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Polyallylamine hydrochloride coating enhances the fluorescence emission of Human Serum Albumin encapsulated gold nanoclusters

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ABSTRACT

Protein encapsulated gold nanoclusters have received much attention due to the possibility of using them as a non-toxic fluorescent probe or marker for biomedical applications, however one major disadvantage currently is their low brightness and quantum yield in comparison to currently used fluorescent markers. A method of increasing the fluorescence emission of Human Serum Albumin (HSA) encapsulated gold nanoclusters (AuNCs) via a Polyallylamide hydrochloride (PAH) coating is described. PAH molecules with a molecular weight of ~17,500 Da were found to enhance the fluorescence emission of HSA-AuNCs by 3-fold when the protein/polymer concentration ratio is 2:1 in solution. Interestingly, the fluorescence lifetime of the AuNCs was found to decrease while the native tryptophan (TRP) fluorescence lifetime also decreased during the fluorescence emission intensity enhancement caused by the PAH binding. Coinciding with the decrease in fluorescence lifetime, the zeta potential of the system was observed to be zero during maximum fluorescence intensity enhancement, causing the formation of large aggregates. These results suggest that PAH binds to the HSA-AuNCs acting as a linker; causing aggregation and rigidification, which results in a decrease in separation between native TRP of HSA and AuNCs; improving Förster Resonance Energy Transfer (FRET) and increasing the fluorescence emission intensity. These findings are critical to the development of brighter protein encapsulated AuNCs.

1. Introduction

Several studies have been undertaken to understand the unique fluorescent properties of protein encapsulated gold nanoclusters (AuNCs) [1–3]. Their red band emission peak, non-toxicity and non-photobleaching nature make them perfectly suited for biological imaging and sensing [4,5]. However, in their current state protein encapsulated AuNCs have very low brightness levels, with quantum yields reported at ~6% [6]. Of all the protein encapsulated AuNCs which have been synthesised, human serum albumin (HSA) AuNCs have received the most attention [7–11]. Serum albumin is the most abundant protein found in mammalian blood, acting as a transportation molecule for a variety of molecules including drugs, thus much research has been undertaken to fully understand the protein characteristics in different environments and conditions [12–17]. Previous research on the native protein offers insights as to why HSA encapsulated AuNC fluorescence characteristics change in differing environments. Previously it has been shown that the fluorescence emission of HSA-AuNCs is quenched in low pH solutions due to the exposure of AuNCs to the solvent as the HSA protein unfolds [18]. The physicochemical characteristics of HSA-AuNCs has also been studied to understand the effects AuNCs synthesis has on the proteins natural characteristics [19]. Of major interest was the large shift in the protein's surface charge to a highly negative charge state at neutral pH values and lends itself to the possibility that positively charged molecules can be bound to the surface; allowing for the modification of the HSA-AuNCs, enhancing the fluorescence emission brightness. Fan et al. successfully enhanced the fluorescence emission brightness of CdSeS/ZnS quantum dots by 2 orders of magnitude via a PMMA-*co*-MMA polymer [20]. To explore these enhancements a positively charged polymer, polyallylamine hydrochloride (PAH), was chosen as a means of coating due to the negative surface charge of the HSA-AuNC complex. PAH is typically used as a means of creating a highly hydrophobic film in conjunction with polysodium styrene sulfonate (PSS), forming a layer by layer negatively and positively charged film, which is typically used in microencapsulation of molecules and nanoparticles [21,22].

2. Materials and Methods

HSA-AuNCs were synthesised using the commonly cited one-pot

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method developed by Xie et al. [23]. 17 mg of $HAuCl_4$ and 250 mg of HSA were dissolved separately in two beakers with 5 ml of water at 37 °C. Once dissolved the two solutions were mixed together for 2 mins. Next 0.5 ml of NaOH at a concentration of 1 M was added to the mixture, increasing the pH above 10. The solution was then stirred and kept at a constant 37 °C for 6 h. After 6 h the sample was then kept at 37 °C in an oven for 24 h. The concentrated solution was then purified after synthesis using 10 kDa dialysis cassettes. Dialysis was carried out in a PBS buffer solution (I = 0.01 M, pH = 7.4). The dialysed HSA-AuNCs solution was diluted to 40 μM with PBS buffer solution (I = 0.01 M, pH = 7.4) for use during all fluorescence based experiments. A stock solution of PAH (Mw = 17,500 Da) was prepared at a concentration of 1 mM for use in all fluorescence based experiments. For Zeta potential and Dynamic Light Scattering (DLS) experiments, the concentration of HSA-AuNCs and PAH stock concentration were diluted by 37.5% to 15.0 μM and 375 μM, respectively. All chemicals were purchased from Sigma Aldrich. Fluorescence emission data was collected using a Horiba Fluorolog 3. Fluorescence lifetime data was collected using a Horiba Deltaflex. All experiments carried out on the Horiba Deltaflex were carried out using a NanoLED pulsed light source (excitation wavelength 295 nm). A Time-to-amplitude converter (TAC) range of 100 ns was used to study tryptophan (TRP) fluorescence lifetimes, a TAC range of 13 μs was used for AuNCs fluorescence lifetimes. Dynamic Light Scattering (DLS) and electrophoretic mobility experiments were carried out on a Malvern Zetasizer NS. UV–Vis measurements were carried out on a Thermofisher Evolution 201 Spectrophotometer.

3. Results and Discussion

To illustrate the maximum fluorescence enhancement of HSA-AuNCs after adding a polymer coating the emission spectra for native HSA-AuNCs and HSA-AuNCs with PAH added (HSA-AuNCs-PAH) excited at 290 nm and 470 nm are shown in Fig. 1.

The fluorescence spectrum of HSA-AuNCs after the 20 μM PAH coating when exciting at 290 nm can be seen to increase 3-fold while the emission peak position is slightly redshifted by 8 nm. When directly exciting the AuNC using 470 nm excitation a fluorescence enhancement is also evident however it is not as substantial as exciting at 290 nm where the AuNC is excited predominately via a Forster Resonance Energy Transfer (FRET) from the single TRP of HSA to the AuNC [9,18]. To find the best ratio of PAH:HSA-AuNCs, small additions of PAH were

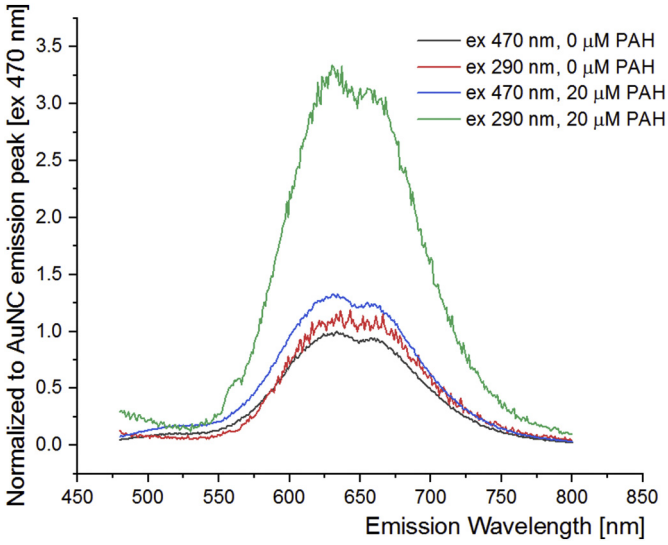

Fig. 1. Fluorescence emission spectra of HSA-AuNCs without PAH coating and HSA-AuNCs with 20 μM PAH coating. Excitation wavelength of 290 nm and 470 nm.

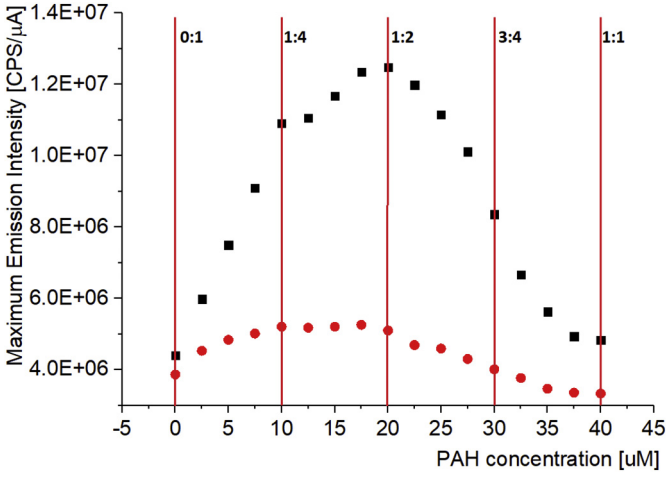

Fig. 2. Maximum emission intensity of HSA-AuNCs as a function of added PAH concentration. Peak emission intensity for 290 nm excitation (square), 470 nm (circle). HSA-AuNCs concentration is kept at a concentration of 40.0 μM throughout. Vertical lines indicate the molecular ratio between PAH:HSA-AuNCs. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

made to a solution of HSA-AuNCs and the maximum emission intensity for both excitation at 290 nm and 470 nm were measured, as shown in Fig. 2.

From Fig. 2 we can see that the addition of PAH coating to HSA-AuNCs results in a gradual increase in fluorescence emission from the AuNCs until a maximum is reached at a concentration of 20 μM of PAH and it returns to the original fluorescence intensity at 40 μM. The fluorescence enhancement when exciting at 290 nm can be explained by an improvement in the FRET process due to the coating of the HSA-AuNC with PAH causing a change in conformation that may bring the TRP and AuNC closer together enhancing the FRET [24–27]. However, no FRET takes place at 470 nm excitation and a different enhancement mechanism must be responsible for the increase in intensity observed. A decrease in collisional quenching due to the PAH shielding the HSA-AuNC complex from solution may account for fluorescence intensity enhancement. Another possibility is the AuNC is rigidified by the PAH interaction, causing an increase in emission via the Au(I) shell [28]. Increasing the PAH concentration from 40 μM to 200 μM resulted in no further changes to the fluorescence emission intensity when exciting at 290 nm and 470 nm. Changes to the peak emission wavelength were also observed upon the addition of PAH to a HSA-AuNC solution, as shown in Fig. 3.

A red shift in the peak emission wavelength of 10 nm when excited at 290 nm and a red shift of 5 nm when excited at 470 nm was observed. The red shift may be explained by changes in the TRP microenvironment brought about by PAH interactions. The red shift of TRP results in a reduced spectral overlap between TRP emission and AuNC absorption. Therefore, improved FRET must arise from a reduced donor-acceptor pair separation. The maximum emission intensity of TRP was also monitored while adding PAH, shown in Fig. 4.

The maximum fluorescence intensity of TRP was observed to decrease (as AuNC emission increased), as a result of increased PAH concentration. This decrease in TRP fluorescence intensity is an indicator of increased FRET efficiency. It is possible that the decrease is also linked to a decrease in quantum yield of TRP due to the presence of the positively charged PAH nearby affecting the electron transfer between the TRP indole ring and acceptor during fluorescence [29]. However, a decrease in donor quantum yield would result in a decrease in FRET efficiency. Therefore the improvement in FRET efficiency observed here may be dominated by the decrease in the donor-acceptor separation. Fluorescence lifetime measurements of the AuNC and TRP