J|A|C|S
COMMUNICATIONS

Published on Web 11/10/2006

Probing Biocatalytic Transformations with CdSe−ZnS QDs

Ron Gill, Ronit Freeman, Jian-Ping Xu, Itamar Willner,* Shira Winograd, Itzik Shweky, and Uri Banin*

Institute of Chemistry and the Center for Nanoscience and Nanotechnology, The Hebrew University of Jerusalem, Jerusalem 91904, Israel

Received September 14, 2006; E-mail: willnea@vms.huji.ac.il

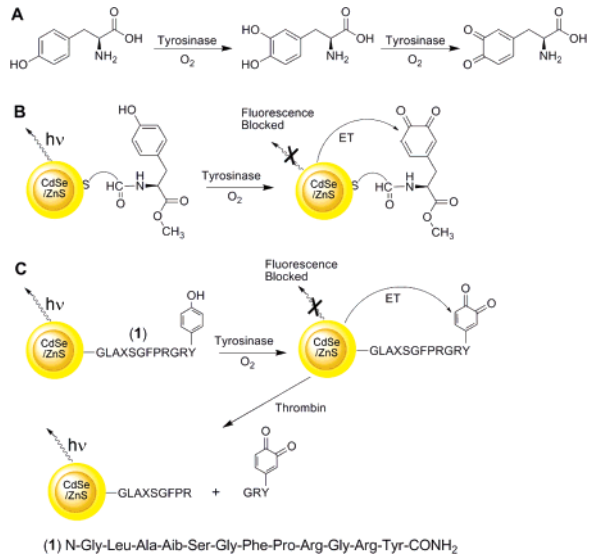
The unique size-controlled photonic properties of semiconductor quantum dots (QDs) find increasing interest for sensing and biosensing.[1] Numerous studies have employed QDs as fluorescent labels for biorecognition events, such as the formation of immunocomplexes[2] or nucleic acid−DNA duplexes.[3] The use of semiconductor QDs as reporter units for biocatalytic processes is, however, scarce, and only few examples demonstrated the application of the photophysical properties of semiconductor QDs to probe biocatalytic reactions. CdS QDs were coupled with biocatalysts, and the resulting photocurrent was employed to follow the enzyme activity.[4] Fluorescence resonance energy transfer (FRET) from CdSe−ZnS QDs to dye units incorporated into replicated DNA or into telomers was used to follow polymerase or telomerase functions.[5] Similarly, the DNase-catalyzed scission of a duplex DNA, consisting of CdSe- and dye-tethered hybridized nucleic acids, was probed by following the FRET quenching of the particle in the duplex structure and the regeneration of the QDs' fluorescence upon the cleavage of the DNA.[6] Recently, the activities of proteolytic enzymes were monitored by fluorescence resonance energy transfer within QD−peptide conjugates.[7,8] Here we wish to report on the monitoring of tyrosinase activity and of thrombin hydrolytic activities by following the fluorescence quenching within CdSe−ZnS QD−peptide conjugates. In contrast to previous studies, we generate by means of tyrosinase the quencher units in the peptide, and we restore the QD fluorescence by the cleavage of the quencher units.

Tyrosinase catalyzes the oxidation of tyrosine to L-DOPA that is subsequently oxidized to the respective *o*-quinone (Scheme 1A).[9] The enzyme oxidizes phenol derivatives; for example, tyramine is oxidized to the catechol derivative. Elevated amounts of tyrosinase were found in melanoma cells, and it is considered as a marker for these cells.[10] Previous studies have demonstrated the optical analysis of tyrosinase through the growth of Au NPs by the L-DOPA product.[11] Scheme 1B depicts the biocatalyzed reactions that were analyzed by probing the fluorescence properties of the CdSe−CdS−ZnS multishell QDs.

CdSe-core CdS(2MLs)/$Zn_{0.5}Cd_{0.5}S$(3MLs)/ZnS(2MLs) multishell QDs, with a diameter of 7.3 ± 1.0 nm (core diameter = 2.6 nm), were prepared according to the literature procedure.[6,12] These QDs were further transformed into water-soluble QDs by ligand exchange with 3-mercaptopropionic acid[6] (MPA). QDs were mixed with either l000-fold excess of tyrosine methyl ester or 100-fold excess of peptide **1**, in a 10 mM HEPES buffer, pH = 7.4, containing 10 mM 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC). The mixtures were placed on a shaker for 2 h and then purified by precipitation of the particles by addition of 2-fold volume acetone, followed by centrifugation at 1500 rpm for 1 min (see details of synthesis and solubilization in Supporting Information).

The loading of the QDs with the tyrosine units was determined spectroscopically, and it corresponded to ca. 60 tyrosine units per particle (Figure S1 in Supporting Information). Interaction of the

Scheme 1[a]



(1) N-Gly-Leu-Ala-Aib-Ser-Gly-Phe-Pro-Arg-Gly-Arg-Tyr-$CONH_2$

[a] (A) Tyrosinase-induced oxidation of tyrosine by O_2. (B) Probing tyrosinase activity through the quenching of the luminescence of the QDs by quinone units. (C) Probing tyrosinase and thrombin activities through the quenching of the luminescence of the QDs by the generation of the quinone-containing peptide and the regeneration of the luminescence of the QDs by the thrombin-induced scission of the peptide.

QDs with tyrosinase/O_2 resulted in the formation of L-DOPA units that are further oxidized to the *o*-quinone residues. The latter products are expected to act as quenchers of the fluorescence of the QDs and thus provide a path for the optical detection of tyrosinase. Figure 1A shows the fluorescence intensity of the QDs prior to the interaction with tyrosinase, curve a, and at time intervals of reaction with the biocatalyst. The fluorescence of the QDs is quenched in the presence of tyrosinase, and as the reaction time intervals are prolonged, the quenching degree is intensified (after 10 min, the fluorescence intensity decreased by 45%). Control experiments revealed that the fluorescence of the QDs was almost unaffected (<10%) by excluding tyrosinase or O_2 from the system. Thus, the biocatalyzed oxidation of the tyrosine units led to the fluorescence quenching of the QDs. Complementary FTIR experiments revealed that the tyrosinase-induced reaction, indeed, generated the *o*-quinone product (characteristic band at 1660 cm^{-1}, Figure S2 in Supporting Information). Also, the monitoring of the lifetime of the CdSe−ZnS QDs revealed that it was shortened as the biocatalyzed oxidation of tyrosine proceeded (Figure S3 in Supporting Information). For example, the lifetime (major component) of the tyrosine-functionalized CdSe−ZnS QDs was 9.4 ns, and after treatment with tyrosinase for 10 min, it was shortened to 6.5 ns (see Supporting Information).

The successful quenching of the fluorescence of the QDs by the biocatalyzed oxidation of tyrosine allowed us to develop an optical

10.1021/ja066636t CCC: $33.50

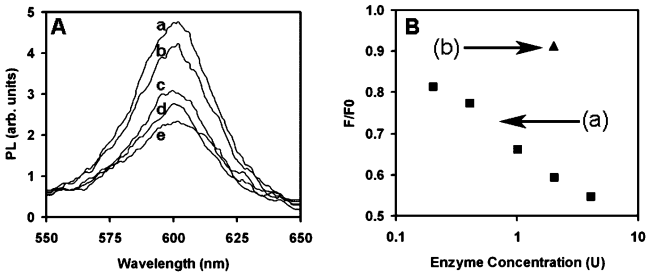


Figure 1. (A) Luminescence spectra of the tyrosine methyl ester-functionalized QDs upon the reaction with tyrosinase, 2 U, for (a) 0 min; (b) 0.5 min; (c) 2 min; (d) 5 min; (e) 10 min. (B) Decrease in the luminescence spectra of the tyrosine methyl ester-functionalized QDs upon the reaction with (a) different concentrations of tyrosinase for a fixed time interval of 6 min; (b) control experiment where 2 U of tyrosinase was employed in the absence of O_2. All data were recorded in 10 mM phosphate buffer solution, pH 6.3, under air (unless otherwise stated) at 25 °C.

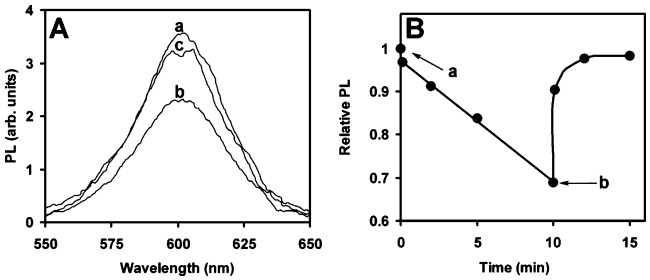


Figure 2. (A) Luminescence spectra of the **1**-functionalized QDs (a) before treatment with tyrosinase; (b) after treatment with tyrosinase, 4 U, for 10 min; (c) after reaction with thrombin, 10 U, for 6 min. (B) Time-dependent luminescence intensities upon reacting the **1**-functionalized QDs with tyrosinase (point a) and thrombin (point b). All data were recorded in 10 mM phosphate buffer solution, pH 6.3, under air (unless otherwise stated) at 25 °C.

assay for tyrosinase activity. Figure 1B shows the fluorescence intensity of the tyrosine-functionalized QDs upon reaction with different concentrations of tyrosinase for a fixed time interval of 6 min. As the concentration of the biocatalyst increased, the quenching efficiency was enhanced. Tyrosinase could be assayed with a sensitivity limit corresponding to 0.2 U of tyrosinase.

In order to generalize the application of semiconductor QDs as optical reporters for biocatalytic transformations, the peptide **1** was covalently coupled to the MPA-modified QDs in the presence of EDC (Scheme 1C). The peptide includes a tyrosine residue that is oxidizable to the respective L-DOPA and *o*-quinone units and, thus, is anticipated to yield the quencher units upon reaction with tyrosinase. The peptide includes, however, the appropriate amino acid sequence that is cleavable by the proteolytic enzyme thrombin. Thus, the hydrolytic cleavage of the *o*-quinone units by thrombin is anticipated to restore the fluorescence properties of the QDs. The loading of **1** on the QDs was determined spectroscopically, and it corresponded to 15. Figure 2A shows the fluorescence properties of the peptide **1**-functionalized CdS−ZnS QDs prior to the reaction with tyrosinase, curve a, and after the tyrosinase-induced oxidation of the tyrosine residue, curve b. The fluorescence intensity is quenched due to the biocatalyzed formation of the *o*-quinone derivative. The fluorescence quenching efficiency (ca. 33%) is, however, substantially lower than that with the tyrosine-functionalized QDs since the peptide introduced a longer distance that separates the QD/quencher pairs.

The QDs modified with the *o*-quinone residues were then reacted with thrombin. Figure 2A, curve c, shows the fluorescence spectrum of the QDs after reaction with thrombin, 10 U, for 6 min. The original fluorescence intensity of the QDs in the absence of the quencher was recovered, implying that the quencher units were cleaved off of the particles. Figure 2B depicts the time-dependent decrease of the fluorescence of the QDs in the presence of tyrosinase and the subsequent recovery of the fluorescence upon the thrombin-induced removal of the quencher units. It should be noted that the tyrosinase-induced oxidation of the peptide **1** is stopped after 10 min of reaction (ca. 30% quenching of the QDs) and subsequently subjected to the scission of the peptide by thrombin, due to the limited stability of the QD−peptide conjugation. Longer time intervals for the oxidation of **1** result in partial precipitation of the QD−peptide conjugates, eliminating the recovery of the QDs' fluorescence upon treatment with thrombin.

In conclusion, we described the successful use of semiconductor QDs as optical probes for biocatalytic transformations. Besides the fundamental significance of the results, the method might be of value for the development of QD-based sensors for following tyrosinase activity.

Acknowledgment. This research was supported by the German−Israeli Program (DIP). We thank Prof. Chaim N. Sukenik, Chemistry Department, Bar-Ilan University, for the FTIR-ATR measurements on our samples.

Supporting Information Available: Synthesis and functionalization of the particles, FTIR data, and transients of the lifetimes of the tyrosine methyl ester-functionalized QDs before and after reaction with tyrosinase. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Medintz, I. L.; Uyeda, H. T.; Goldman, E. R.; Mattoussi, H. *Nat. Mater.* **2005**, *4*, 435−446. (b) Costa-Fernandez, J. M.; Pereiro, R.; Sanz-Medel, A. *Trends Anal. Chem.* **2006**, *25*, 207−218.
(2) Goldman, E. R.; Medintz, I. L.; Whitley, J. L.; Hayhurst, A.; Clapp, A. R.; Uyeda, H. T.; Deschamps, J. R.; Lassman, M. E.; Mattoussi, H. *J. Am. Chem. Soc.* **2005**, *127*, 6744−6751.
(3) (a) Mitchell, G. P.; Mirkin, C. A.; Letsinger, R. L. *J. Am. Chem. Soc.* **1999**, *121*, 8122−8123. (b) Parak, W. J.; Gerion, D.; Zanchet, D.; Woerz, A. S.; Pellegrino, T.; Micheel, C.; Williams, S. C.; Seitz, M.; Bruehl, R. E.; Bryant, Z.; Bustamante, C.; Bertozzi, C. R.; Alivisatos, A. P. *Chem. Mater.* **2002**, *14*, 2113−2119.
(4) Pardo-Yissar, V.; Katz, E.; Wasserman, J.; Willner, I. *J. Am. Chem. Soc.* **2003**, *125*, 622−623.
(5) Patolsky, F.; Gill, R.; Weizmann, Y.; Mokari, T.; Banin, U.; Willner, I. *J. Am. Chem. Soc.* **2003**, *125*, 13918−13919.
(6) Gill, R.; Willner, I.; Shweky, I.; Banin, U. *J. Phys. Chem. B* **2005**, *109*, 23715−23719.
(7) Medintz, I. L.; Clapp, A. R.; Brunel, F. M.; Tiefenbrunn, T.; Uyeda, H. T.; Chang, E. L.; Deschamps, J. R.; Dawson, P. E.; Mattoussi, H. *Nat. Mater.* **2006**, *5*, 581−589.
(8) Shi, L.; De Paoli, V.; Rosenzweig, N.; Rosenzweig, Z. *J. Am. Chem. Soc.* **2006**, *128*, 10378−10379.
(9) Korner, A. M.; Pawelek, J. M. *Science* **1982**, *217*, 1163−1165.
(10) Angeletti, C.; Khomitch, V.; Halaban, R.; Rimm, D. L. *Diagn. Cytopathol.* **2004**, *31*, 33−37.
(11) Baron, R.; Zayats, M.; Willner, I. *Anal. Chem.* **2005**, *77*, 1566−1571.
(12) Xie, R.; Kolb, U.; Basche, T.; Mews, A. *J. Am. Chem. Soc.* **2005**, *127*, 7480−7488.

JA066636T