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Albumin–CdTe Nanoparticle Bioconjugates: Preparation, Structure, and Interunit Energy Transfer with Antenna Effect

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ABSTRACT

Conjugates of bovine serum albumin and CdTe nanoparticles capped with L-cysteine have been synthesized via a one-pot glutaric dialdehyde cross-linking procedure. Diads (1:1) with some amount of 2:1 albumin–nanoparticle assemblies preferably form in this reaction, as evidenced by gel electrophoresis. Circular dichroism spectroscopy demonstrates that the tertiary structure of the protein remains largely intact after the conjugation. Attachment of protein moieties result in a significant increase of CdTe emission, which is attributed to the resonance energy transfer from the tryptophan moieties of albumin to CdTe nanoparticles acting as receptors for the protein antennae.

Colloidal semiconductor nanoparticles (NPs) attracted a lot of interest in the past decade.[1−7] Their optical and electrical properties are controllable through the particle size because of the quantum confinement of the electronic states.[8,9] Although the thrust of the nanoparticle (NP) research is focused on their optical, electrical, and magnetic properties and corresponding electronic, opto-, and nanoelectronic devices,[1−5,10,11] a new direction of biological surface modification of semiconductor[12−19] and metal NPs[20−24] with antibodies[12−15,19] and peptides[20−23] received a lot of attention lately. Biomodified NPs from a variety of inorganic materials can be used in life sciences for luminescence tagging, drug delivery, and implantable microdevices as well as for assembling hybrid protein−NP units for molecular electronics. Further advances of this research field depend on the understanding of specificity and capabilities of biomolecule−

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NP coupling techniques and on the development of methods of characterization and separation of the conjugates.

Here, we report on the conjugation of luminescent CdTe NPs with a simple protein, bovine serum albumin (BSA). The purpose of this paper is 3-fold: (1) to present a simple method of protein−NP conjugation utilizing amino acid-coated NP and discuss its limitations, (2) to report on the structure of protein−NP conjugates, and (3) to describe their optical properties. BSA is the most studied protein, whose structure and property are known to the smallest detail. This makes it a convenient model for the fundamental studies of protein−NP conjugates. Despite low biospecificity, BSA has a strong affinity to a variety of organic molecules binding to different sites, which makes possible utilization of BSA-decorated NPs in a variety of supramolecular assemblies. Simultaneously, since albumin is a typical plasma protein, albumin-coated NPs are important for the study of their interactions with live blood cells and the immune response of the organism to their intravenous administration. To prepare the conjugates, we used glutaric dialdehyde (G) linker applicable to the majority of proteins. The NPs were stabilized with a hydrophilic stabilizer L-cystein. As compared to the previously reported procedures of protein−NP composites,[25,26] this method overrides the stages of silica coating and/or stabilizer exchange, which makes it simpler. At the same time, we want to point out that the protective encapsulation of NPs is necessary for prevention of their slow decomposition and nonspecific luminescence quenching. Two findings we consider to be important for the current research on biomolecular modification of NPs are (1) CdTe NPs predominantly form 1:1 BSA:CdTe conjugates with some presence of 2:1 BSA:CdTe conjugate and (2) excited states of BSA and NP interact via resonance energy transfer mechanism, which opens interesting possibilities for optical applications of biospecific assemblies of NPs.

The synthesis of L-cysteine-stabilized CdTe NP followed the standard technique of arrested precipitation in aqueous solution reported previously.[27−29] We used a one-step G linkage procedure, which was shown previously to work well for the protein−protein conjugation[30−32] and for the preparation of self-assembled monolayers.[33−35] One aldehyde group of G (O═CH−$(CH_2)_3$−HC═O) forms a Schiff base linkage with the L-cysteine amino group on the surface of NPs, while the other side forms an identical bond with predominantly lysine moieties on BSA. The selection of proper conditions and reagent ratios[36] results in a minimal formation of oligomers, which is often mentioned as one of the disadvantages of G coupling as compared to *N*-hydroxysulfosuccinimide-based conjugation reactions.

The cigar-shaped ellipsoidal molecules of BSA with hydrodynamic dimensions of 40 Å (short axis) by 140 Å (long axis) are often used in gel electrophoresis assay as a molecular weight standard (M_w 67 kDa),[37] and the products of BSA−G−CdTe conjugation can be conveniently analyzed by this technique (Figure 1).[38] In all samples, free CdTe NPs ($M_w \sim 18$ kDa, as estimated for a 2 nm CdTe core covered with a monolayer of L-cysteine), compact and highly negatively charged, traveled far ahead of BSA and were

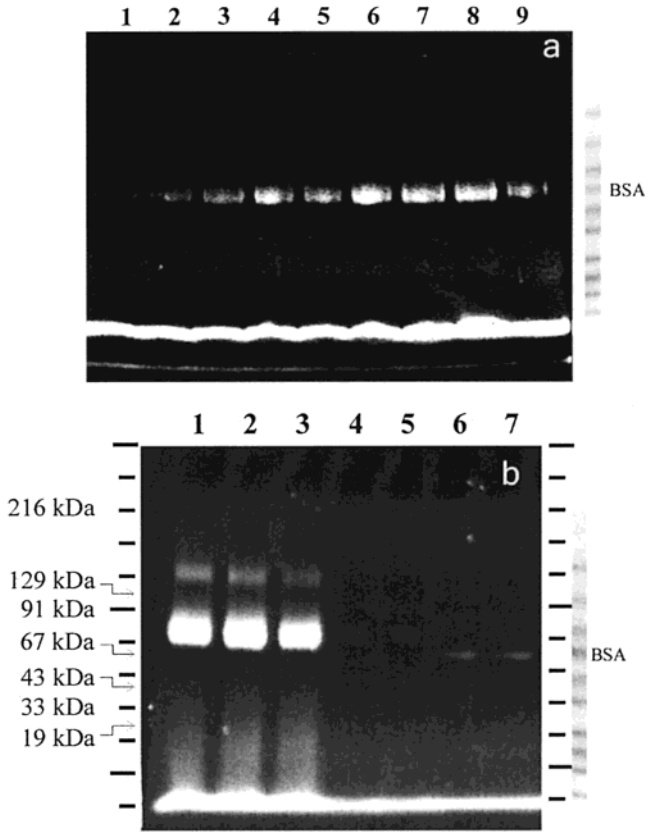

Figure 1. (a) Luminescence image of the SDS PAGE assay of BSA−G−CdTe conjugates. Wells: (1) CdTe; (2) BSA; (3) CdTe + BSA (no G); (4) CdTe + G + BSA heated at 25 °C 1 h; (5) CdTe + G + BSA heated at 40 °C for 1 h; (6) CdTe + G + BSA heated at 50 °C for 1 h; (7) CdTe + G + BSA heated at 60 °C for 1 h; (8) CdTe + G + BSA heated at 60 °C for 1.5 h; (9) CdTe + G + BSA heated at 60 °C for 2 h. The standard protein ladder with BSA spot marked was prepared on the same gel plate and visualized by staining with Coumassie Blue. (b) Luminescence image of the native gel electrophoresis assay of BSA-G-CdTe conjugates. Wells: (1−3) (all the same) CdTe + G + BSA heated at 50 °C for 1 h; (4−5) CdTe + BSA (no G); (6−7) BSA. The molecular weight scale on the left was graduated by the positions of reference proteins in the standard protein ladder given on the right with BSA spot marked.

typically found at the lower edge of the gel plate with other components traveling with the electrophoretic front. The bands of BSA were always positioned in the middle of the gel plate and could be clearly identified by the comparison with the standard protein ladder (Figure 1). CdTe NPs used here were strongly luminescent with a quantum yield of 15−20%, while that for BSA under the best conditions was about 3%. Hence, the emission intensity of SDS−PAGE electrophoresis spots was used for the optimization of conjugation conditions. The yield of BSA−G−CdTe conjugate gradually increased with the increasing temperature of the reaction (Figure 1a, wells 3−6). It reached the maximum at approximately 50 °C for 1 h reaction time, as indicated by the strongest luminescence of the BSA band (Figure 1a well 6). For longer times and higher temperatures, the yield of BSA-G-CdTe decreased due to oligomerization reactions (Figure 1a, wells 7−9). Unfolding of BSA occurs above 65 °C and is quite reversible unless extreme pH conditions are used.[37]

More detailed information about the products of the conjugation reaction could be obtained from native gel electrophoresis data.[38,39] Contrary to SDS−PAGE, the denaturation of the protein caused by the addition of SDS does not occur in this technique and the protein retains its original charge, which makes it more informative. When BSA was mixed with NPs without coupling agent (Figure 1b, wells 4 and 5), the luminescence of the BSA band was very weak regardless the time of exposure, while all NP were run off the gel plate. Importantly, this demonstrated that there is very little, if any, nonspecific binding of NPs to BSA. The BSA bands in wells 4 and 5 were slightly shifted to higher molecular weights as compared to pure BSA in NP-free buffer (Figure 1b, wells 6 and 7). This occurred due to the complexation of Cd^{2+} ions present in colloidal solution of CdTe by metal-binding sites for which BSA is well-known.[37,40] The net charge per one BSA molecule is −19 at pH 7.4.[37] The addition of Cd^{2+} reduced the negative charge of BSA and resulted in lowering its electrophoretic mobility. As expected, this shift disappeared when the protein was denaturated by sodium dodecyl sulfate in the SDS−PAGE electrophoresis experiment (Figure 1a). Cd^{2+} ions may be present in solution both due to incomplete removal by the dialysis as well as due to slow decomposition of the NPs caused by the exposure to light and oxygen. Regardless of the origin, for any biomedical applications of NPs it is important to minimize the presence of heavy metals because of their cytotoxicity.

Two new strong luminescent bands appeared after addition of G and incubating the reaction mixture at 50 °C for 1 h (Figure 1b, wells 1−3). By using the standard protein ladder as a reference, the molecular weights of the conjugation products were determined to be 82 and 150 kDa. The position of the band at 82 kDa matched the sum of masses of BSA (67 kDa) and NP (18 kDa), and therefore, should be attributed to 1:1 BSA:CdTe conjugate (∼85 kDa). At the same time, the mobility of the conjugate has not significantly changed in respect to the BSA−CdTe mixture (Figure 1b, wells 4 and 5). It may be suggested that the added weight and high negative charge associated with the formation of the BSA−G−CdTe diad partially compensate each other. The band at 150 kDa should be attributed to a 2:1 BSA−CdTe conjugate with an estimated mass of 152 kDa. Unlike the case of NP−DNA conjugates,[41] the attachment of a greater number of BSA units to CdTe did not occur, most likely due to the steric hindrances related to the difficulty with the accommodation of bulky protein units around relatively small NPs (2 nm). We ran the electrophoresis experiment in a variety of conditions and saw no further separation of the luminescence bands. Although there is no direct evidence of formation of conjugates with two or more CdTe attached to one albumin unit, the possibility of their formation should also be considered and they may, in fact, contribute to broadenning the 1:1 conjugate band. Importantly, virtually no byproducts, such as $(BSA−G−NP)_n$ oligomers, were present in solution, as could be seen by the absence of luminescent bands for masses above 150 kDa, which was one of the goals of the optimization of reaction conditions.

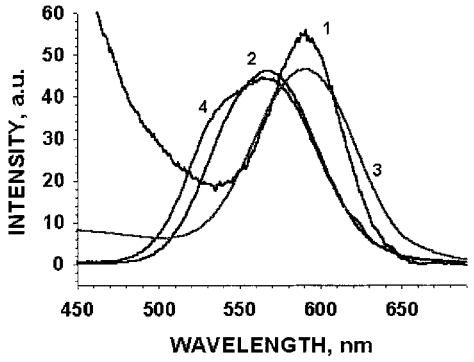

Figure 2. Luminescence spectra of (1) 82 kDa band cut out from the native state electrophoresis gel plate, (2) CdTe as synthesized, (3) CdTe + G (no BSA) heated at 50 °C for1 h, and (4) CdTe (no BSA, no G) heated at 50 °C for1 h. Excitation wavelength λ_{ex} = 360 nm.

To confirm the binding of CdTe to BSA, the luminescence spectrum of the 82 kDa band in the native gel electrophoresis was obtained.[42] The luminescence spectrum of the gel piece showed a characteristic band-edge emission peak of CdTe red-shifted by ∼25 nm from its original position in the NP solution (Figure 2). As we learned in a series of additional experiments, this shift was caused by the growth of NP due to the reaction with G (Figure 2, trace 3). Heating at 50 °C per se did not result in the luminescence spectral shift (Figure 2, trace 4). The quantum yield of NP excitonic emission for 360 nm excitation did not change appreciably after the reaction with G or BSA and decreased by 1−3% during heating without conjugation.

Circular dichroism spectra (Figure 3) of BSA and BSA−G−CdTe indicate a small disturbance of the BSA conformation observed as a curve shift in 210−214 nm range, which is to be expected when highly charged species, such as NPs, are conjugated to protein molecules. Besides that, the circular dichroism spectra coincided well with each other and with reported BSA data.[43] This shows that the tertiary structure of BSA remains mostly intact, which is essential for the preparation of protein-based assemblies of NPs.

Interestingly, the produced BSA−G−NP conjugates demonstrated effective interaction between the excited states of the biological and the inorganic parts. This effect should be attributed to the spatial closeness of BSA and NP in the tightly bound covalent supramolecule. In aqueous solution, free BSA emitted at 340 nm (Figure 4, trace 1). This emission peak originated from the luminescence of two aromatic tryptophan moieties present in BSA amino acid sequence: Trp134 and Trp 214.[37] Simple addition of nonbound CdTe NPs reduced the maximum intensity of the 340 nm BSA luminescence band by ca. 2.5 times, while the NP luminescence did not significantly change (Figure 4, trace 2). This can be understood as a response to, among other factors, the change in pH and presence of Cd^{2+} ions, which alters the presentation of Trp moieties to water and the efficiency of quenching their luminescence by ambient oxygen.

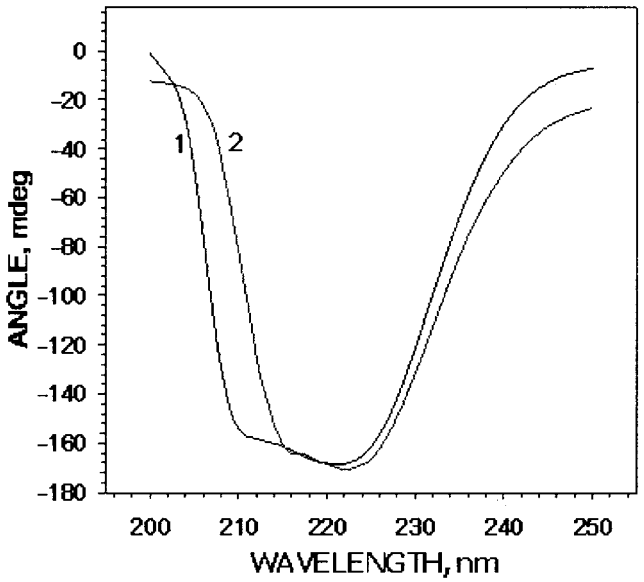

Figure 3. Circular dichroism spectra of BSA before (1) and after (2) the conjugation to CdTe NP. The spectra were recorded on a JASCO J-500A spectropolarimeter. BSA sample was dissolved in 0.01 M PBS buffer. A sample of CdTe, cysteine-stabilized nanoparticles, and the conjugate sample were at pH 7.4. The initial solutions were diluted to approximately 1 mg/mL concentration of BSA immediately before the spectra were taken. A JASCO cell of path length 0.10 cm was used.

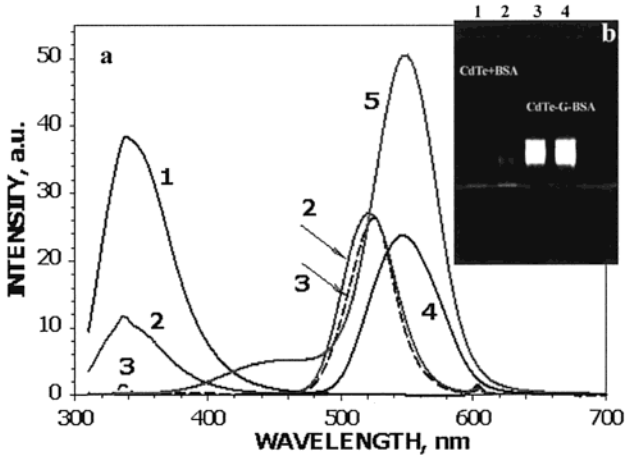

Figure 4. (a) Luminescence spectra of (1) BSA, (2) BSA + CdTe (no G), (3) CdTe heated at 50 °C for 3 h (no G, no BSA), (4) CdTe + G heated at 50 °C for 1 h, and (5) BSA + CdTe + G heated at 50 °C for 1 h. Excitation wavelength $\lambda_{ex} = 290$ nm. The concentration of NPs and their optical density at 290 nm were kept the same for all the experiments. Luminescence spectra were recorded in solutions at room temperature in 1 cm × 1 cm quartz cuvettes. (b) Gel electrophoresis of BSA + CdTe (wells 1 and 2) and BSA−G−CdTe conjugate (wells 3 and 4) carried out in 18% Tris−HCl Gel S (Bio-Rad, 18% resolving gel, 4% stacking gel) for 45 min to resolve the band of the NP and to avoid gel edge effects.

The emission intensity of both biological and inorganic components changed dramatically in the BSA−G−CdTe conjugates. The luminescence of BSA was quenched completely, while the excitonic peak of CdTe increased ca. 2-fold in amplitude and 2.8-fold in the area under the curve (Figure 4, trace 5). Note that all the spectra were taken for 290 nm excitation, where both BSA and NP absorb light. Taking into account that there was no major change in the quantum yield for long wavelength excitation, such a strong increase in luminescence efficiency of NPs is quite remarkable. The strong increase of the luminescence after the conjugation can also be seen in the gel-electrophoresis taken for the short running times (Figure 4b). The bands of the conjugates that are not yet separated are noticeably brighter (wells 3 and 4) than the band of unconjugated NP (wells 1 and 2). Note that, in this image, one can also see that the relative amount of unconjugated NP for BSA−G−CdTe is negligible.

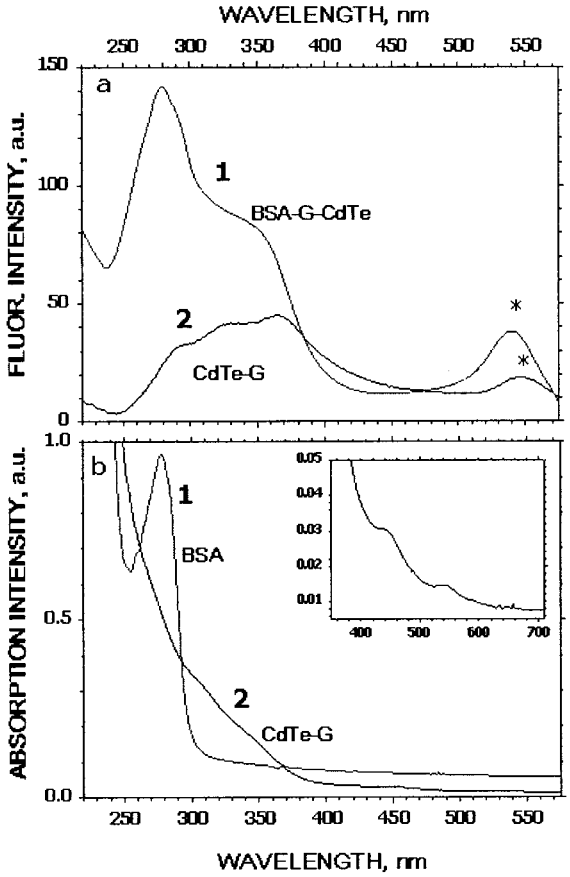

Figure 5. (a) Excitation spectra of (1) BSA−G−CdTe conjugate and (2) CdTe heated with G at 50 °C 1 h. Registration wavelength $\lambda_{reg} = 580$ nm. Peaks marked with a star correspond to the excitonic transition in CdTe NPs. (b) UV−vis absorption spectra of (1) BSA and (2) CdTe heated with G at 50 °C 1 h. The inset shows the UV−vis absorption spectrum of BSA−G−CdTe conjugate in the visible region of the spectrum.

We associate the luminescence enhancement effect with the concentration of adsorbed energy collected by NP from protein unit(s) through the resonance energy transfer[44] analogous to the classical Förster energy transfer.[45] The excitation energy transfer from protein to the excitonic state of NP can be confirmed by the excitation spectrum of BSA−G−CdTe conjugates registered in the emission band of CdTe (Figure 5). The excitation peak of the BSA−G−CdTe conjugate at 280 nm (Figure 5a, trace 1) coincided very well with UV−vis absorption peak of BSA (Figure 5b, trace 1). Concomitantly, there was no similar band in the excitation spectrum of CdTe reacted with G (Figure 5a, trace 2) or in their UV−vis absorption spectrum (Figure 5b, trace 2). These data indeed indicate that the quanta of light absorbed by BSA

are emitted by the CdTe. For conjugates bearing two BSA units, the NP collects the light from four Trp moieties. Similar antenna effects had been observed for some metal complexes and polymers.[46−52] However, to the best of our knowledge, this is the first observation of such phenomena for NPs.

In summary, protein−NP conjugates were made by using glutaric dialdehyde as a cross-linking agent in conjunction with L-cystein-capping of CdTe NPs. This procedure is expected to be applicable to other proteins with exposed Lys moieties primarily targeted by G. While simplifying the protein−NP conjugation procedure, it also shows the necessity of a protective encapsulation of NP to prevent particle growth and photoinduced decomposition. Importantly, it was found that the coupling process yields preferably BSA−NP 1:1 diads with some amount of 2:1 aggregates. These and analogous products can be used as building blocks in more complex protein-based NP supramolecules.

One of the interesting aspects of this work is the demonstration of the efficient excitation energy transfer between the protein units and core levels of NPs. This can be considered as a communication link between the biological and inorganic components of the conjugate. The significance of this observation is that it demonstrates a possibility of the integration of protein-based self-assembly and information exchange functionalities for protein−NP conjugates, which can be used in NP-based molecular electronic circuits and bioinformatics.

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(27) 125 mL of the nitrogen-saturated aqueous solution containing 2.35 mmol (0.731 g) of $Cd(ClO_4)_2$·2H2O and 5.7 mmol (0.69 g) of L-cysteine was reacted with H_2Te gas at pH 11.2. H_2Te was generated by the reaction of Al_2Te_3 with 0.05 M H_2SO4. The molar ratio of Cd^{2+}:Te^{2-}:L-cysteine was 1:0.47:2.43. The solution was refluxed for ca. 1 h to promote growth of CdTe. The resulting NPs were 2 nm in diameter with band edge emission at 520 nm and quantum efficiency 15%. The final concentration of CdTe NP was ca. 0.135 mmol, assuming the agglomeration number equal to 65 for 2 nm crystallites.
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(39) The sample buffer for native state gel electrophoresis was prepared from 15.5 mL of 1 M Tris−HCl pH 6.8, 2.5 mL of a 1% solution of bromophenol blue, 7 mL of water, and 25 mL of glycerol. The electrophoresis running buffer was made by dissolving 3.0 g of Tris base and 14.4 g of glycine in water and adjusting the volume to 1 L at pH 8.3.
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