RNA-mediated fluorescent Q-PbS nanoparticles

ARTICLE *in* LANGMUIR · APRIL 2007

Impact Factor: 4.46 · DOI: 10.1021/la0628975 · Source: PubMed

2 AUTHORS:

Anil Kumar
Indian Institute of Technology Roorkee
87 PUBLICATIONS **1,017** CITATIONS
SEE PROFILE

Anshuman Jakhmola
University of Kentucky
11 PUBLICATIONS **165** CITATIONS
SEE PROFILE

Langmuir
The ACS Journal of Surfaces and Colloids

MARCH 13, 2007
VOLUME 23, NUMBER 6

Letters

RNA-Mediated Fluorescent Q-PbS Nanoparticles

Anil Kumar* and Anshuman Jakhmola

Department of Chemistry and Centre of Nanotechnology, Indian Institute of Technology Roorkee, Roorkee − 247667, India

Received October 3, 2006. In Final Form: December 26, 2006

RNA-mediated fluorescent PbS nanoparticles have been synthesized in the quantum-confined region of a face-centered cubic phase. The binding of RNA to the surface of PbS nanoparticles has been exploited to tailor its size and to improve the stability and electronic properties. These particles display excitonic features and a relatively strong narrow emission band (fwhm 70 nm) at 675 nm with a broad excitation range extending from 330 to 620 nm. The manipulation of experimental conditions could control the relaxation dynamics of charge carriers in the illuminated particles. The multifunctionality of the RNA structure contributes to the observed electronic properties in a cooperative manner. Such biopolymeric nanostructures may find tremendous applications in the fabrication of solar cells, fluorescence imaging, and detection devices.

Progress in the processing of colloids has offered the advantage of synthesizing size- and shape-controlled nanoparticles in solution, enabling one to fine tune their optical, photophysical, and electronic properties with ease.[1−4] The functionalization of nanoparticles using a 3D network of biomolecules[5,6] may allow their controlled synthesis with remarkably different physicochemical properties. In view of the small band gap (0.41 eV) and large exciton Bohr radius (18 nm) of PbS,[1] in recent years a number of reports have focused on the investigation of their optical, photophysical, and nonlinear optical properties mediated by biomolecules. DNA- and nucleotitide-capped PbS quantum dots exhibit weak excitonic features and display the onset of the absorption and emission bands in the NIR region.[7,8] It would be interesting to control the growth and manipulate the electronic properties of these particles by using other biomolecules as templates. An analysis of their interactions with different components of biomolecules may provide a complete understanding of these systems.

In the present work, RNA (yeast) has been employed as a template to produce PbS nanoparticles. Certain metal ions bind RNA relatively more tightly and are better localized because of discrete interactions.[9] Pb^{2+} is among such metal ions that are observed to bind to the specific sites of RNA[10] and thus could be helpful in observing the size quantization effect and analyzing its interactions with PbS. Because RNA is a structured biopolymer having a naturally occurring repeat unit, it could serve as an effective matrix for nucleation and growth. TEM and XRD analyses indicate that PbS nanoparticles on the RNA matrix are produced in the face-centered cubic (fcc) phase. These particles display prominent excitonic peaks at 350 and 575 nm and exhibit

* Corresponding author. E-mail: anilkfcy@iitr.ernet.in. Fax: +91-1332-273560.

(1) Fick, J.; Martucci, A. In *Encyclopedia of Nanoscience and Nanotechnology*; Nalwa, H. S., Ed.; American Scientific Publishers: Stevenson Ranch, CA, 2004; Vol. 4, p 481.
(2) (a) Alivisatos, A. P. *J. Phys. Chem.* **1996**, *100*, 13226. (b) Bruchez, M., Jr.; Moronne, M.; Gui, P.; Weiss, S.; Alivisatos, A. P. *Science* **1998**, *281*, 2013.
(3) Zhang, J. Z. *J. Phys. Chem. B* **2000**, *104*, 7239.
(4) Sargent E. H. *Adv. Mater.* **2005**, *17*, 515.
(5) Gugliotti, L. A.; Feldheim, D. L.; Eaton, B. R. *Science* **2004**, *304*, 850.
(6) Alivisatos, A. P. *Nat. Biotechnol.* **2004**, *22*, 47.
(7) Levina, L.; Sukhovatkin, V.; Musikihin, S.; Cauchi, S.; Nisman, R.; Bazett-Jones, D. P.; Sargent, E. H. *Adv. Mat.* **2005**, *17*, 1854.
(8) Hinds, S.; Taft, B. J.; Levina, L.; Sukhovatkin, V.; Dooley, C. J.; Roy, M. D.; MacNeil, D. D.; Sargent, E. H.; Kelley, S. O. *J. Am. Chem. Soc.* **2006**, *128*, 64.
(9) Felg, A. L.; Uhlenbeck, O. C. In *The RNA World*, 2nd ed.; Gesteland, R. F., Cech, T. R., Atkins, J. F., Eds.; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, 1999; p 287.
(10) Ennifar, E.; Walter, P.; Dumas, P. *Nucleic Acids Res.* **2003**, *31*, 2671.

10.1021/la0628975 CCC: $37.00
Published on Web 02/08/2007

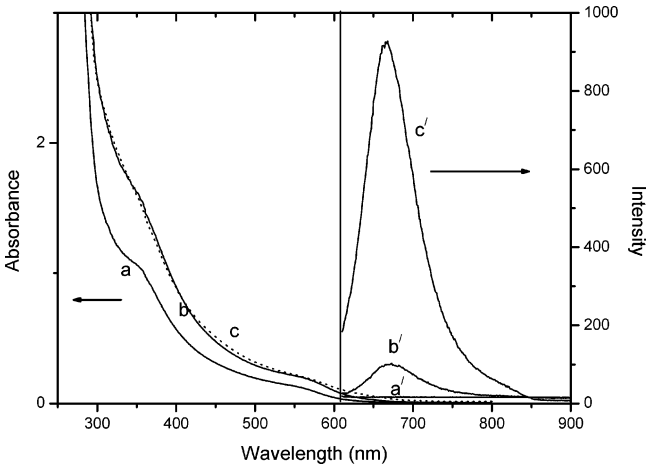


Figure 1. Electronic and fluorescence spectra of PbS with a Pb/S molar ratio of 1.0 (a, a′) 2.0; (b, b′) 2.0; (c, c′) aged particles at pH 9.8; $\lambda_{ex} = 610$ nm.

a strong band gap narrow fluorescence band (fwhm 70 nm) in the quantum-confined region at 675 nm. The interaction of Q-PbS with RNA has been analyzed using electronic, fluorescence, and NMR spectroscopy. The fluorescence lifetime is employed to understand the functionalized surface and the dynamics of charge carriers in this system. Aging of this colloidal solution results in the reorganization of the particles in a different pattern.

For the formation of Q-PbS mediated by RNA, experimental conditions were optimized by varying the pH, temperature, and [RNA] and [Pb^{2+}]. An increase in [RNA] caused a blue shift in the onset of absorption and fluorescence maxima (Supporting Information, Figures S1 and S2). At a particular [RNA], a change in the pH from 9.0 to 10.5 did not influence the emission characteristics appreciably, and above pH 11, the absorption spectra due to PbS did not show any excitonic features and the intensity of fluorescence was also reduced. The best characteristic absorption and fluorescence features were obtained by synthesizing them at 4 °C; they contained 0.017 g/100 mL RNA and had a Pb/S molar ratio of 2 at pH 9.8 (Figure 1). The absorption spectrum of PbS particles in the RNA matrix exhibits the onset of optical absorption in the visible range corresponding to a band gap of 1.86 eV with prominent excitonic bands at 350 and 575 nm. The fluorescence from these particles lies in the visible range, peaking at 675 nm at room temperature. The quantum efficiency of fluorescence of these particles was <1%. Interestingly, a change in the energy of exciting light from 2.0 to 3.8 eV did not influence the energy of fluorescence, although an increase in the energy of excitation reduced the intensity of the 675 nm fluorescence band to about one-fourth its orignal value despite the high extinction coefficient of PbS at higher energies.

TEM images show the size of the particles to depend on [Pb^{2+}] (Figure 2). The average size of the particle decreased from 23 to 5 nm by varying the Pb/S molar ratio from 1 to 3.3. A typical electron micrograph of aged PbS particles, containing a Pb/S molar ratio of 2, is shown in Figure 2c. A comparison of parts b and c of Figure 2 reveals that the aging of the colloidal solution results in the reorganization of particles with a broad size distribution (2−24 nm) compared to the fresh particles that have a relatively narrow size distribution (3−13 nm), although a large number of particles in the two cases still lie in the same range, resulting in an average size that remains virtually the same at ∼6 nm (Supporting Information, Figure S3). The long-time aging at 4 °C gave very similar changes in the particle size and size distribution to those observed at room temperature. The selected area diffraction patterns (SAED) of fresh particles show them to be crystalline (Figure 2d). For aged particles, the SAED pattern indicates them to be polycrystalline (Figure 2e). The XRD pattern displayed peaks having 2*d* values (Å) of 3.20 (111), 2.92 (200), 2.04 (220), 1.79 (311), 1.72 (222), and 1.48 (400), which correspond to PbS (galena). The indexing of the SAED pattern correlated to three diffraction planes (111), (200), and (220) that identical to those observed in XRD, thus confirming the structure of the produced particles to be face-centered cubic.

The absorption and emission characteristics of the RNA-capped PbS nanoparticles, having a Pb/S molar ratio of 2, were monitored over a period of 6 months at 4 °C as well as at room temperature. For similar electronic changes in this system, it took about 5 days at room temperature as compared to 2 months at 4 °C. It was curious that during aging neither excitonic peak at 350 nor 580 nm showed any appreciable change in absorption except that upon aging the onset of absorption was increased from 670 to about 800 nm; thereafter, it did not cause any further change in the spectrum. The fluorescence spectrum, however, demonstrated a more than 9-fold increase in the intensity of the 675 nm band without causing any appreciable change in the nature of the emission spectrum (Figure 1). The aging enhanced the quantum efficiency of fluorescence significantly and was estimated to be 5.1 ± 0.1%.

To investigate the interaction of RNA with PbS nanoparticles, we recorded the proton NMR spectra of pure RNA, RNA containing Pb^{2+}, and PbS nanoparticles capped with RNA (Figure 3). The NMR spectrum of pure RNA exhibited all of the characteristic features corresponding to protons of the sugar moiety (H2′, H3′, H4′, H5′, H5″); the aromatic protons of cytosine and uracil (H6), guanine and adenine (H8), and adenine (H2) resonated between 5 and 6.3 ppm and 7−9 ppm, respectively. In a D_2O medium, the NMR spectrum due to RNA depicted a little better resolution. All of these features agreed with the literature values of RNA reported previously.[11] The addition of Pb^{2+} to RNA also exhibited the characteristic NMR spectrum corresponding to different moieties of RNA except that the NMR peaks were finely resolved and protons corresponding to different moieties resonated at slightly higher frequencies. Also, the peak assigned to the 2′ proton of the hydroxyl group could now be observed, possibly because of the reduced exchange of protons due to complexation with Pb^{2+}. NMR of RNA-capped PbS particles demonstrated a further increase in the chemical shift due to different protons of all of the moieties. Obviously, there is an increased shielding of protons attached to different nuclei upon complexation with Pb^{2+}, which increases further upon the formation of PbS. It clearly indicates that Pb^{2+} in PbS binds selectively to RNA through purine bases, pyrimidines, and the sugar moiety as shown in structure 1.

To analyze the interaction of PbS with specific components of RNA further, the above procedure was employed to synthesize PbS nanoparticles using different individual components of RNA and their mixtures as a template under the abovementioned optimized conditions, viz., adenosine monophosphate, guanosine monophosphate, cytosine monophosphate, and uracil monophosphate separately as well as various combinations of their mixtures. None of these individual components or their mixture produced PbS particles with the observed optical features, and these systems depicted negligible emission that, too, in other wavelength ranges compared to that of particles capped with RNA.

The fluorescence due to Q-PbS decays in three exponential processes (Figure 4). Changes in [Pb^{2+}] and [RNA] influence

(11) Fürtig, B.; Richter, C.; Wöhnert, J.; Schwalbe, H. *ChemBioChem* **2003**, *4*, 936.

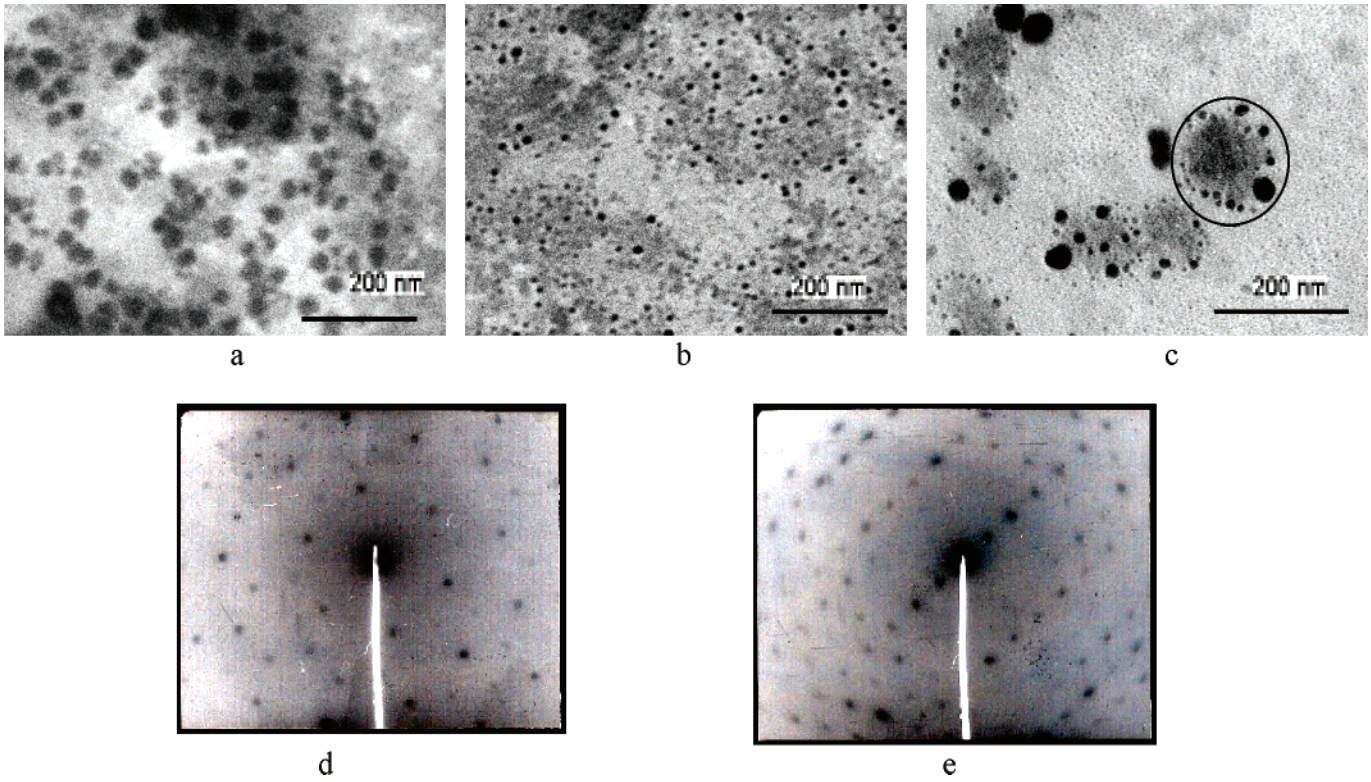


Figure 2. TEM micrographs of Q-PbS with the following Pb/S molar ratios: (a) 1; (b) 2; (c) 2 (aged particles). SAED pattern of Q-PbS with a Pb/S molar ratio of 2: (d) fresh and (e) aged.

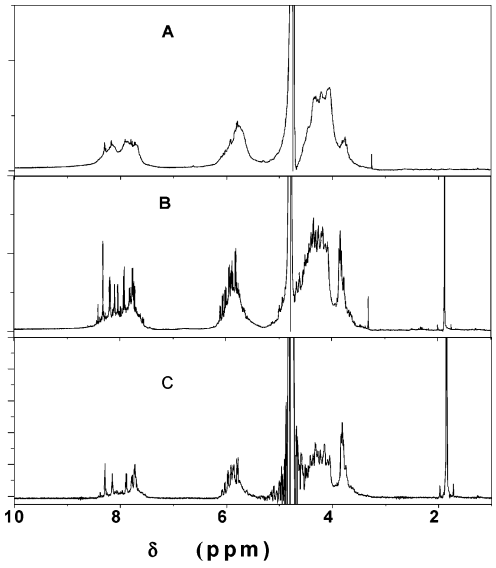


Figure 3. NMR spectra of: (A) pure RNA, (B) RNA containing Pb^{2+}, and (C) PbS grown RNA matrix with excess Pb^{2+}.

the average fluorescence lifetime $\langle\tau\rangle$ in a complex manner. An increase in the Pb/S molar ratio from 1 to 3.3 enhanced $\langle\tau\rangle$ from 10.6 to 75.0 ns. On the contrary, increasing [RNA] from 0.0067 to 0.023 g/100 mL reduced it from 74.4 to 31.1 ns. A decrease in the energy of excitation from 3.06 to 2.04 eV enhanced $\langle\tau\rangle$ from 33.1 to 61.8 ns. For the aged particles, the latter value of $\langle\tau\rangle$ is further increased to 0.1 μs time domain. This finding is in accordance with steady-state measurements in which the intensity of 675 nm fluorescence was found to increase with the decreasing energy of the excitation light.

The above electronic features of Q-PbS are fairly different from those of bulk PbS, which has a band gap of 0.41 eV. The observed blue shift in the absorbance band edge and the band

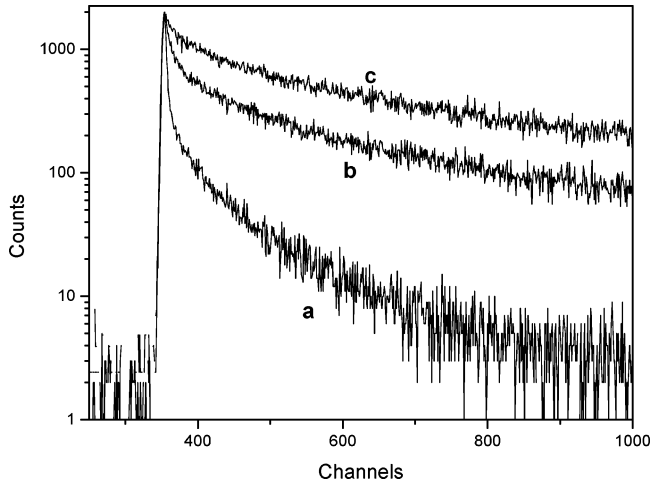
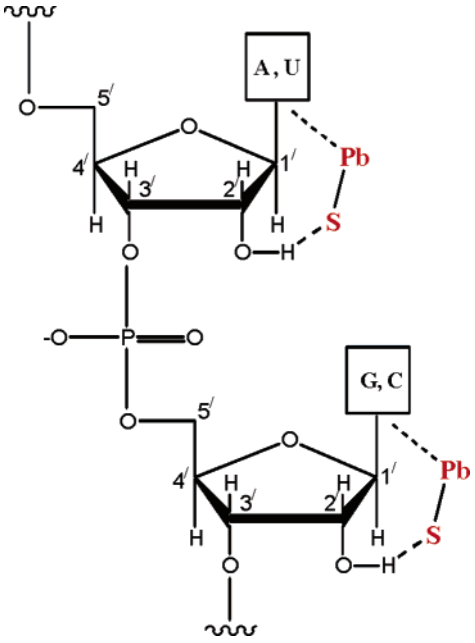


Figure 4. Fluorescence decay curves of PbS nanoparticles with Pb/S molar ratios of (a) 1, (b) 2, and (c) 2 (aged particles, $\lambda_{ex} = 605$ nm, $\lambda_{em} = 680$ nm at pH 9.8). The time calibration is 2.2308×10^{-10} s/channel.

gap fluorescence evidently demonstrates the electronic properties of RNA-capped PbS to be consistent with the quantum confinement (Supporting Information Figures S1 and S2 and Figure 1).[12−14] The enhanced photostability with an increase in the molar ratio of Pb^{2+} to RNA suggests an increased surface passivation for these nanoparticles. The reorganization of particles upon aging is reflected in the TEM micrograph, SAED patterns, and absorption and emission studies (Figures 1 and 2). The observation of a small change in the absorption behavior but a significant increase in the fluorescence intensity and fluorescence

(12) Patel, A. A.; Wu, F.; Zhang, J. Z.; Torres-Martinez, C. L.; Mehra, R. K.; Yang, Y.; Risbud, S. H. *J. Phys. Chem. B* **2000**, *104*, 11598.
(13) Warner, J. H.; Thomsen, E.; Watt, A. R.; Heckenberg, N. R.; Rubinsztein-Dunlop, H. *Nanotechnology* **2005**, *16*, 175.
(14) Kumar, A.; Jakhmola, A. *J. Colloid Interface Sci.* **2006**, *297*, 607.



lifetime upon aging suggests the modification of the localized trap states of Q-PbS through the surface passivation of sites responsible for the fluorescence properties. The availability of a large number of anchoring sites on the surface of RNA-mediated particles results in their reorganization without depicting any significant change in the average size; therefore, no variation was exhibited in their optical and fluorescence peak positions upon aging, unlike those of bare inorganic colloids. Because the intensity of the 675 nm fluorescence band and the emission lifetime is increased upon reducing the energy of the exciting light, it is obvious that surface states at high energy occupy the shallow traps and are not involved in radiative recombination and these energy levels relax to the ground states largely through a radiationless process. This phenomenon may also arise from multiple exciton generation by absorption of photon of energy that is greater than the band gap energy, as has been observed recently with PbSe and PbS nanoparticles.[15] This may result in the nonradiative recombination of excitons, thereby reducing the fluorescence intensity and lifetime at higher energies. The electronic features of these particles are quite different from those mediated by DNA. PbS quantum dots grown on DNA exhibit a featureless electronic spectrum having an absorption threshold in the NIR region. DNA- and nucleotide-directed growth of PbS does not depict any fluorescence in the visible region but results in the strong photoluminescence at 1100 nm.[7,8]

Because none of the individual components of RNA, viz., AMP, GMP, CMP, and UMP, is able to show a similar effect regarding the optical and electronic behavior observed in the present work, it is apparent that these moieties when present in RNA display this effect in a cooperative manner. Obviously, the multifunctional 3D structure of RNA contributes to this effect. Such systems could be useful as biological probes.[6,16]

In summary, RNA serves as an effective matrix for the nucleation and growth of nanostructured PbS particles. It produces quantized PbS, which exhibits relatively high solubility, photostability, and a narrow emission band in the visible region over a broad excitation range. This is the first report on a PbS system demonstrating such a high quantum efficiency of emission in the visible region obtained upon excitation by light having a wavelength >600 nm. The quantum efficiency of fluorescence and the fluorescence lifetime are correlated to each other and are significantly increased upon aging compared to that of freshly prepared colloidal PbS solution due to the reorganization of particles. Q-PbS could be stored for more than 6 months at 4 °C without losing its optical characteristics. The observed electronic features make them the promising material for solar cells,[17] biological labeling,[6] fluorescence imaging,[16] sensors, and visible detector technology.

Acknowledgment. The financial support of DST, New Delhi is gratefully acknowledged in undertaking this work. A.J. is thankful to CSIR, New Delhi for the SRF award. Thanks are also due to the Director, AIIMS, New Delhi and Head, IIC, IIT Roorkee for providing us with TEM and single photon counting facilities, respectively.

Supporting Information Available: Experimental section containing materials, equipment, and methodology. Effect of [RNA] on electronic and fluorescence spectra of PbS. Size histograms of electronic micrographs given in Figures 2b,c. This material is available free of charge via the Internet at http://pubs.acs.org.

LA0628975

(15) Schaller, R. D.; Sykora, M.; Pietryga, J. M.; Klimov, V. I. *Nano Lett.* **2006**, *6*, 424.

(16) Michalet, X.; Pinaud, F.; Lacoste, T. D.; Dahan, M.; Bruchez, M. P.; Alivisatos, A. P.; Weiss, S. *Single Mol.* **2001**, *4*, 261.

(17) Plass, R.; Pelet, S.; Krueger, J.; Grätzel, M.; Bach, U. *J. Phys. Chem. B* **2002**, *106*, 7578.