

Photosynthetic reaction center protein in nanostructures

Kata Hajdu¹, Tibor Szabó¹, Melinda Magyar¹, Gábor Bencsik², Zoltán Németh³, Krisztina Nagy⁴, Arnaud Magrez⁵, László Forró⁵, György Váró⁴, Klára Hernádi³, and László Nagy^{*,1}

¹Department of Medical Physics and Informatics, University of Szeged, 6720 Szeged, Hungary

²Department of Physical Chemistry and Materials Science, University of Szeged, 6720 Szeged, Hungary

³Department of Applied and Environmental Chemistry, University of Szeged, 6720 Szeged, Hungary

⁴Institute of Biophysics, BRC, Szeged, 6726 Szeged, Hungary

⁵Institute of Physics of Complex Matter, Ecole Polytechnique Fédérale de Lausanne, 1015 Lausanne, Switzerland

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* Corresponding author: e-mail lnagy@sol.cc.u-szeged.hu, Phone/Fax: 36-62-544121

Photosynthetic reaction center (RC) is one of the most important proteins, because it is Nature's solar battery converting light energy into chemical potential in the photosynthetic membrane assuring conditions for carbon reduction in cells. Although it is developed in nanometer scale, and is working in nanoscopic power, this is the protein that assures the energy input practically for the whole biosphere on Earth. The

extremely large quantum yield of the primary charge separation (close to 100%) in the RC offers a big challenge to use it in nanodevices. Results of structural (AFM, EM), optical, and electro chemical investigations on RC bio-nanocomposite materials based on different carrier matrices (e.g., CNTs, ITO) will be presented.

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1 Introduction Photosynthetic reaction center (RC, Fig. 1) is one of the most important proteins, because it is Nature's solar battery converting light energy into chemical potential in the photosynthetic membrane assuring conditions for carbon reduction in cells [1, 2]. Although it is developed in nanometer scale, and is working in nanoscopic power, this is the protein that assures the energy input practically for the whole biosphere on Earth. The extremely large quantum yield of the primary charge separation (close to 100% [3]) in the RC offers a big challenge to use it in nanodevices. Details of the pigment–protein complex organization and kinetic components of charge separation and stabilization are given earlier [1, 4, 5].

It is known that biological materials keep the extreme sensitivity only in their original environment. When they are isolated from the living organisms their activity usually decreases. However, there are evidences that it is possible to attach them to artificial materials so that they are able to retain their activity. Other unique possibility is to attach them to nanomaterials. These unique systems are called bio-nanocomposites. In these materials there is a chance to combine the advantageous properties of the two

components. These new types of materials might open so many possible directions for new generations of practical applications, e.g., energy conversion and storage, integrated optoelectronics in analytical, memory, and microimaging devices, etc. For review see, e.g., Giardi and Pace [6].

For example, several organic materials, as well as light sensitive biological systems were immobilized on inorganic carrier surfaces, like semiconducting nanoparticle aggregates, silicon nanorods, or transition metal-oxides, metal, or carbon nanowires showing functional activities (for typical references see, e.g. [7–9]). The key questions of researches these days are: new applications, reproducibility of the measurements, and stability – mainly that of the biological component.

It was shown recently [10, 11] that RCs can be attached to carbon nanotubes (CNTs) and there is an electronic connection between the two types of materials. In our studies we aimed to show the physico chemical properties (photochemical activity, electric conductivity) of the bio-nanocomposite materials prepared by RCs and different carrier matrices. The carrier matrices in our experiments are the isolating borosilicate glass, conducting indium tin oxide

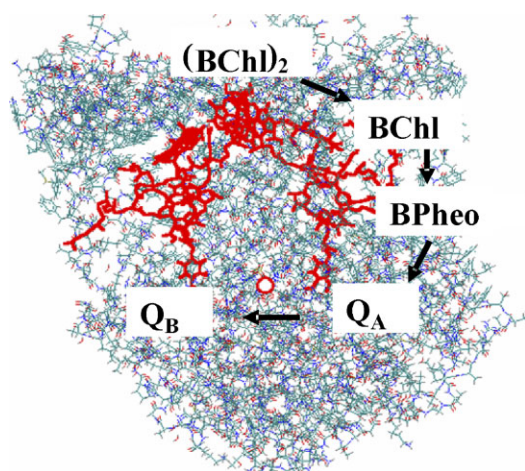


Figure 1 (online color at: www.pss-b.com) The protein frame and the redox active cofactors of the photosynthetic RCs. (BChl)₂: primary electron donor bacteriochlorophyll dimer; BChl: bacteriochlorophyll monomer; BPheo: bacteriopheophytine; QA: primary quinone; QB: secondary quinone. Black arrows: the electron transport route.

(ITO), none-functionalized, and NH₂-functionalized multi-walled carbon nanotubes (MWNT) and conductive artificial materials.

Results of structural (atomic force, AFM and electron microscopy, EM), optical and electro chemical investigations on RC bio-nanocomposite materials based on different carrier matrices (e.g., simple glass, CNTs, ITO) will be presented here.

2 Experimental

2.1 Sample preparation Reaction centers from *Rhodospirillum rubrum* R-26 were prepared by LDAO (N,N-dimethyldodecylamine-N-oxide, Fluka) solubilization and purified by ammonium sulfate precipitation, followed by DEAE Sephacel anion-exchange chromatography [12]. Calculated amount of RCs were bound to SWNT or MWNT by dialyzing out the detergent as described earlier [10] or alternatively dried on the surface of glass or ITO.

For binding the RCs to functionalized CNTs after dialysis calculated amount of carbodiimide and succinimide were added to the RC and stirred for 2 h for activation of the RC carboxyl groups. After activation, amino-functionalized MWNT were added to the sample and stirred for a day.

2.2 Measurements In order to visualize the binding of the protein to CNTs transmission electron microscopy (TEM) and atomic force microscopy (AFM) investigations were carried out. For TEM investigations the material was dried on a Cu-grid and the pictures were taken 100 kV acceleration voltage by Philips CM10 instrument [13].

Atomic force microscopy images were taken with an MFP-3D atomic force microscope (Asylum Research, Santa Barbara, CA). The scanning was performed in AC (tapping) mode with an Olympus cantilever AC160 (Tokyo, Japan) [10, 14].

Flash-induced absorption change was measured routinely by a single-beam kinetic spectrophotometer of local design. The P/P⁺ redox changes of the primary bacteriochlorophyll dimer, the electrochromic response of the absorption of bacteriopheophytins associated to the formation of the Q_A⁻Q_B and Q_AQ_B⁻ states were detected at 860 and 430 or 771 nm, respectively [10, 15].

Light induced change in the conductivity of RC film deposited on ITO surface was measured by a Keithley 2400 s multimeter with seven digits resolution and four points connection. A constant 10 mA current was set and the voltage was measured. Data are represented in ohms. The whole measurement was regulated through the USB port of a computer by using the home developed software (<http://www.noise.physx.u-szeged.hu/>). Sample was illuminated by tungsten lamp filtered by bandpass filter centered at 400 nm.

3 Results and discussions

3.1 RCs on different carrier matrices The decay of absorption change measured at 430 nm after single short pulse of saturating excitation light, reflects the redox state of the primary electron donor (BChl)₂ within the RCs. Decay curves and kinetic parameters are summarized on Fig. 2 and Table 1, respectively.

Different carrier matrices were checked. We found that the RC photochemistry on conductive matrices was different compared to the one measured on isolating glass.

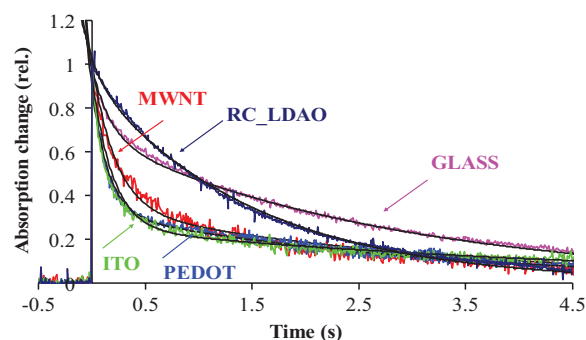


Figure 2 (online color at: www.pss-b.com) The decay of absorption change of RCs measured at 430 nm after single short pulse of saturating excitation light by using different carrier matrices as indicated. PEDOT: Poly(3,4-ethylenedioxythiophene). Solid lines are calculated according to the parameters listed in Table 1.

Table 1 Fitting parameters of the absorption change decay curves of measured samples indicated on Fig. 2.

parameters	LDAO	glass	NT/RC	PEDOT	ITO
A _f (%)	89.7	67.7	33.7	35.6	26.3
k _f (s ⁻¹)	0.7	0.4	0.3	0.3	0.2
A _s (%)	10.3	32.3	66.3	64.4	73.7
k _s (s ⁻¹)	8.0	5.9	4.6	6.7	5.3

A_f and A_s are the contribution, k_f and k_s are the life times of the fast and slow components, respectively.

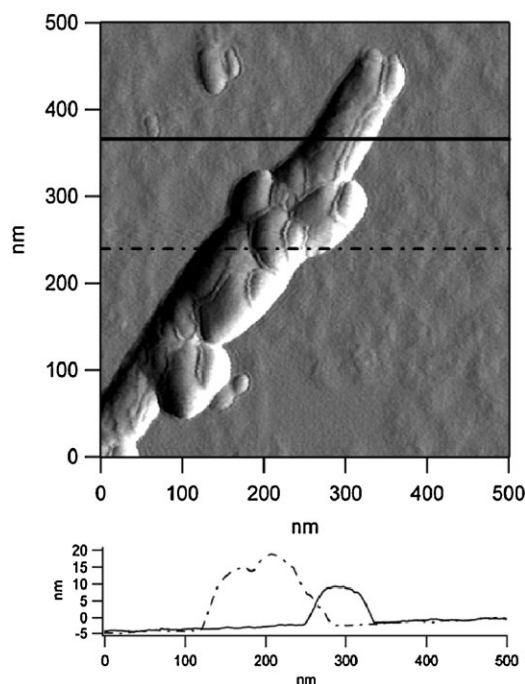


Figure 3 Amplitude image and height section of photosynthetic RCs sitting on MWNT.

On conductive surface the biphasicity is more pronounced and the rate of the slower component is smaller.

3.2 RCs bound to NH₂-functionalized MWNTs

Experiments were carried out by RCs bound either by physisorption to none-functionalized (Fig. 3) or by carbodiimide activation to NH₂-functionalized MWNT (Fig. 4).

First we checked by fluorescamine, which is a fluorescent dye specific to amine groups, whether the NH₂-groups on MWNTs are accessible for the RC binding or not (data are not shown), then the protocol for chemical binding was carried out. Although, until now we do not have a suitable protocol of precise calibration to amine group determination, we can conclude that CNTs are overloaded by the NH₂-groups which can be accessible to the RCs.

Steady state absorption spectra proved that there is a dramatic change in the environment of the donor within the protein. The absorption at 860 nm, which is specific to the primary donor, is shifted to the direction of shorter wavelength and the half-bandwidth is smaller compared to the case in detergent solution (Fig. 5). In addition, the absorption characteristic to the bacteriochlorophyll monomer (at 800 nm) is also narrower in the MWNT/RC complex. The alteration in the absorption at 800 and 860 nm indicate that there is a dramatic change in the electrostatic interactions around the (BChl)₂ and the BChl within the protein after the binding to MWNT.

3.3 Interaction between the RCs and conductive glass – ITO Figures 6 and 7 show that there is an electric interaction between the RCs and the conductive ITO

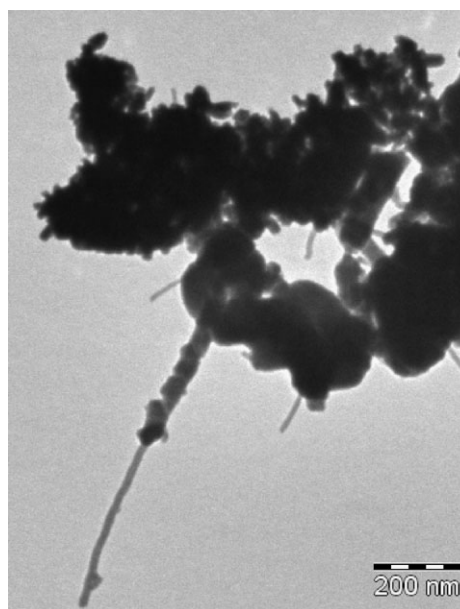


Figure 4 TEM image of MWNT/RC complexes. RCs were bound to NH₂-functionalized MWNT after carbodiimide activation.

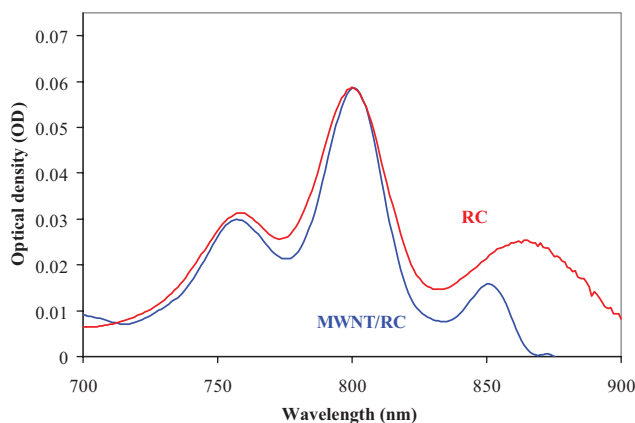


Figure 5 (online color at: www.pss-b.com) Steady state absorption spectra of the RC and the MWNT/RC suspension in the near IR, as indicated.

layer. The electric resistance decreases if the pure ITO is illuminated. However, this decrease is smaller if RCs are deposited on the surface and reaches saturation. This level can be corresponded to more or less single layer on the ITO if we assume tightly packed layer and calculate using 10 nm diameter of the RC [10, 16]. After reaching the saturation of the ITO surface, i.e., multiple layers started to form by the protein, additional effects can be accounted. One reasonable possibility is that the resistance increases mainly due to increasing temperature because of heat dissipation.

The very fast submillisecond phase of the absorption change at 771 nm reports the electronic structure of the RC protein around the BPheo monomer. Due to the electron transfer after charge separation there is a structural rearrangement of the hydrogen bonding network around the acceptor site.

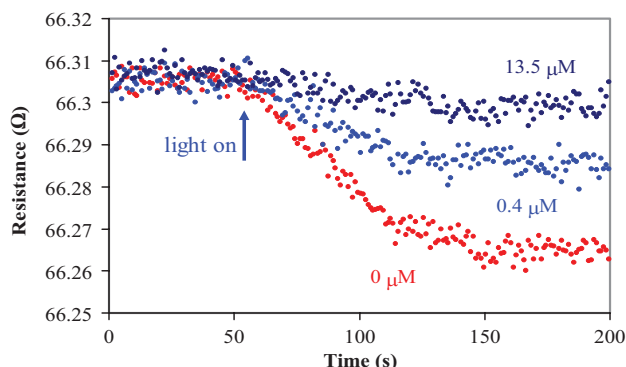


Figure 6 (online color at: www.pss-b.com) Resistance of ITO with or without RCs as a function of time under light excitation. The concentration of RCs and the time when the light was switched on are indicated.

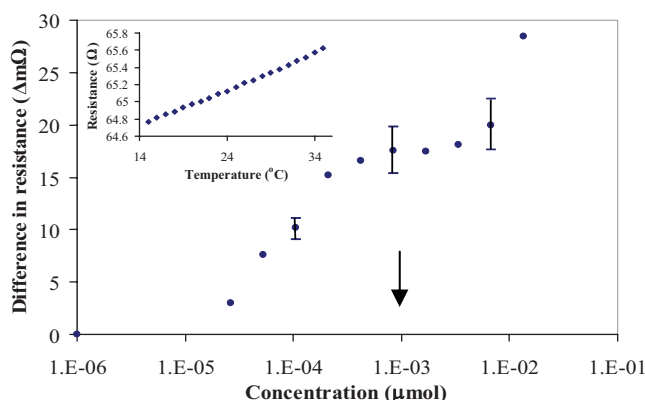


Figure 7 Change of the resistance of ITO as a function of the RC concentration. In order to estimate the temperature effect insert shows the resistance of ITO as a function of the temperature. Insert shows the temperature dependence of electric resistance of ITO, arrow indicates the calculated saturation concentration.

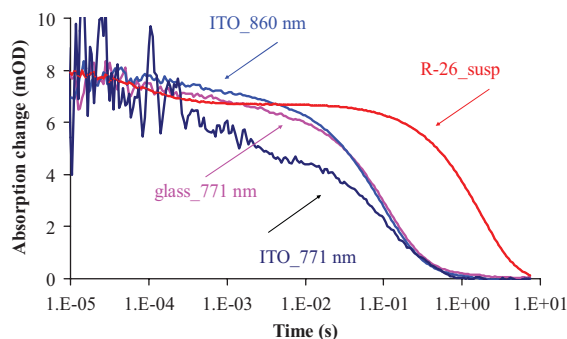


Figure 8 (online color at: www.pss-b.com) The absorption change of RCs after single saturating laser pulse in detergent suspension (R-26_susp), on the surface of borosilicate glass and ITO at 771 and 860 nm as noted. For better comparison the transient at 860 nm is inverted. The suspension contained LDAO at the concentration of cmc.

Figure 8 shows that the amplitude of the pheophytin shift, measured at 771 nm, is about two times larger if the RC is deposited on the ITO as compared to the glass surface, however, the life time of the transient did not change considerably.

4 Summary Our results indicate that it is possible to bind photosynthetic RCs to different carrier matrices. After the binding there is only a partial loss of the RC photochemical activity. The recombination kinetics after the charge separation becomes highly biphasic and the life time of the slow component is slower compared to the case in detergent solution. If the carrier matrix is a conductive material, like CNTs, ITO, conductive artificial polymer, an electrostatic interaction also exists between the RCs and the carrier inorganic material.

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