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Purple-bacterial photosynthetic reaction centers and quantum-dot hybrid-assemblies in lecithin liposomes and thin films

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ABSTRACT

Quantum dots (QDs) absorb ultraviolet and long-wavelength visible light energy much more efficiently than natural bacterial light-harvesting proteins and can transfer the excitation energy to photosynthetic reaction centers (RCs). Inclusion of RCs combined with QDs as antennae into liposomes opens new opportunities for using such hybrid systems as a basis for artificial energy-transforming devices that potentially can operate with greater efficiency and stability than devices based only on biological components or inorganic components alone.
RCs from *Rhodobacter sphaeroides* and QDs (CdSe/ZnS with hydrophilic covering) were embedded in lecithin liposomes by extrusion of a solution of multilayer lipid vesicles through a polycarbonate membrane or by dialysis of lipids and proteins dispersed with excess detergent. The efficiency of RC and QD interaction within the liposomes was estimated using fluorescence excitation spectra of the photoactive bacteriochlorophyll of the RCs and by measuring the fluorescence decay kinetics of the QDs. The functional activity of the RCs in hybrid complexes was fully maintained, and their stability was even increased. The efficiency of energy transfer between QDs and RCs and conditions of long-term stability of function of such hybrid complexes in film preparations were investigated as well. It was found that dry films containing RCs and QDs, maintained at atmospheric humidity, are capable of maintaining their functional activity for at least some months as judged by measurements of their spectral characteristics, efficiency of energy transfer from QDs to RCs and RC electron transport activity. Addition of trehalose to the films increases the stability further, especially for films maintained at low humidity. These stable hybrid film structures are promising for further studies towards developing new phototransformation devices for biotechnological applications.

1. Introduction

Photosynthetic reaction centers (RCs) are naturally occurring nano-sized structures, with dimensions on the order of 10 nm [1–3]. Porphyrins—(bacterio)chlorophylls, (B)Chls, and (bacterio)pheophytins, (B)Pheo—are the major co-factors participating in the transformation of solar light energy into an electrochemical potential (i.e., charge separation) in these naturally membrane-embedded pigment–protein supercomplexes. Other co-factors include carotenoids, quinones and cytochromes. Solar energy-conversion proceeds with an efficiency of almost 100%. Theoretical calculations indicate that the photoconversion efficiency of hybrid assemblies on the basis of native photosynthetic RCs may potentially increase the efficiency of silicon batteries by 17%–20% [4,5]. However, for the successful assembly of efficient hybrid energy-converting devices based on photosynthetic RCs, several problems remain to be solved.

First, photoprotection and repair mechanisms are absent—or at least, much less efficient—*in vitro*. Thus, stability of isolated RCs has to be considerably improved. Second, the efficiency of light harvesting in the region of the solar spectrum can possibly be substantially increased by filling the absorption gap of the RC spectra between the near-ultraviolet/blue and the near-infrared absorption regions of BChls (350–740 nm). Moreover, monolayers of isolated RCs have very low optical densities. Therefore, approaches using hybrid assemblies, i.e. consisting of plasmonic materials and/or semiconductor nanocrystals and RCs appear to be promising [6,7].

Quantum dots (QDs)—fluorescent semiconductor nanocrystals—have unique optical properties, which render them attractive for a wide range

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of applications in biology and medicine, as reviewed, e.g. in Oleynikov et al. [8]. The most important properties are

i) large absorption cross-sections (their extinction coefficients are several ten times higher than those of organic dyes)
ii) extremely high photostability
iii) broad absorption spectra
iv) narrow fluorescence spectra
v) the fluorescence quantum yield of QDs is, moreover, on the order of 70% [8,9].

Notably, the emission maximum of QDs depends on their diameter. For cadmium selenide (CdSe/ZnS) nanocrystals with core diameters of 2.5 to 6 nm, which are covered by zinc sulphide the fluorescence spectrum can be tuned in the range between 480 and 600 nm [8,10]. For cadmium telluride (CdTe) optical tunability from 600 to 1000 nm can be achieved. Covering QDs with an additional shell of bi- or tri-functional polymers can render them water-soluble. Moreover, these functional groups increase the QDs interaction with biomolecules [11,12].

Recent studies show that QDs may be used as light-harvesting antennae for native RCs with a very high (up to 90%) efficiency of excitation energy transfer by a Förster-type resonance mechanism. Fluorescence of the acceptors (RCs and other photosynthetic pigment–protein complexes) can be enhanced by a factor of 4 to 5, potentially even up to 300 [6,13,14]. Therefore, QDs (and other nano-structured materials) can be used to increase the light-conversion efficiency of hybrid devices based on RCs.

One promising approach to increase the stability (over time, at elevated temperatures, etc.) of such hybrid systems may be their inclusion into liposomes. Liposomes are thought to mimic the native environment of membrane proteins (such as RCs) due to interaction with membrane lipids. The lipid bilayer will protect membrane proteins from denaturation and provides the required environment for effective functions and mutual interactions of proteins. Lipids are known to influence the activity of membrane-bound enzymes [15,16]. This also holds for membrane-bound photosynthetic pigment–protein complexes, such as light-harvesting antenna complexes and RCs. However, the sensitivity of structure and function of transmembrane helices to lipid properties such as the lateral packaging, the width of the hydrophobic region as well as to the charge of the head groups should be taken into account. Moreover, also the association of transmembrane protein segments and their tilt with respect to the surface normal of the bilayer may be altered [17].

Phosphatidylcholin is the main lipid component of bacterial photosynthetic membranes, even through their lipid composition can vary considerably depending on the growth conditions, in particular, oxygen contents [18–20]. Following even intensive purification, some lipids, in particular, cardiolipin, phosphatidylcholin and glucosylgalactosyldiacylglycerol still remain closely associated with the protein subunits of the RCs of *Rhodobacter (Rb.) sphaeroides* [21]. This suggests specific interactions of these phospholipids with the RCs. Moreover, the thermodynamics and kinetics of electron transport via the quinone acceptor of the purple bacterial RCs depends of the presence of physiologically important lipids in its vicinity—phosphatidylcholin, phosphatidylglycerol, phosphatidylethanolamin and cardiolipin [22,23]. The mechanism by which lipids influence the photosynthetic energy conversion processes is not understood in detail. Probably, electrostatic effects of anionic phospholipids influence the long-lived charge-separated state at the level of the primary quinone acceptor, as previously suggested by Agostiano et al. (2005). Our work also indicates that the electron transport processes within the RCs, in particular, those involving the quinone acceptor, are substantially influenced by the dynamic state of the RC. Intermolecular mobility of different regions of RCs (which can be considered as molecular probes) is correlated to the kinetics of electron transport within a particular component of the photosynthetic electron transport chain. Such conformational changes are revealed by the temperature and the hydration state dependencies [24–26].

An alternative approach to achieve functional stability of hybrid light-converting systems *in vitro* is to use slightly dehydrated samples (thin films) which can be generated, in particular, by the addition of substances such as trehalose to stabilize the protein structures. Such substances are known to confer high stability against denaturation and help to retain functional activity of the proteins, at least to some extent [27].

In the current study, photosynthetic RCs from the purple bacterium *Rb. sphaeroides* and QDs were incorporated into liposomes based on phosphatidylcholin (lecithin). The functional properties of these hybrid complexes were investigated with regard to their potential applications in biotechnology. Conditions to achieve long-term functional stability of hybrid RC–QD complexes in thin film samples are also reported in the following.

2. Materials and methods

2.1. Preparation of reaction centers

Cells of wild type non-sulfur purple bacteria *Rb. sphaeroides*, were grown under anaerobic conditions in a luminostat at a temperature of 30 °C for 5–6 days. Cells were disrupted with an ultrasonic disintegrator. Chromatophores were collected by centrifugation and incubated for 30 min at 4 °C in 10 mM sodium phosphate buffer, pH 7.0, containing 0.5% lauryldimethyl ammonium oxid (LDAO). Following the centrifugation for 90 min at 144,000 *g* and 4 °C the supernatant containing the RCs was subjected to hydroxyl-apatite column chromatography as described in detail by Zakharova and Churbanova [28]. RCs were resuspended to a concentration of 10 μM in 10 mM sodium phosphate buffer, pH 7.0, containing 0.05% LDAO. The photoreactivity of the sample was assayed by light-induced absorption changes in the Q_y absorption band of photoactive BChl at 870 nm (P870).

2.2. Quantum dots

In the current study, CdSe/ZnS QDs with a hydrophilic polymer coating with carboxyl groups (Rusnanotech-Dubna, Russia) and emission maximum photoluminescence at 530 nm and 580 nm were used.

2.3. Preparations of liposomes

To prepare liposomes with L-α-lecithin from soybean (Sigma, USA) two different methods were used. The first was a comparatively new method of preparing single-walled vesicles by repeated pressing (extrusion) of a multilayer lipid vesicle suspension through a porous polycarbonate membrane (in our case, the pore size was 0.1 μm) in a special device—Extruder (Avanti Polar Lipids, Inc., USA). In another method, both a detergent and dialysis were used (D–D).

The method of preparing vesicles using detergent and dialysis (D–D) included the following steps:

1. 50 mg lecithin was dissolved in 0.5 ml of chloroform and evaporated
2. the lipid film was covered with 2 ml of 50 mM Tris–HCl buffer (pH 8) containing 2% sodium cholate
3. the lipid suspension was sonicated with an ultrasonic disintegrator until it became clear
4. a 0.5-ml aliquot of the resulting lipid solution in Tris buffer was supplemented with 0.2 ml of Tris buffer, sonicated for 5 s, and used for preparing lecithin liposomes
5. a 0.5-ml aliquot of the resulting lipid solution in Tris buffer was supplemented with 0.1 ml of Tris buffer and 0.1 ml of RCs (42 μM), sonicated for 5 s, and used for preparing RC-containing proteoliposomes