Effective Reduction of Nonspecific Binding by Surface Engineering of Quantum Dots with Bovine Serum Albumin for Cell-Targeted Imaging

Bingbo Zhang,*† Xiaohui Wang,* Fengjun Liu,‡ Yingsheng Cheng† and Donglu Shi*‡§

The Institute for Biomedical Engineering & Nano Science, Tongji University School of Medicine, Shanghai 200092, P. R. China

Medical Imaging Center, the Tenth People’s Hospital, Tongji University, Shanghai 200072, P. R. China

School of Electronic and Computing Systems, University of Cincinnati, Cincinnati, Ohio 45221, United States

Supporting Information

ABSTRACT: Quantum dots (QDs) have been widely used as fluorescent probes in cell-targeted imaging. However, nonspecific binding to cellular membranes has been a major challenge. In this study, a new approach is developed for effective reduction of nonspecific binding by bovine serum albumin (BSA)-coated QDs in cell targeting. The experimental results show efficient transfer of hydrophobic QDs from organic to aqueous phase in the presence of BSA aqueous solution under ultrasonication. This ultrasonication-based approach is facile, rapid, and efficient. Stabilization of QDs is mainly achieved by multiple mercapto groups in BSA macromolecules as multidentate ligands and partially by hydrophobic interaction between BSA and pending fatty ligands on QDs. The water solubility of QDs is enhanced by the surface amino and carboxyl groups, which also provide reaction sites for conjugation of targeting ligands. The BSA-coated QDs, with an overwhelming majority of hydrodynamic diameter size of ca. 18 nm, are colloidally stable under both acidic and basic conditions and found to exhibit strong fluorescent intensities. The nonspecific cellular binding is effectively reduced by BSA-coated QDs, compared with the mercaptopropionic acid (MPA)-coated CdTe QDs. BSA-coated QDs are further functionalized with cyclic Arg-Gly-Asp (cRGD) peptide. The cell assays indicate their high target-selectivity in integrin αvβ3-expressed cell imaging.

INTRODUCTION

Quantum dots (QDs) are known for their monochromatic emissions, tunable wavelength, broad absorption cross section, large molar extinction coefficients, high quantum yields (QYs), and excellent photostability.1–3 For these unique properties, QDs have been widely used in biomedical labeling, molecule tracking, and imaging.4–8 However, some critical issues still remain to be addressed on their colloidal properties such as water solubility, hydrodynamic size, and colloidal stability.9,10 Nonspecific binding is a major challenge in biodetection.11,12 QDs generally attach onto the cell membranes, proteins, and other matrix materials nonspecifically, resulting in a high level of background fluorescence that degrades the signal-to-noise ratio and limits detection sensitivity. False positives are likely as a result of nonspecific binding.

Different ways have been developed to address the issue in recent years. Nie et al. reported hydroxyl (–OH)-coated QDs for minimizing nonspecific cellular binding.11 Their experimental results indicated that the hydroxyl-coated QDs showed significantly reduced nonspecific binding compared to those functionalized with carboxylate, streptavidin, and poly(ethylene glycol). Further study implied that nonspecific binding was mainly associated with the surface conditions of the nano-particles such as chemical groups, surface charge, and molecular weight of surface ligands. Therefore, generating hydroxyl groups from negatively charged carboxyl groups has become a main approach for achieving lower surface charges. PEG, known for its hydrophilicity and biocompatibility, is often linked to the surfaces of nanoparticles for reduction of nonspecific binding and improving water solubilization.12–14 These previous studies showed that pegylated QDs with nearly neutral surface charges had significantly reduced nonspecific binding.12–17 Unfortunately, although achieving amazing development, these modification processes are generally tedious, high cost, and environmentally unfriendly, spoiling their advantages in biomedical applications.

BSA is an extensively used and commercially available biomacromolecule in biological applications, for its capability of reducing nonspecific binding in immunoassay.18,19 In this study, a facile water solubilization approach was developed based on BSA surface engineering, aiming at reducing nonspecific binding with fine nanoparticle sizes. BSA is an important

dx.doi.org/10.1021/la302758g | Langmuir 2012, 28, 16605−16613

© 2012 American Chemical Society

Published: November 12, 2012

Received: July 9, 2012

Revised: October 30, 2012

16605
blood protein containing one single cysteine and eight pairs of disulfide bonds.20,21 These sulphydryl compounds acting as multidentate ligands can replace the original hydrophobic ligands of QDs. Ligand exchange with multidentate compounds has become a promising trend in improving water-solubilization of hydrophobic nanoparticles, since they exhibit much stronger interactions with QD surfaces compared with that of single or dithiol ligands.22–25 Dihydropropionic acid (DHLA) ligands can enable stable interactions with QD surfaces due to the bidentate chelate effect afforded by the dithiol groups.26 However, aggregation and nonspecific binding were observed, when these QDs were mixed with cationic polymer or nanoparticles in acidic solutions.27 These behaviors are associated with the loss of water compatibility once the carboxylic acid end groups on the DHLA-coated QDs are no longer ionized in the acidic solution.

Furthermore, as a zwitterionic polymer, the colloidal stability of QDs can be enhanced well by BSA coating, due to the surface amino and carboxyl groups, in both acidic and basic environments. The surface charges of QDs are also well-balanced for reduction of nonspecific binding. The phase transfer of QDs is induced by ultrasonication. This process is facile, reproducible, and rapid within 5 min. The BSA-coated QDs developed in this study have been found to exhibit good colloidal stability, high quantum yields, and pronounced reduction of nonspecific binding. Upon conjugation with cRGD peptide,28–30 they are highly selective for targeted integrin α5β1-expressed cell imaging.
Cell-Targeted Imaging. For comparison, U87 MG (integrin $\alpha_v\beta_3$ overexpressed) and MCF-7 (integrin $\alpha_v\beta_3$ low expressed) cells were used in this study. Preseed $0.2 \times 10^5$ U87 MG cells in each glass-bottomed microwell dish were incubated overnight. Parallel experiments using integrin $\alpha_v\beta_3$-negative MCF-7 cells were carried out as a control. The cell culture medium was aspirated from the dish and the cells were washed three times with 0.01 M PBS (3−5 min each). A 2% BSA blocking solution was added and incubated for 20 min. After the solution was aspirated, BSA-coated or cRGD-functionalized BSA-coated QDs in the blocking solution were added and incubated with the cells for 30 min. The cells were then washed three times with 1× PBS to remove the QDs. Finally, the cells were imaged under fluorescence microscopy.

Characterization. Transmission Electron Microscopy (TEM). The BSA-coated QDs were dispersed in deionized water and dried onto carbon-coated copper grids before examination. TEM images were obtained with a Philips Tecnai G² F20 TEM operating at an acceleration voltage of 200 kV.

Spectrum Characterizations. The absorbance and emission spectra of hydrophobic QDs in chloroform and BSA-coated QDs in deionized water were measured on a Cary 50 spectrophotometer (Varian) and a F-4500 spectrophotometer (Hitachi), respectively. The dispersion property of BSA-coated QDs in deionized water was measured using a particle size analyzer (Nano ZS, Malvern). The fluorescence QYs of the QDs in deionized water were measured using Rhodamine 6G as fluorescence standard.

RESULTS AND DISCUSSION

Phase Transfer of QDs. The schematic illustration of the one-pot facile QD phase-transfer process is shown in Scheme 1. The phase transfer is accomplished by ultrasonication. QD/chloroform solution is slowly injected into the BSA/water solution during ultrasonication. BSA as a natural biomacromolecule has one free sulfhydryl and eight pairs of disulfide bonds. Although sulfhydryl and disulfide bonds can replace the hydrophobic surfactants and bind onto the surfaces of QDs, they are inside the BSA macromolecules, resulting in impeditive contacts with QDs. This steric obstruction can be reduced by ultrasonication. Under the external physical force, the BSA macromolecules are stretched and the sulfhydryl and disulfide bonds are exposed to QDs. Ultrasonication has been found to be a precondition for phase transfer of QDs. Once all the sulhydryl and disulfide bonds bind onto the surfaces of QDs, the multidentate interaction is much stronger than that of the single or dithiol ligands.

DHLA is a commonly used dithiol chemical in the phase transfer of QDs. Currently, dithiol derivatives based on DHLA have been commonly used for enhancement of colloidal stability of modified QDs. In this study, commercially available BSA protein was selected for coating of QDs instead of chemical synthesis of multithiol compounds for low cost and time savings.

The successful phase transfer of QDs from organically soluble to water-soluble was achieved in this study. Instead of chloroform, the as-prepared QDs can only be solubilized in aqueous solution. Subsequent TEM analysis further confirms that a large proportion of the BSA-coated QDs are well-dispersed on the copper mesh after drying from water (Figure 1a) except some aggregated clusters (see Figure S1). As shown in Figure 1b, the hydrodynamic size of BSA-coated QDs is slightly larger than that observed from TEM. This is due to BSA macromolecules being capped on QDs. The original QD core used in this study is about 8.0 nm in size, and BSA molecule is about 5.0 nm in water. Thus, the hydrodynamic diameter of BSA-coated QDs is about 18.0 nm (8.0 + 2 × 5.0), which coincides with the DLS analysis. The presence of large-
sized fractions of BSA-coated QDs shown in TEM and DLS will be discussed below.

The degree of ligand exchange (phase transfer efficiency) was preliminarily evaluated by spectrum characterization. The change of QDs local surface microenvironment arising from ligand exchange can cause spectrum fluctuations. Figure 2a shows the photoluminescence spectra of the as-prepared samples with different BSA/QD mole ratios. Consistent but respectable blue shifts (ca. 5 nm) are observed for samples of higher BSA/QD mole ratios of 500 and 1000, respectively. A smaller blue shift (ca. 3 nm) is observed at the mole ratio of 250. No further blue shift is observed for BSA/QD mole ratios greater than 500. The effect of phase transfer on absorption spectra is less pronounced as shown in Figure 2b. The as-prepared samples with BSA/QD mole ratios below 250 aggregate easily (see Figure S2). One possible explanation for this aggregation is that the hydrophobic surfactants are not efficiently replaced by limited BSA macromolecules at low BSA/QD mole ratios. The fluorescence QYs of the original hydrophobic QDs in chloroform and the BSA-coated QDs (with 500 of BSA/QDs mole ratio) in deionized water are 45% and 35%, respectively.

In order to conjugate sulphydryl and disulphide moieties on BSA accessible for covalent binding to QD surfaces, BSA was denatured by adding sodium borohydride, and the surface ligand exchange experimental results show similar capability of the reduced BSA in the phase transfer of QDs. To simplify the chemical procedures, intact BSA was used in this study.

**Mechanism of Phase Transfer.** To identify the phase transfer mechanism, hydrophobic magnetic nanoparticles, OA-coated QDs, and 1-dodecanethiol (DDT)-coated CuInS2/ZnS QDs were synthesized, respectively, in this study. The same approach was found to apply well on phase transfer of the hydrophobic magnetic nanoparticles. The only difference is the clustering of the magnetic nanoparticles (see Figure S3). Quite similar phase transfer behaviors were found between the OA-coated and the OA/ODA/TOPO-coated QDs. DDT, as the capping agent for CuInS2/ZnS QDs, has one free thiol group, which binds on the surface of CuInS2/ZnS QDs. This binding force is stronger than that of OA, ODA, and TOPO with QDs, which gives rise to inefficient ligand exchange by BSA. Although some CuInS2/ZnS nanoparticles are efficiently coated by BSA (see Figure S4), a large fraction of the modified CuInS2/ZnS QDs is found to aggregate after storage for a period of time (see Figure S2).

Fatty ligands are originally capped on the surfaces of QDs and magnetic nanoparticles. However, the results of water solubilization are found to be drastically different between BSA-coated QDs and magnetic nanoparticles under ultrasonication. The QDs treated by BSA are individually dispersed in water solution (ca. 18 nm of hydraulics diameter, except a tiny fraction of aggregated clusters; see Figure S1), while magnetic nanoparticles (also treated by BSA) are nanoparticle clusters formed by tens of single magnetic nanoparticles (ca. 90 nm of hydraulics diameter, Figure S3). These results indicate higher affinity of BSA macromolecules on the surface of QDs than on the magnetic nanoparticles. It should be noted that the physicochemical properties of surface capping ligands on QDs and magnetic nanoparticles are nearly the same. Thus, a hypothesis is put forward that BSA macromolecules could replace the original organic ligands on the surface of QDs, but not on the magnetic nanoparticles. This difference is understood by higher affinity between the -SH, -S-S- groups, and Cd/Zn, when compared with the Fe atoms.

The chemical -SH group has been extensively reported as an excellent affinitive ligand for aqueous QDs synthesis and on ligand exchange of hydrophobic QDs. However, there have been few reports on the possibility of -S-S- groups being responsible for original organic ligand exchange on the surface of QDs. In this study, the following experiment was conducted to address this issue. Thioctic acid was used as a disulphide compound model for phase transfer of QDs according to the procedures described in this study. Interestingly, hydrophobic QDs were found to transfer successfully into the water phase under ultrasonication, while phase transfer cannot be achieved under magnetic stirring (see Figure S5). The -S-S- bonds cannot be reduced to -SH by ultrasonication, which is confirmed with an Ellman’s Test Kit. It is noted that all the observed results in this study present both BSA and ultrasonication as necessary for QD phase transfer. A hypothesis is consequently put forward that radical formation (S•) occurs transiently during sonication, but the -S-S- bridge reforms afterward. We are considering verifying this hypothesis with experimental evidence. However, the thiotic acid modified QD precipitate appeared after one day storage, which indicates that thiotic acid modified QDs are not stable. In terms of BSA, it has one free mercapto group and eight pairs of disulphide bonds. These sulphydryl compounds, acting as multidentate ligands, have high affinity for Cd/Zn atoms. BSA is quite a bit larger than thiotic acid in molecular structure, suggesting it has multidentate sites for conjugation with QDs. Thus, these render good colloidal stability of BSA-coated QDs (Figure 3). Although ligand exchange is executed under ultrasonication conditions, a tiny fraction of aggregates remains (Figure S1), and these aggregates are also shown in the DLS data. This aggregation could be attributed to insufficient ligand exchange and subsequent hydrophobic interactions between BSA and the pendant fatty acid chains on the surface of QDs.

Figure 2. Photoluminescence spectra (a) and UV–vis absorption spectra (b) of BSA-coated QDs prepared with different QDs/BSA mole ratios.
The aggregated QDs are smaller than 50 nm, and also have quite good colloidal stability.

For Fe₃O₄ magnetic nanoparticles, however, the original organic ligands are hardly replaced by BSA macromolecules due to weak interactions between -SH/-S-S- bonds and Fe atoms. Fe₃O₄ nanoparticles can be made water-soluble via hydrophobic interactions with BSA macromolecules. BSA has been reported to exhibit high affinity with fatty acids, hematin, and bilirubin. It is also known for a broad affinity with small, negatively charged aromatic compounds via a combination of hydrophobic and electrostatic interactions. The hydrophobic fatty acids, coated on the Fe₃O₄ magnetic nanoparticles, can also bond with BSA proteins. This bonding evolves into clustering of magnetic nanoparticles under ultrasonication in the presence of the BSA/water phase. The clustering process is also facile, reproducible, and rapid.

**Stability Study of BSA-Coated QDs.** Figure 3 shows the colloidal stability of the BSA-coated QDs at various pH values and ionic strengths. As expected, the BSA-coated QDs are stable over a wide range of pH values (pH 2.5−12) showing no signs of sedimentation. This indicates the dependence of the BSA-coated QDs on electrostatic stabilization from the amino and carboxyl groups. As is well-known, the BSA macromolecules can be ionized in both acidic and alkaline solutions. The large quantity of amino and carboxyl groups on BSA macromolecules renders BSA with effective buffering capacity that posts significant resistance to harsh chemical environments.

Figure 4 shows the effects of pH values and ionic strengths on the photoluminescence of BSA-coated QDs. Overall, the emission intensity of the BSA-coated QDs increases with increasing pH value. QDs in acid solution exhibit slight erosion, that is detrimental to the local surface environment of the QDs, leading to reduced fluorescence intensity. The decrease of photoluminescence at pH 5.6 and 6.1 is observed, respectively, attributable to the isoelectric point of BSA in this pH range. The structure of BSA is found to change at these pH values, responsible for variations of fluorescence. Little or no change in photoluminescence intensity is observed at various ionic strengths (see Figure 4b). These experimental results indicate good stability of QDs, capped with the multidentate BSA in high ionic strength conditions. Moreover, these results are directly applicable to intracellular and in vivo studies, where the ionic concentration is known to be high.

**Nonspecific Cellular Binding of BSA-Coated QDs.** BSA is frequently used as a nonspecific binding blocking agent. BSA macromolecules can be absorbed onto the interspaces of ligands on the surface of nanoparticles. In this way, the nanoparticles are blocked by BSA to lower nonspecific binding when labeled with targeting ligands, such as antibodies, peptides, and other proteins. In this study, BSA as multidentate ligands is used to exchange the original organic ligands on the surface of QDs to reduce nonspecific cellular binding. The role of the BSA is twofold: one as the ligand-exchange agent for water-solubilization of QDs, and the other for reduction of nonspecific cellular binding.

Nonspecific cellular binding was both qualitatively and quantitatively evaluated by fluorescent microscopy and
fluorescence measurement plate reader of cell cultures grown on 96-well plates. Figures 5 and 6 show significant amounts of nonspecific cellular binding of MPA-coated QDs on all selected cell lines, while no obvious nonspecific cellular binding is found for BSA-coated QDs. Although BSA used for cell blocking can adsorb on MPA-coated QDs for reduction of its nonspecific binding with the involved cells, the resulting nonspecific signals are much higher than that of BSA-coated QDs. This suggests that the BSA adsorption on MPA-coated QDs is less efficient than ultrasonication, which could explain the difference observed between BSA-coated QDs and MPA-coated QDs on nonspecific cellular binding. For further comparison of the performance of BSA-coated and MPA-coated QDs on nonspecific cellular binding, cell experiments have been performed in the absence of serum protein blocking (Figure S6). It also shows that BSA-coated QDs have superior performance on reduction of nonspecific cellular binding.

BSA are known for nonspecific binding reduction, and are widely used in biological detection.42,43 As indicated by these results, BSA macromolecules as multidentate ligands are well-coated on the surface of QDs, showing excellent reduction of nonspecific cellular binding. Interestingly, the degree of nonspecific cellular binding of MPA-coated CdTe QDs on HeLa cells is higher than that on 3T3 cells. This is consistent with a previous report.12 Rosenthal et al. found the degrees of nonspecific binding of QDs to be dependent on cell types. In their study, nonspecific binding of QDs on 3T3 cells was found to be much lower than that on other cell types.

Nonspecific cellular binding of QDs is a complex issue. It can be attributed to hydrophobic interactions between the ligands of QDs and lipids on the cell membranes. It is also associated with the electrostatic interactions between the cells and the negatively charged groups on the surfaces of QDs.11,12,44 Different cell types express a variety of proteins or lipids on their surfaces that allow for interactions with exposed hydrophobic regions of QDs. Thus, the complete coating of QDs is critical to nonspecific cellular binding. In this study, hydrophobic surfactants on the surfaces of QDs are mainly exchanged as a result of multidentate BSA ligands, as evidenced in the cellular binding experiments.

Nonspecific cellular binding by electrostatic interaction was studied in a previous study.47 Nonspecific cellular binding was remarkably reduced by hydroxylation of carboxylated QDs. Hydroxylation reduces surface charges of QDs, thus weakening the electrostatic interactions between QDs and cell membranes. The surface charge is directly dependent on the chemical groups of QDs. MPA-coated QDs have abundant carboxyl groups, contributing to high negative surface potentials. The...
amino and carboxyl groups on QDs surfaces, which are oppositely charged, give rise to partial neutralization of surface potentials. This is clarified in Table 1, showing that, in the same buffer, BSA-coated QDs hold lower zeta potential (−19.5 mV) than MPA-coated QDs (−28.8 mV).

Table 1. Properties of the BSA-Coated QDs and MPA-Coated QDs Used in This Study

<table>
<thead>
<tr>
<th>QDs</th>
<th>hydrodynamic diameter (nm)</th>
<th>zeta potential (mV)</th>
<th>buffer</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSA-coated</td>
<td>18.0</td>
<td>−19.5</td>
<td>borate saline buffer (50 mM, pH 8.2)</td>
</tr>
<tr>
<td>MPA-coated</td>
<td>6.0</td>
<td>−28.8</td>
<td>borate saline buffer (50 mM, pH 8.2)</td>
</tr>
</tbody>
</table>

The effective reduction of nonspecific cellular binding is also observed with fluorescent BSA-coated gold (Au) nanoclusters (Au NCs) (see Figure S7 and S8). The Au NCs were prepared via biomimernalization by using BSA as a scaffold. The fluorescent Au NCs are capped by BSA. Due to combined effects of surface coating and partial neutralization, the BSA-coated QDs and Au NCs are particularly effective in reducing nonspecific cellular binding.

**Targeted Cellular Imaging of BSA-Coated QDs.** BSA-coated QDs with significantly reduced nonspecific cellular binding have valuable applications in biomedical detection, particularly in cell-targeted imaging. In this study, targeted cellular imaging using BSA-coated QDs was carried out by functionalization with cRGD peptide. The successful conjugation was demonstrated by agarose gel electrophoresis (see Figure S9). Under excitation, there are two distinguishable narrow and bright bands, due to the difference in electrophoresis characteristics between cRGD-functionalized and unfunctionalized BSA-coated QDs. The remarkably narrow bands suggest that the BSA-coated QDs have quite homogeneous distributions of sizes and charges. The peptide of cRGD has a high binding affinity with integrin αβ3, and it is extensively used as the targeting ligand for integrin αβ3-expressed cells or vascularization imaging. Figure 7 shows cRGD-functionalized BSA-coated QDs having different binding affinities between MCF-7 and U87 MG cells. U87 MG cells overexpress integrin αβ3, while MCF-7 cells exhibit low expressions. As a result, many more QDs are captured by U87 MG cells. Unfunctionalized BSA-coated QDs exhibit insignificant binding to U87 MG cells. These results indicate little or no nonspecific cellular binding of the BSA-coated QDs to U87 MG cells. Otherwise, the QD emission on cells is not distinguishable between nonspecific binding and specific targeting. These cellular specific imaging data indicate excellent reduction of nonspecific binding by BSA-coated QDs.

The cytotoxicity test was conducted for the BSA-coated QDs on normal L929 cells. No significant cytotoxicity was found at the concentrations indicated (Figure S10).

**CONCLUSIONS**

In conclusion, we have developed a facile QD phase transfer approach by surface engineering of QDs with BSA macromolecules under ultrasonication conditions for reduction of nonspecific cellular binding and cell-targeted imaging. The BSA-functionalized QDs exhibit excellent colloidal stability with fine hydrodynamic size distribution in a wide range of pH and ionic strength values. In particular, the BSA-coated QDs are experimentally shown to be effective in the reduction of nonspecific cellular binding. Furthermore, BSA-coated QDs are labeled with cRGD peptides for targeted integrin αβ3 imaging. The advantages of BSA-coated QDs include straightforward synthesis, high colloidal stability, and significantly reduced nonspecific binding. These are therefore proved to be ideal nano systems for biomedical labeling, targeting, and imaging.

**ASSOCIATED CONTENT**

Supporting Information

Synthesis procedures of MPA-coated CdTe QDs, oleic acid capped QDs, hydrophobic iron oxide magnetic nanoparticles, CuInS2/ZnS QDs, and AuNCs; TEM of BSA modified QDs, iron oxide, and CuInS2/ZnS nanoparticles under ultrasonication condition; colloidal stability study of BSA-coated different surfactant-capped QDs after storage; digital image of the thioctic acid-coated QDs by ultrasonication; spectral characteristic and nonspecific cellular binding of AuNCs; gel electrophoresis analysis of the cRGD conjugation to BSA-coated QDs; MTT protocol and MTT data. This material is available free of charge via the Internet at http://pubs.acs.org.

![Figure 7](http://dx.doi.org/10.1021/la302758g)
This work was supported by the National Natural Science Foundation of China (51073078, 81271629); the Nanotechnology Program of Shanghai Science & Technology Committee (11nm0504500); the Program for Young Excellent Foundation of China (51003078, 81171393, 81271629); the National Natural Science Funds for the Central Universities.

ACKNOWLEDGMENTS

The authors declare no competing financial interest.

REFERENCES


reagents via successive ion layer adsorption and reaction. J. Am. Chem. Soc. 2003, 125 (41), 12567–12575.


