Surfactant-Assisted Synthesis of Water-Soluble and Biocompatible Semiconductor Quantum Dot Micelles

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Received January 4, 2005; Revised Manuscript Received February 4, 2005

ABSTRACT

We report a simple, rapid approach to synthesize water-soluble and biocompatible fluorescent quantum dot (QD) micelles by encapsulation of monodisperse, hydrophobic QDs within surfactant/lipid micelles. Analyses of UV−vis and photo luminescence spectra, along with transmission electron microscopy, indicate that the water-soluble semiconductor QD micelles are monodisperse and retain the optical properties of the original hydrophobic QDs. The QD micelles were shown to be biocompatible and exhibited little or no aggregation when taken up by cultured rat hippocampal neurons.

Semiconductor quantum dots (QDs) with superior optical properties are a promising alternative to organic dyes for fluorescence-based bioapplications. The potential benefits and use of conjugated QD probes to monitor cellular functions has prompted extensive efforts to develop methods to synthesize water-soluble and biocompatible QDs that can be widely used for fluorescent-based bioimaging.1−11 In general, the key to develop QDs as a tool in biological systems is to achieve water solubility, biocompatibility and photostability, and, importantly, to provide flexible QD surface chemistry/functionality that will enable efficient coupling of these fluorescent inorganic probes to reagents capable of targeting and/or sensing ongoing biological processes.

We recently developed a method to prepare gold nanocrystal (NC) micelles in aqueous media.12 The method is to incorporate monosized, hydrophobic gold NCs into the hydrophobic interiors of surfactant micelles. The uniform gold NC size and self-assembled monolayer-like nature of its surface resulted in the formation of monosized gold NC micelles composed of a gold NC core and a hybrid bilayer shell with precisely defined primary and secondary layer thicknesses. Here, we extend this technique to the synthesis of water-soluble, biocompatible semiconductor QD micelles. This synthetic approach avoids complicated multistep procedures1,6 and promises to add to the versatility of QD-based probes for investigating intracellular transport and other cellular signaling pathways in living cells.

Monodisperse CdSe and CdSe/CdS core/shell QDs were synthesized according to previous methods13,14 using trioctylphosphine (TO) and oleylamine (OA) as stabilizing agents. In a typical QD micelle synthesis procedure, a concentrated suspension of QDs in chloroform is added to an aqueous solution containing a mixture of surfactants or phospholipids with different functional headgroups such as ethylene glycol (−PEG) and amine (−NH2). PEG is used to improve biocompatibility and amine groups provide sites for bioconjugation. Addition of the QD chloroform suspension into the surfactant/lipid aqueous solution under vigorous stirring results in the formation of an oil-in-water microemulsion.15 Evaporation of chloroform during heating (40−80 °C, ~10 min) transfers the QDs into the aqueous phase by an interfacial process driven by the hydrophobic van der Waals interactions between the primary alkane of the stabilizing ligand and the secondary alkane of the surfactant, resulting in thermodynamically defined interdigitated bilayer structures (Scheme 1d). Based on evaluation of water solubility and stability using ultraviolet−visible spectroscopy
UV−vis, transmission electron micrograph (TEM), and fluorescent spectra, optimized lipids were those with at least 6−8 carbons in their hydrophobic alkane chains.

QD-micelle solutions exhibited the same visible and emission colors as hydrophobic QDs, as shown in the optical micrograph (Figure 1A). The formation of individual CdSe/CdS QD micelles was further characterized by UV−vis spectroscopy shown in Figure 1B. We observed no difference in the position or width of the absorbance bands at ~480 nm and ~590 nm from hydrophobic QDs and QD micelles, which suggests that QD micelles maintain their optical properties in water. Formation of ordered hexagonal close packing shown in TEM (Figure 1C), as expected for individual monosized QDs, further confirmed the monodispersity of QD micelles. Photoluminescence (PL) spectra of OA-stabilized hydrophobic QD and QD micelles prepared using 1,2-dioctanoyl-sn-glycero-3-phosphocholine (C8-lipids) are shown in Figure 1D. PL of QD micelles exhibited no obvious shift in emission wavelength. Studies of photostability of these water-soluble NC micelles under long-time laser irradiation showed that these QD micelles exhibited no loss of PL intensity in water (Figure 2A inset). The stability of QD micelles in a cellular aqueous environment was confirmed by incubation with rat hippocampal neurons (see below).

The advantage of our method to provide water-soluble QD micelles is its capability to utilize a wide variety of surfactants/lipids with different functionally terminated groups, which can be used to improve biocompatibility and to enable bioconjugation. The encapsulation is accomplished in a single, rapid (10 minute) step of solvent evaporation. In our method, the stabilization of hydrophobic QDs in water relies on the thermodynamically favorable interdigitation of surfactants and QD stabilizing ligands formed after solvent evaporation.

Figure 2. (A) Persistence curves from CdSe/CdSe in toluene, RB in ethanol, and CdSe/CdS micelles in water solution prepared by using C8-lipid. (Inset) PL intensity CdSe/CdS micelles in water solution vs laser irradiation time.
evaporation, enabling rapid efficient transfer of the QDs into the aqueous phase. The QD micelles preserve the optical properties of the original source QDs, such as PL intensity and lifetime etc., as the encapsulation process involves no chemical substitution reactions. In contrast, in other methods, such as the ligand exchange method, chemical substitution occurs at the QD interface. This usually results in QD aggregation and changes in QD physical properties.17,18 Additionally, our method can effectively prevent QD micelles from aggregation due to the interdigitated “bilayer.”

To evaluate the biocompatibility of CdSe/CdS QD micelles, we performed an initial study to examine their uptake in cultured rat hippocampal neurons. While the route through which QDs are taken up by cells has yet to be resolved fully, they do appear to accumulate in intracellular vesicular compartments19–21 in several cell types, suggesting uptake may be mediated, at least in part, through endocytosis. Because synaptic vesicle recycling is tightly coupled to neurotransmitter release,22 endocytosis can be manipulated by depolarization of the plasma membrane to excite neurons, evoking exocytosis for neurotransmitter release followed by endocytic retrieval of synaptic vesicle membrane from nerve terminals. As shown in Figure 3A, QD micelles (emission 592 nm, red) accumulate in neurons, distinguished by immunohistochemical counterstain for NeuN (green), after exposure for 16 h in basal serum-free growth media. A three-dimensional reconstruction of confocal images confirmed the intracellular localization of QD fluorescence after long-term exposure (see Supporting Information, movie 1). The observation that QDs retain their fluorescence character after a fairly long incubation suggests that QDs can accumulate without aggregation and a general compatibility of the micelle coating within a cellular environment.

To obtain a more detailed view of QD uptake, we used pulse-chase labeling to expose hippocampal cultures to QD micelles during membrane depolarization (induced by addition of 55 mM KCl) to trigger neurotransmitter release and coupled exo/endocytosis, followed by a return to basal media. After a 10 min exposure to QDs during depolarization, robust QD fluorescence was detected by confocal microscopy in a widespread, yet clustered pattern surrounding NeuN-positive neurons (Figure 3, panel B), consistent with a diffuse, punctate localization and uptake in neuronal processes and synapses. Interestingly, following exchange for basal media without QDs, there was an apparent time-dependent increase in the perinuclear accumulation of QD fluorescence accompanied by a decrease in the more widespread distribution seen immediately after KCl depolarization (Figure 3, Panels C, D). By 5 h, the majority of QD fluorescence appeared to locate to a perinuclear compartment adjacent to NeuN stained neuronal nuclei. The results indicate that under these conditions QDs were effectively taken up by an endocytotic mechanism that was promoted by increased synaptic vesicle recycling and potentially followed by transport to lysosomes. Trafficking of QDs to lysosomal compartments may reflect autophagy, a vesicular-mediated process that underlies catabolic recycling and degradation of macromolecules, including both cellular and xenotypic agents, such as bacteria and viral pathogens.23 Autophagy may be a particularly critical mechanism for maintaining the cellular integrity of neurons, which are long-lived and generally show little or

Figure 3. Images of CdSe/CdS micelles, prepared using modified phospholipids including aPEG, DPPC, and HDA, in cultured rat hippocampal neurons. In this case where aPEG and DPPC are less water soluble, aPEG and DPPC were directly added into QD chloroform solution and water was added after evaporation of chloroform to prepare a QD micelle aqueous solution. Dispersed cultures of hippocampal neurons, prepared from 3 day old rat pups and grown on polylysine/collagen coated glass coverslips for two weeks in Neurobasal A media with B-27 supplement (Invitrogen/GIBCO), were exposed to QD micelles emitting at 592 nm. After incubation, the cells were fixed in 4% paraformaldehyde/4% sucrose, permeabilized and stained with antibodies to neuronal specific marker NeuN (Chemicon, Temecula, CA), followed by FITC (Panel A) or Alexa Fluor 488 (Panel B–D) labeled antimouse secondary antibody (Molecular Probes, Eugene OR). Panel A, fluorescent microscopy image of QD accumulation (red, arrows) after 16 h of incubation (~1 mg/ml QD micelles) in neurons identified by nuclear staining for NeuN (green). Panels B–D, confocal microscopy of pulse-chase labeling with QD micelles. Incubation with QD micelles during depolarization with 55 mM KCl for 10 min (panel B) shows dispersed, punctate staining (indicated by asterisks) characteristic of neurite processes; whereas after chase in basal media without QD micelles for 1 h (panel C) or 5 h (panel D) shows increasing perinuclear localization (arrows) with a corresponding decrease in the punctate stain seen in the 10 min labeling. Scale bar, 10 microns, for panels B–D. Images of 1 μm thick optical sections were obtained with a Zeiss Meta System Confocal Microscope using a 40× objective. QDs were imaged using 405 nm excitation and 560–615 nm emission; Alexa 488 NeuN staining with 488 nm excitation and 505–530 nm emission.
no turnover in most regions of the nervous system, and could play a role in neuronal cell death under certain conditions. The ability to use QDs to probe the trafficking of autophagic vacuoles promises to provide a useful tool to track this cellular process, but whether this tool itself elicits an autophagic response, precipitating cell death over time, will require further study.

In conclusion, we report a simple and rapid synthesis of water-soluble QD micelles through encapsulation of hydrophobic QDs into surfactant/lipid micelles. The resulting water-soluble QD micelles retain the optical properties of the original hydrophobic QDs. In addition to bioapplications, the water-soluble QD micelles are suitable building blocks for further self-assembly into ordered arrays in inorganic matrices needed for enhanced optical properties and for achieving mechanical robustness in nanodevices.

Acknowledgment. We thank Drs. John Guzowski and Teiko Miyashita for training and help with fluorescent microscopy. This work was partially supported by the U.S. Department of Energy (DOE) Basic Energy Sciences Program, Sandia National Laboratory’s Laboratory Directed R&D program, the Air Force Office of Scientific Research, NSF/UNM Science and Learning Center (SLC) Catalyst/R&D program, the Air Force Office of Scientific Research, Department of Energy’s National Nuclear Security Administration’s multiprogram laboratory operated by Sandia Corporation, a Lockheed Martin Company, for the United States Department of Energy’s National Nuclear Security Administration under Contract DE-AC04-94AL85000.

Supporting Information Available: Confocal images of QD accumulation. This material is available free of charge via the Internet at http://pubs.acs.org.

References