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Evidence of Energy Transfer from Tryptophan to BSA/HSA Protected Gold Nanoclusters

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Abstract

This work reports on the chromophores interactions within protein-protected gold nanoclusters. We conducted spectroscopic studies of fluorescence emissions originated from gold nanoclusters and intrinsic tryptophan (Trp) in BSA or HSA proteins. Both, steady state fluorescence and lifetime measurements show a significant Forster resonance energy transfer (FRET) from Trp to the gold nanocluster. Tryptophan lifetimes in the case of protein-protected gold nanoclusters are 2.6ns and 2.3ns for BSA and HSA Au clusters while 5.8ns for native BSA and 5.6 for native HSA. The apparent distances from Trp to gold nanocluster emission center, we estimated as 24.75Å⁰ for BSA and 23.80Å⁰ for HSA. We also studied a potassium iodide (KI) quenching of protein-protected gold nanoclusters and compared with the quenching of BSA and HAS alone. The rates of Trp quenching were smaller in BSA-Au and HSA-Au nanoclusters than in the case of free proteins, which is consistent with shorter lifetime of quenched Trp(s) and lower accessibility for KI. While Trp residues were quenched by KI, the emissions originated from nanoclusters were practically unquenched. In summary, for BSA and HSA Au clusters, we found 55% and 59% energy transfer efficiency respectively from tryptophan to gold clusters. We believe this interaction can be used to our advantage in terms of developing resonance energy transfer based sensing applications.

1. Introduction

Efforts towards finding novel fluorophores have fueled a growth and application of nanotechnology in the fluorescence community. Along with quantum dots and fluorescent nanoparticles such as nanodiamonds, fluorescent metal nanoclusters are one such outcome of research in this area. Specifically, protein protected metal nanoclusters are attractive due to an ease of preparation, small size, lack of toxicity and tunable emission. BSA, HSA,

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lysozyme, trypsin and ferritin family proteins have been used to synthesize metal nanoclusters[1–5]. They have been applied successfully in the areas of microscopy, imaging, catalysis, chemical and biological sensing[6–12].

Despite many publications on their applications in chemical and biological sciences, there are fewer reports on their role in FRET based studies. Pu et al reported fluorescence resonance energy transfer between oligomeric silsequioxane and red emitting clusters[13]. Raut et al showed use of BSA Au clusters as resonance energy transfer donors and acceptors to/from organic fluorophores [14]. Wang et al have reported quenching of CdTe quantum dots of different diameters by BSA gold clusters [15]. Xavier et al touched upon possibility of fluorescence resonance energy transfer from tryptophan to clusters formed within lactoferrin in their report [16]. Nonetheless, interaction of tryptophan residues in proteins with gold clusters formed within remains to be revealed. We selected two proteins- HSA and BSA as protective supports for gold nanoclusters. HSA contains a single tryptophan (Trp-214), buried deeply in protein structure, and BSA contains two tryptophan residues, Trp-134 and Trp-213, with the latter being more exposed to the solvent. In this report we studied FRET from Trp donor to gold nanocluster.

In general, resonance energy transfer has been used to develop a number of sensing applications. Applicability of donor to acceptor intensity ratio becomes a valuable tool because these measurements turn out to be mostly independent of the overall intensity. We believe this interaction between tryptophan and clusters can be used to our advantage in terms of developing resonance energy transfer based sensing applications.

2. Material and Methods

2.1 Synthesis of BSA Au Nanoclusters

The Au NCs used in this study were synthesized using an approach developed by Xie *et al* [4]. Typically, 5 mL of 10 mM HAuCl₄ was mixed with 5 mL of 50 mg/mL BSA with 0.5mL of 1M NaOH and kept at 37 °C overnight in the incubator. The light brown solution of clusters was further dialyzed (2000 MWCO membrane) against de-ionized water for at least 12 hr with periodic change of water to remove any small impurities. Dialyzed cluster solution was filtered using 0.02µm syringe filter and used for subsequent measurements.

2.2 Spectroscopic Measurements

UV-Vis absorption and fluorescence spectra were obtained using a Cary 50 bio UV–visible Spectrophotometer (Varian Inc.) and Cary Eclipse spectrofluorometer (Varian Inc.) respectively. All the measurements were done in 1cm X 1cm cuvettes at room temperature with optical density below 0.05 unless mentioned otherwise. Fluorescence lifetime was measured on a FluoTime 200 fluorometer (PicoQuant, Inc.) using a 280nm LED. The fluorometer is equipped with an ultrafast microchannel plate detector (MCP) from Hamamatsu, Inc. The fluorescence lifetimes were measured in the magic angle condition and data analyzed using FluoFit4 program from PicoQuant, Inc (Germany) using multi-exponential fitting model;

$$I(t) = \sum_i \alpha_i e^{-t/\tau_i} \quad (1)$$

Where, α_i is the amplitude of the decay of the i th component at time t and τ is the lifetime of the i th component. The intensity weighted average lifetime (τ_{avg}) was calculated using following equation;

$$\tau_{avg} = \sum_i f_i \tau_i \quad \text{Where, } f_i = \frac{\alpha_i \tau_i}{\sum_i \alpha_i \tau_i} \quad (2)$$

Moreover, total photon counts were calculated using integration of fluorescence intensity decays obtained from time resolved fluorescence measurements in Origin graphing software version 8.0. Forster distances for each pair were calculated using following equation given by [17–19].

$$R_0 = 8.79 \times 10^3 \left(\kappa^2 n^{-4} Q_D J \right)^{1/6} \quad (3)$$

Where, κ^2 is a factor describing the relative orientation in space of the transition dipoles of the donor and acceptor, n is the refractive index of the medium, Q_D is the quantum yield of the donor in the absence of the acceptor, and J is the overlap integral expressing the degree of spectral overlap between the donor emission and acceptor absorption (in $M^{-1} cm^3$). The transfer efficiencies were calculated using fluorescence lifetime measurements using following equation,

$$E = 1 - (\tau/\tau_0) \quad (4)$$

Where, τ fluorescence lifetime of donor in presence of acceptor and τ_0 is fluorescence lifetime of donor in absence of acceptor.

3. Results and Discussion

This investigation was undertaken in order to study the interaction of native tryptophans in BSA/HSA and gold clusters formed within these proteins. Size of these protein protected clusters is less than 10nm and suggests possible electronic interaction between tryptophan and clusters within the protein template. Based on spectroscopic data of fluorescent BSA protected clusters (λ_{exc} : 360nm and λ_{ems} : 650nm, distance between cluster and tryptophan: <10nm and spectral overlap), we hypothesized that tryptophan can behave as resonance energy transfer donor. The excitation spectra of BSA/HSA gold clusters are significantly enhanced in UV region (Figure 1) suggesting that tryptophan moieties play a significant role in the clusters excitation process. However, it is difficult to conclude on FRET from clusters UV excitation spectra because absorption of tryptophan is heavily overlapped with the cluster absorption. In order to test the tryptophan-cluster FRET process, we studied clusters and free proteins emission spectra at different excitations and investigated KI (potassium

iodide) quenching. The rigorous time-resolved (lifetime) measurements were used to estimate FRET efficiencies.

Figure 2 A shows the emission spectra of tryptophan from native BSA and BSA Au clusters of equi-molar concentration. It is evident that tryptophan emission is distinctly quenched compared to native BSA. Moreover, enhanced acceptor (gold clusters) emission can be seen when excited at 280 nm as compared to the direct excitation of gold clusters at 310nm. Similarly, decreased donor emission and enhanced acceptor emission was observed for clusters formed within HSA (figure 3). Figure 2 B shows normalized emission spectrum of tryptophan from native BSA and tryptophan from BSA Au clusters. Peak emission of tryptophan in BSA Au clusters is blue shifted by 10nm suggesting the presence of tryptophans in more hydrophobic environment compared to the native BSA protein. A similar observation was made for HSA Au clusters (figure 3 B). We do not know the exact position and orientation of these tryptophan residues in protein structure once the clusters are formed. Moreover, it is also unknown as to why BSA and HSA behave similarly?

Fluorescence lifetimes of tryptophan in native proteins and BSA/HSA Au clusters were measured by exciting with 290nm LED. Figure 4 shows the fluorescence intensity decays of tryptophan residues. Average fluorescence lifetime of tryptophan in native BSA is 5.8ns; however, it was 2.6ns in BSA Au clusters. Similarly, in case of HSA Au clusters tryptophan lifetime was 2.3ns as opposed to 5.6ns in native HSA (Figure 5). Table 1 shows the fluorescence intensity decay parameters of these measurements. Both of these observations suggest energy transfer from tryptophan to BSA/HSA Au clusters. Figures 4B and 5B show the spectral overlap and calculated R_0 . The energy transfer efficiencies calculated using lifetime measurements are 0.55 and 0.59 for BSA Au clusters and HSA Au clusters respectively. BSA has two tryptophan residues (Trp-134 and Trp-213), however HSA has only one tryptophan residue at position Trp-214. The average distances of tryptophan to gold cluster calculated from transfer efficiencies are 24.75Å and 23.80Å for BSA Au and HSA Au clusters respectively.

It is understood that clusters are formed inside the protein core and the protein template wraps around the gold cluster core. Presence of tryptophans in both BSA and HSA in the hydrophobic environment compared to their native counterparts suggests close association between them and formed gold clusters inside the protein. Furthermore, we also tested the accessibility of tryptophan residues in native protein and BSA/HSA Au clusters by performing KI quenching experiment. Figure 6 shows the Stern-Volmer plots of tryptophan quenching in native BSA and BSA Au clusters using KI. It is observed that tryptophan residues in native proteins are more accessible than BSA Au clusters supporting our earlier observation of tryptophan being present deep inside the protein structure. A similar observation was made for native HSA and HSA Au clusters (figure 7).

Moreover, we wanted to check if we can quench the fluorescence of gold clusters using 500mM KI. Figure 8 shows the emission spectra of BSA Au clusters when excited using 280nm with and without KI. Although the change is small, one can observe the decreased emission intensity in a spectrum with KI at tryptophan emission wavelength (~345nm) as well as gold clusters emission wavelength (~650nm). In order to test the possibility of direct

quenching of clusters via KI, we excited BSA Au clusters at 500nm and found that clusters emission cannot be quenched even at a high (0.5M) concentration of KI. Above observations were also applicable to HSA Au clusters as shown in figure 9. The quenching of the clusters emission observed in Figure 8A (left panel) and 9A (left panel) is an effect of Trp quenching, followed by less efficient FRET from Trp to the clusters.

4. Conclusions

Overall, tryptophan showed 55% FRET efficiency for BSA Au clusters, and 59% FRET efficiency for HSA Au clusters. We have shown that the interaction between tryptophan residues and gold clusters formed within BSA/HSA proteins leads to a significant energy transfer. KI quenching studies and lifetime measurements of tryptophan in native proteins and in BSA/HSA Au clusters proved that tryptophan acts as energy donor to gold clusters. This kind of interaction within protein template clusters (ratio-metric approach and FRET) can be used to develop biochemical assays for drugs, enzymes and bio-molecules using an advantage of long lifetimes of gold clusters to reduce an unwanted background.

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Figure 1.

Left panel (A) shows the excitation and emission spectra of the BSA Au clusters as measured showing enhanced excitation in UV region. Right panel (B) shows the normalized emission and excitation spectra of BSA Au clusters in visible wavelength region.

Figure 2.

Left panel (A): Emission spectrum from native BSA (solid blue), emission spectrum of BSA Au (solid red) at the excitation of 280nm, and emission spectrum of BSA Au with 310nm Ex (dashed red). Equi-molar concentration of native BSA and BSA Au were used. Right panel (B): Tryptophan emission spectra in native BSA and BSA Au clusters using 280nm exc.

Figure 3.

Left panel (A) Emission spectrum from native HSA (solid blue), emission spectrum of HSA Au (solid red) at 280nm excitation, and emission spectrum of HSA Au with 310nm Ex (dashed red). Equimolar concentration of native HSA and HSA Au were used. Right panel (B): Tryptophan emission spectra in native HSA and HSA Au clusters using 280nm exc.

Figure 4.

Left panel (A): Fluorescence intensity decays of tryptophan in native BSA (blue) and tryptophan in BSA Au clusters (red). Right panel (B): Spectral overlap of tryptophan emission and BSA Au clusters deconvoluted absorption marked by horizontal lines.

Figure 5.

Left panel (A): Fluorescence intensity decays of tryptophan in native BSA (blue) and tryptophan in BSA Au clusters (red). Right panel: Spectral overlap of tryptophan emission and HSA Au clusters deconvoluted absorption marked by horizontal lines. Right panel (B): Spectral overlap of tryptophan emission and HSA Au clusters deconvoluted absorption marked by horizontal lines.

Figure 6.
Stern-Volmer plot showing tryptophan quenching in BSA and BSA Au clusters.

Figure 7.
Stern-Volmer plot showing tryptophan quenching in HSA and HSA Au clusters.

Figure 8.

A) Emission spectrum of BSA Au cluster in presence and absence of KI (0.5M) using 285 nm excitation. **B)** Emission spectrum of BSA Au cluster using 500 nm excitation in presence and absence of KI (0.5M).

Figure 9.

A) Emission spectrum of HSA Au cluster in presence and absence of KI (0.5M) using 285 nm excitation. **B)** Emission spectrum of HSA Au cluster using 500 nm excitation in presence and absence of KI (0.5M)

Table 1

Sample	Lifetime (ns)			Amplitudes		Average Lifetime (ns)		χ^2_R
	τ_1	τ_2	τ_3	α_1	α_2	τ_{INT}	τ_{AMP}	
BSA native	6.7	2.8		0.90	0.10	6.3	5.8	0.880
BSA Au Clusters	4.9	1.4	0.44	0.66	0.34	3.7	2.6	0.878
HSA Native	7.1	2.1		0.89	0.11	6.5	5.6	0.919
HSA Au Clusters	4.6	1.1	0.09	0.68	0.32	3.5	2.3	0.853