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Conductive Wiring of Immobilized Photosynthetic Reaction Center to Electrode by Cytochrome *c*

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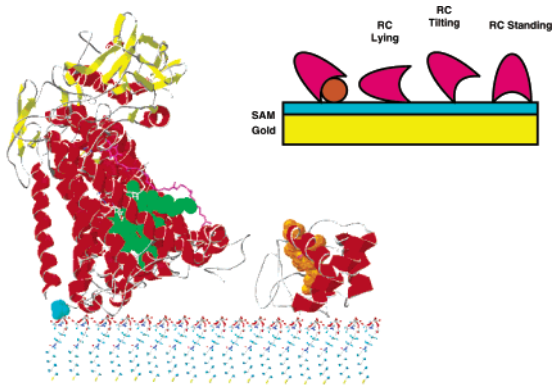
The photosynthetic reaction center (RC) is one of the most advanced photovoltaic devices developed by nature with quantum conversion efficiency ~100%.[1−3] This has led to many attempts to use it for the construction of artificial bio-photovoltaic and bio-photoelectronic devices.[4−7] However, despite the extremely high efficiency of primary charge separation in native photosynthetic membranes, the external power conversion efficiency after immobilization of the RC on electrodes is relatively low.[8] The reason for this is not clear.

Recently we initiated a series of studies to understand this problem. Using a genetically modified RC protein and specifically synthesized organic linkers we were able to construct oriented and aligned protein monolayers on various electrodes, including gold, indium tin oxide (ITO), and carbon with surface coverage of 75−80%.[9,10] By comparing of the efficiency of electron transfer (ET) at donor and acceptor sides of the RC protein we were able to conclude that the major factors decreasing the efficiency of ET between the RC and electrode might be the buried location of ET molecules inside RC protein.[10] This leads to a rather long e-tunneling distance between them and the electrode, and thus inefficient ET.

In photosynthetic organisms, cytochrome *c* acts as a diffusible ET mediator to the RC primary donor. Precisely adapted by evolution for the interaction with the RC, cytochrome *c* penetrates inside the RC protein at the side of primary donor (special pair, P) leading to a high efficiency of ET between these two proteins.[3,11−14] At the same time, because of a relatively small size, cytochrome can efficiently exchange electrons with electrodes at either protein orientation after immobilization on electrode surface.[15−18] Therefore, if a RC-cytochrome complex can be formed on an electrode it might open a possibility for an efficient electrical connection between RC and the electrode.

In the present work we tested the possibility for the construction of a multiprotein ET chain (a supracomplex of the RC and cytochrome) on an electrode and the use of cytochrome for improving the electrical connection between photosynthetic RC and the electrode. To measure the RC-induced photo-ET, we constructed, oriented, and aligned monolayers of RC protein on gold electrodes. Initially, carboxyl terminated alkanethiol self-assembled monolayers (SAMs) were formed and then converted to nitrilotriacetic acid (NTA) terminated SAMs (see Supporting Information for experimental details). After chelating this surface with Ni^{2+}, an oriented RC layer with the primary donor facing the electrode was assembled on the top of the SAM layer. An RC protein from *Rb. sphaeroides* containing 7 added histidines at the C-terminal end of M-subunit was used for these experiments.[9,10,19] The monolayer thicknesses were estimated by ellipsometry.

Scheme 1. Schematic Presentation of RC and Cytochrome *c* on NTA SAM[a]



[a] Red = α-helixes, yellow = *β*-strands, green = chlorophylls, brown = heme, blue = his-tag). Inset shows the possible RC orientation relative to SAM surface (violet = RC, brown = cytochrome)

The illumination of constructed surfaces demonstrated their ability to generate photocurrent with the direction of electron transfer from the electrode to protein and thus confirmed the expected protein orientation with the primary donor facing the electrode (Scheme 1). After the addition of cytochrome *c* (horse heart, Sigma) in either oxidized or reduced form[19,20] to the electrolyte we observed a time-dependent improvement of the photocurrent which after few minutes of incubation reached an intensity of 20−40 times higher than the initial value (Figure 1). The increase in photocurrent was observed both with oxidized and reduced forms of cytochrome *c*. Washing out nonbound cytochrome after the photocurrent reached saturation (by rinsing the electrode with fresh buffer and changing the electrolyte) did not reduce it to the initial low level, indicating the irreversible nature of the changes induced by cytochrome in the system.

To identify the possible origin of the cytochrome-induced activation of ET between RC and electrode, we performed calculations of ET reactions in RC-cytochrome−SAM-electrode and RC−SAM-electrode complexes at different RC configurations. The calculations were performed using Marcus' theory,[21] and the approach developed in Gray's, Dutton's, and Onuchic's laboratories.[22−24] The structures of RC, cytochrome, and RC-cytochrome complex were taken from the Protein Data Bank (http://www.rcbs.org/pdb/) as estimated by crystallographic analysis.[25−27] In the RC-cytochrome−SAM-electrode complex, cytochrome was considered as sitting on SAM surface and the RC was assumed to have a single point of contact with SAM (around the polyHis-Ni−NTA

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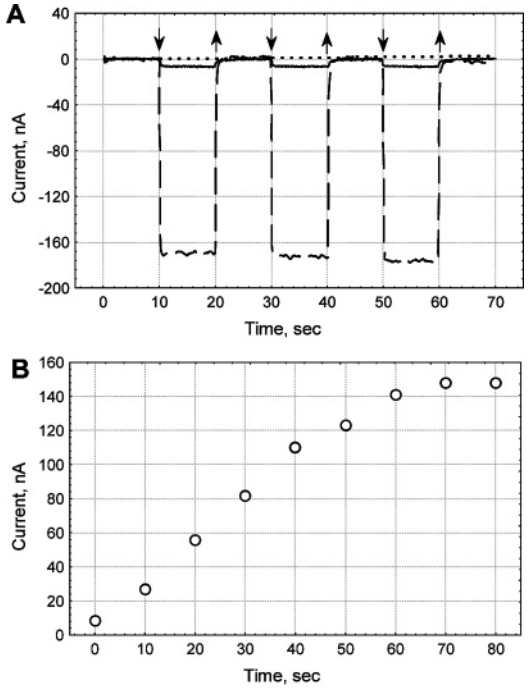

Figure 1. (A) Steady-state photocurrent at RC−NTA-electrode with (− − −) and without (—) 1 μM cytochrome *c* and NTA electrode incubated with cytochrome *c* without RC (···). The arrows indicate light on (↓) and off (↑). Panel B shows the time course of increase in the photocurrent of RC−NTA−EL electrode after the addition of 1 μM cytochrome *c* to the electrolyte under continuous illumination (λ > 700 nm, 0.1 W/cm^2).

Table 1. Calculated Rates (Squares of the Matrix Elements of Overlapping Wave Functions) and Their Ratio for Electron Transfer between an Electrode and RC in the Presence and Absence of Cytochrome *c* at Different RC Orientations

RC orientation	k_{et}+ (with cytochrome)	k_{et}− (without cytochrome)	k_{et}+/k_{et}−
tilting	1.16×10^2	5.68×10^{-5}	2.04×10^6
lying		1.99×10^{-6}	5.79×10^7
standing		1.04×10^2	1.11

linker) (see Scheme 1). For the cytochrome-free RC−SAM-electrode complex, we considered three possible RC orientations (Scheme 1, inset). In the first case, we considered RC lying on the SAM surface. In the second case, RC was oriented to SAM in the same way as if cytochrome is present. In the third case, we considered RC standing up on the surface of SAM and facing it by the primary donor. In all cases the open area surrounding RC protein was considered as filled with water. The calculations have shown that without cytochrome ET is possible only when the RC is close to the standing position (the third case). However in the presence of cytochrome, the rate of ET should increase by orders of magnitude depending on the initial position of RC (Table 1). Cytochrome cannot touch both the RC and SAM at the RC lying configuration.

The following possible mechanism can explain the observed effect. Being bound to the SAM at a single point on the protein surface, the RC protein can tilt, rotate, or even lie down on the SAM. Cytochrome *c*, after diffusion and adsorption from the solution onto the SAM surface, can bind to the RC. Electrostatic interaction between the two proteins leads to the formation a proper ET complex at the P-side of the RC protein. The advantage of ET through cytochrome compared to either the rather long-distance e-tunneling through the RC protein or through the cavity filled with water seems to be in (1) the division of the electron tunneling pathway into two relatively short steps (from SAM to heme and from heme to special pair) and (2) the possible decrease in the distance of total tunneling by the size of the heme because of electron delocalization within the porphyrin ring.

In conclusion, our results show that electron transfer between an immobilized RC and a gold electrode is significantly improved by the incorporation of another ET protein, cytochrome *c*, into the system. This effect does not depend on the initial redox state of cytochrome and seems to be the result of the formation of a RC−cytochrome complex on the surface of the RC−SAM-electrode. Utilization of cytochrome as a conductive wire to the RC special pair opens a possibility for the analysis of ET properties of the special pair in RC protein on the electrode that is deeply buried inside the protein globe and is not easy accessible for EC analysis on the electrode surface. Similar approaches might be useful for the analysis of buried ET components in other proteins.[15]

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Supporting Information Available: The details of SAM fabrication, the ellipsometric estimation of monolayer thicknesses, the measurements of photocurrent, and the calculation. This material is available free of charge via the Internet at http://pubs.acs.org.

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