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Emission transformation in CdSe/ZnS quantum dots conjugated to biomolecules

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ABSTRACT

The variation of photoluminescence (PL) spectra in CdSe/ZnS quantum dots (QDs) at the conjugation to antibodies (ABs) has been investigated and discussed in this paper. Two types of CdSe/ZnS QDs with different CdSe core sizes (5.4 and 6.4 nm) and emissions (605 and 655 nm) were studied before and after the conjugation to anti–Interleukin-10 (IL-10) and anti-Pseudo rabies virus (PRV) ABs. The PL high energy shift and asymmetric shape of PL bands have been detected in bioconjugated QDs. Note that the bioconjugation impact on spectral characteristics of CdSe/ZnS QD emission has been not studied yet in details.

The surface enhanced Raman scattering (SERS) effect is revealed in bioconjugated CdSe/ZnS QDs. The SERS effect testifies that the excitation light used at the Raman study generates the electric dipoles in AB molecules. At the same time, the permanent position of LO-phonon Raman lines in Raman spectra of nonconjugated and bioconjugated QDs confirms that QD materials do not change at the bioconjugation. It is shown as well that the compressive strains do not play any role in the PL high energy shift in bioconjugated QDs.

PL spectra of pure anti IL-10 ABs, anti PRV ABs, a phosphate buffer saline (PBS) and PL spectrum dependences versus excitation light intensities have been investigated as well. Finally, the PL spectrum transformation in bioconjugated QDs is attributed to varying the quantum confinement effect in CdSe/ZnS QDs and the energy band profiles in QD cores. Both these effects are stimulated by the electromagnetic field of excited AB dipoles. The obtained results can be useful for sensitivity improving the QD bio-sensors.

1. Introduction

In the last two decades an enormous attention has been attracted to the core/shell quantum dots (QDs) of II-VI group semiconductors (CdSe/ZnS, CdSeTe/ZnS, …) with the high photoluminescence (PL) quantum yield ($\zeta \leq 75\%$) and excellent chemical stability. This interest is connected mainly with fundamental scientific aspects and promising QD applications in optoelectronics, photonics, biology and medicine [1–5]. The known luminescent markers - organic dyes or fluorescent proteins - are characterized by the wide emission bands, low photo stability and the material degradation [6]. The excellent bright emission and chemical stability of CdSe/ZnS QDs permit them to compete and even overcome the parameters of luminescent organic dyes. This makes II-VI QDs interesting for biology and medicine, where multi-target protein sensors and bright imaging are required. In addition, II–VI QD applications in medicine can produce the major advances in diagnostic [1], gene technology [2], toxin detection [3], drag delivery [4], bio-imaging [5] etc.

The core/shell QDs of II–VI group semiconductors with interface passivating are stable against bleaching and degradation. However, the bioconjugation impact on the spectral characteristics of II–VI QD emission has been not studied yet in details. It was revealed early that the PL intensity of QDs decreased [7,8], increased [9,10] or remained constant [11] at the conjugation to biomolecules. PL varying was assigned to the energy exchange between QDs and biomolecules [12]. The QD emission intensity is sensitive to a number of conjugated biomolecules that promising the QD applications as a bio-sensor [12,13]. Recently it was shown that the sensitivity of enzyme-linked immunosorbent assay (ELISA) cancer testing can be improved by using the PL high energy spectral shifts detected in bioconjugated CdSe/ZnS QDs [14].

To explain the PL high energy shift in PL spectra of bioconjugated CdSe/ZnS QDs, a set of physical reasons was discussed. These reasons were related the application of compressive strains to QDs [14], the oxidation or degradation of QD materials in a bio-conjugated state [15], changing a quantum confined effect [16,17], or dominating the emission of excited state excitons [18] in bioconjugated QDs. Despite on the intensive discussion the physical mechanism of PL spectral shifts

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in bioconjugated QDs is unclear yet. To understand the physical reasons of PL spectral shift in bioconjugated QDs, it is desirable to study PL spectrum varying versus QD sizes and AB types. This study permits to obtain the additional information concerning the PL spectral shift in bioconjugated QDs and its results, as expected, will be useful for understanding this effect and for sensitivity improving the QD biosensors.

2. Materials and Methods

Commercial colloidal core/shell CdSe/ZnS QDs (Molecular Probes Inc.), covered by amine-derivatized polyethylene glycol (PEG) polymer, were used at the study. CdSe/ZnS QDs with different CdSe core sizes (5.4 and 6.4 nm) and emissions (605 and 655 nm) (catalog numbers Q22001MP and Q22021MP, respectively) were diluted in a phosphate buffer saline (PBS) with a volumetric ratio 1:200. The amine-thiol cross-linker (SMCC) was used for the QD conjugation reaction, owing to the fast and efficient triol coupling to reactive maleimide groups presented on the QD surface. The detail protocol for the QD bio-conjugation process can be found elsewhere [19].

The volume (1 ml) of each QD conjugation kits was divided on three equal parts. The first part of QD solutions was left non-conjugated and PL spectra studied for these QD ensembles are named 605N or 655N. The second parts of 605 nm and 655 nm QD kits have been conjugated to anti–Interleukin-10 (**IL-10**) ABs (*antihuman IL10, Rt IgG1, stock concentration of 1 mg/ml in PBS, clone JES3-9D7, quantity1ml, code RHCIL1000, Accesolab Co.*). These QD ensembles are named **605-IL-10 or 655- IL-10**. The third parts of 605 nm and 655 nm QD kits were conjugated to anti Pseudo rabies virus (**PRV**) ABs (*immunoglobulin G antibodies, affinity purified with Protein G-Cepharose from rabbit antiserum to Pseudorabies virus, stock concentration of 1 mg/ml in PBS, quantity 1 ml*). These QD ensembles are named in the text as **605-PRV or 655-PRV**.

Optical measurements were performed on the dried droplets (d = 5 mm) of PBS solutions of nonconjugated or bioconjugated CdSe/ZnS QDs located on the Si substrate. The dried state permits to obtain the maximum value of PL high energy spectral shifts in PL spectra of bioconjugated QDs [13,14,20].

PL spectra were measured at an excitation by a He-Cd laser, with a 325 nm light wavelength and a beam power of 76 mW at 300 K using a setup on a base of spectrometer SPEX500 [21,22]. Raman scattering spectra were measured in a backscattering geometry in a Jobin-Yvon LabRAM HR 800UV micro-Raman system at an excitation by solid state light-emitting diode with a wavelength of 785 nm [20].

3. Results and Discussion

PL spectra of non-conjugated QDs are characterized by the Gaussian shape PL bands related to the exciton emission in CdSe cores of different sizes (Figs. 1a and 2a). PL spectra have varied essentially in bioconjugated QDs: the PL intensity decreases on 10–50% and the PL high energy spectral shift appears (Figs. 1b,c and 2b,c). Simultaneously, the full width at half maximum (FWHM) of PL bands increases and the PL band shape becomes asymmetric with essential high energy tails (Figs. 1b,c and 2b,c). The PL peak positions and PL high energy shifts detected in bioconjugated QDs are summarized in Table 1. It is clear that the PL energy shift is higher for 655 nm QDs (d_{CdSe} = 6.4 nm) in comparison with one for 605 nm QDs (d_{CdSe} = 5.4 nm) at the bioconjugation to the same antibody types (Table 1). PL high energy shifts for both QD types are higher essentially at the conjugation to anti IL-10 ABs in comparison with those for QDs conjugated to anti PRV ABs (Table 1).

A set of physical and chemical processes in the QD core, shell, interface or surface can lead to varying the QD energy levels and manifest itself in the PL spectral shift. To investigate the emission impact of pure ABs and PBS, the PL spectra of anti-IL-10 ABs, anti-PRV ABs, and PBS (without QDs) have been measured at UV excitation

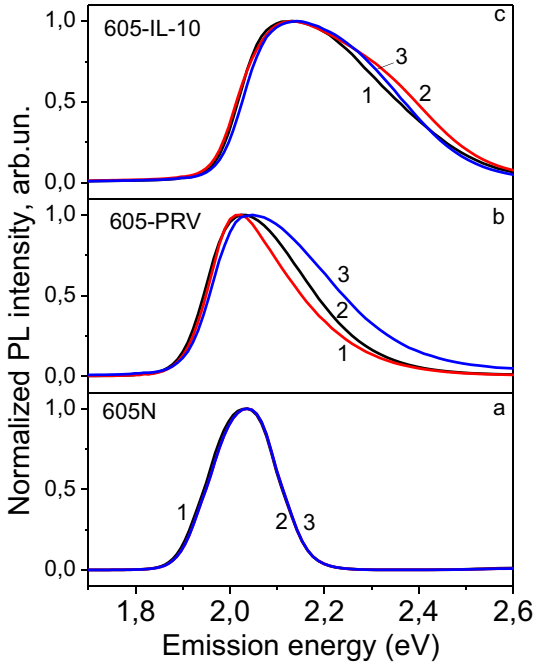

Fig. 1. PL spectra of three non-conjugated 605N (a) and three bio-conjugated 605-PRV (b) and 605-IL-10 (c) QD ensembles.

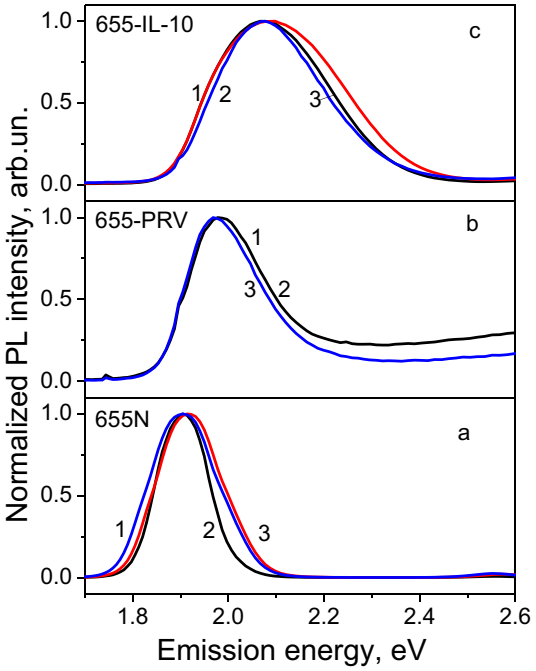

Fig. 2. PL spectra of three non-conjugated 655N (a) and three bio-conjugated 655-PRV (b) and 655-IL-10 (c) QD ensembles.

(Fig. 3). PL spectra of PBS and anti PRV ABs are characterized by wide PL bands within the spectral range of 2.0–3.2 eV (Fig. 3, curve 1, 2). The emission intensities of PBS and PRV ABs are smaller fifteen-fold than the PL intensity detected in studied QDs. Hence the PL spectrum modification in bioconjugated QDs cannot be explained by the emission impact of anti PRV ABs or PBS. The PL intensity of anti IL-10 ABs is higher (Fig. 3, curve 3) and this emission can modify a little the high energy side of PL spectra only of 605 nm QDs.

To obtain the additional information concerning the structure of CdSe/ZnS QDs the Raman scattering spectra have been studied. Raman scattering spectra of nonconjugated 655N QDs, as well as bioconjugated