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Highly efficient energy transfer from quantum dot to allophycocyanin in hybrid structures

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ABSTRACT

Excitation energy transfer (EET) is observed in hybrid structures that composed of allophycocyanin and CdSe/ZnS core-shell quantum dot (QD). We demonstrate that the EET efficiency in such systems could be significantly increased under conditions inducing monomerization of allophycocyanin trimers. For these purposes, the EET efficiency was estimated under different experimental conditions (pH, high temperature or the presence of NaSCN) for self-assembled hybrid structures. Additionally, the hybrid structures were stabilized by covalent coupling which resulted in approximately 20-fold enhancement of allophycocyanin fluorescence upon excitation of QDs. The observed effect provides new opportunities for the practical implementation of hybrid systems as fluorescent markers.

1. Introduction

Fluorescent labeling of biological molecules is one of the commonly used approaches in modern biotechnology. Excitation energy transfer (EET) between two types of fluorescent dyes via Förster Resonance Energy Transfer (FRET) provides an opportunity to screen protein–protein interactions, ligand-receptor binding and other processes [1]. However, the scope of organic fluorophores is limited due to their low stability, narrow excitation or broad emission bands [2,3].

Quantum dots (QDs) are semiconductor nanocrystals, which have unique optical characteristics. QDs absorb light in a broad optical range from ultraviolet to near-infrared range, whereas their fluorescence spectrum is narrow, symmetric and the peak position is determined by the diameter of the nanocrystal [4–6]. Modern methods allow obtaining water-soluble biocompatible QDs with high fluorescence quantum yield, which are able to interact with different kinds of molecules [7,8]. Nowadays QDs are widely used in biology and medicine as an alternative to organic fluorescent dyes. Moreover, QDs can be energetically coupled with molecules, increasing their effective absorption cross-section. For instance, QDs are able to form hybrid structures with photosynthetic light-harvesting complexes [9–12] and can even substitute native antenna complexes of photosystem II [10]. Thus, QDs may be considered as artificial antennae.

Native light-harvesting antenna complexes are pigment-protein structures, the main role of which is to absorb a quantum of light and transfer their energy to the photosynthetic reaction center. Therefore, the antennas increase an absorption capacity of the photosystem. Cyanobacteria and red algae have special light-harvesting complexes called phycobilisomes composed of several types of water-soluble phycobiliproteins, which allow them to expand the range of wavelengths available for photosynthesis in the 530–620 nm region, inaccessible to chlorophyll *a* absorption [13]. Structure of the phycobiliproteins is conservative and relatively stable, they can easily be purified and are characterized by a high fluorescence quantum yield (~0.6). For these reasons, the phycobiliproteins are widely used as model objects for protein research [14] and as fluorescent markers in DNA microarray and flow cytometry [15,16]. Allophycocyanin (APC) is one of the phycobiliproteins, which forms trimers in solution and emits fluorescence at 660 nm [17]. The APC monomer consists of two subunits containing chromophores — the phycobilins [18–20]. Interaction of the phycobilins from different monomers within the trimer causes a characteristic increase of absorption at 650 nm [21]. However, APC is capable of monomerization under the influence of high temperatures, low or high values of pH or the presence of chaotropic agents like NaSCN [21,22]. A decrease of the fluorescence quantum yield and a blue shift of the emission as well as disappearance of the exciton peak at 650 nm in the absorption spectrum are typical for the APC monomerization

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[20]. It is assumed that chemically cross-linked APC trimers are the most efficient for different applications such as cell sorting, high-throughput screening and microscopy [23,24]. However, relatively low Stokes shift and low extinction values in the blue-green region limit the range of light sources suitable for excitation of APC.

The goal of this work was to obtain hybrid structures of QDs and APC with EET which increase the effective absorption cross-section of APC in the blue-green region of the spectrum. We present an approach that allowed us to improve the effectiveness of EET in self-assembling hybrid structures of QDs and APC via electrostatic interactions and especially by stabilizing them with a help of chemical coupling.

2. Materials and Methods

2.1. Materials

CdSe/ZnS core-shell QDs with a peak fluorescence emission at 620 nm, water soluble due to the amphiphilic polymer coating and containing carboxyl groups on the surface, were obtained from Nanotech Dubna (Russia). The fluorescence quantum yield of QDs was estimated by comparison with that of Rhodamine 6G (Sigma Aldrich) and was equal to 0.62. Purified allophycocyanin was purchased from Sigma Aldrich (USA). APC and QDs were mixed in 0.03 M potassium phosphate buffer (pH 7.3) and also in 0.3 M sodium acetate buffer (pH 5.4; 4.6; 3.6).

2.2. Optical Methods & Software

Absorption spectra of QD and APC were measured using a L25 UV/Vis/NIR spectrophotometer (Perkin Elmer, USA). To calculate APC concentration we used extinction coefficient ε equal to 700,000 $l \cdot mol^{-1}\ cm^{-1}$ at 652 nm [25]. For CdSe/ZnS QDs the extinction coefficient was calculated on the basis of the empirical dependence [26] and was equal to 258,000 $l \cdot mol^{-1}\ cm^{-1}$ at 600 nm.

In order to register emission and excitation fluorescence spectra, we used Fluoromax 4 (Horiba Jobin Yvon, France). Steady-state fluorescence emission spectra were measured in the range from 420 to 750 nm with excitation at 405 nm. Fluorescence excitation spectra were measured at 670 nm emission, whereas excitation light was changed over the range from 400 to 650 nm. On the basis of fluorescence excitation spectra, APC fluorescence enhancement factor A was calculated according to the equation:

$$A = (I_{AD} - I_A - I_D)/I_A, \quad (1)$$

where I_{AD} is the APC fluorescence intensity in the presence of QD, I_A is the APC fluorescence intensity in the absence of QD, and I_D is the QD fluorescence intensity in the absence of APC [27].

Time-resolved emission spectra were obtained using the time-correlated single photon counting system based on the SPC-130 module, PML-16-C detector (Becker & Hickl, Germany) and a 405 nm laser diode (InTop, Russia) delivering 13 pJ, 26 ps (FWHM) pulses driven at 10 MHz. Fluorescence decay curves were approximated by a sum of exponential decay functions in SPCImage software package (Becker and Hickl, Germany). To compare different decay curves we calculated average fluorescence lifetime according to the following expression:

$$\tau_{av} = \sum a_i \cdot \tau_i, \quad (2)$$

where τ_i and a_i are the lifetime and the amplitude of the i-th fluorescence decay component, respectively (the amplitude is normalized to unity: $\sum a_i = 1$).

FRET parameters were calculated in PhotochemCAD software (LindseyLab, USA). Additionally, the isoelectric point of APC pI = 5.2 was calculated by the Isoelectric Point Calculator [28].

For data evaluation, we used OriginPro 9.1 (OriginLab, USA). Graphical visualization of protein structure was performed in PyMol v.1.3 software.

2.3. Calculation of FRET Parameters

Non-radiative energy transfer between QDs and pigment-protein complexes is sufficiently described by the Förster theory [2]. According to the Förster theory, one of the main factors determining the efficiency of EET is an overlap between donor's (QDs) fluorescence and acceptor's (APC) absorption spectra which is expressed by the corresponding integral I:

$$I = \int F_D(\lambda) \cdot \varepsilon_\alpha(\lambda) \cdot \lambda^4 \cdot d\lambda, \quad (3)$$

where $F_D(\lambda)$ is the normalized fluorescence spectrum of the donor, $\varepsilon_\alpha(\lambda)$ is the molar extinction coefficient of the acceptor, λ — the wavelength. Using characteristic absorption and fluorescence spectra of the hybrid system components (Fig. 1**A**) and PhotochemCAD software, the integrals of the overlap were estimated to be equal to $2.98 \cdot 10^{-12}\ cm^6$ and $3.11 \cdot 10^{-12}\ cm^6$ for the hybrid complexes of QDs and APC in trimeric and monomeric forms, respectively. Values of the overlap integrals were used to calculate the Förster distance R_0, which characterizes the distance between donor and acceptor of the excitation energy, when the energy migration efficiency is equal to the efficiency of intramolecular relaxation processes. The Förster distance R_0 is determined by the equation:

$$R_0 = \left(8.8 \cdot 10^{23} \cdot \kappa^2 \cdot \varphi_D \cdot I\right)^{1/6}, \quad (4)$$

where φ_D is the fluorescence quantum yield of donor in the absence of acceptor, κ^2 — the dipole orientation factor, which can vary from 0 to 4 but assumed to be equal to $^2/_3$ for solutions and disordered systems [29]. R_0 was found to be equal to 103 Å and 107 Å for hybrid complexes of QDs and APC in trimeric and monomeric forms, respectively.

EET from QD to APC is followed by the quenching of the QD fluorescence (Fig. 1**B**). The EET efficiency E was quantified by a reduction of donor's lifetime:

$$E = 1 - \tau_{DA}/\tau_D = R_0^6/(R_0^6 + r^6), \quad (5)$$

where τ_{DA} and τ_D are the fluorescence lifetimes of QD in the presence and the absence of APC, respectively; r is the real characteristic distance between the QD and APC chromophores [4].

2.4. Chemical Coupling of the QD-APC Hybrids

Chemical coupling of APC with QDs was conducted via random chemical coupling method [30] using 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide iodide (EDC) as a catalyst and N-hydroxysulfosuccinimide (sulfo-NHS) as a stabilizer of the EDC intermediate (all reagents were purchased from Sigma Aldrich). Conjugation was carried out in 0.03 M sodium phosphate buffer (pH 7.3) at a QD to APC molar ratio of 1:1. The final EDC and sulfo-NHS concentrations were 400 μM and 500 μM, correspondingly. QD solution was mixed with EDC and then sulfo-NHS was added to the reaction tube. After 5 min of incubation with gentle shaking, the APC was added. The APC monomerization was induced prior to the coupling either by incubating the sample for several hours at 50 °C, or by addition of NaSCN up to the final concentration of 0.25 M [20]. After the coupling the sample was centrifuged at 12,000 rpm for 30 min at 20 °C to remove uncoupled APC. The supernatant was removed and the sediment was dissolved in a sodium phosphate buffer. The series of controls were carried out to estimate the effect of crosslinking chemicals on individual properties of QDs and APC. We did not observe significant influence of these chemicals on the QD and APC optical properties.