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Short Communication

Controlled influence of quantum dots on purple membranes at interfaces

Sergei Yu. Zaitsev*, Eugeni P. Lukashev, Daria O. Solovyeva, Alexander A. Chistyakov, Vladimir A. Oleinikov

Laboratory of Nano-Bioengineering, National Research Nuclear University "Moscow Engineering Physics Institute", 115409 Moscow, Russian Federation

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ABSTRACT

The development of bio-sensitized nanofilms engineered from biomembrane components and inorganic nanoparticles is a promising field of colloid and interface science and technologies. Recent nano-bioengineering approaches employing quantum dots (QDs) permit the enhancement of the purple membrane (PM) "light-harvesting capacity" compared to native PMs. The influence of QDs on the PM properties, especially the bacteriorhodopsin (bR) photocycle, has been found that has both fundamental (mechanisms of photoreception) and applied implications (including the fabrication of hybrid bionano-materials). Samples of PM–QD complexes capable of energy transfer and characterized by increased rates of M-intermediate formation and decay have been obtained. The modified bR photocycle kinetic parameters may be explained by changes in the PM interface upon QD adsorption. The increase and decrease in absorption at 410 nm (or photopotential) for PM–QD complexes are, on average, several times more rapid than for PM suspensions or PM dry films. These results provide a strong impetus for the development of nanomaterials with advanced properties.

1. Introduction

The development and study of bio–nano hybrid systems engineered from membrane proteins and inorganic nanoparticles constitute a fascinating modern field at the interface of chemistry, biology, physics, and nano-biotechnology [1–4]. A number of photosensitive proteins have been explored in terms of sensoring, bioelectronic, and optical applications, but bacteriorhodopsin (bR), a photosensitive membrane protein from purple membranes (PMs) of *Halobacterium salinarium*, has attracted the most attention [5–9]. bR possesses unique physicochemical properties and has three main molecular functions: photoelectric, photochromic, and proton-transporting [8–11]. Native PMs (without light-harvesting complexes) can utilize only 0.5% of the solar light. Recent nano-bioengineering approaches employing quantum dots (QDs) and bR permit the enhancement of the PM "light-harvesting capacity," thus providing a strong impetus for the development of novel nano-materials [4]. It is known [1–4,12,13] that illumination of QDs in the region of their absorption causes some specific effects, including Förster resonance energy transfer (FRET) in the presence of an acceptor, such as a photosensitive protein. This effect in a hybrid bio-nanosystem depends on the properties of the PM–QD system and is used in numerous applications [2–4,11,14].

One of the first PM–QD hybrid systems was studied by Li et al. [15]. Although some of their experimental results were inconclusive, the authors developed a theoretical model explaining how QDs could act as "nanoscaled light sources" (embedded in PMs) to promote the generation of a stationary photocurrent [15]. In general, the main advantage of this system is the possibility of FRET, which improves the bR function in a hybrid material consisting of PMs and QDs [2–4]. However, inorganic nanocrystals, being in direct contact with PMs, may have some effects on bR that are not directly related to FRET. We expected that QDs as additional parts of the composition (which have a specific charge and an intrinsic dipole moment) would significantly influence the bR photocycle.

Here, we studied the QD effect on PMs, in particular, on the bR photocycle. This effect may be of both fundamental and applied importance, especially for the development of new bio–nano hybrid materials.

2. Experimental

PMs were isolated from *H. salinarum* by the standard procedure [3]. The concentration of the PM dispersion was 6 mg/ml as

* Corresponding author. Tel.: +7 4953779539; fax: +7 4953774939.
E-mail addresses: s.y.zaitsev@mail.ru, szaitsev@mail.ru (S.Yu. Zaitsev).

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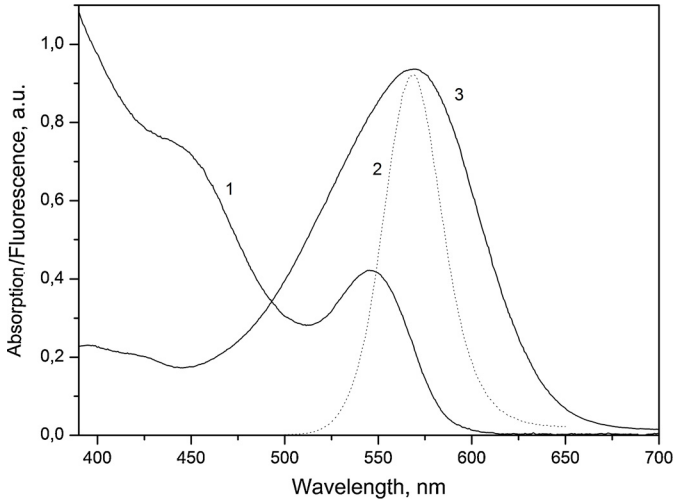

Fig. 1. Spectra of (1) absorption and (2) fluorescence of the quantum dots used (QD_{570}) and (3) the absorption spectrum of the PM suspension.

measured by light absorbance at 570 nm (the molar extinction coefficient was 63,000 M^{-1} cm^{-1}). PMs are generally used more often than bR, because the isolation of bR from PMs considerably decreases both the biochemical and thermodynamic stabilities of the protein.

The changes in the PM light absorbance were measured using a laboratory flash-photolysis setup with the probing beam passing a double monochromator after excitation of the sample with a Nd-YAG laser (532 nm, 8 ns, 15 mJ). Films based on pure PM fragments or their mixtures with QD_{570} were obtained on a transparent conductive electrode (PET-ITO) by means of electrophoretic deposition (EPD) and measured according to the procedure described earlier [16]. Ni foil was used for the second (top) electrode, which could be tightly pressed against the smooth surface of the oriented PM films.

CdSe/ZnS quantum dots emitting at a wavelength of 570 nm were synthesized. These QDs were covered with trioctylphosphine oxide (TOPO) as an amphiphilic ligand for particle stabilization [17]. The TOPO molecules were replaced with poly(ethylene glycol) at the QD surface as described earlier [3,17]. The fluorescence intensity and lifetime of the QDs were measured by means of a FLUOROLOG-3 spectrofluorimeter (Horiba Jobin Yvon) using the TCSPC option. All measurements were carried out in a 100 mM sodium phosphate buffer solution containing 100 mM KCl (pH 7.5). The lifetimes (τ, obtained from fluorescence and photopotential measurements) were evaluated by multiexponential fits and presented as average lifetimes (τ_{avr}) in all cases. The τ_{avr} value was calculated as the sum of the products of each τ value by the respective amplitude divided by the sum of all amplitudes.

All standard chemicals of analytical grade were purchased from Sigma–Aldrich.

3. Results and discussion

QDs are energy converters that absorb light in a wide range of photon energies (Fig. 1, curve 1) and can fluoresce (Fig. 1, curve 2) and transfer the harvested energy to bR (in PMs) with a high efficiency [2–4] because of a wide overlap between the QD fluorescence (Fig. 1, curve 2) and PM absorption (Fig. 1, curve 3) spectra.

The spectral properties of all the QD batches synthesized were studied. The overlap between the bR absorption spectrum and the QD fluorescence spectrum was the largest for QD_{570} (with fluorescence at 570) (Fig. 1). An important result of the experiments with photoactivation of PM–QD_{570} mixtures is that the energy harvested

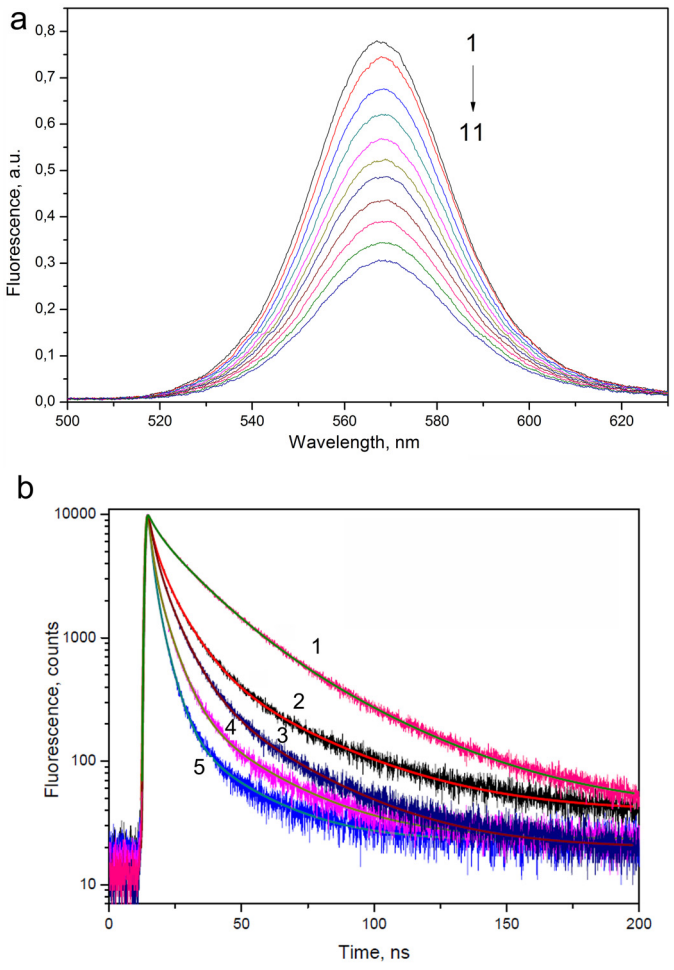

Fig. 2. (a) The change in the fluorescence intensity of a QD_{570} solution (1 μM) after the addition of a PM suspension: curve 1 (top), QD_{570} without PMs; curve 11 (bottom), the same QD_{570} in the presence of PMs at the maximum concentration (about 6 μM of bR). (b) Kinetics of the photoluminescence intensity: curve 1, QD_{570} feed solution ($\tau_{avr} = 18.5 \pm 0.5$ ns); curve 2, QD_{570} films ($\tau_{avr} = 7.8 \pm 0.3$ ns); curve 3, EPD films from a suspension with a QD to BR molar ratio of 2:9 ($\tau_{avr} = 5.7 \pm 0.1$ ns); curve 4, EPD films from a suspension with a QD to BR molar ratio of 1.4:9 ($\tau_{avr} = 3.7 \pm 0.1$ ns); curve 5, EPD films from a suspension with a QD to BR molar ratio of 1:9 ($\tau_{avr} = 2.2 \pm 0.1$ ns).

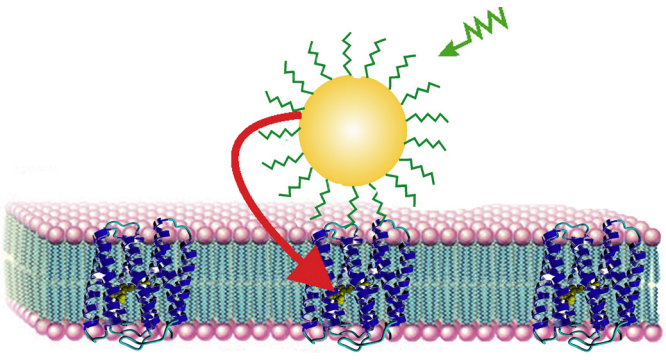

Fig. 3. Förster resonance energy transfer (FRET) in a system of purple membranes (PMs) and quantum dots (QDs), where illumination of the donor (QD) in the region of its absorption leads to FRET to the acceptor (PM), which changes the optical properties of photosensitive membrane proteins.