LIGHT-HARVESTING BIOCOMPOSITES USING POROUS SILICON AND PHOTOSYNTHETIC REACTION CENTERS

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SUMMARY

Porous silicon microcavity (PSiMc) structures were used to immobilize photosynthetic reaction center (RC) purified from the purple bacterium Rhodobacter sphaeroides R-26. Two different binding methods were compared by specular reflectance and absorption kinetic measurements. The surface of the PSiMc was characterized by Scanning Electron Microscopy (SEM) and Atomic Force Microscopy (AFM).

1. INTRODUCTION

Photosynthetic reaction center (RC) is a protein of high interest, because it is nature’s solar battery converting light energy into chemical potential in the photosynthetic membrane, hereby assuring carbon reduction in cells. Although RC functions on nanometer scale, with 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 presents a great challenge to use it in artificial light harvesting systems. Porous silicon is a potential carrier matrix as a biocompatible semiconductor material. The pores size and optical properties are adjustable during the wet electrochemical etching process used to fabricate it. In this work RC was immobilized to porous silicon microcavities via two different methods: covalent and peptide binding. In both cases the RC preserved its activity but the efficiency of the two methods is obviously different.

2. EXPERIMENTAL RESULTS AND DISCUSSION

Rb. sphaeroides R-26 cells were grown photoheterotrophically.2,3 RCs were prepared by LDAO (N,N-dimethyldodecylamine-N-oxide, Fluka) solubilisation and purified by ammonium sulfate precipitation, followed by DEAE Sephacel (Sigma) anion-exchange chromatography.4 Highly boron-doped silicon wafers were treated to obtain a multilayered stacks of low and high refractive indices and of different layer thickness (SQI Inc: P+ type, 0.01-0.02 Ω·cm1, <100> oriented).5 The obtained structures were characterized using AFM and SEM. RC protein binding within the PSiMC scaffolds was performed via two different binding methods.5 One is the covalent binding through a three-step conjugation method with the glutaraldehyde (GTA) crosslinker molecule. The first step is the silanization of the surface with APTES that ensures free amine groups on the surface. GTA being an amine-targeted homobifunctional crosslinker molecule activates the amine groups. The RC binds to the free aldehyde group of the GTA. This way it is possible to form a chemical attachment between the two materials. The second method is the peptide functionalization. RC is bound to PSiMc by strong physical binding through a hydrophobic peptide layer (SPGLSLVSHMQT). This peptide, elaborated via phage display technology reveals a high binding affinity for the p+ Si material.6 The reflectance spectra of the PSiMc were recorded after each modification step by a Bruker 66V Fourier Transform Infrared spectrometer. Functionalization and protein binding causes a red shift in the reflectance spectra that depends on the RC concentration.5 Figure 1 shows the efficiency of RC binding via the two different methods.
In order to test the activity of RC immobilized on the surface of PSiMc, time resolved flash-induced absorption change measurements were carried out at 860 nm, in a reflection arrangement. Without an externally added electron donor, the RC performs a single turnover after light excitation and a pair of positive (P⁺) and negative (Q⁻ or Q₅⁻) charges is formed within the protein. Comparing the two samples important conclusions can be drawn. When the RC is bound to PSiMc through peptide functionalization, the signal amplitude appears larger. When GTA crosslinker is used, the signal amplitude is about 2/3rd of that of the peptide sample and the decay kinetics become biphasic revealing a fast and a slow component.

The pathway and kinetics of the overall electron transfer through the RC in living organisms and in reconstituted systems are coupled to the oxidation of cytochrome c₂ (the native electron donor) on the donor side and to the redox cycle of quinones on the acceptor side of the protein. Direct optical detection of cytochrome photooxidation in the cytochrome cycle is a reliable method of tracking the steps of the RC photocycle. The oxidation of cytochrome c can be followed by the gradual decrease in the absorption at 551 nm after flash excitation. Both the kinetics and the magnitude of the absorption change are strongly dependent on the size (i.e. the redox state) of all components of the pool and the interaction between them.

3. CONCLUSIONS

We demonstrated successful infiltration of RCs into PSiMc photonic structure and that the protein largely preserves its photochemical activity. The light induced charge recombination showed highly biphasic character in GTA method and monophasic in peptide coating, but the Q₅ site could be reconstituted as shown by multiple turnover experiments. After reconstitution of the donor and acceptor sites the RC photocycle was also restored and the accessibility of the secondary quinone site to exchangeable cofactors was not blocked in the PSiMc matrix.

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