Immobilization of Quantum Dots in Multiple Responsive Microgels for Biomedical Applications

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ABSTRACT

A novel type of smart hybrid materials based on the in situ immobilization of quantum dots (QDs) on a responsive microgel template was prepared and investigated. Firstly, a temperature and pH dual responsive hybrid microgel was developed through the in-situ immobilization of CdS QDs in the interior of a copolymer microgel of poly(N-isopropylacrylamide-acrylamide-acrylic acid) [p(NIPAM-AAm-AA)]. The amino groups of the pAAm segments in the microgels are designed to sequester the precursor Cd^{2+} ions for in situ formation of CdS QDs in the interior of the microgels and stabilize the CdS QDs embedded in the microgels. We demonstrated that the carboxyl groups on the p(NIPAM-AAm-AA)-CdS hybrid microgels can be used for further coupling with 3-aminophenyl boronic acid for optical glucose sensing. The glucose concentration change can induce a reversible swelling/shrinkage of the hybrid microgels, which can further modify the physicochemical environment of the QDs immobilized inside the microgels, resulting in a reversible quenching/antiquenching in photoluminescence (PL). The method is extendable to other QDs with different emission wavelengths and other targeting ligands, thus it is possible to develop multifunctional hybrid micro-/nano-gels for additional important biomedical applications.

Keywords: Optical glucose sensing, quantum dots, smart hybrid microgels

1. INTRODUCTION

Responsive hybrid materials, based on semiconductor quantum dots (QDs) immobilized in smart polymer carriers, have recently attracted intensive interests.[1-9] Fluorescent QDs possess broad absorption, narrow emission, intensive brightness, and good photostability relative to organic dyes,[3,10] while smart polymers can undergo a volume phase transition in response to external stimuli, such as temperature,[11-12] pH,[13-14] or glucose level,[15] to modify the physicochemical environment of the QDs immobilized inside. Thus, the hybrid materials with QDs embedded in smart microgels, combining the properties from both QDs and responsive polymers, can offer the possibilities for external switching and manipulation when applied to sensors, electronic/optical devices, and catalytic reactions. Different methods have been recently developed to immobilize QDs into thermosensitive microgels based on poly(*N*-isopropylacrylamide) (PNIPAM) and its derivatives. Gong et al.[4] incorporated the CdTe NPs into the PNIPAM microgels through the hydrogen bonding between the ligands capped on the CdTe NPs with the PNIPAM chains. Agrawal et al.[8] covered the PNIPAM microgels with the CdTe NPs through the covalent bonding between the ligands capped on the CdTe NPs and the surface functional groups of the microgels. All these methods require pre-synthesized QDs capped with functional ligands. A simple and facile method is in-situ template synthesis of QDs. Kumacheva's group[1] has firstly developed an in-situ template method to synthesize CdS QDs randomly distributed in the interior of PNIPAM-based microgels. The temperature tunable photoluminescence (PL) of CdTe or CdS nanocrystals have been observed in these hybrid materials.[1,4,5,8]

In this work, we aim to develop a novel optical glucose sensor through the in-situ immobilization of CdS QDs in the interior of a copolymer microgel of poly(N-isopropylacrylamide-acrylamide-acrylic acid) [p(NIPAM-AAm-AA)], followed by coupling with glucose-sensitive 3-aminophenyl boronic acid (APBA). The resultant p(NIPAM-AAm-PBA)-CdS hybrid microgels demonstrate different morphology and glucose-sensitive PL property in comparison with our previous p(NIPAM-AAm-PBA)-CdS hybrid microgels synthesized in a different method.[7] Such designed glucose sensing system can meet the ever growing demand for continuous, non-invasive or minimally invasive glucose monitoring. Considering the advantages of easy functionalization, porous network structure, uniform size distribution, tunable dimension, potential biocompatibility, and a very short response time of microgels, the present route to the smart

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polymer-QD hybrid microgels could potentially extend to simultaneous cellular imaging and controlled drug delivery applications.

2. EXPERIMENTAL SECTION

2.1 Materials

D(+)-Glucose was purchased from ACROS, and all other chemicals were purchased from Aldrich. NIPAM was recrystallized from a hexane-acetone (a 1:1 volume ratio) mixture and dried in vacuum. AA was purified by distillation under reduced pressure to remove inhibitors. Cadmium perchlorate hydrate ($Cd(ClO_4)_2 \cdot H_2O$), thioacetamide (CH_3CSNH_2), AAm, *N,N′*-methylenebisacrylamide (MBAAm), ammonium persulfate (APS), sodium dodecyl sulfate (SDS, anionic), APBA, and *N*-(3-dimethylaminopropyl)-*N′*-ethyl-carbodiimide hydrochloride (EDC) were used as received without further purification. The water used in all experiments was of Millipore Milli-Q grade.

2.2 Synthesis of p(NIPAM-AAm-AA) Microgels

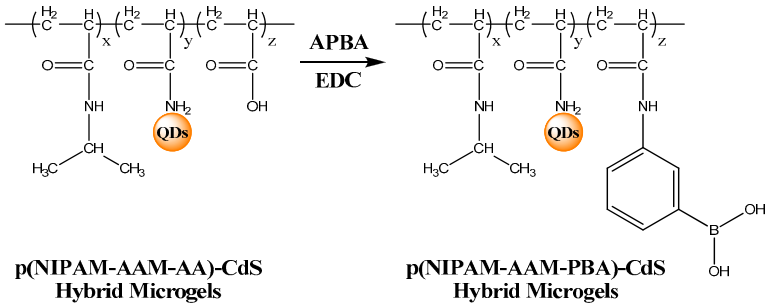
The p(NIPAM-AAm-AA) microgels were prepared by free radical precipitation copolymerization of NIPAM, AAm, AA, and MBAAm using APS as an initiator. A mixture of NIPAM (1.405 g), AAm (0.080 g), AA (0.078 g), MBAAm (0.101 g), SDS (0.050 g), and water (95 mL) was poured into a 250 mL three-neck round-bottom flask equipped with a stirrer, a nitrogen gas inlet, and a condenser. The mixture was heated to 70 °C under a N_2 purge. After 30 min, 5 mL of 0.089 M APS was added to initiate the polymerization. The reaction was allowed to proceed for 5 h. The obtained p(NIPAM-AAm-AA) copolymer microgels were purified by centrifugation (Thermo Electron Co. SORVALL® RC-6 PLUS superspeed centrifuge, 20000 rpm, 27 °C, 20 min), decantation, and then washed with water. The resultant microgel was further purified by 3 days of dialysis (Spectra/Por® molecularporous membrane tubing, cutoff 12000-14000, the same below) against very frequently changed water at room temperature (~ 22 °C).

2.3 In Situ Synthesis of CdS QDs in p(NIPAM-AAm-AA) Microgels

The mixture of 0.1431 g $Cd(ClO_4)_2 \cdot H_2O$ with 20 mL p(NIPAM-AAm-AA) microgel suspensions was stirred at room temperature for 1 day in a 100 mL round-bottom flask under a N_2 purge. After that, excess $Cd(ClO_4)_2$ was removed by centrifugation (20000 rpm, 10 min), decantation, and dialysis against water for 2 days. In the whole process, the solution was kept in acidic condition of pH ~ 4.2. The microgels loaded with Cd^{2+} ions were poured into a 250 mL round-bottom flask equipped with a stirrer, a nitrogen gas inlet, and a condenser. The mixture was heated to 60 °C under a N_2 purge. After a 30 min, a thioacetamide solution (0.073 g in 5 ml water) was dropwisely added. The temperature was raised to 85 °C, and the color gradually turned to brilliant yellow. The mixture was further stirred for 1 hour under N_2 atmosphere. The resulted microgels incorporated with CdS QDs were then purified by centrifugation, decantation, and dialysis against very frequently changed water at room temperature for 3 days. The obtained hybrid microgels were coded as p(NIPAM-AAm-AA)-CdS.

2.4 Synthesis of p(NIPAM-AAm-PBA)-CdS Microgels

The p(NIPAM-AAm-AA)-CdS hybrid microgels can be further functionalized with glucose-sensitive moieties through the coupling of –COOH groups with APBA (Scheme 1). 0.231 g of APBA and 0.231 g of EDC were dissolved in 35 mL of water. The solution was cooled in an ice bath, and then 20 mL of purified p(NIPAM-AAm-AA)-CdS microgels was added. The reaction mixture was kept in an ice water bath for 4 h. The resultant products were purified by dialysis against very frequently changed water for at least 1 week.



Scheme 1. Synthesis of Glucose-Sensitive p(NIPAM-AAm-PBA)-CdS Hybrid Microgels.

2.5 Characterization

The FTIR spectra were recorded with a Nicolet Instrument Co. MAGNA-IR 750 Fourier transform infrared spectrometer. The microgel suspensions were dried on Spectra-Tech IR sampling cards with a PE substrate. The PL spectra of the hybrid microgel dispersions at different glucose concentrations were obtained on a JOBIN YVON Co. FluoroMax®-3 Spectrofluorometer equipped with a Hamamatsu R928P photomultiplier tube, calibrated photodiode for excitation reference correction from 200-980 nm. The pH values were obtained on a METTLER TOLEDO SevenEasy pH meter. The transmission electron microscopy (TEM) images were taken on a FEI TECNAI transmission electron microscope at an accelerating voltage of 120 kV. Approximately 10 μL of the diluted microgel suspension was air-dried on a carbon-coated copper grid for the TEM measurements. Dynamic light scattering (DLS) was performed on a standard laser light scattering spectrometer (BI-200SM) equipped with a BI-9000 AT digital time correlator (Brookhaven Instruments, Inc.). A He-Ne laser (35 mW, 633 nm) was used as the light source. In DLS, the Laplace inversion of each measured intensity-intensity time correlated function can result in a characteristic line width distribution $G(\Gamma)$.[16] For a purely diffusive relaxation, Γ is related to the translational diffusion coefficient D by $(\Gamma/q^2)_{C\to 0,q\to 0} = D$, where $q = (4\pi n/\lambda)\sin(\theta/2)$ with n, λ, and θ being the solvent refractive index, the wavelength of the incident light in vacuo, and the scattering angle, respectively. $G(\Gamma)$ can be further converted to a hydrodynamic radius (R_h) distribution by using the Stokes-Einstein equation, $R_h = (k_B T/6\pi\eta)D^{-1}$, where T, k_B, and η are the absolute temperature, the Boltzmann constant, and the solvent viscosity, respectively.

3. RESULTS AND DISCUSSION

3.1 Structure and Volume Phase Transition of Glucose Sensitive p(NIPAM-AAm-PBA)-CdS Hybrid Microgels

In our previous report, the fluorescent CdS QDs were incorporated into the glucose-sensitive p(NIPAM-AAm-PBA) microgels in situ to achieve optically glucose sensing.[7] Herein, we report another new route by functional modification of the p(NIPAM-AAm-AA)-CdS hybrid microgels with the glucose-sensing moiety PBA, via the EDC-catalytic reaction of carboxylate groups in hybrid microgels with amino groups in APBA molecules (Scheme 1). Covalent conjugation can only occur when both the APBA and the EDC molecules diffuse sufficiently close to a COOH group in the platform microgel network to react. In general, the conjugation efficiency of the –COOH in microgels with APBA increases as the COOH group distribution in the platform microgel becomes more radially surface localized and chain delocalized.[17] These effects can be rationalized on the basis of both steric and diffusive factors. Although the p(NIPAM-AAm-AA)-CdS hybrid microgel has a nearly-uniform radial –COOH-rich domain distribution and a highly average separation of the –COOH groups within the polymer chains, both the hydrophilicity of AAm segments and the rigid nature of the CdS QDs would provide significant steric inhibition to the conjugation of relatively hydrophobic APBA molecules into the interior of the hybrid microgels. The –COOH groups near the more lightly cross-linked microgel surface region are more easily available for the APBA and EDC molecules to conjugate with. Thus, it could happen that more PBA groups rich on the surface region of the p(NIPAM-AAm-PBA)-CdS hybrid microgels. Concurrently, the increased steric crowdedness from the hydrophobic PBA groups leads to the gradual outward diffusion of the CdS QDs from the inner part of the microgels, resulting in the hybid microgels with CdS QDs rich on the surface region (Figure 1A).

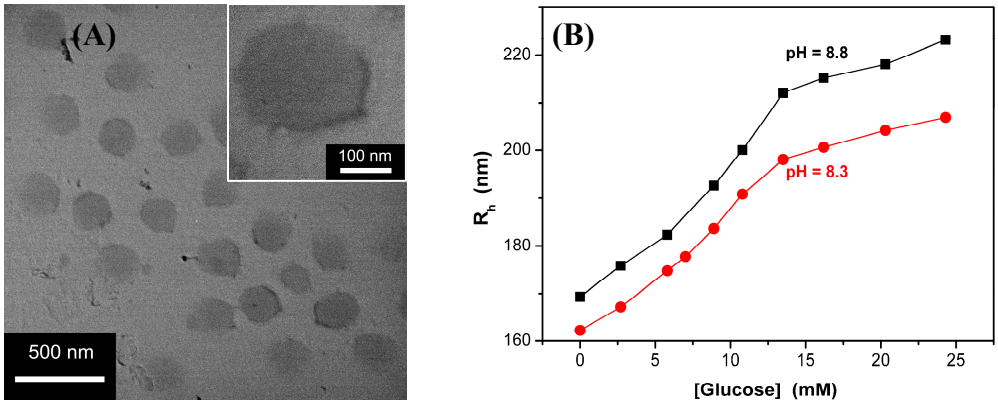
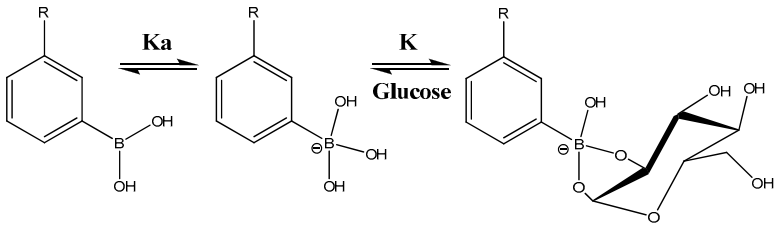


Figure 1. (A) TEM images of the p(NIPAM-AAm-PBA)-CdS hybrid microgels; (B) Glucose dependent average R_h value of the p(NIPAM-AAm-PBA)-CdS hybrid microgels at pH = 8.3 (●) and pH = 8.8 (■), respectively, measured at 22.1 °C and a scattering angle θ = 60°.



Scheme 2. Complexation Equilibrium between Phenylboronic Acid Derivative and Glucose.

The introduction of PBA groups makes the resultant p(NIPAM-AAm-PBA)-CdS hybrid microgels glucose-sensitive. As shown in Scheme 2, PBA is in equilibrium between the undissociated (or uncharged) and the dissociated (or charged) forms in aqueous solution. Both forms react reversibly with 1,2-*cis*-diols such as glucose. It was found that the glucose sensors made from the phenylborated polyacrylamide hydrogels embedded with crystalline colloid array are only responsive to glucose concentration change in the pH range of $7.0 < pH < 9.5$.[18] At $pH < 7.0$, the majority of PBA groups are in the uncharged form. The complexation of the uncharged form with glucose is unstable because it is highly susceptible to hydrolysis, but the binding with glucose causes the thermodynamically more favorable charged form. For a microgel modified with PBA group, the binding of glucose increases the degree of ionization on the hydrogel and builds up a Donnan potential for the microgel swelling. We have found that the glucose-sensitivity of the microgels increases with the increase in pH, reaches a maximum at $pH \approx 8.8$, and then decreases at higher pH.[15] While at $pH > 9.5$, almost all of the PBA groups are transferred to the charged form. As a result, the PBA-based glucose-sensors lose their sensitivity under these conditions. In the present system, the glucose-sensitive properties were thus mainly studied in a 0.005 M phosphate buffer solution (PBS) of pH = 8.8. As shown in Figure 1B, the glucose-induced swelling of the p(NIPAM-AAm-PBA)-CdS hybrid microgels is significant. The glucose-sensitivity of the hybrid microgel at a lower pH value of 8.3 also shows the same trend, indicating the repeatability of the glucose-induced volume phase transition. However, the hybrid microgel has a smaller size in the pH = 8.3 buffer than that in the pH = 8.8 buffer at all glucose concentrations. Based on these glucose-responsive volume phase transition properties, hybrid microgels bearing PBA moieties allows us to change the interactions of the CdS QDs immobilized inside the polymer networks and their local surface environments. The obtained p(NIPAM-AAm-PBA)-CdS hybrid microgels were very stable. In our design, the pAAm segments are also used to increase the hydrophilicity of the hybrid microgels after coupling the PBA to the –COOH groups of AA units in the microgels. No sediment was observed after 2 months' placement at room temperature, standing in vivid contrast against the easy aggregate of p(NIPAM-PBA) microgels[15] under similar conditions. As a result, the hybrid microgels with hydrophilic pAAm segments can reach the equilibrated swelling/collapsing limit without aggregation.

3.2 Glucose-Sensitive PL Properties of the p(NIPAM-AAm-PBA)-CdS Hybrid Microgels

The PL spectra for the p(NIPAM-AAm-PBA)-CdS hybrid microgels were collected at room temperature (Figure 2A). Two PL emission components were clearly observed. The PL spectra of the APBA solution were also presented for a comparison, showing a weak peak at 416 nm and a broad emission band in the range of 400-500 nm. Clearly, the p(NIPAM-AAm-PBA)-CdS hybrid microgels are superior in PL intensity to the free APBA solution. In order to probe the glucose-sensitive PL property of p(NIPAM-AAm-PBA)-CdS hybrid microgels, we have plotted the evolution of PL emissions in the low energy region (centre at 600 nm) in Figure 2B and 2C, corresponding to two different pH values of 8.8 and 8.3, respectively. Quenched fluorescence was clearly observed in the presence of variable concentration of glucose. Figure 2D summarized the fluorescence quenching of the CdS QDs in the p(NIPAM-AAm-PBA)-CdS hybrid microgels as a function of glucose concentration. The comparison of Figure 1B with Figure 2D indicates that the luminescence quenching of the QDs is conspicuously enhanced as the glucose concentration increases, and then reach nearly a constant at nearly the same glucose concentration when the microgel network chains stretch to a maximum. These results are in good agreement with our previous results of p(NIPAM-AAm-PBA)-CdS hybrid microgels with in situ synthesized CdS QDs in the interior of the p(NIPAM-AAm-PBA) microgels,[7] implying that the optical variation phenomena can be fully reproducible, although two different methods were employed to synthesize the hybrid microgels. It is well known that the optical property of a material is closely associated with its electronic structure. We believe that there are three ways to explain how the reversible swelling/shrinking of the microgels could trigger the fluorescence change of QDs immobilized in the microgels: (1) the change of the bonding interaction between the ligand molecules with the surface of QDs, (2) the local refractive index around the QDs,[19] and (3) the reduced number of surface defects of the QDs due to the shrinking of the microgels. When a strong chemical bond is formed, it will not only change the

electronic structure of the adsorbate itself, but also influence to some extent the surface electronic structure of CdS QDs. This may cause a change of the local optical electric field at the QD surface.[20] The local optical electric field can also be altered by an increase in the refractive index of the microgel due to the shrinking. On the other hand, the nonradiative energy loss paths are highly dependent on the nature of the environment around the QDs.[21] The cross-linkage of the polymer chains hindered the volume expansion at high swelling state, creating elastic tensions localized at the cross-linking points. Because of the bonding between the polymer and CdS QDs, the CdS QDs could also act as cross-linking points, introducing an elastic tension in the bond that could stretch the polymer/CdS interface, and creating surface states that could quench the PL.

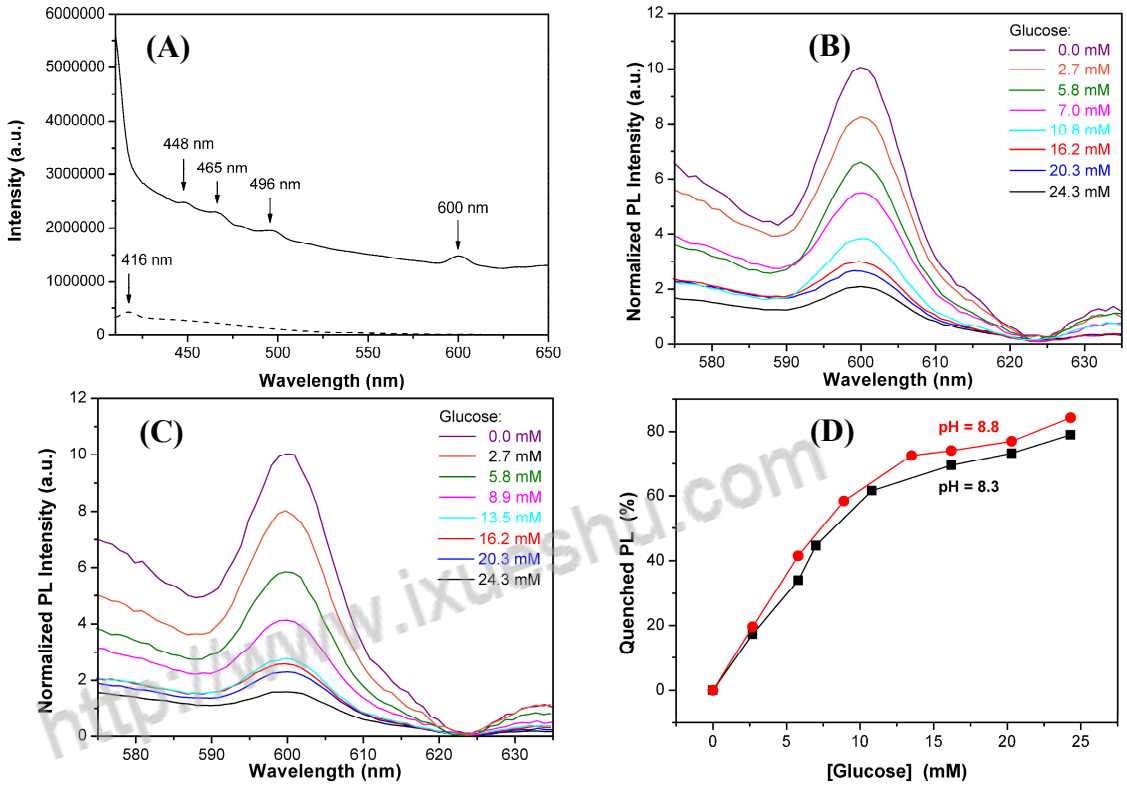


Figure 2. (A) Typical PL spectrum of p(NIPAM-AAm-PBA)-CdS microgels. The PL spectrum of the APBA solution was also presented in (A) (dash lines) showing a weak peak at 416 nm, but no other peaks. (B,C)Glucose-dependent normalized PL spectra at 600 nm region and pH = 8.8 (B) and pH = 8.3 (C), respectively. (D) Quenched PL at 600 nm as a function of glucose concentration. The excitation wavelength was 366 nm.

4. CONCLUSIONS

Quantum dots can be synthesized in situ in the interior of p(NIPAM-AAm-AA) microgels. The amino groups of the pAAm segments in the microgels are important for the uptake of precursor Cd^{2+} ions and stabilize the resultant CdS QDs inside the microgels. The carboxyl groups in the p(NIPAM-AAm-AA)-CdS hybrid microgels can be further functionalized with glucose sensitive moieties of APBA for optical glucose sensing. The glucose induced reversible swelling/shrinking of the p(NIPAM-AAm-PBA)-CdS hybrid microgels can modify the physicochemical environment of the QDs immobilized inside, resulting in a reversible quenching/antiquenching in PL intensity. We believe that it is promising to develop novel fluorescent probes involving an active role for the chemical and biological sensors based on the tightly anchoring of fluorescent QDs in the responsive microgels. Through the use of other emission wavelength-specific QDs or other targeting molecules, multifunctional nanoparticles may potentially be developed for additional important medical and optoelectronic applications.

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