Enzyme mediated synthesis of phytochelatin-capped CdS nanocrystals

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We reported the enzyme mediated synthesis of CdS nanocrystals by immobilized phytochelatin synthase, which converts glutathione into the metal-binding peptide phytochelatin (PC). Formation of CdS nanocrystals were observed upon the addition of $CdCl_2$ and Na_2S with PC as the capping agent. By varying the reaction times, different compositions of PCs (form PC2 to PC3) can be synthesized, resulting in the formation of highly stable nanocrystals with tunable sizes (from 2.0 to 1.6 nm diameter). This approach may be generalized to guide the *in vitro* self assembly of a wide range of nanocrystals with different compositions and sizes. © *2010 American Institute of Physics*. [doi:10.1063/1.3485295]

Semiconductor nanocrystals, also known as quantum dots (QDs), are nanometer-sized crystals of semiconductor with unique optical and electrical properties due to the quantum confinement effect.[1,2] Their unique attributes such as extremely bright photoluminescent, broad absorption yet sharp emission spectra, good photostability and chemical stability are ideal for light-emitting diodes, solar cell and laser applications.[3–5] Moreover, there is increasing interest in applying semiconductor QDs as fluorescent labels for biological imaging and detection in place of organic fluorophores.[6,7] QDs are typically synthesized in an organic solvent at high temperature resulting in hydrophobic nanoparticles coated with organic capping ligands such as trioctylphosphine oxide that are not suitable for biological applications.[8] In recent years, thiol-containing molecules such as 2-mercaptoethanol, thioglycolic acid, 1-thioglycerol and L-cysteine have been used as capping agents for the direct synthesis of CdS, CdSe, CdTe, and HgTe QDs in aqueous solution at lower reaction temperature (80–100 °C).[9–12] The resulting nanocrystals are water soluble and have excellent biocompatibility for use in biological imaging.

In addition to synthetic thiol-containing molecules, naturally occurring cysteine-rich peptides, which are employed as chelating agents to remove a variety of heavy metals, have also been employed for QD synthesis.[13] One example is the use of the tripeptide glutathione (GSH) as the capping agent for the synthesis of ZnS, ZnSe, CdS, CdSe, and CdTe nanocrystals.[9,14–17] However, most of them showed poor stability and high polydispersity because of the relatively weak binding affinity of GSH (containing one cysteine).

Recent studies have shown that peptides containing two or more cysteines are more effective in capping QD growth than those with a single cysteine.[18] Improvements on colloidal stability have been reported by using either dithiol ligands or multidentate thiol ligands, to achieve tighter binding to the nanocrystals.[7,19] The naturally occurring metal-chelating peptide, phytochelatin (PC) possesses the general structure $(\gamma\text{–Glu–Cys})_n\text{–Gly}$ (PC2 to PC11, n=2–11) (Ref. 20) that are capable of coordinating Cd^{2+} through the multiple thiol moieties. Early reports using PCs for CdS nanocrystals synthesis resulted in more uniformly sized particles compared with GSH-capped CdS nanocrystals.[21] In addition, cross-linked GSH capped CdS nanocrystals have been shown to have enhanced stability due to the formation of PC-like crosslinked-GSH cappings,[22] suggesting the benefits of using PCs as the capping ligand. In all these cases, PCs were either purified from Cd-exposed yeast culture using time-consuming gel-filtration and ion exchange chromatography or were produced using expensive chemical synthesis before nanocrystal preparation. Because of the unusual γ bond presence in PCs, they are naturally synthesized through the transfer of γ–Glu–Cys from GSH to another GSH or other PCs by the enzyme PC synthase (PCS).[20,23] We recently reported the use of genetically modified *E. coli* expressing SpPCS, a PCS originated from the fission yeast *S. pombe*,[24] as a potentially low-cost approach for nanocrystal synthesis. However, since the precursors $CdCl_2$ and Na_2S must go through the cell membrane, accessibility becomes a problem in controlling the properties of the nanocrystals. Moreover, other precursors such as Pb^{2+} or NaHSe cannot be readily transported, making it difficult to expand this method for other more interesting nanocrystals. Here, we suggest an alternative way for *in vitro* PC-based CdS nanocrystal synthesis using immobilized PCS (Fig. 1).

To produce PC, a gene encoding SpPCS (Ref. 25) was introduced into *E. coli* strain JM109. A hexa-histidine tag

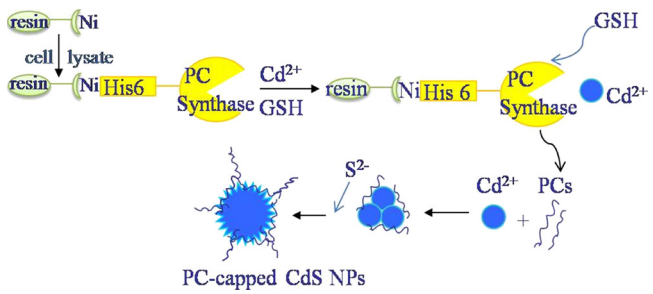

FIG. 1. (Color online) Schematic representation of the strategy for the PC template synthesis of CdS nanocrystals using purified PCS.

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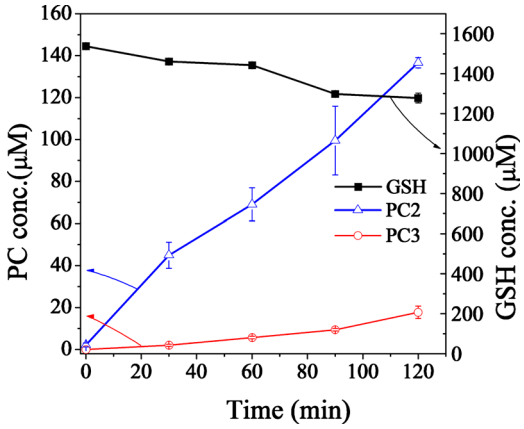

FIG. 2. (Color online) Time course of thiol peptides, PC and GSH measured by RP-HPLC. The initial concentration of GSH was 2 mM.

was added to the N-terminal to facilitate immobilization. Cells induced with IPTG were harvested and lysed by sonication. SpPCS was separated from the cell lysates by mixing with nickel resins based on nickel-hexa-histidine affinity interaction. SpPCS bound resins were collected by centrifugation and resuspended in the buffer. A band corresponding to SpPCS was removed from the cell lysates after binding as shown by SDS-PAGE (Ref. 27). Enzyme immobilization was confirmed by eluting bound proteins with imidazole (Ref. 27); only a single band corresponding to SpPCS was detected, indicating that this batch method was efficient for immobilization.

PC production was initiated upon addition of $CdCl_2$ (0.5 mM) and GSH (2 mM) (Fig. 1) since the enzymatic conversion of GSH into PCs is activated by heavy metals. PC synthesis was analyzed using monobromobimane, a fluorescent dye selectively reactive to thiol peptide[26] and measured by high-performance liquid chromatography (HPLC). As shown in Fig. 2, the PC concentration increased gradually with time and was accompanied by a decrease in the GSH content. The amount of PC2 (134 μM) and PC3 (17 μM) detected after 2 h was in good agreement with the amount of GSH (280 μM) consumed.

At various PC synthesis times, Na_2S at an equal molar ratio to Cd was added to the PC-Cd complexes to investigate the ability to guide the formation of CdS nanocrystals. Independent of the PC synthesis times, a characteristic absorption peak around 315 nm emerged after the addition of Na_2S indicating the presence of PC-capped CdS nanocrystals, while PC-Cd complex alone exhibited no absorption (Fig. 3). The presence of CdS nanocrystals was further supported by a blueshifted absorption edge at 350 nm compared to the absorption onset of bulk CdS material (band gap of the bulk material: 2.41 eV; absorption edge at $\lambda = 515$ nm), indicating a good quantum confinement. An emission peak was detected at 380 nm for the PC-capped nanocrystals, which is significantly blueshifted compared to that obtained with GSH-capped nanocrystals synthesized using the same 4:1:1 molar ratio of GSH:Cd:S, indicating that PCs were primarily used as the capping agents even in the abundance of remaining GSH [Fig. 3(a)]. This can be explained by the higher metal-binding affinity of PC as demonstrated by the formation of only PC-Cd complexes even when GSH was in tenfold excess in a mixture of GSH and PCs.[21] The full-width at half maximum of the PC-CdS emission band (~64 nm) was

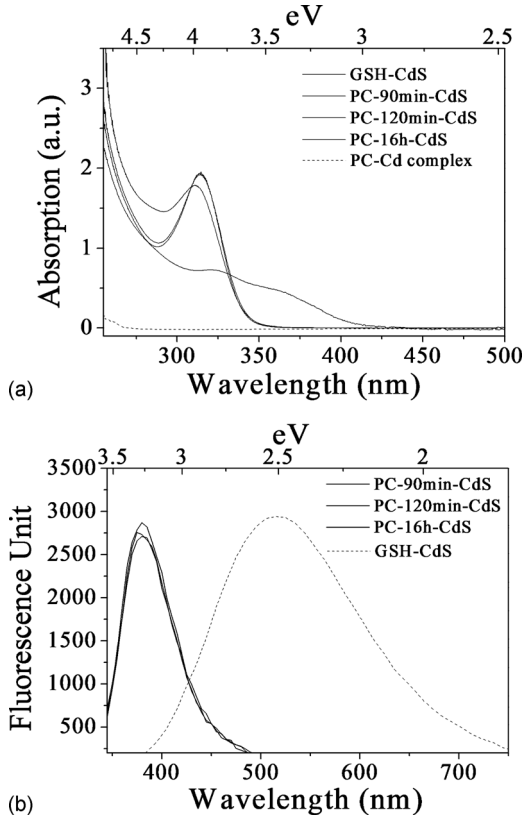

FIG. 3. (a) UV-VIS spectra of CdS nanocrystals at different PC synthesis time points (solid line) and PC-Cd complexes before sulfide addition (dotted line). (b) Emission spectra of CdS nanocrystals at different PC synthesis time points (solid line) and GSH capped CdS nanoparticles (dotted line). The excitation wavelength was fixed at 315 nm.

much smaller than GSH-CdS (~150 nm), which also implies that PC mediated CdS nanoparticles have a fairly uniform size distribution.

We have previously demonstrated that smaller CdS nanocrystals can be obtained *in vivo* using PC4 as the capping agent rather than using PC2.[23] To see whether a similar level of size control can be achieved, we first investigated the ability to synthesize different forms of PC. From the initial PC synthesis results, PC2 dominated the PC population when the reaction time was shorter than 120 min. However, the PC3 level increased with time from a ratio of PC3/PC2 of 0.1 at 90 min to 0.17 at 120 min, suggesting the gradual conversion of PC2 to PC3. After 16 h, most of the existing PC2 were converted into PC3 and a PC3/PC2 ratio of 1.7 was detected. After Na_2S addition, the absorption and emission peak were both blueshifted [Figs. 3(a) and 3(b)], suggesting the presence of CdS nanocrystals of smaller sizes.

The formation of nanocrystals was confirmed by high-resolution transmission electron microscopy (HR-TEM) [Fig. 4(a) and Ref. 27]. The inset of Fig. 4(a) showed a single PC-capped CdS nanocrystal. Lattice fringes of a single nanocrystal revealed an inter-planar spacing of 0.2087 nm, corresponding to the unique spacing of (110) planes in the hexagonal structured CdS. Elemental analysis also confirmed the formation of CdS nanocrystals [Fig. 4(a)]. HR-TEM images confirmed that PC-capped nanocrystals had a smaller average particle size of 2.0 nm at 90 min reaction time (Ref. 27) and 1.9 nm at 120 min reaction time [Fig. 4(b)] than the GSH-capped nanocrystals of 2.7 nm (Ref. 27), in agreement with the results from the fluorescence and absorption spectra.

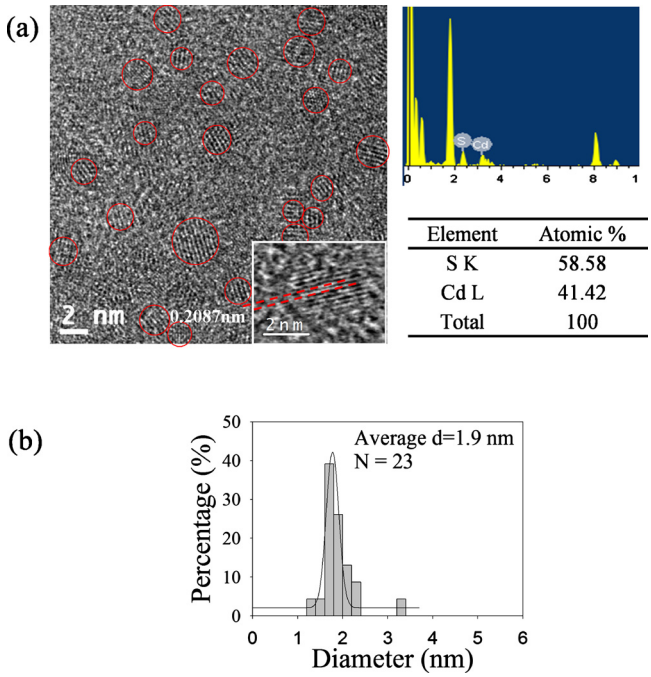

FIG. 4. (Color online) (a) HR-TEM micrograph and EDX analysis of PC-capped CdS nanocrystals formed at 120 min reaction time. The inset showed a single nanocrystal. (b) A histogram of the nanocrystal size distribution, N is the number of nanocrystals analyzed.

The PC capped CdS nanocrystals also had a narrower size distribution with less polydispersity. The average size of the 16 h nanocrystals was further reduced to 1.6 nm (Ref. 27), a result consistent with the use of PC3 as the main capping ligand. Moreover, PC capped nanocrystals were stable even after 1 week's dialysis against deionized water while GSH capped nanocrystals were unstable with visible precipitation after one to two days of dialysis. This result is consistent with previous observations showing improved nanocrystal stability by using dithiol ligands or multidentate thiol ligands.[7,19]

To confirm that the synthesized CdS nanocrystals were capped by PCs, we extracted the capping peptides and determined the concentration by HPLC. As shown in Table I, no GSH was detected confirming that only PCs were used as the capping agent. As expected, mostly PC2 were detected for nanocrystals obtained at 90 and 120 min. However, the PC2/PC3 ratio detected was lower than that in the solution indicating the preferred binding to Cd by the higher binding affinity of PC3. The derived peptides from the nanocrystals formed at 16 h showed that mostly PC3 were used as the capping agent, a result consistent with the smaller size particles and the corresponding shift in fluorescence and emission spectra. These results confirm our ability to control the size of the nanocrystrals produced by controlling the capping PC population.

TABLE I. The concentrations of PCs extracted from PC-CdS nanocrystals which were dialyzed against deionized water for three to six days. A 1000 Da cut-off membrane was used.

Sample	PC concentration after extraction (μM)			PC3/PC2 ratio
	GSH	PC2	PC3	
PC-90 min-CdS	0	11.31	2.18	0.19
PC-120 min-CdS	0	15.90	4.46	0.28
PC-16 h-CdS	0	1.29	79.04	61.27

In summary, we demonstrated the *in vitro* enzyme mediated synthesis of CdS nanocrystals using immobilized SpPCS. The formation of CdS nanocrystals was directly mediated by PCs synthesized by PCS. PC-capped CdS nanocrystals had smaller size, higher stability and less polydispersity compared to CdS nanocrystals capped by GSH. Unlike the cell-based approach we reported previously,[24] this *in vitro* approach allows the use of other precusors such as Pb^{2+} or NaHSe and be easily extended to the synthesis of zinc sulfide (ZnS) or lead sulfide (PbS) as well as cadmium selenide (CdSe), cadmium telluride (CdTe), or lead selenide (PbSe) nanocrystals.

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[27]See supplementary material at http://dx.doi.org/10.1063/1.3485295 for Figs. S.1 (lane 1 and lane 2) and S2–S4.