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Short communication

A highly sensitive system for urea detection by using CdSe/ZnS core-shell quantum dots

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Abstract

An original and novel assay system with urease as a catalyst and CdSe/ZnS quantum dots (QDs) as an indicator has been developed for quantitative analysis of urea. By mixing urease and QDs, the determination of urea can be performed in a quantitative manner. The detection is based on the enhancement of QD photoluminescence (PL) intensity, which is correlated to the enzymatic degradation of urea. By controlling the buffer concentration and pH, PL enhancement due to the degradation of urea is linear in the urea concentration ranging from 0.01 to 100 mM. This property makes the urease/QDs system to be a promising urea-biosensing system. The newly developed system is a superior design and possesses many advantages, including its simple preparation, low cost, no enzyme immobilization required, high flexibility, and good sensitivity.

Keywords: Assay; Quantum dots; Photoluminescence; Biosensor; Urea; Urease

1. Introduction

Quantum dots (QDs) or semiconducting nanoparticles are highly luminescent, photostable fluorophores that have recently been used for sensing applications. QDs have much higher photoluminescence (PL) quantum efficiency than their bulk counterparts (Bhargava et al., 1994; Bruchez et al., 1998; Kulkarni et al., 2005). Fluorescence emission wavelength can be tuned by modifying the size of QDs particles, and the type of capping molecules, which are responsible for modification of the surface charges for bimolecular coupling. The hybrid systems containing QDs coupled with various bio-molecules stimulate growing interests in the research areas of biotechnology and nanotechnology. Through the bioconjugation of QDs, hybrid materials have demonstrated both the unique optical properties of QDs and high specificities toward biomolecules, such as oligonucleotides and proteins (Chan and Nie, 1998). In addition to the imaging application, QDs were recently demonstrated to be good "indicators" for enzymatic hydrolysis of paraoxon (Ji et al., 2005). It was found that a subtle change of the surface property of QDs can result in a dramatic change in their optical properties. In principle, this novel feature of QDs can be extended for detecting specific analytes if appropriate conditions are established.

Urea in blood or in urine is an important substance in the diagnosis of renal and liver diseases. The detection of urea is performed frequently in the medical care. The normal level of urea in serum is in the range of 2.5–6.7 mM. In patients suffering from the renal insufficiency, the urea concentration in serum may be as high as 30–80 mM. With such a urea level, the hemodialysis is required (Chen et al., 2003). For urea detection, biosensors based on the potentiometry and amperometry were previously investigated and reported (Vostiar et al., 2002; Campanella et al., 1990; Singhal et al., 2002; Sehigutollari and Uslan, 2002; Mizutani et al., 1997; Luo and Do, 2004). However, the complexity of sensor fabrication is a common drawback often mentioned in the literature. Herein, we describe a simple scheme of preparation of a urea-sensing system, which is composed of water-soluble mercaptosuccinic acid (MSA)-QDs and urease; it allows for effective and quantitative detection of urea.

2. Materials and methods

2.1. Instruments and chemicals

A Hitachi U-3010A UV–vis spectrophotometer and a Jobin-Yvon Spex Fluolog-3 spectrophotometer were used to

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investigate the fluorescence characteristics of the samples. The pH meter (IQ120) was purchased from IQ Scientific, USA. All chemicals were of analytical grade or highest purity available. Chloroform, methanol (all anhydrous, Aldrich), cadmium oxide (CdO, Aldrich), selenium (Se, Aldrich), tri-*n*-butylphosphine (TBP, Showa), *n*-hexadecylamine (HDA, Lancaster), stearic acid (SA, Lancaster), diethyl zinc (1 M solution in toluene, Aldrich), sulfur powder (S, Aldrich), D,L-mercaptosuccinic acid (MSA, Acros), and tetramethylammonium hydroxide 25% by weight in methanol (TMAOH, Acros) were used as received. Urease (E.C. 3.5.1.5.) was purchased from Sigma.

2.2. Synthesis of water-soluble MSA-capped CdSe/ZnS QDs

Luminescent CdSe/ZnS QDs were synthesized according to the previously reported procedure (Peng et al., 2000; Hines and Guyot-Sionnest, 1996; Dabbousi et al., 1997). However, MSA was selected as the surface capping reagent to form water-soluble QDs. Thirty milligrams of CdSe/ZnS QDs were dissolved in 10 ml of methanol and transferred to a reaction vessel. Then, a separate 50 ml methanolic solution of 1 M MSA was added to the above-mentioned QD-containing solution and the pH was adjusted to 11 by adding tetramethylammonium hydroxide pentahydrate. The mixture was then heated at 60 °C overnight with refluxing under N_2, and the sample was stored in the dark. The QDs were precipitated with anhydrous ether, centrifuged at 6000 rpm, and the supernatant was decanted to remove the organic solvent. Methanol was added to wash the precipitates four times. Finally, the precipitate was redissolved in a phosphate buffer solution, and stored in the dark (Gerion et al., 2001; Aldana et al., 2001).

2.3. Assay condition and PL measurement

For studying the pH effect on the PL intensity of the MSA-QD, a series of samples with different basicity (pH 8, 8.4, 9.5, 10.3, 11.0, 11.5) were prepared by adding various amount of NaOH (0.1 M) and water to a 200 μl MSA-QDs solution (20 mM phosphate buffer, pH 8) to reach a final volume of 300 μl and the amount of MSA-QDs employed was controlled by the absorption at 565 nm with final 0.6 OD. For urea determination, the assay solutions containing urease (1.33 units), MSA-QD (final 0.3 OD observed at 565 nm), and various concentrations of urea (0–120 mM) were prepared in 300 μl of phosphate buffer (20 mM, pH 8.0). Reactions were performed for 10 min before spectrophotometric analysis and acidity (pH) measurements. The PL intensity was measured by using the Jobin-Yvon Spex Fluolog-3 spectrophotometer with 365 nm He/Ne light source to excite MSA-QDs.

3. Results and discussion

3.1. Optical characterization of MSA capped CdSe/ZnS

The water-soluble, MSA functionalized, CdSe/ZnS QDs were successfully synthesized according to the protocol described in the experimental section. The size of the particles was analyzed by TEM and found to be about 5 nm (data not shown). The optical property of MSA-QDs exhibited a broad absorption spectrum (covering the range of wavelength <600 nm) with a characteristic peak at 560 nm and a narrow emission band centered at 580 nm, which was obtained when sample was irradiated with 365 nm lights. The narrow emission spectrum indicated that high degree of monodispersity of QDs present.

3.2. Influence of pH on luminescence of MSA-CdSe/ZnS

It has been addressed that the PL intensity of the water-soluble MSA-CdSe/ZnS sensitizes with the acidity (or basicity) of the environment (Wang et al., 2004). Our investigation of the PL intensity of MSA-CdSe/ZnS under different pH conditions confirmed this finding, as shown in Fig. 1. The PL intensity (monitored at 580 nm) was found to be enhanced by 1.97-fold when the pH was varied from 8 to 11.5. The observed enhancement very likely results from reduced non-radiative recombination by minimizing the surface defects of QDs and thus confines the wave function of electron-hole pairs inside the nano-crystals. The emission peak of MSA-CdSe/ZnS was found to red-shift (∼5 nm) slightly when pH value is varied from 8 to 11.5, indicating the occurrence of the surface smoothing of QDs (Wang et al., 2004). The relationship of the PL intensity (measured at 580 nm) with response to pH value was plotted and shown as the inset of Fig. 1.

3.3. Photoluminescence spectroscopy for the detection of urea

CdSe/ZnS QDs are potentially useful in sensor design, as they possess a significant response in fluorescence and a high surface/volume ratio. Since the PL intensity of QDs was found to correlate with the acidity (or basicity) of the environment, we expect that QDs can be used as an indicator in monitoring biochemical reactions associated with pH change. For examining

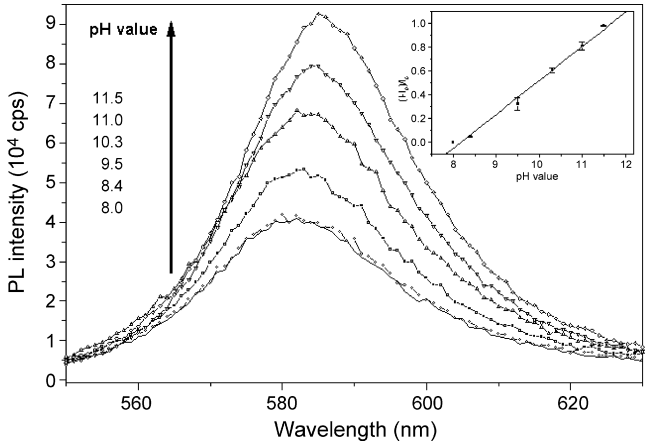

Fig. 1. PL spectra of MSA-CdSe/ZnS QDs at different pH values. The inset figure exhibited the correlation of PL enhancement of QDs with pH conditions. $(I - I_0)/I_0$ represents the ratio of PL enhancement. I_0 is the PL intensity observed at 580 nm at pH 8.0, whereas I is that at other pH value. Note that the PL intensity of MSA-CdSe/ZnS QDs at pH 8.0 and 8.4 are nearly superimposed.

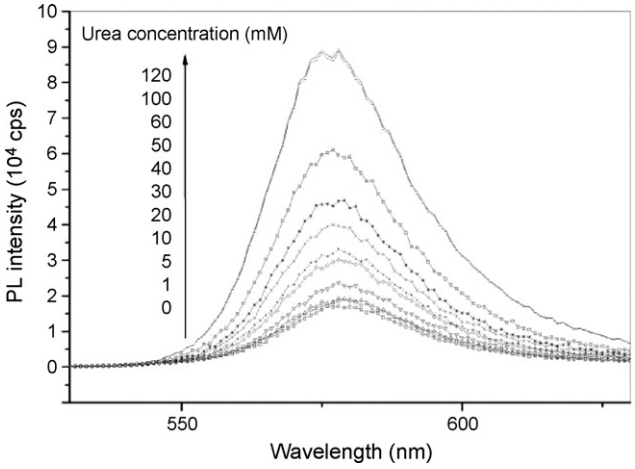

Fig. 2. The variation of PL spectra of urease/QDs with urea concentration. Reactions took place in 20 mM pH 8.0 buffers. Note that, the spectra obtained from the reactions with 100 and 120 mM of urea are nearly superimposed.

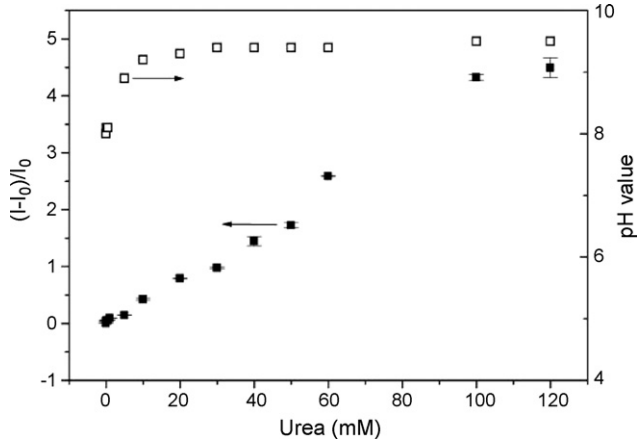

Fig. 3. The variation of pH value and PL intensity with urea concentration. Urea was analyzed by the urease/QDs sensing system in 20 mM pH 8.0 buffers, in which the pH value (□) and the PL perturbation (■) were determined. I_0 and I represent the PL intensity of MSA-CdSe/ZnS observed at 580 nm with the reaction at zero time and at the end-point, respectively.

the feasibility of such an application, we have designed a system of enzymatic analysis of urea. Since the urease-catalyzed hydrolysis of urea releases NH_4^+, OH^- and HCO_3^- ions as products (see Eq. (1)), the pH value of the assayed sample gradually increases as the urea degradation occurs.

$$(NH_2)_2CO + 3H_2O \xrightarrow{\text{urease}} 2NH_4^+ + OH^- + HCO_3^- \quad (1)$$

Fig. 2 exhibits the variation of PL intensity of MSA-QDs as a function of urea concentrations (0–120 mM) under urease catalysis in 20 mM phosphate buffer with pH 8.0. The emission spectra were recorded using the excitation wavelength (λ_{ex}) of 365 nm. The results indicated that the PL intensity of MSA-QDs was enhanced with increasing urea concentration. Fig. 3 was plotted by the ratio of PL enhancement $(I - I_0)/I_0$, versus urea concentration. An approximately linear relationship can be observed with the urea concentration lower than 100 mM. Since the PL intensity is perturbed by the acidity, it is of interest to measure both the change of PL intensity and the final acidity of a reaction, simultaneously. The response of acidity measured by pH meter was found to be linear at low urea concentration (0–15 mM) and to maintain at pH 9.5 when the substrate concentration higher than 20 mM. However, interestingly, the PL intensity of MSA-QDs showed approximately linear response for urea concentration up to 100 mM. Clearly, when the acidity of an assay system approaches its thermodynamic steady-state, the micro-environment of MSA-QDs is continuously changed. One of the possible situations is that the MSA moiety on the surface of QD may be removed through the substitution reaction of hydroxide, which is produced from the urea degradation. Consequently, the PL intensity is enhanced owing to the increased amount of less MSA-coated QDs, which has been shown to be stronger in fluorescence intensity (Wuister et al., 2004; Aldana et al., 2001).

In order to evaluate other possible interferences in this system, urease, urea, NaCl, and NH_4^+ were incubated with MSA-QDs separately. No significant perturbation on PL intensity was observed (data not shown) when urease, urea, NaCl and NH_4^+ were added up to 2.5 units and 100, 1000, 20 mM, respectively. We concluded that the change of PL intensity of our system is indeed solely owing to the product(s) of the catalytic reaction.

With this newly developed system, urea concentration can be analyzed in a broad range (0.01–100 mM). The designed system is very promising for urea analysis in biological systems as compared with many other currently existing methods. Table 1 summarizes the detecting limits of urea with various methodologies including amperometry, potentiometry and optical spectroscopy and the present study. Clearly, the current method exhibits great potential for urea sensing with a wide detection range.

Table 1
Comparison of performance of different urea sensors

Type	Sensing matrix	Linear range (mM)	Limiting detection (mM)	Reference
Amperometry	Polytoluidine blue film	0.02–0.8	0.02	Vostiar et al. (2002)
	PVC ammonium electrode	15–80	15	Campanella et al. (1990)
Potentiometry	PNVK/SA/urease LB film	0.5–68	0.5	Singhal et al. (2002)
	Polyvinylalcohol/2-fluoro-1- methylpyridiniumtoluene-4-sulphonate/urease	0.089–1.1	0.089	Sehigutollari and Uslan (2002)
	Urease + polyurethane-acrylate	0.2–6	0.2	Mizutani et al. (1997)
Optical method	Urea/MSA-QDs	0.01–120	0.01	This work

4. Conclusions

We have demonstrated that the water-soluble MSA-QDs can be utilized as a reliable agent for the enzymatic determination of urea concentration. The MSA-QDs exhibit linear response in PL intensity when urea with concentration ranging from 0.01 to 100 mM was analyzed. As compared to other urea sensors reported in the literature, the MSA-QD-based biosensor exhibits several advantages, such as the ease of its fabrication, low cost, no enzyme immobilization process required, high flexibility, and good sensitivity.

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References

Aldana, J., Wang, Y.A., Peng, X., 2001. J. Am. Chem. Soc. 123, 8844–8850.
Bhargava, R.N., Gallaghar, D., Hong, X., 1994. Phys. Rev. Lett. 72, 416–419.
Bruchez, M., Moronne, M., Gin, P., Weiss, S., Alivisatos, A.P., 1998. 281, 2013–2016.
Chan, W.C.W., Nie, S.M., 1998. 281, 2016–2018.
Chen, J.C., Chou, J.C., Sun, T.P., Hsiung, S.K., 2003. Sens. Actuators B. 91, 180–186.
Campanella, L., Mazzei, F., Sammartino, M.P., Tommassetti, M., 1990. Bioelectrochem. Bioenerg. 23, 195–202.
Dabbousi, B.O., Rodriguez-Viejo, J., Mikulec, F.V., Heine, J.R., Mattoussi, H., Ober, R., Jensen, K.F., Bawendi, M.G., 1997. J. Phys. Chem. 101, 9463–9475.
Hines, A.M., Guyot-Sionnest, P., 1996. J. Phys. Chem. 100, 468–471.
Gerion, D., Pinard, F., William, S.C., Parak, W.J., Zanchet, D., Weiss, S., Alivisatos, A.P., 2001. J. Phys. Chem. B. 105, 8861–8871.
Ji, X., Zheng, J., Xu, J., Rastogi, V.K., Cheng, T.C., 2005. J. Phys. Chem. B 109, 3793–3799.
Kulkarni, S.K., Ethiraj, A.S., Kharrazi, S., Deobagkar, D.N., Deobagkar, D.D., 2005. Biosens. Bioelectron. 21, 95–101.
Luo, Y.C., Do, J.S., 2004. Biosens. Bioelectron. 20, 15–23.
Mizutani, F., Yabuki, S., Sato, Y., 1997. Biosens. Bioelectron. 12, 321–328.
Peng, X.G., Manna, L., Yang, W.D., Wickham, J., Scher, E., Kadavanich, A., Alivisatos, A.P., 2000. 404, 59–61.
Singhal, R., Gambhir, A., Pandey, M.K., Annapoorni, S., Malhotra, B.D., 2002. Biosens. Bioelectron. 17, 697–703.
Sehigutollari, A., Uslan, A.H., 2002. Talanta 57, 1039–1044.
Vostiar, I., Tkac, J., Sturdik, E., Gemeiner, P., 2002. Bioelectrochemistry 56, 113–115.
Wang, Y., Tang, Z., Correa-Duarte, M.A., Pastoriza-Santos, I., Giersig, M., Kotov, N.A., Liz-Marzan, L.M., 2004. J. Phys. Chem. B. 108, 15461–15469.
Wuister, S.F., Donega, C.-de., Meijerink, M.A., 2004. J. Phys. Chem. B 108, 17393–17397.