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Charge stabilization by reaction center protein immobilized to carbon nanotubes functionalized by amine groups and poly(3-thiophene acetic acid) conducting polymer

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A large number of studies have indicated recently that photosynthetic reaction center proteins (RC) bind successfully to nanostructures and their functional activity is largely retained. The major goal of current research is to find the most efficient systems and conditions for the photoelectric energy conversion and for the stability of this bio-nanocomposite. In our studies, we immobilized the RC protein on multiwalled carbon nanotubes (MWNT) through specific chemical binding to amine functional groups and through conducting polymer

(poly(3-thiophene acetic acid), PTAA). Both structural (TEM, AFM) and functional (absorption change and conductivity) measurements has shown that RCs could be bound effectively to functionalized CNTs. The kinetics of the light induced absorption change indicated that RCs were still active in the composite and there was an interaction between the protein cofactors and the CNTs. The light generated photocurrent was measured in an electrochemical cell with transparent CNT electrode designed specially for this experiment.

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1 Introduction It would be hard to contradict that converting light energy into chemical potential by photosynthesis is one of the most important processes in nature because it ensures the free energy for the energy consuming on every level in the biological systems – from the cell organelles to the biosphere [1]. The very first steps of the energy conversion, take place in the so-called reaction center protein (RC, Fig. 1), which is the minimal nanoscopic photoconverter in the photosynthetic membrane that catalyzes the conversion of solar light in energy readily usable for the metabolism of the living organisms.

The research of the RC protein to several possible technical applications is a real challenge today. Even though the RC represents only nanometers (about 10 nm size) and nanopower in the cells, it works with such efficiency that could not have been imitated by human so far. The RC owns such technical

properties that different applications are possible – for example, its use in the nanostructures or in the optoelectronic systems: (i) Characteristic light absorption in the near infrared (700–1000 nm) of the spectrum; (ii) after the excitation there are components from the few femtoseconds to seconds inside the protein; (iii) the quantum yield of the primary charge separation is extremely large (close to 100% [2]); (iv) the created redox centers inside the protein can be in interaction with their environment and ideally can be made to work.

One of the most challenging tasks is to use different type of RC proteins (PS-I and PS-II of plants and bacterial RCs) in different systems as energy converters in photovoltaic devices [3–7].

It was shown recently that RCs can be attached to carbon nanotubes (CNTs) and there is an electronic connection between the two materials [8–11].

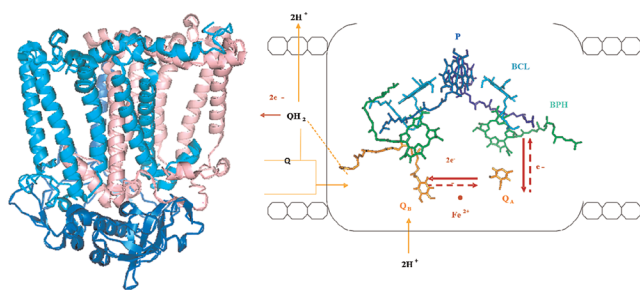


Figure 1 (online color at: www.pss-b.com) The protein subunits (left) and cofactors (right) of the photosynthetic RCs. The helix structures indicate the membrane spanning region of the protein. P, primary electron donor bacteriochlorophyll dimer; BCL, bacteriochlorophyll monomer; BPH, bacteriopheophytine; Q_A , primary quinone; Q_B , secondary quinone; QH_2 , reduced quinone in the membrane pool. Red arrows indicate the e^- -transfer routes within the protein, orange arrows indicate the role of the quinone in the proton and e^- -translocation processes.

In our experiments, we have bound RCs to multiwalled carbon nanotubes (MWNTs) through specific chemical binding to amine functional groups and through conducting polymer (PTAA). By combining the two systems, efficient vectorial charge transfer can be driven in the composites. Results of the optical and electrochemical investigations on bio-nanocomposite materials made of RCs, MWNTs, and conducting polymer, (PTAA) will be presented here.

2 Experimentals

2.1 RC preparation Reaction centers were prepared routinely from *Rhodobacter sphaeroides* R-26 purple bacteria. The cell membrane was solubilized by detergent (LDAO, N,N-dimethyldodecylamine-N-oxide, Fluka) and purified by ammonium sulfate precipitation, followed by anion-exchange chromatography (DEAE Sephacel, Sigma) [12]. The fractions of OD280/OD800 smaller than 1.50 were used for our experiments.

2.2 Preparation of PTAA solution PTAA powder was prepared by a simple chemical synthesis, by oxidizing the 3-thiophene-acetic-acid (TAA). The monomer, anhydrous iron-chloride ($FeCl_3$) and nitrobenzene (NB) were purchased from Sigma-Aldrich. The water content of nitrobenzene was determined by coulometric Karl-Fischer titration, and it was kept below 30 ppm. All solid chemicals have been dried in Büchi Glass Oven B-580 vacuum system before use [13]. The polymer was then dissolved in 0.1 M phosphate buffer solution (pH = 8.0) by ultrasonic treatment, to get a 1 mg ml^{-1} concentration solution.

2.3 Preparation of PTAA covered ITO electrode ITO covered glass was cleaned by oxygen plasma cleaner, than it was immersed into phosphate buffer (0.1 M, pH 8.0) for 5 min and into PDAA solution (poly(diallyldimethylammonium chloride), $c = 1.9 \text{ mmol dm}^{-3}$, pH = 1.0 HCl) for 10 min. Following this pre-treatment the electrode was

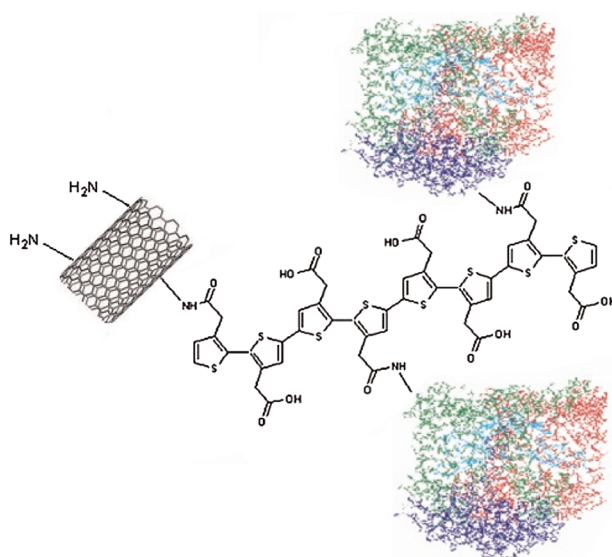


Figure 2 (online color at: www.pss-b.com) The schematic representation of the RC/PTAA/MWNT complex.

immersed into PTAA solution for 15 min. The appearance of the light absorption at 450 nm is indicative of the formation of the monolayer, which was detected by UV-Vis spectrophotometer (Agilent 8453).

2.4 Binding RCs to PTAA/MWNT complex Amino functionalized MWNTs (0.14 mg ml^{-1}) were mixed with PTAA solution (1 mg ml^{-1} in 0.1 M phosphate, pH = 8.0) and incubated for 2 h at room temperature. PTAA-MWNT complexes were separated by ultracentrifuge ($30\,000 \times g$, 20 min). RC was added to the PTAA/MWNT complex at a final concentration of $65 \mu\text{M}$ and the detergent was dialyzed out. Finally the RC/PTAA/MWNT complex (Fig. 2) was separated by ultracentrifuge ($30\,000 \times g$, 20 min).

2.5 Electrochemical measurements A three-electrode electrochemical cell was designed especially for our experiments. The working, the counter, and the reference electrodes were ITO, platinum, and Ag/AgCl/3M NaCl, respectively. RCs were bound to the working electrode by a conducting polymer, PTAA which chemical served also as a cross-linker and a conductive wiring between the MWNT and the RCs. Water soluble UQ-0 (2,3-dimethoxy-5-methyl-1,4-*p*-benzoquinone) was used as an electron transport mediator. This chemical is widely used as an artificial electron acceptor to the RC protein instead of the natural UQ-10 (2,3-dimethoxy-5-methyl-6-decylsoprenyl-1,4-benzoquinone, cf. Fig. 1). For driving the photochemistry during the electrochemical measurements the sample was illuminated by tungsten lamp (150 W).

2.6 Flash-induced absorption change measurements Flash-induced absorption change was measured in the millisecond to second time scale by a single-beam kinetic spectrophotometer of local design. The

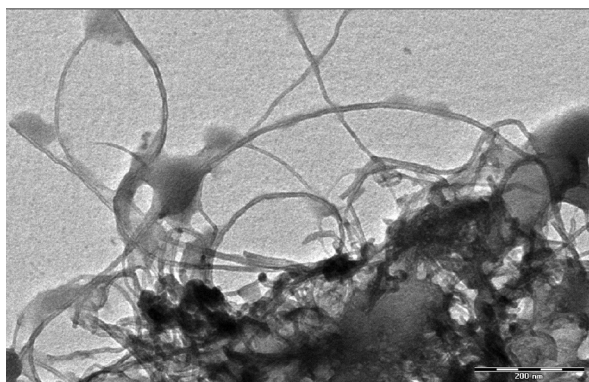


Figure 3 The TEM image of the RC/PTAA/MWNT complex.

redox changes of the primary bacteriochlorophyll dimer were detected at 860 nm [12, 14].

3 Results and discussions

3.1 Preparation of the RC/PTAA/MWNT composite TEM image (Fig. 3) shows that after the preparation there are dense plaques around the MWCNTs, corresponding to inhomogeneous coverage by the PTAA polymer. The characteristic peak in the absorption spectrum at 450 nm (Fig. 4) shows that PTAA forms a homogenous monolayer on the ITO covered by PDDA polyelectrolyte. Flash induced absorption change (Fig. 5) indicates that the RC/PTAA/MWNT complex can also be fixed on the ITO surface by PDDA and the RCs are still active after the preparation.

Figure 5 also shows that the efficiency of the binding was highly affected by the detergent concentration. The decay curves could be analyzed by a multi-exponential fit: $A(t) = \sum A_{(0,i)} \exp(-t/\tau_{(i)})$, where $A_{(0,i)}$ and $\tau_{(i)}$ are the contribution and the life time of the i^{th} components, respectively. The fitting parameters are summarized in Table 1. The monophasic decay in the absence of detergent indicates the homogenous binding of the RC to the complex.

However, it was heterogeneous in the presence of LDAO, indicated by the biphasic decay curves. The fact

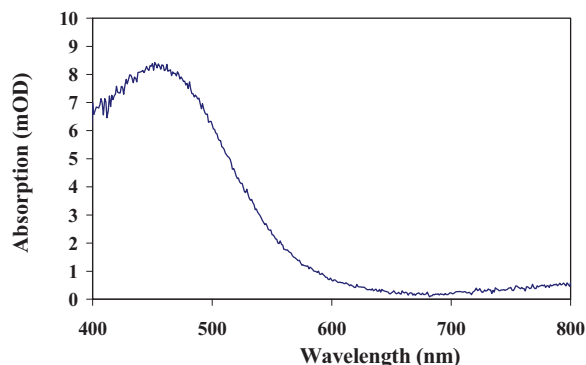


Figure 4 (online color at: www.pss-b.com) The absorption spectrum of the PTAA monolayer on ITO surface. The absorption spectrum was measured against the PDDA/ITO electrode as a reference.

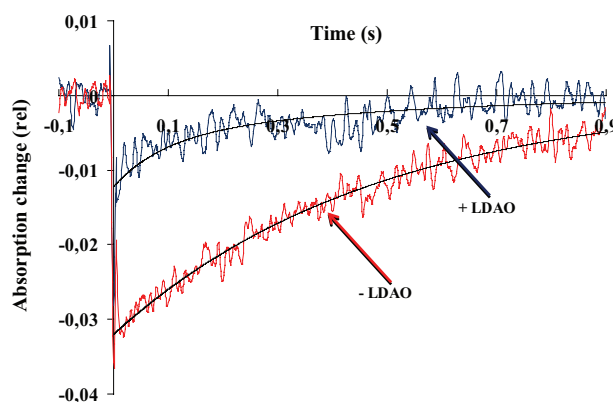


Figure 5 (online color at: www.pss-b.com) The absorption change of RC/PTAA/MWNT complex after flash excitation (blue curve: using detergent during the preparation; red curve: detergent is dialyzed out). Black solid line was calculated by the multi-exponential fit according to the equation described in the text (Section 3.1).

Table 1 Fitting parameters of the decay curves of absorption changes shown in Fig. 5.

	A_1 (%)	τ_1 (ms)	A_2 (%)	τ_2 (ms)
+LDAO	72	90	28	476
-LDAO	—	—	100	480

that the slow phase is almost the same in the two decay curves ($\tau \approx 480$ ms) indicates that there is a RCs population that bind to the complex in uniform way.

3.2 Electrochemistry of the RC/PTAA/MWNT complex on the ITO surface All measurements were done in a three-electrode electrochemical cell designed especially for our experiments as described in the “Experimentals” section.

All measurements were done at the dark equilibrium open circuit potential. After the system has reached its equilibrium it was illuminated with the white light of the

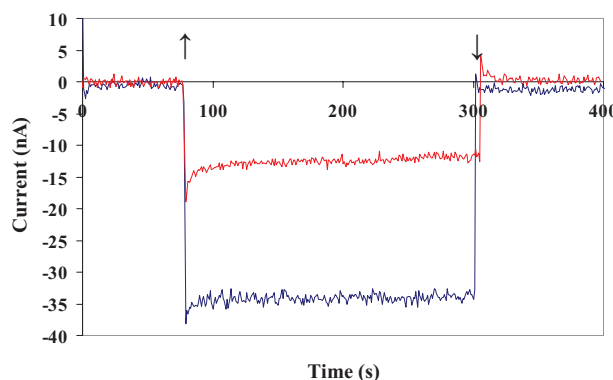


Figure 6 (online color at: www.pss-b.com) Light induced photocurrent of the electrochemical cell using PTAA covered ITO working electrode without (blue) and with quinone (UQ-0) mediator (red). The time when the light was switched on ($\uparrow$) and off ($\downarrow$) is indicated.

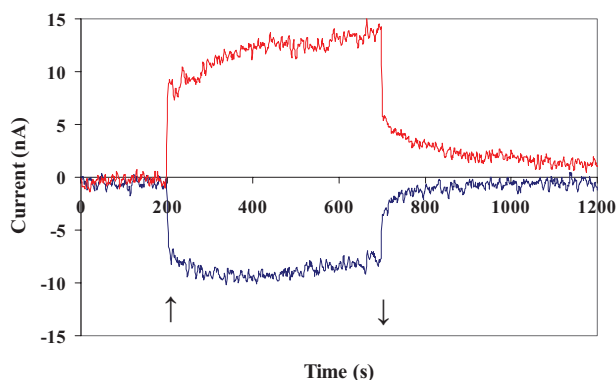


Figure 7 (online color at: www.pss-b.com) Light induced photocurrent of the electrochemical cell using PTAA and RC covered ITO electrode without (blue) and with quinone mediator (red). The time when the light was switched on (↑) and off (↓) is indicated.

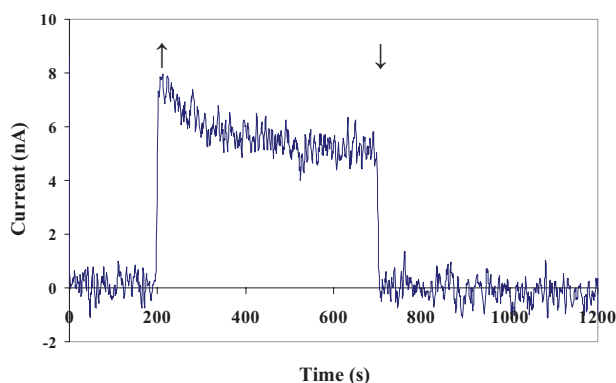


Figure 8 (online color at: www.pss-b.com) Light induced photocurrent of the electrochemical cell using ITO working electrode covered by RC/PTAA/MWNT complex. The time when the light was switched on (↑) and off (↓) is indicated.

tungsten lamp (150 W). Figures 6–8 show the photoactivity of the PTAA electrode alone, PTAA–RC complex and RC/PTAA/MWNT complex on ITO surface, respectively.

Figure 6 shows that if the PTAA/ITO electrode is illuminated alone (i.e., without the RC protein) by white light, photocurrent can be measured but is affected by the UQ-0 mediator.

If RC/PTAA complex covers ITO, the characteristics of the photocurrent is changed considerably in the presence of UQ-0 (Fig. 7). The cathodic photocurrent (measured in the absence of UQ-0) becomes anodic if the mediator is present.

In another set of experiments, the photochemical activity of the RC/PTAA/MWNT complex was tested. The ITO electrode was covered by RC/PTAA/MWNT and the light induced current was measured in the electrochemical cell in the absence of UQ-0.

Figure 8 shows that a photocurrent was induced in this system as well, however, the direction of the current is the opposite of the expected one on the basis of the experiment shown in Fig. 7. Indeed, an anodic current instead of a cathodic is measured.

4 Summary We managed to bind purple bacterial RCs to amino functionalized MWNTs supported by PTAA conducting polymer. The RCs remain active after the preparation of this nanocomposite. It can clearly be demonstrated by flash induced absorption change. The efficiency of the binding was highly affected by the detergent concentration applied during the preparation. Photocurrent could also be measured on RC/PTAA and RC/PTAA/MWNT complexes, which is affected by the redox mediator UQ-0, a chemical that participates in the electron transport path of the RC protein.

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