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A new approach for quantitative determination of glucose by using CdSe/ZnS quantum dots

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Abstract

Quantitative detection of glucose is an important issue in food industry, various biotechnological processes and medication of metabolic disorders. Many ways have been developed to approach this target. In this study, we demonstrated a simple assay system containing glucose oxidase (GOD) and CdSe/ZnS quantum dots (QDs) for specific and quantitative detection of glucose. The role of QDs functioned as an "indicator" to reveal the acidity change of the assay system resulting from the enzymatic oxidation of glucose. By controlling the initial buffer conditions of the assay system at 10 mM or 30 mM phosphate, pH 8.0, the quench ratio of PL intensity of QDs at 586 nm was found to be linear to the tested glucose in the range of 0.2–10 mM or 2–30 mM, respectively. The assay system also provides a convenient way to determine the glucose concentration by visualizing the color change of QDs fluorescence. As compare to most of the existing methods, this newly developed system possesses many advantages, including simplicity, low cost, high flexibility, and good sensitivity. Furthermore, no complicated chemical modification and enzyme immobilization were needed in this system.

Keywords: Assay; Quantum dots; Photoluminescence; Glucose determination

1. Introduction

Low-dimensional semiconductor nanoparticles, also known as quantum dots (QDs), have gained great interests in bio-detection in recent years [1]. Owing to the quantum-size confinement effects, occurring in the small particle with size in 1–10 nm, QDs have unique optical properties including high luminescence and photostability as compared with traditional organic dyes. In a recent study, QDs were demonstrated to be good "indicators" for enzymatic hydrolysis of paraoxon [2]. It was found that a subtle perturbation of the surface property of QDs could result in a dramatic change in their fluorescent emission properties. In principle, this novel feature of QDs can be extended for detecting specific analytes if appropriate conditions are established.

Determination of glucose concentration takes a relevant place in controlling various food and biotechnological processes as well as in diagnosing many metabolic disorders. Among the various methods employed to this aim, the utilization of glucose oxidase (GOD) to produce hydrogen peroxide from glucose and further couple with the applications of potentiometry, amperometry, or optical method to analyse the amount of hydrogen peroxide is the most common strategy [3–5]. An alternative approach to glucose sensing was recently developed by using fluorescence technique. For example, Schultz and coworkers developed a competitive glucose assay that does not consume glucose by the optical detection [6,7]. The sensing principle was based on fluorescence resonance energy transfer (FRET) between a fluorescence donor and an acceptor, each covalently linked to concanavalin A (ConA) or dextran. The presence of glucose resulted in its competitive binding to ConA, which was consequently released from the dye-labeled dextran, thus, decreasing in the FRET efficiency. Although the early results of this technique have gained considerable enthusiasm for fluorescence sensing of glucose [7–9], the complexity of chemical modification on protein is the evident drawback. Another case of glucose detection by FRET technique was designed based on the spontaneous formation of viologens/pyramine complex, in which the boronic acid-substituted 4,7-phenanthrolinium viologens was used to quench the fluorescence of pyranine. In the presence of glucose, the complex dissociates, resulting in a large increase in the fluorescence [10]. Unfortunately, some compli-

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cated procedures are required for synthesizing the counterparts of the complex.

In this study, a new approach for glucose analysis was designed by using QDs as a sensitive "indicator". An assay system containing glucose oxidase (GOD) and CdSe/ZnS quantum dots (QDs) was developed for quantitative analysis of glucose without chemical modification on protein or QDs.

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 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. Glucose oxidase (E.C. 1.1.3.4.) and D-glucose were 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 procedures [11–13]. However, MSA was selected as the surface-capping reagent to form water-soluble QDs. Thirty milligram of CdSe/ZnS QDs were suspended in 10 mL of methanol and transferred to a reaction vessel. Then, a separated 50 mL methanolic solution of 1 M MSA was added to the above-mentioned QD-containing solution and the pH of the solution was adjusted to 11 with tetramethylammonium hydroxide pentahydrate. The mixture was then refluxed at 60 °C overnight 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 precipitant four times. The precipitant was re-suspended in a phosphate buffer (10 mM or 30 mM, pH 8.0) and stored in the dark [14,15].

2.3. Assay conditions and PL measurements

For all tests and reactions, the experiments were repeated at least three times to ensure the accuracy of the measurement. To study the effect of pH on the PL intensity of the MSA-CdSe/ZnS QDs, a series of samples with different pH values (pH 3.5–11.6) were prepared by adding various amount of HCl (0.1 M) or NaOH (0.1 M) and water to a 200 μL MSA-CdSe/ZnS QDs solution (10 mM phosphate buffer, pH 8.0) to reach a final volume of 300 μL. Glucose oxidase and glucose solution were prepared in high concentration in phosphate buffer (10 mM or 30 mM, pH 8.0) for further study. For all experiments, the total volume of the sample was 300 μL and the amount of MSA-CdSe/ZnS QDs employed was controlled by the absorption at 574 nm with final 0.3 OD. For assay system, glucose oxidase (13.5 U) was added to the solution containing QDs and various concentration of glucose. Reactions were performed for 30 min before spectrophotometric analysis and acidity (pH) measurements. The PL intensity was monitored at 586 nm through out all experiments. The Jobin-Yvon Spex Fluolog-3 spectrophotometer with excitation wavelength set at 365 nm (He/Ne light source) was employed.

3. Results and discussion

3.1. Characterization of MSA-capped CdSe/ZnS

The water-soluble, MSA functionalized, CdSe/ZnS QDs were successfully synthesized according to the protocol described in the Materials and methods section. The particle sizes of QDs were measured to be about 5 nm by transmission electron microscopy (Fig. 1). The optical property of MSA-CdSe/ZnS QDs was characterized and the results are shown in Fig. 2, wherein QDs exhibited a broad absorption spectrum with a characteristic peak at 574 nm. A narrow emission band centered at 586 nm was observed when QDs were irradiated with 365 nm light. The narrow emission spectrum indicated that high degree of monodispersity of QDs present.

3.2. Influence of GOD on photoluminescence of MSA-CdSe/ZnS QDs

For understanding the possible influence of GOD on the PL intensity of QDs, the fluorescence of GOD was investigated. With excitation at 365 nm, a broad fluorescence spectrum (410–600 nm) with two peaks centered at 450 and 520 nm was observed. These characteristic bands confirmed the presence of

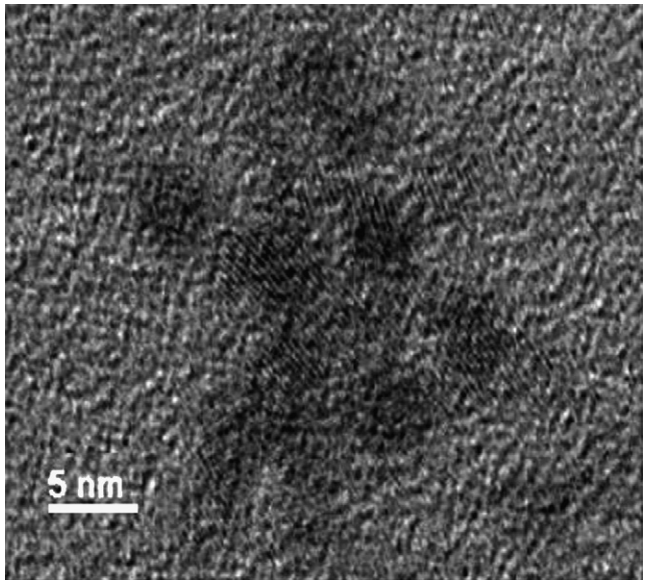

Fig. 1. TEM images of the MSA-CdSe/ZnS QDs. The average diameter of MSA-QDs was measured to be about 5 nm.

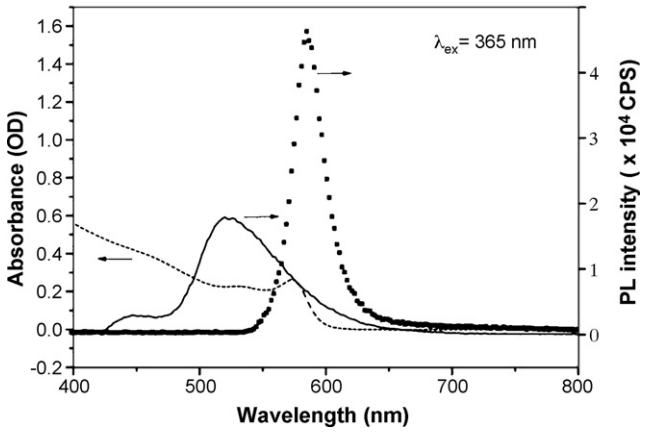

Fig. 2. UV–vis and PL spectra of MSA-CdSe/ZnS QDs and GOD. The absorption spectrum of QDs (- - -), emission spectrum of QDs (. . .), and emission spectrum of GOD (—) were shown. All measurements were performed in 10 mM phosphate buffer, pH 8.0.

flavin adenine dinucleotide (FAD, a prosthetic group) in GOD [16–20]. Since the emission spectrum of FAD was observed to overlap with the absorption band of QDs, the fluorescence resonance energy transfer (FRET) should be taken into account in the assay system. A simple examination was performed to evaluate the influence of GOD to the PL intensity of QDs. As anticipated, the presence of GOD significantly enhanced the PL intensity of QDs. Fig. 3 demonstrates such linear relationship with the increment of 2300 cps per unit of GOD. Although the PL enhancement may not be solely due to the process of FRET, it is closely related to the presence of GOD.

3.3. Influence of acidity on the luminescence of MSA-CdSe/ZnS QDs

It has been proposed that the PL intensity of the water-soluble MSA-CdSe/ZnS was sensitive to the acidity (or basicity) of the environment [21]. Our investigation confirmed this finding. A series of fluorescence spectra of QDs in different pH conditions

Fig. 3. Effect of GOD on PL enhancement of MSA-CdSe/ZnS QDs represented by plotting $(I - I_0)$ vs. GOD concentration.

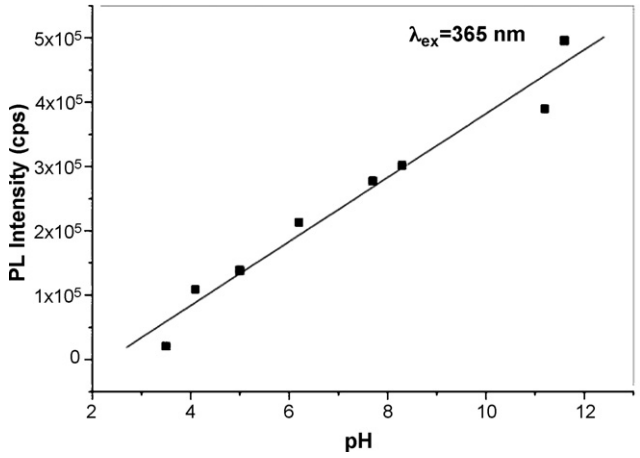

Fig. 4. The correlationship between the PL intensity monitored at 586 nm of QDs and pH value.

(3.5–11.6) were measured and the results showed that the PL intensity and the emission spectra were significantly perturbed by pH condition. As pH value varied from 8.3 to 3.5, the PL intensity of QDs was quenched and the emission spectrum was blue-shifted (data not shown). The quenching phenomenon and the blue shift of emission peak (∼10 nm) were very likely due to the etching process occurring on the surface of QDs. On the other hand, the PL intensity was found to increase when the pH was increased from 8.3 to 11.6. The observed PL enhancement might result from the reduction of the non-radiative recombination by minimizing the surface defects of QDs, which consequently confined the wave function of electron-hole pairs inside the nanocrystals. Fig. 4 exhibits the PL intensity of QDs, which was found to linearly correlate to the pH value with a factor of ∼5000 cps/pH unit.

3.4. Photoluminescent spectroscopy of the detection of glucose

Since the PL intensity of QDs was found to be sensitively influenced by the acidity (or basicity) of the environment, we proposed that the MSA-CdSe/ZnS QDs should be potentially useful for assays that produce acidic or basic product. To examine the feasibility of such proposal, we designed an assay system specifically for glucose analysis. In this system, GOD was used to catalyze the oxidation of glucose to release H_2O_2 and gluconolactone. The latter was then rapidly hydrolyzed to form D-gluconic acid and, consequently, lowered the pH value of the assay system. The catalytic reactions can be depicted in Eqs. (1) and (2) as represented below

$$\text{D} - \text{glucose} + \text{O}_2 \xrightarrow{\text{GOD}} \text{D} - \text{gluconolactone} + \text{H}_2\text{O}_2 \quad (1)$$

$$\text{D-gluconolactone} + \text{H}_2\text{O} \rightarrow \text{D-gluconic acid} \quad (2)$$

Fig. 5 exhibits the variation of fluorescence spectra of various assay reactions as a function of glucose concentration (0.2–10 mM). Note that, the fluorescence spectra, with excitation at 365 nm, were taken at the end-point of the enzymatic reaction that occurred in 10 mM of phosphate buffer, pH 8.0. The

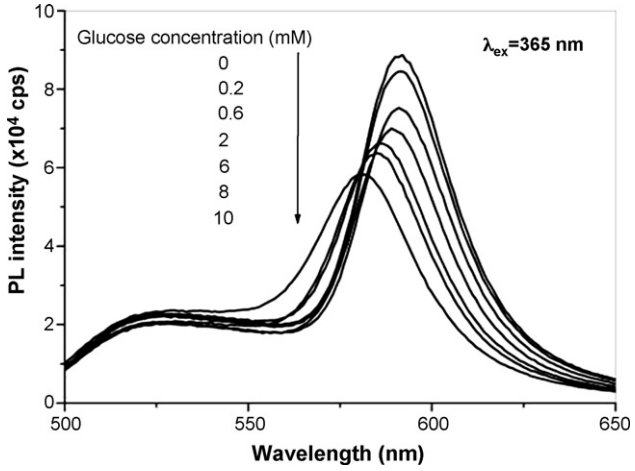

Fig. 5. The PL spectra of the analytes at the end-point of the reaction. The assay system contained GOD, QDs, and the tested glucose with concentration 0 mM, 0.2 mM, 0.6 mM, 2 mM, 6 mM, 8 mM and 10 mM. Reactions were performed in 10 mM phosphate buffer, pH 8.0 for 30 min.

quenching of PL intensity of MSA-CdSe/ZnS QDs was observed with increasing glucose concentration. The catalytic oxidation of glucose also resulted in spectral shifts toward shorter wavelengths in the PL spectra. Since acids were found to be able to slowly etch QDs [22], the blue-shifted emission spectra were presumably attributed to the particle size reduction as more acid was produced. Fig. 6 represents the experiments of PL quenching and the pH values as a function of glucose concentrations oxidized in two different buffer systems, 10 mM and 30 mM phosphate, pH 8.0. The data obtained from the assay system of 10 mM phosphate, pH 8.0 were directly derived from Fig. 5. The quench ratio of PL intensity was defined as 100 $(I_0 - I)/I_0$, where I_0 and I represented the PL intensity of QDs observed at 586 nm with the reaction at zero time and at end-point, respectively. When the assays were performed in 10 mM phosphate, the quench ratio of PL intensity $[(I_0 - I)/I_0]$, was found to be proportional to glucose concentration in the range of 0.2–10 mM,

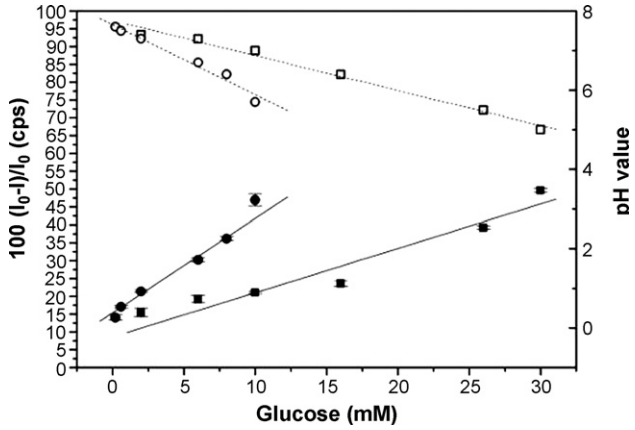

Fig. 6. Degree of fluorescence quenching and the pH variation as a function of the glucose concentration. Open circle (◯) and open square (□) were the pH measurements of the reactions assayed in 10 mM and 30 mM phosphate, respectively. The filled circle (●) and filled square (■) represent the quench ratio of PL intensity obtained in 10 mM and 30 mM phosphate systems, respectively.

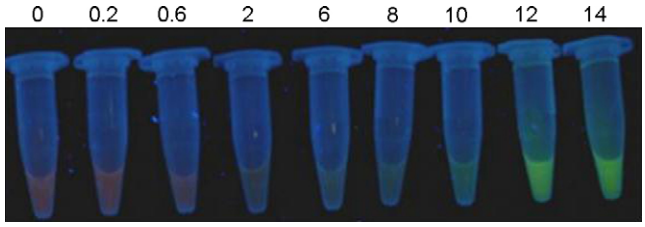

Fig. 7. Fluorescence images of analytes taken using 365 nm UV radition.

while at higher glucose concentration (>15 mM) the quenching effect was leveled-off (data not shown), suggesting that the capacity of QDs be insufficient to reveal the existing acid. To solve such a problem one could use higher concentration of QDs. Alternatively, increasing buffer concentration was suggested. For that, the gluconic acid produced at early stage of reaction will be consumed by the buffer and allow QDs to effectively function as an indicator to track and reveal the later stage of reaction. The assay system using 30 mM phosphate, pH 8.0 was found to be suitable for glucose concentration ranging from 2 to 30 mM. The pH value of each reaction in both the10 mM and 30 mM buffer systems also consistently showed the correlation between the fluorescence quenching and the amount of glucose measurement.

Our assay system can also provide a convenient way for the determination of glucose concentration by visualizing the color change of QDs fluorescence. Fig. 7 exhibits the series of fluorescence images for various glucose analytes obtained from 0 to 14 mM in the 10 mM buffer system. The fluorescence of the reaction samples, irradiated with 365 nm light, resulted in the color change from pale orange to bright, yellowish green when glucose concentration was increased from 0 to 14 mM. Two breaking points between 0.6 mM–2 mM and 10 mM–12 mM were clearly observed, respectively. With appropriate control of the buffer system, it is feasible to develop a simple assay kit for semi-quantitative determination of glucose without using expensive instrumental set-up.

4. Conclusions

This study demonstrated an assay system containing water-soluble MSA-QDs and glucose oxidase for quantitative analysis of glucose. With this new approach, glucose can be successfully analysed with wide range of concentration. As compared to other glucose assay methods, the MSA-CdSe/ZnS QDs-based detection exhibits several advantages, such as the ease of reagent preparation, low cost, no enzymatic immobilization, high flexibility, and good sensitivity. It offers a persuasive way to determine the glucose concentration without using complicate instrumental application.

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