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Immobilization effects on the photocatalytic activity of CdS quantum Dots-Horseradish peroxidase hybrid nanomaterials

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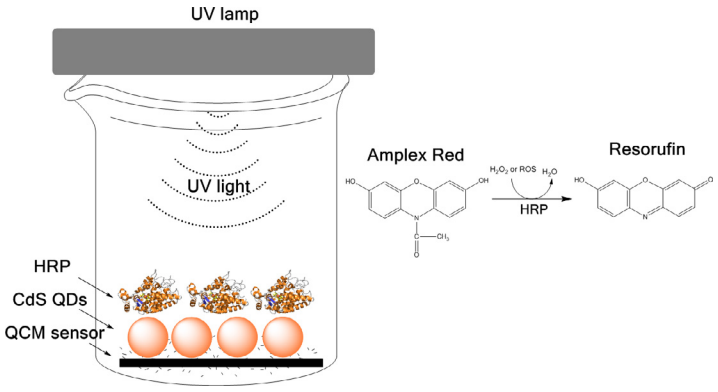
GRAPHICAL ABSTRACT



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ABSTRACT

The potential use of hybrid nanomaterials based on inorganic optically active nanoparticles known as quantum dots (QDs) and horseradish peroxidase (HRP) has been proposed by several authors as light-controllable nanocatalyzers, moreover, the immobilization within or over silica based supports represents an advantage over bulk-dispersed systems. However, the implications of the immobilization of such hybrid photoactivatable catalyzing systems have not been clarified with detail. Here, we present a thorough study of the functional photoactive efficiency and recycling of immobilized CdS QDs and HRP systems with different configurations, immobilized over silanized silica quartz crystal microbalance (QCM) sensors, allowing an accurate measure of the immobilized mass of each component and its correlation with the initial reaction rate of conversion of Amplex Red (AR) to resorufin. As well, the conversion efficiency is compared between the different systems and also to non-immobilized QD-HRP complexed systems.

1. Introduction

Hybrid nanomaterials combining inorganic nanoparticles and bioactive molecules have shown to be attractive functional prospects with a great number of applications including biosensing, biolabeling, therapy and diagnosis, energy systems, photocatalysts,

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etc. [1–3]. Special attention has been paid to quantum dot/enzyme hybrid systems due to the unique photoelectrical properties of the former and the highly specific biocatalytic performance of the latter [1,3–5]. Quantum dots (QDs) are colloidal nanocrystals of metal chalcogenides [6]. As a consequence of their nanometric size, below the Bohr radius, QDs have a special interaction with light, manifested by a broad absorption band and a narrow emission peak tunable in the UV–vis and near infrared regions of the spectrum [7]; besides, QDs have an outstanding photostability and can be tagged with biomolecules, which makes them natural substitutes for traditional fluorophores and photosensitizers [8]. Optical and electronic properties of QDs are mainly determined by their chemical composition, size and surface chemistry (stabilizing agents). Upon light excitation, QDs can follow two different pathways of energy conversion, either fluorescence or electronic exchange with the surrounding medium [9]. In the presence of molecular oxygen, QDs, including CdS QDs, are capable to generate reactive oxygen species (ROS), namely superoxide radical ($O_2^{\bullet}$), hydroxyl radical ($OH^{\bullet}$), singlet oxygen ($^1O_2$), and hydrogen peroxide (H_2O_2), as previously demonstrated by Niemeyer et al. [10,11].

On the other hand, peroxidase enzymes are biomolecules capable of conducting chemical reactions with high specificity and efficiency by employment of H_2O_2 or other reactive oxygen species (ROS).[11-12] Horseradish peroxidase (HRP) is a heme-containing enzyme with different commercial uses such as clinical diagnosis kits and immunoassays [13], and many more potential applications such as wastewater and soil treatment [14] organic synthesis [15] and gene-directed enzyme/prodrug therapy, which consists in the introduction of a vector containing a therapeutic gene, which encodes a foreign enzyme in tumor cells, then, a specific prodrug is administered, and consequently converted into a cytotoxic drug by the enzyme [16]. For instance, the association of HRP with the plant hormone indole-3-acetic acid has been proposed as an enzyme/prodrug system for cancer therapy. *In vitro* studies have demonstrated that in HRP-transfected human cells, activation of the prodrug and the generation of cytotoxic products of indole-3-acetic acid was efficient in tumor treatment [17].

Despite all their virtues, enzymes are relatively unstable, and it is technically very difficult to recover the active enzyme from the reaction mixture when used in solution [18]. Immobilization and confinement of enzymes have many advantages as compared to the use of enzymes in a dispersed form. For example, some of their properties such as catalytic activity or thermal stability are enhanced [12]. The modification of this properties can be explained by changes in the intrinsic activity of the immobilized enzyme or because the interaction between the immobilized enzyme and the substrate takes place in a microenvironment that differs from the free QD-HRP solution [18]. Also, a reconfiguration of the three-dimensional conformation of the enzyme caused by the immobilization/confinement in the solid support might be the reason for the unusual stabilization and improved catalytic performance.

Herein, with the aim to determine the immobilization and confinement effects on hybrid bio-photocatalytic system formed by CdS QDs and HRP, we have investigated the adsorption phenomena of mercaptoacetic acid-capped CdS QDs and the enzyme HRP on SiO_2 supports previously functionalized with a (3-aminopropyl) triethoxysilane (APTES) monolayer. Different approaches to immobilize QD/HRP hybrid systems were followed, including layer-by-layer and pre-complexation and further adsorption. The adsorption phenomena, as well as the quantities of QDs and HRP adsorbed were carefully studied by means of a Quartz Crystal Microbalance (QCM), providing a very close approximation to the actual mass of each component of the system immobilized on APTES functionalized SiO_2 sensors. We have determined the efficiency of the QD/HRP hybrid systems to catalyze, in a controllable manner, the reaction of a model substrate, Amplex Red (AR), upon UV illumination. The systems were also compared with a non-immobilized system in solution (free QD-HRP system). Potential applications of immobilized QDs/HRP hybrid systems include light-controllable sensors [19], microfluidic based bioassay detection kits [20], detection/quantification of pollutants,[4] cancer treatment and diagnosis [21–22], and photo-triggered enzymatic activation of prodrugs for cancer treatment [17].

2. Materials and methods

Horseradish peroxidase (isozyme VI), cadmium perchlorate hydrate ($Cd(ClO_4)_2{\cdot}xH_2O$), sodium disulfide nonahydrate ($Na_2S{\cdot}9H_2O$), heptane, isopropanol, butanol, ethanol, methanol, pyridine, mercaptoacetic acid (MAA), dioctylsulfosuccinate sodium salt (AOT), (3-aminopropyl)triethoxysilane (APTES), were from Sigma-Aldrich. Potassium dihydrogen phosphate and dipotassium hydrogen phosphate were from JT Baker. Amplex Red (*N*-acetyl-3,7-dihydroxyphenoxazine) was from Thermo Scientific. SiO_2 quartz crystal microbalance sensors were acquired from Biolin Scientific. All aqueous solutions were prepared using deionized water (DI water, resistivity > 18 MΩ cm). All the other reagents were from the highest purity available.

2.1. Synthesis of CdS quantum dots (QDs)

CdS quantum dots were synthesized by a slightly modified reverse-micellar method previously reported [10]. Briefly, a reverse micelles system was prepared by dissolving 2.8 g of AOT (dioctylsulfosuccinate sodium salt) in 40 mL of heptane and subsequent addition of 0.8 mL of deionized water under continuous stirring. After stabilization of the micellar dispersion, it was divided in two parts of 24.4 mL and 16.4 mL; thereafter 96 μL of cadmium perchlorate (1 M in water) and 64 μL of sodium sulfide (1 M in water) were added to each one of the portions, respectively. Both dispersions were stirred for 15 min, sonicated for 1 min in a sonication bath at 25 °C and stirred for additional 15 min for stabilization (both solutions must be completely clear). Then, both dispersions were mixed under N_2 atmosphere and stirred for 60 min inside an ice cold bath. Right after mixing, the dispersion changed from colorless to a yellowish color, indicating QDs formation. To this mixture, 128 μL of mercaptoacetic acid (MAA) were added and stirred for 8 h. The QDs were precipitated by the addition of about 10 mL of pyridine, filtered and washed sequentially with heptane, butanol, ethanol and methanol. Subsequent to vacuum drying, the precipitated nanoparticles were redispersed in phosphate buffer (PB, 50 mM, pH 7.4). To eliminate the excess of unattached MAA, the sample was dialyzed against PB for 24 h. The nanoparticle concentration was determined by UV–vis spectroscopy using the molar extinction coefficient of $2 \times 10^4\ M^{-1}\ cm^{-1}$ [10].

2.2. QDs and HRP characterization

Size and zeta potential of QDs and HRP were determined by Dynamic Light Scattering, in a Zetasizer NS (Malvern, England) at 25 °C and using PB pH 7.4 as dilution solvent. Size measurements were done by triplicate with a run time of 60 s, using disposable polystyrene cuvettes. Zeta potential of QDs and HRP was measured using disposable folded capillary cells, and was measured by triplicate. UV–vis and fluorescence spectra of QDs and HRP were recorded with a Varian Cary 50 (Agilent Technologies, USA) and an Infinite 200 PRO multimode reader (Tecan, Switzerland), respectively.