



Long term stabilization of reaction center protein photochemistry by carbon nanotubes

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The long term stability and the redox interaction between single walled carbon nanotubes (SWNTs) and photosynthetic reaction center proteins (RCs) purified from purple bacterium *Rhodospirillum rubrum* R-26 in the SWNT/RC complex has been investigated. The binding of SWNT to RC results in an accumulation of positive (the oxidized primary electron donor, P^+) and negative (semiquinone forms, Q_A^- and Q_B^- , the reduced primary and secondary quinones, respectively) charges followed by slow reorganization of the protein structure after excitation. The photochemical activity of the SWNT/RC

complexes remains stable for several weeks even in dried form. In the absence of SWNT the secondary quinone activity decays quickly as a function of time after drying the RC onto a glass surface. Polarography measurements substantiate the idea that there is an electronic interaction between the RCs and SWNTs after light excitation, which was suggested earlier by optical measurements. The special electronic properties of the SWNT/protein complexes open the possibility for several applications, e.g., in microelectronics, analytics, or energy conversion and storage.

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1 Introduction On the basis of their advantageous properties and abundant possibilities for applications nanomaterials are today in the focus of emerging research technologies. Beside promising technical applications, such as in nano(molecular)electronics as diodes, transistors, logic gates, and memory bits, the convincing demonstrations that biological (macro)molecules (e.g., proteins, nucleic acids, and lipids) interact specifically with single walled carbon nanotubes (SWNTs) have opened up new directions for devices based on bio-matter (bio-nanocomposites) [1, 2].

The main advantage when nano- and bio-materials are associated is that, by combining their advantageous properties, the new (bio-nano-) material exhibit exceptionally new characteristics with (perhaps predictable) designable parameters. For example, SWNTs have exceptional, unidirectional electronic and structural properties in the microscopic length dimension, as do the photosynthetic reaction center proteins (RCs) of plants or bacteria as well.

By combining the two systems a very efficient vectorial charge transfer can be driven in the SWNT/RC composites (note that the quantum yield of the primary charge separation in the RC alone is close to one [3]). The new feature of this material is that the charge transfer can be driven by light in the visible or infrared region over a broad time scale (from few ps to seconds or minutes) in nanoscopic dimensions [4].

Photosynthetic RC is a pigment protein complex, a redox-active enzyme, which uses sunlight to power a chain of intraprotein electron transport events that leads to the separation of electrical charges. The capture of light energy by bacteriochlorophylls in photosynthetic bacteria is followed by a separation of positive and negative charges in the state of P^+BPheo^- and then $P^+Q_A^-$. Here P^+ is the oxidized primary donor, a specialized bacteriochlorophyll dimer (BChl)₂, BPheo is the first electron acceptor, a monomer bacteriopheophytin, and Q_A^- is the reduced quinone type primary electron acceptor. The separated charges are then

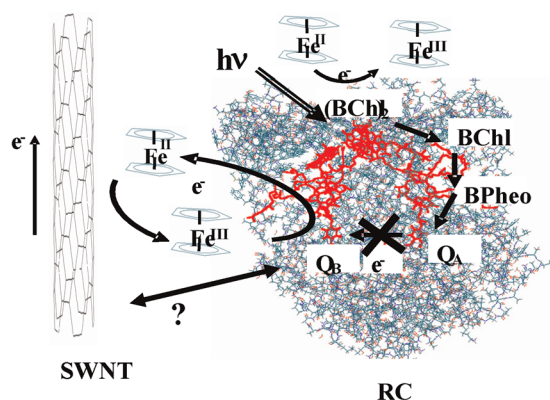


Figure 1 (online color at: www.pss-b.com) Schematic presentation of the positions of cofactors and the path of the electron flow in the RC protein and of the possible interaction between the carbon nanotube and the RC. X indicates the electron transport step, which is blocked by the inhibitor, terbutryn.

further stabilized in the form of a P^+QB^- , where Q_B^- is the reduced secondary quinone.

Recently we have shown that (i) RCs can be attached to SWNTs without specific modifications, (ii) the attachment induces changes in the intraprotein electron transport (the change is the stabilization of the light induced charge pairs), (iii) there is a possible electronic “communication” between the SWNTs and RCs [5]. For any further possible application the long term stability of the system, which depends on many internal and external factors is a very important question. The positions of cofactors and the path of the electron flow in the RC protein and the possible interaction between the carbon nanotube and the RC is demonstrated in Fig. 1. Besides the indirect optical proofs a direct evidence for the redox interaction between the SWNTs and RCs should be given.

In our study we aimed to show that (i) besides the indirect proofs of optical measurements a direct evidence for the redox interaction between the SWNTs and RCs can be given, e.g., by polarography experiments. (ii) For any further possible application the long term stability of the system, which depends on many internal and external factors is a very important question. The long term photochemical stability of the RC-composites will be determined by using different carrier matrices and environment, like humidity, temperature.

2 Experimental

2.1 Sample preparation Reaction center proteins were prepared by detergent solubilization of the photosynthetic membrane, and further purified by ammonium sulfate precipitation, followed by anion-exchange chromatography. After preparation the RC Q_B -site was reconstituted by the addition of ubiquinone-10 (UQ-10), and concentrated by centrifuge filter to 80–100 μ M. Calculated amount of RCs were dried under a stream of N_2 on the surface of the carrier matrices like SWNT, glass, ITO, Pt-electrode then the sample was incubated at different conditions as will be shown in “Results” section [5].

2.2 Optical spectroscopy Flash-induced absorption changes were measured by a single-beam kinetic spectrophotometer of local design. The stabilized light of 55 W halogene lamp was passed through a Joben Yvon monochromator and the sample. Excitation was achieved by a xenon flash lamp (type EG&G FX200, $t_{1/2} = 8.5 \mu$ s) passed through a 760 nm cut-off filter. The photomultiplier (Hamamatsu R928) was protected by a Corning 4-96 blue filter. The analog signal of the photomultiplier was converted with an A/D converter card (Advantech PCL818) attached to an IBM computer [6].

2.3 Conductivity measurements In order to measure flash induced conductivity changes a two electrode electrochemical cell was used. The current between the sample containing working Pt and the $Ag^+/AgCl$ reference electrode was measured and converted to potential while 0.1 M KCl in 10 mM TRIS buffer solution was flowing slowly above the surface of the $Ag^+/AgCl$, which was separated by a celluloid membrane from the Pt (Fig. 2). Applying -700 mV polarization voltage against the Pt the photochemical reaction was driven by ten short light flashes placed 0.1 s apart by a Stroboslave (Type 1539-a General Radio Concord Mass, USA). Data were stored by digital oscilloscope (Hitachi VC-6025) and transferred to an IBM PC for further processing.

3 Results and discussion

3.1 Kinetics of the absorption change Figure 3 shows examples for the kinetics of the single saturating flash-induced absorption change of RCs of *Rhodobacter sphaeroides* R-26 detected at 430 nm for RCs (Fig. 3, upper part) and SWNT/RC complexes (Fig. 3, lower part) dried on the surface of glass in the course of the incubation time.

The decay kinetics was decomposed assuming two exponential components. The lifetime and the contribution of the components were analyzed. The fast component has a lifetime of about 120 ms and is generally assigned to $P^+Q_A^- \rightarrow PQ_A$ charge recombination and was affected by the drying. The lifetime of the slow component, which is attributed to the $P^+Q_B^- \rightarrow PQ_B$ charge recombination is about 1–1.5 s in detergent solution, increased for longer times. In

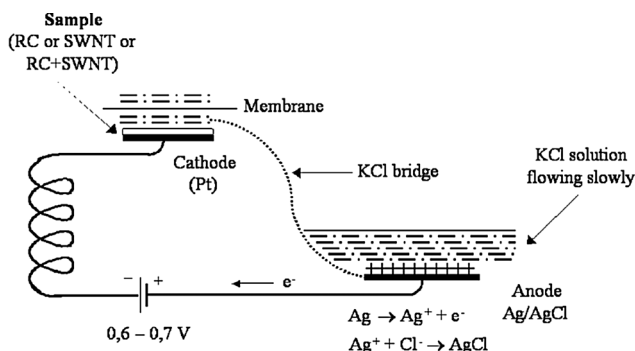


Figure 2 Schematic representations of the electrode arrangement and reactions for polarography measurements.

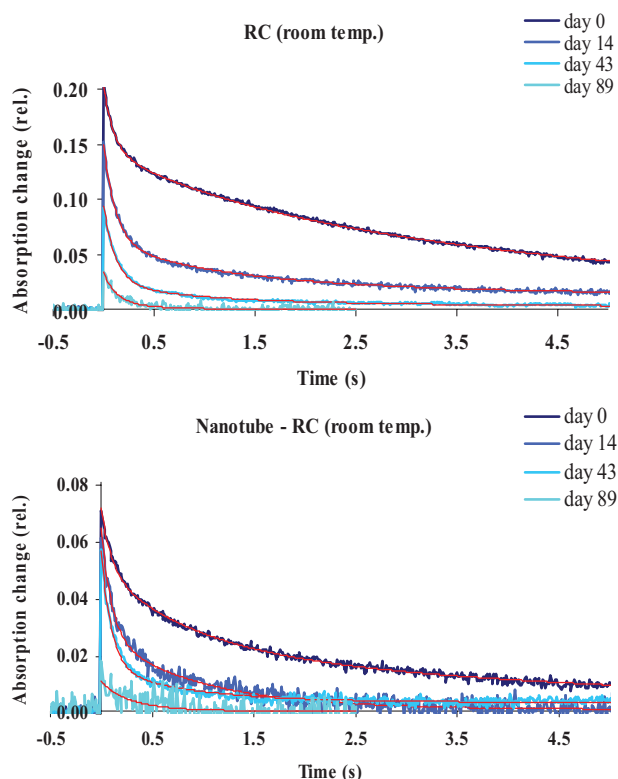


Figure 3 (online color at: www.pss-b.com) The absorption change of reaction centers after single saturating excitation flash as a function of time. Purified RCs (upper part) and SWNT/RC samples (lower figure) were dried on glass surface and incubated at room temperature. Measurements were taken at 430 nm as described in the “Experimental” section.

our earlier works we found that both the lifetime and the contribution of the slow component decreased quickly after the sample preparation for purified RCs. Here we found larger stability of this component; however it was more sensitive to the incubation time compared to the SWNT/RC sample.

3.2 Effect of the humidity on the stability of the RC dried on glass surface Before the stability of the SWNT/RC complexes was investigated we measured the effect of temperature and the relative humidity on RCs dried only on glass. This information was very important to find the best conditions for measuring the characteristics of the SWNT/RC complexes. The light induced absorption change at 430 nm was measured; the decay curves were decomposed into two exponential components. The maximum amplitude was normalized to that of calculated after 1 day incubation time. The lifetimes and the contributions of the exponential components in percentage were calculated. The results are summarized in Fig. 4.

If we follow the change of the maximum amplitude of the signal as a function of the incubation time no effect of the humidity above 10% and of the temperature was found. Both the contribution and the lifetime of the slow component

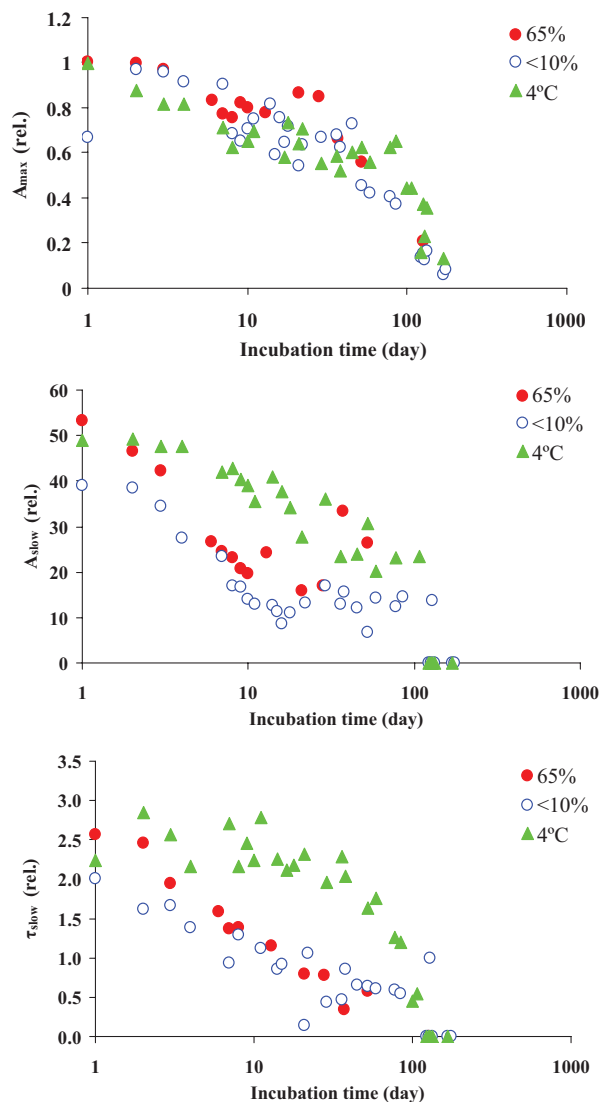


Figure 4 (online color at: www.pss-b.com) The maximum relative amplitude ($A_{\max}$, up), contribution (A_{slow} , middle), and lifetime (τ_{slow} , down) of the slow component of the flash induced absorption decay curves after incubation of the dried RC samples for several days. The humidity was 10 or 65% at room temperature and 10% at 4 °C, as indicated.

showed the same degradation at room temperature regardless the humidity was 10 or 65%. If the incubation temperature was reduced to 4 °C the degradation slowed down as indicated both by the contribution and the lifetime of the slow component.

Since, the humidity did not have an effect on these parameters of the slow component we used 65% humidity in the subsequent experiments for SWNT/RC complexes.

3.3 Effect of the incubation temperature on the SWNT/RC complexes Figure 5 shows that after 3 months the stability drops very suddenly in every samples. However, in the absence of CNTs the decay is faster at room temperature.

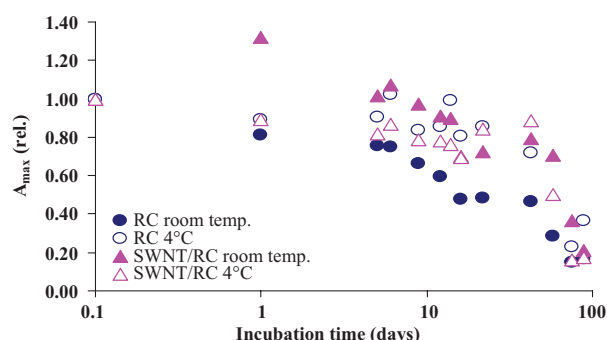


Figure 5 (online color at: www.pss-b.com) The maximum amplitude ($A_{\max}$) of the flash induced absorption change after incubation of the dried RC and SWNT/RC samples at room temperature and 4 °C as a function of time as indicated.

3.4 Redox interaction between RCs and SWNT

In an earlier work, we have shown by optical absorption change and electron paramagnetic resonance (EPR) measurements that there is a possible electronic communication between the RC and SWNTs [6]. However, there is a need to prove it with direct assays, e.g., with conductivity measurement. For this purpose we used a two electrode electrochemical cell, with a working Pt, and a non-polarizable Ag^+/AgCl electrode (see Section 2). RC with fully reconstituted Q_B site bound or unbound to the SWNT, and SWNT as a reference were investigated after drying them on the surface of the Pt. After excitation by ten saturating, short light flashes the control sample showed no

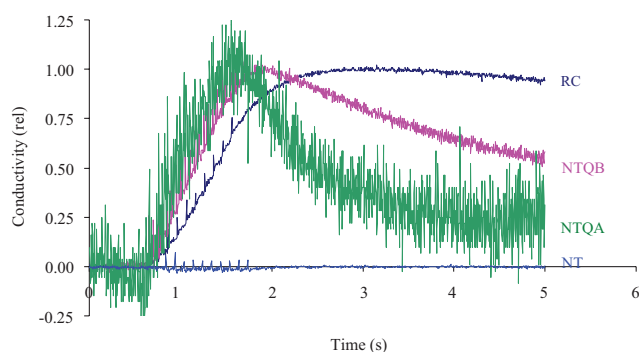


Figure 6 (online color at: www.pss-b.com) The relative conductivity induced by ten short repetitive flashes fired with a 10 Hz repetition frequency on RC and SWNT/RC complexes on the surface of Pt-electrode. (Symbols: RC: RCs with fully reconstituted Q_B site; NTQB: SWNT/RC complexes done by fully reconstituted Q_B site; NTQA: SWNT/RC complexes done by RCs where only the QA site was active; NT: only the SWNT sample, for comparison.)

response to the light excitation; however, the samples containing RC did show characteristic changes (see Fig. 6).

On the repetitive excitation phase, the current increased quickly in RC-samples and reached a saturation by the end of the series of flashes. The main difference was in the dark relaxation after the flash excitation was stopped. If the RCs were dried on the Pt, the current intensity, and consequently the conductivity, remained at a high level and decayed very slowly, over more than 40 s, probably due to the accumulation of the reduced quinone pool. The SWNT/RC sample decayed faster (with a lifetime about 4.7 s). In our interpretation this is due to the shortcircuit between the SWNT and the Pt electrode. If the quinones were depleted, there was a disruption in the e^- -flow between the SWNT and the RCs.

4 Summary

The difference in electric conductivity between the SWNT/RC and pure RC after light excitation can be explained by direct redox interaction between the RC protein and SWNT. If the humidity is higher than 10%, the RC stability on the surface of borosilicate glass is not significantly different. At 4 °C, both the contribution and the lifetime of the slow phase is more stable as compared to the case of room temperature. We have shown first time that, if the RC is bound to SWNT by physical sorption the activity remains stable for longer time at room temperature.

The effects of different parameters (orientation (chemical binding), other components (lipids, proteins)) are under investigation.

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