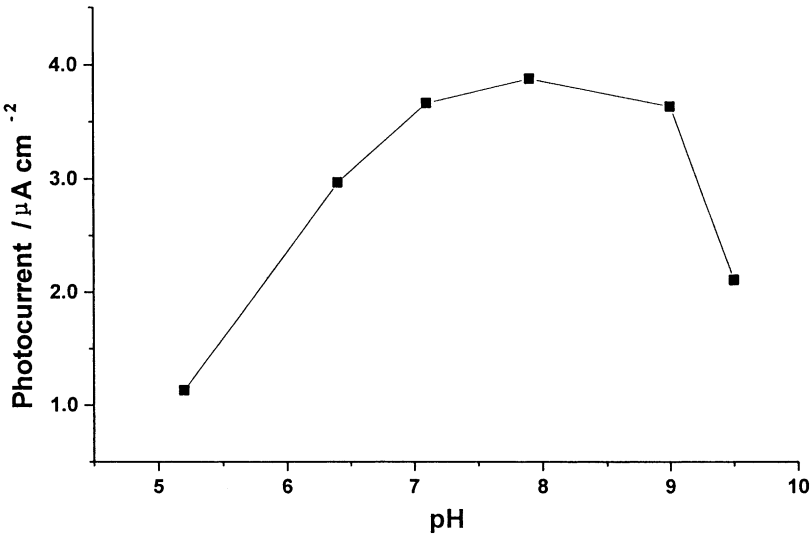

Fig. 4. The relation of photocurrent and pH of the electrolyte. The testing potential was about −0.05 V and the RC electrode used was the same as in Fig. 1.

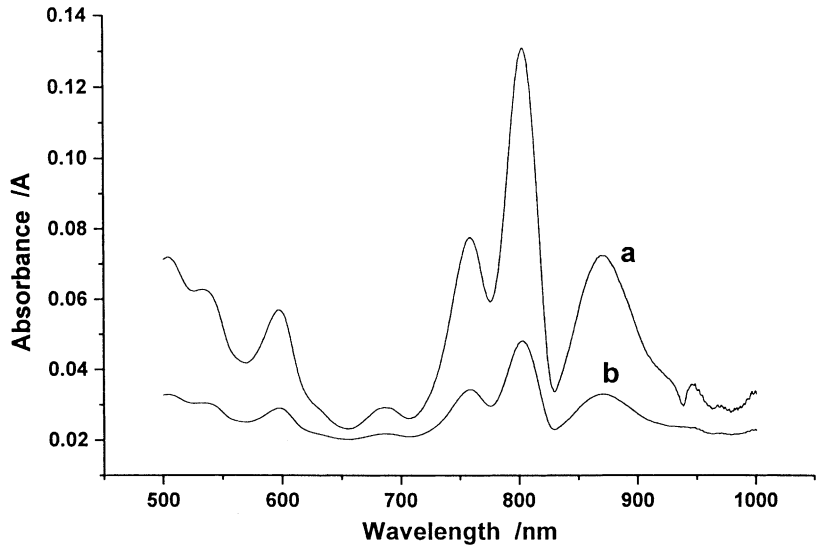

Fig. 5. Absorption spectra of (a) the 0.5 mg dm^{-3} RC in the Tris–HCl buffer and (b) RC-Al_2O_3 film immobilized on the quartz slides.

The CD spectra in the far ultraviolet bands (far UV, 185–240 nm) provide information about the secondary structure of the protein [30,31]. The spectra were characterized by the presence of two minima at 206 and 223 nm, see Fig. 6. It might be accounted for by the similar conformations of relatively bonded protein molecules in RC film and RC-Al_2O_3 film, accounting to the similar shape of CD spectra and the double negative peaks existing, which was characteristic of α-helical structure [30], in the far ultraviolet band.

Near-UV CD between 240 and 350 nm was a probe for protein tertiary structure changes that affect the environment of aromatic side chains and disulfide bonds [32]. Tyrosine and tryptophan side chains, along with cysteine disulfides, are the three major contributors to the near-UV CD of proteins [33,34]. A strong absorption band at 283 nm was tentatively ascribed to tyrosine. Tryptophan was tentatively assigned to an absorption band at 292 nm. However, tryptophan side chains may also contribute somewhat to the strong band at 283 nm [33]. The negative peak between 290 and 350 nm in near-UV CD spectrum changed apparently in RC-Al_2O_3 film, which indicated some disruption of the tight packing of the other core residues in the RC-Al_2O_3 film. This may be because of the interaction of the negative charged protein and the positive charged Al_2O_3 matrix.

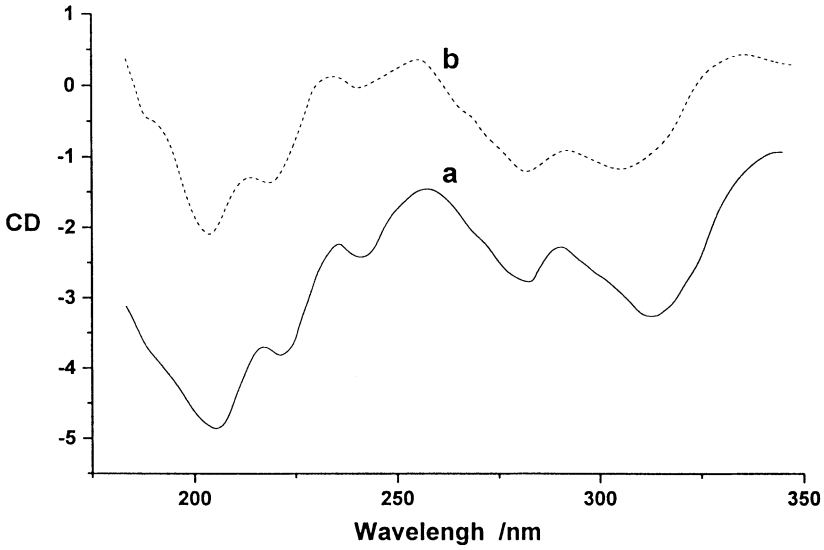

Fig. 6. The CD spectra of RC film on the quartz slides. (a) RC film; (b) the RC-Al_2O_3 gel film.

4. Conclusions

Bacterial photosynthetic RC from *Rhodobacter sphaeroides* strain RS601 was immobilized in positively charged Al_2O_3 gel. The optimum conditions for RC preparations appeared to be H_2O/Al (mol/mol) ratio of 200:1 and at 4 °C. The effects of pH and the RC concentration on photoelectrochemical responses were investigated. The absorption and CD spectra and photocurrent results showed that the structure and activity of protein were kept in Al_2O_3 gel film. It is anticipated that the properties of photosynthetic RC in sol–gel film would be useful for a better understanding the mechanism of photo-driven charge transfer and essential for the device of the biomimic solar-cell.

Acknowledgements

The authors thank the National Science Foundation of China (projects 39,970,195, 29,905,001, 39,730,040 and G1998,010,100), the Young Key Teacher Fund and EYTP from the MOE, P.R. China and Shanghai Governmental Photo Fund.

References

[1] G. Feher, J.P. Allen, M.Y. Okamura, D.C. Rees, Nature 339 (1989) 111.
[2] C. Kirmaier, D. Gaul, R. Debey, D. Holten, C.C. Schenck, Science 251 (1991) 922.
[3] E. Katz, J. Electroanal. Chem. 365 (1994) 157.
[4] O. Lev, M. Tsionsky, L. Rabinovich, V. Glezer, S. Sampath, I. Pankratov, J. Gun, Anal. Chem. 67 (1995) A22.
[5] J. Wang, Anal. Chim. Acta 399 (1999) 21.
[6] A.K. Williams, J.T. Hupp, J. Am. Chem. Soc. 120 (1998) 4366.
[7] J. Blum, A. Rosenfeld, N. Polak, O. Israelson, H. Schumann, D. Avnir, J. Mol. Catal. A: Chem. 107 (1996) 217.
[8] Z.J. Liu, B.H. Liu, J.L. Kong, J.Q. Deng, Anal. Chem. 72 (2000) 4707.
[9] Z.J. Liu, J.Q. Deng, D. Li, Anal. Chim. Acta 392 (1999) 135.
[10] M. Hara, J. Miyake, J. Goc, D. Frackowiak, J. Photochem. Photobiol. A: Chem. 124 (1999) 15.
[11] A.P. Goc, M. Hara, J. Miyake, J. Photochem. Photobiol. A: Chem. 122 (1999) 33.
[12] X.H. Zeng, H. Yu, Y.Q. Wu, M.J. Wu, J.M. Wei, H. X Shong, C.H. Xu, Acta Biochim. Biophys. Sinica 29 (1997) 46.
[13] N.A. Kalabina, S.Yu. Zaitsev, V.P. Zubov, E.P. Lukashev, A.A. Kononenko, Biochim. Biophys. Acta 1284 (1996) 138.
[14] A.A. Solov'ev, E.Yu. Katz, V.A. Shuvalov, Y.E. Erokhin, Bioelectrochem. Bioenerg. 26 (1991) 29.
[15] D.A. Moss, M. Leonhard, M. Bauscher, W. Mäntele, FEBS. Lett. 283 (1991) 33.
[16] A. Ivancich, K. Artz, J.C. Williams, J.P. Allen, T.A. Mattioli, Biochemistry 37 (1998) 11812.
[17] S. Keller, Y. Riou, J.M. Laval, W. Leibl, FEBS Lett. 487 (2000) 213.
[18] J.S. Huebner, H.T. Tien, J. Membr. Biol. 11 (1973) 47.
[19] J.L. Kong, W.L. Sun, X.L. Wu, J.Q. Deng, Z.Q. Lu, Y. Lvov, R.Z.B. Desamero, H.A. Frank, J.F. Rusling, Bioelectrochem. Bioenerg. 48 (1999) 101.
[20] G. Alegria, P.L. Dutton, Biochim. Biophys. Acta 1057 (1991) 239.
[21] A.R. Crofts, C.A. Wraight, Biochim. Biophys. Acta 726 (1983) 149.
[22] A.W. Rutherford, M.C.W. Evans, FEBS Lett. 110 (1979) 257.
[23] M.R. Gunner, Curr. Top. Bioenerg. 16 (1991) 319.
[24] M.Y. Okamura, G. Feher, Annu. Rev. Biochem. 61 (1992) 861.
[25] L. Kálámn, T. Gajda, P. Sebban, P. Maróti, Biochemistry 36 (1997) 4489.
[26] T. Miyasaka, K. Koyama, Thin solid films 210/211 (1992) 146.
[27] D.C. Arnett, C.C. Moser, P.L. Dutton, N.F. Scherer, J. Phys. Chem. 103 (1999) 2014.
[28] N.J. Greenfield, Trac-Trends Anal. Chem. 18 (1999) 236.
[29] M.V. Encinas, L.R. Olsen, J.F. Díaz, J.M. Andreu, H. Goldie, E. Cardemil, Biochim. Biophys. Acta 1252 (1995) 23.
[30] I.T. Arkin, S.I. Sukharev, P. Blount, C. Kung, A.T. Brunger, Biochim. Biophys. Acta 1369 (1998) 131.
[31] E. Sedlák, M. Antalík, Biochim. Biophys. Acta 1434 (1999) 347.
[32] A. Fatouros, B. Sjöström, Int. J. Pharm. 194 (2000) 69.
[33] M.H. Alaimo, E.D. Wickham, H.M. Farrell Jr., Biochim. Biophys. Acta 1431 (1999) 395.
[34] W. Haas, R. MacColl, J.A. Banas, Biochim. Biophys. Acta 1384 (1998) 112.