Chalcogenide Bioconjugated Nano and Microcrystals

Semiconductor NCs have demonstrated several remarkable and attractive characteristic features especially suited to analytical applications in the biochemical field. Bioconjugated NCs have become the focus of intensive research due to their applications in drug delivery, biological labeling, luminescence tagging, etc. Semiconductor chalcogenides have been widely used as optical filters, optical recording materials, thermoelectric cooling materials, sensors, solar cells, superionic materials, and laser materials. However, in order to synthesize biomaterials involving biomolecules, it is desirable to have relatively low temperature (<100 °C) synthesis where biofunctionalities could be retained. Herein, we report the use of BSA, a low molecular weight protein in the preparation of bioconjugate PbSe and CdSe semiconducting nanomaterials via aqueous phase chemical reduction approach. Researchers have already used similar low molecular weight water soluble proteins to generate different kinds of nanomaterials with biofunctionalities. BSA is a low molecular weight, water soluble, and well characterized protein. Due to its extraordinary stabilizing behavior, it has been frequently used in the synthesis and stabilizing of biocojugated NPs. Apart from this, it has also been found to be a good shape directing agent. The shape controlled effects have been significantly influenced by the folded or unfolded state of BSA, where unfolded state has been found to produce ordered geometries. Synthesis of monodisperse ordered chalcogenide morphologies is an important area of material science where such morphologies have been used for the device formation. We have tried to explore this behavior of BSA to produce well defined morphologies of PbSe and CdSe bioconjugated chalcogenide NCs. All reactions were conducted at 85 °C to ensure that BSA is in its fully unfolded state so as to achieve maximum stabilizing ability of BSA. BSA in its fully unfolded state behaves like an amphiphilic species and hence possesses ability for the interfacial adsorption to control the crystal growth. Thus, the evolution of different morphologies of chalcogenides has been discussed to obtain nano- to micro-scale biocojugated materials.

Results and Discussion

3.1 Microscopic Studies

The synthesis of PbSe and CdSe nanoparticles is described in the experimental section and Figure 3.1a shows the FESEM images of PbSe particles synthesized in the presence of BSA = 1 × 10⁻⁴ g/mL. Small polyhedral NCs along with well-defined NRs were obtained. The size distribution histograms for NCs
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(Figure 3.2a) and NRs (Figure 3.2b) provide their respective sizes as 123 ± 18 nm and 3.1 ± 0.5 (aspect ratio). Some interesting observations were revealed while performing the EDX analysis on both kinds of particles. Polyhedral NCs were made up of PbSe, while NRs were of only Se. Focusing the electron beam only on the polyhedral NCs (the area shown in a square box in Figure 3.1a) produced the EDX spectrum demonstrated in Figure 3.1b with 1:1 atomic % of Pb and Se. On the contrary, when the beam was focused only on a group of NRs as shown in dark field image in Figure 3.1c, it produced EDX spectrum (Figure 3.1d) with maximum emission from Se (48 atomic % relative to other elements) but no emission due to Pb was observed. It suggests that the NRs are only made up of Se. When the same sample was prepared under identical conditions at pH = 7, we obtained similar morphologies and compositions (Figure 3.3). Again polyhedral particles were made up of PbSe (Figure 3.3a) and NRs (Figure 3.3b) were made by Se only. As the amount of BSA was increased to $5 \times 10^{-4}$ g/mL (sample PbSe 2, Table 1), the NCs formation was drastically reduced, and all geometries were either well-defined NRs or bundles of long threads. Figure 3.1e shows both kinds of geometries. NRs of $10.2 \pm 2.5$ aspect ratio (Figure 3.4a) can very well be differentiated from the bundles of threads in this figure due to their singly dispersed nature. Again, carefully selecting a bundle of long threads as shown in Figure 3.1f for the EDX analysis, the spectrum (Figure 3.1g) was produced with emissions due to Pb and Se. It means that such bundles are made up of PbSe. On the other hand, if only NRs are selected (Figure 3.1h, dark field image), we obtained Se with 17 atomic % relative to other elements but without any Pb contents (Figure 3.1i). The XRD patterns of this sample show narrow prominent peaks of cubic phase of PbSe as well as trigonal Se (Figure 3.4b). It is to be noted that for XRD, the sample contains the mixture of PbSe and Se particles which cannot be separated out at microscopic level. Further increase in the amount of BSA up to $10 \times 10^{-4}$ g/mL (sample PbSe 3, Table 1) converts the bundles of threads of PbSe into bundles of thick microrods (MRs). Figure 3.6a clearly shows a bundle of MRs of several micrometers long. Elemental mapping of such MRs clearly demonstrates the EDX emission due to Pb (Figure 3.6b), Se (Figure 3.6c), C (Figure 3.6d), and O (Figure 3.6e), where Pb and Se are in their 1:1 atomic % ratio. Hence, the MRs are in fact homogenously made up of PbSe. The elemental emission of C and O could be due to the presence of conjugated BSA. This sample also produces well-defined rounded Se NRs of high
Figure 3.1. Part (a) shows FESEM of polyhedral PbSe NCs prepared with BSA = $1 \times 10^{-4}$ g/mL at 85 °C, square box shows the electron beam focused on the polyhedral NCs. (b) shows EDX spectrum with clear peaks due to Pb and Se with 1:1 atomic % ratio. (c) shows FESEM of a group of Se NRs. (d) shows EDX spectrum with maximum emission from Se, and no Pb contents are observed. (e) shows FESEM of PbSe NCs prepared with BSA = $5 \times 10^{-4}$ g/mL at 85 °C, showing well-defined NRs or bundles of long threads. (f) shows bundles of long threads of PbSe NCs and (h) represents dispersed Se NRs. (g), (i) show EDX spectra of samples (f) and (h), respectively.
aspect ratio (19.1 ± 8.2, Figure 3.5). Figure 3.7a and 3.7b demonstrate the corresponding FESEM and TEM images of such NRs. A careful analysis of a single NR (Figure 3.7c) suggests that it is again purely made up of Se. We further tried to evaluate the Se NRs formation by carrying out the reaction at 40 °C, where BSA is considered to be in its native state\textsuperscript{13-16}. Even at 40 °C, we observed the crystalline Se NRs formation (Figure 3.7e) though they were not as well-defined as we observed at 85 °C (Figure 3.7a). High temperature obviously causes the annealing effect\textsuperscript{17,18}.

![Figure 3.2](image-url)

**Figure 3.2.** Size distribution histograms of (a) PbSe NCs & (b) Se NRs with $[\text{BSA}] = 1 \times 10^{-4}$ g/mL at 85°C.
**Figure 3.3.** (a) shows FESEM of same PbSe NCs with BSA = $1 \times 10^{-4}$ g/mL synthesized at pH = 7 & 85°C. (c) represents its EDX spectrum. (b) shows FESEM of Se NRs produced by sample (a). (d) represents its EDX spectrum.

**Figure 3.4.** (a) Size distribution histograms of Se NRs & (b) XRD pattern of PbSe NCs with BSA = $5 \times 10^{-4}$ g/mL. * represents crystal planes of Se NRs. This sample contains the mixture of PbSe and Se particles which cannot be separated out at microscopic level.
**Figure 3.5.** Size distribution histogram of Se NRs with BSA = $10 \times 10^{-4}$ g/mL.

**Figure 3.6.** (a) FESEM of PbSe NCs prepared with BSA = $10 \times 10^{-4}$ g/mL at 85 °C, the area in the square box of a bundle of MRs has been selected for elemental analysis. (b), (c), (d) and (e) elemental maps due to Pb, Se, C, and O, respectively.
Figure 3.7. Parts (a) and (b) represent FESEM and TEM images, respectively, of Se NRs produced by sample (a) of the Figure 3.6 with BSA = $10 \times 10^{-4}$ g/mL at 85 °C. (c) shows FESEM image of a single Se rod for EDX analysis. (d) shows EDX spectrum with clear major peak only due to Se. (e) illustrates FESEM image of crystalline Se NRs, prepared with BSA=10 $\times 10^{-4}$ g/mL at 40 °C.
Figure 3.8. (a) FESEM of CdSe NCs synthesized with BSA = $1 \times 10^{-4}$ g/mL at 85 °C, dotted circles demonstrate meshes of small NPs and block arrows show the long threads of Se with wavy surfaces. (b) and (d) represent the area selected for EDX analysis of a mesh of small NPs and a long thread, respectively, of sample (a). Parts (c) and (e) show EDX spectra of (b) and (d), respectively. Part (f) demonstrates FESEM of CdSe NCs prepared with BSA = $1 \times 10^{-4}$ g/mL at 40 °C, inset (upper) shows a SAED pattern of randomly oriented NCs, and inset (lower) represents EDX spectrum of this sample.

The same method was used for the synthesis of CdSe and Se particles. Figure 3.8 shows the images of both kinds of particles for sample CdSe1 (Table 1) synthesized in the presence of $1 \times 10^{-4}$ g/mL of BSA. The XRD pattern of this sample shows narrow prominent peaks of CdSe as well as Se (Figure 3.9d). Figure 3.8a shows the interconnected threads with wavy surfaces of several hundred nanometers
long and thick (indicated by block arrows). They appear to carry a mesh of small NPs shown in dotted circles. After carefully selecting the mesh of small particles and avoiding long threads (Figure 3.8b), it has been realized that they are in fact CdSe NCs. Corresponding EDX spectrum (Figure 3.8c) demonstrates the emissions due to Cd and Se in 1:1 atomic % ratio. However, when threads were selected for EDX spectrum (Figure 3.8d), we obtained the emission only due to Se (Figure 3.8e). To further confirm the presence of CdSe NCs, we carried out the same reaction at 40 °C which would allow BSA to be in its native state and hence would alter its capping ability. It is to be mentioned that the surface activity of proteins is directly related to the overall shape and structure of their native state. Globular proteins, such as BSA,

**Figure 3.9.** (a) FESEM showing meshes of small NPs of CdSe and the long threads of Se synthesized at pH=7 and 85 °C with BSA = 1 × 10^{-4} g/mL. (b) & (c) represent single Se thread and its EDX spectrum respectively. (d) XRD pattern of Figure 3.3(a).
Figure 3.10. Part (a) demonstrates FESEM of crystalline Se MRs, produced along with CdSe NCs with BSA = $5 \times 10^{-4}$ g/mL at 85 °C. Part (b) shows FESEM of groups of CdSe NCs synthesized. Part (c) represents the area selected for EDX analysis of both CdSe NCs and Se MRs. Parts (d) and (e) represent EDX spectra of (c). Part (g) shows FESEM of CdSe NCs synthesized with BSA = $10 \times 10^{-4}$ g/mL at 85 °C. Part (f) shows FESEM of sharp needles of Se.
are considered to be less surface active than fibrous proteins in its native form, i.e., 40 °C, but its unfolded form at 80 °C becomes considerable surface active which in turn acts as a good capping agent. Similar groups of NCs of ≈ 2-3 nm are obtained (Figure 3.8f) at 40 °C, which shows BSA is not effectively showing its capping ability toward CdSe NCs (the fact will be discussed later in the Growth Mechanism section). The corresponding SAED patterns depict rings from randomly oriented NCs of nanometric dimensions. EDX analysis fully confirms the presence of Cd and Se (inset). The same reaction produced similar morphologies and compositions when conducted at pH = 7 under identical conditions (Figure 3.9), which is probably due to the self-buffering effect of BSA in the presence of salts. Increase in the amount of BSA to 5 × 10^{-4} g/mL, produced well-defined Se MRs with fine edges which are still interconnected in a star like arrangement (Figure 3.10a) while groups of CdSe NCs are not as much associated as observed in Figure 3.8a. They are essentially scattered around (Figure 3.10b). Carefully selecting both kinds of morphologies (see dark field image Figure 3.10c) for an EDX analysis indicated that MRs are purely made up of Se (Figure 3.10e) while groups of NCs are of CdSe (Figure 3.10d). With further increase in the concentration of BSA = 10 × 10^{-4} g/mL, the fine MRs turned into sharp needles which are still present in the form of star-shaped bundles (Figure 3.10f).
Corresponding TEM image is shown in Figure 3.11, along with associated groups of CdSe NCs. EDX analyses of single bundle (Figure 3.10f, inset) at couple of places give 31.8 ± 3.0 atomic % of Se. Similar analyses of groups of NCs (Figure 3.10g) provide Cd and Se in 1:1 ratio within ±4% error. The major difference between the morphologies of PbSe and CdSe indicated no rod formation of CdSe with respect to an increase in the amount of BSA, as it happened in the case of PbSe.

3.2 Reaction Mechanism (Formation of Se NRs)

The occurrence of Se MRs looks to be quite strange in the first place. The reaction is proposed to proceed through the following steps. Lead/cadmium acetate and sodium selenite are expected to dissociate into their respective ions as soon as they are dissolved in water (equations 3.1 and 3.2, respectively). Hydrazine will convert selenite ions into selenium ions by following a redox reaction (equation 3.3). Once selenium ions are produced, they will undergo neutralization with lead/cadmium ions to generate lead selenide/cadmium selenide (equations 3.4 and 3.5, respectively). Small nucleating centers will achieve colloidal stability in the presence of BSA which will act as capping/stabilizing agents. At 85 °C, BSA is in unfolded state and hence provides a better capping ability in comparison to native unfolded state. Further growth should follow the autocatalytic process.

\[
Pb(CH_3COO)_2(s) \rightarrow Pb^{2+} (aq) + 2CH_3COO^- (aq) \quad (3.1)
\]

\[
Na_2SeO_3 (s) \rightarrow 2Na^+ (aq) + SeO_3^{2-} (aq) \quad (3.2)
\]

\[
3N_2H_4 (aq) + SeO_3^{2-} (aq) \rightarrow 2Se^{2-} (aq) + 3N_2 (g) + 6H_2O (l) \quad (3.3)
\]

\[
Pb^{2+} (aq) + Se^{2-} (aq) \rightarrow PbSe (s) \quad (3.4)
\]

\[
Cd^{2+} (aq) + Se^{2-} (aq) \rightarrow CdSe (s) \quad (3.5)
\]

The large amount of hydrazine is also expected to simultaneously convert the SeO_3^{2-} ions into Se nucleating centers (reaction 3.6).

\[
N_2H_4 (aq) + SeO_3^{2-} (aq) \rightarrow Se (s) + N_2 (g) + 2OH^- (aq) + H_2O (l) \quad (3.6)
\]
The capping ability of BSA helps to attain the colloidal stability and eventually leads to long rod formation. We always got long 1D Se rods of several hundred nanometers in both the cases of PbSe and CdSe. There are many reports of t-Se long rod formation in the literature\textsuperscript{20-25}, which is the most stable allotropic form of Se with semiconducting properties. Ma \textit{et al.}\textsuperscript{26} have reported the formation of t-Se nanotubes from non-ionic surfactant micellar solutions. They proposed that nucleation occurs at the circumferential edges of hexagonally or trigonally faceted seeds, leading to the formation of t-Se nanotubes. Since t-Se is a thermodynamically stable form of Se and hence the growth is favoured only in the \textit{<100>} direction and that leads to the formation of mostly 1D nanostructures. Increasing amounts of BSA produce well-defined Se rods as greater amount of BSA is available for an effective capping. This is not only true in the case of Se, but it is also observed for a progressive PbSe MRs formation from Figures 3.1 and 3.6.

3.3 Protein Estimation

A systematic protein assay with respect to reaction time has been carried out to determine the amount of BSA associated with nanomaterials on the basis of the Bradford method\textsuperscript{27}. Each sample was first properly purified to remove unassociated BSA and then dried slowly at 40 °C in a vacuum oven. A constant amount of dried sample (1mg) was used for protein assay in triplicate. The amount of conjugated BSA (BSA\textsubscript{C}) thus estimated in each sample is shown in Figure 3.12. As expected, the amount of BSA\textsubscript{C} increases with the increase in the time of reaction up to 25 hours and then tends to constant. It seems that such a long time is required for complete annealing effect. At 85 °C, aqueous BSA is considered to be in its denatured state. The denatured BSA has already been found to be a better capping/stabilizing agent in comparison to native BSA in the case of PbS NCs\textsuperscript{28}. Thus, an increase in the amount of BSA\textsubscript{C} with respect to time simply indicates its enhanced association with the NP surface to obtain well-defined geometry. Figure 3.12 therefore indicates that this is achieved in all cases within 25 hours of the reaction time.

3.4 Growth Mechanism

As far as the crystal growth is concerned, there is a distinct difference between the growth of PbSe and CdSe morphologies. An increase in the amount of BSA clearly converts the polyhedral nanomorphologies of PbSe into thread like bundles of MRs which ultimately end up in cubic faced MRs (Figures 3.1 and 3.6). This is
demonstrated in a systematic manner in Scheme 1. This is not observed in the case of CdSe. From low to high amounts of BSA used, CdSe NPs (≈ 2-3 nm) always exist in large scattered groups (Figures 3.8 and 3.10). FESEM images do not show any significant difference between their overall morphologies with respect to the amount of BSA used. It means that BSA has a significant control over the crystal growth of PbSe (Scheme 1) than CdSe. PbSe has rock salt crystal structure which is much different from wurtzite (hexagonal) of CdSe. Conversion of polyhedral nanomorphologies (Figure 3.1a) of PbSe into cubic shaped MRs (Figure 3.6) indicates a selective adsorption of unfolded BSA in comparison to that on CdSe particles. This selective adsorption should be more favorable on the \{100\} crystal planes of cubic PbSe geometry so as to convert it into rod shape geometry by promoting the growth at \{111\} crystal planes. Two factors are expected to be participating in this mechanism. First, unfolded BSA would have less steric compulsions during its adsorption on

\[\text{Figure 3.12. Plot of estimated amounts of } BSA_c (\mu g) \text{ versus reaction time (hr) for PbSe and CdSe samples (see Table 1 for sample specifications).}\]
SCHEME 1

[BSA] = 1 x 10^{-4} \text{ g/ml}

polyhedral nanoparticles

[BSA] = 5 x 10^{-4} \text{ g/ml}

bundles of threads

[BSA] = 10 x 10^{-4} \text{ g/ml}

micro rods
{100} facets of cubic geometry in comparison to that on wurtzite (hexagonal). Second, {100} crystal planes of rock-salt structure should have higher atomic density, (6, 6)-coordination, in comparison to that of wurtzite structure, (4, 4)-coordination. Thus, higher atomic density planes in the formal case would provide favorable platform for better surface adsorption and thereby a better crystal growth control. Recently, Roach et al.\textsuperscript{29} have explained that both surface chemistry and topography play key roles in determining the structure of bound proteins. A high surface curvature in fact reduces the probability of an effective adsorption. Thus, relatively less ordered adsorption would eventually provide poor capping ability with little shape controlled effects and that could be the consequence of practically no shape change of CdSe NCs. In addition, small CdSe NCs of 2-3 nm will obviously provide high curvature in comparison to PbSe MRs which in turn will prove less receptive in accepting ordered interfacial arrangement of BSA.

However, pure Se particles are always produced as rods with dimensions ranging from nano to micro range with respect to the amount of BSA. BSA also acts as a polyelectrolyte to attract Se\textsuperscript{2-}/SeO\textsubscript{3}\textsuperscript{2-} ions to be adsorbed electrostatically on unfolded BSA. Whereupon they are reduced by hydrazine into nucleating centers, which in turn start the autocatalytic process. Trigonal arrangement can be achieved by spiral chains of Se atoms associated with each other through van der Waal interactions in a hexagonal lattice\textsuperscript{30} that can be easily provided by the BSA soft template effect. Such an arrangement provides a unidirectional growth tendency and that is why there are many reports on \(r\)-Se long rods formation in the literature\textsuperscript{20-25}. In other words, a thermodynamically stable form of Se (\(r\)-Se) would favour the growth along <100> direction and ultimately leads to the formation of 1D nanostructures. An effective capping ability of BSA helps to attain well-defined rod shape geometries.

**Conclusions**

A low molecular weight, water-soluble carrier protein BSA has been used in the concentration range of 1-10 \(\times\) 10\(^{-4}\) g/mL as a capping/stabilizing agent to synthesize PbSe, CdSe, and Se nanobiomaterials at relatively mild temperature in aqueous phase following hydrazine reduction. It has proved to be a fine shape-directing agent in the case of PbSe, whereas no significant influence has been observed on CdSe morphologies with respect to its amount. Se rods are obtained as
reaction byproduct in both reactions of PbSe and CdSe, but their overall morphologies remain same in both reactions though they become quite well-defined at high amounts of BSA used. The shape directing ability of BSA has been explained on the basis of its selective interfacial adsorption at liquid-solid (PbSe) interfaces. A correlation between the conversion of polyhedral geometries into long microrods, with the amount of BSA, suggests its favorable adsorption at \{100\} crystal planes of cubic PbSe geometry which shifts the overall growth at \{111\} planes in order to produce microrods. This is probably not happening in the case of CdSe NPs with wurtzite (hexagonal) geometries because no significant change is observed in their morphology within the range of BSA used. Nevertheless, BSA proved to be an excellent stabilizing agent for colloidal CdSe NCs. Thus, BSA, a low molecular weight protein, is a good capping/stabilizing agent for semiconducting nanomaterials and can be easily used in an aqueous phase to synthesize nanobiomaterials.
References


