Interaction of colloidal AgTiO$_2$ nanoparticles with bovine serum albumin

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1. Introduction

One of the most important biological functions of albumins is their ability to carry drugs as well as endogenous and exogenous substances [1]. The binding capacity and sites of albumins have been characterized [2]. Serum albumins are the most abundant proteins in plasma [3]. As the major soluble protein constituents of the circulatory system, they have many physiological functions [4]. Among the serum albumins, BSA has a wide range of physiological functions involving binding, transport and delivery of fatty acids, porphyrins, bilirubin and steroids, etc. It is home to specific binding sites for metals and pharmaceutical dyes [5]. The binding properties of BSA with various drugs have been fully investigated by many researchers [6–10], which are useful for understanding the reaction mechanism as well as for providing guidance for the application and design of new drugs [11].

Bovine serum albumin (BSA) has been selected as our protein model due to its water-soluble nature which is important for interaction studies [12,13]. It contains 582 amino-acid residues with a molecular weight of 69,000, and two tryptophan moieties at positions 134 and 212 as well as tyrosine and phenylalanine [14]. The intrinsic fluorescence of BSA is due to aromatic amino-acid residues. Nanoparticle probes act as biosensors in the chemical and biochemical fields, and their applications are becoming more extensive. These probes have been applied to ultrasensitive detection of proteins, DNA sequencing, clinical diagnostics etc. Recently we reported the interaction of colloidal TiO$_2$ with bovine serum albumin using fluorescence spectroscopy [15].

Metal semiconductor nanoparticles have become an attractive topic of research because of their potential applications in different fields, such as charge-transfer processes [16], optoelectronics [17] and medicine [18]. Among such nanocomposite structures, AgTiO$_2$ has more attention paid to it because silver is an extremely attractive noble metal on the nanoscale due to its remarkable catalytic activity [19], size and shape-dependent optical properties, promising applications in chemical and biological sensing [20], and also due to its antimicrobial activity [21]. Elechiguerra et al. studied metal nanoparticle interactions with the human immunodeficiency virus-1 (HIV-1), and silver nanoparticles of the size 1–10 nm were attached to HIV-1, and this prevented the virus from binding to host cells [22].

Based on the above strategy we have investigated the interaction of BSA with colloidal AgTiO$_2$ nanoparticles (Scheme 1). This is the first challenge made to investigate the mode of interaction of colloidal AgTiO$_2$ nanoparticles with BSA.

BSA absorbs light energy around 280 nm, which is not absorbed by TiO$_2$. The excited state energy of BSA is transferred to the ground state BSA–AgTiO$_2$ complex. Upon excitation of BSA, colloidal AgTiO$_2$ nanoparticles effectively quenched the intrinsic fluorescence of BSA. The number of binding sites ($n = 1.06$) and apparent binding constant ($K = 3.71 \times 10^4$ M$^{-1}$) were calculated by the fluorescence quenching method. A static mechanism and conformational changes of BSA were observed.

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2. Experimental

2.1. Materials

Bovine serum albumin and titanium-(triethanolaminito) isopropoxide were purchased from Aldrich. All measurements were performed at ambient temperature.

2.2. Methods

2.2.1. Preparation of colloidal AgTiO₂ nanoparticles

The method of preparation of colloidal AgTiO₂ nanoparticles in water is similar to the one reported earlier [23]. Colloidal AgTiO₂ nanoparticles were prepared by a one pot synthesis that involved reduction of metal ions and hydrolysis of titanium-(triethanolaminato)isopropoxide [TTEAIP] in dimethylformamide. The solvent, DMF, plays an important role in reducing the Ag⁺ ions first, followed by the slow hydrolysis of TTEAIP to form colloidal AgTiO₂ nanoparticles.

2.2.2. Instrumentation

Samples for spectroscopic measurements were prepared by dissolving bovine serum albumin in water and administering the appropriate concentration of colloidal AgTiO₂ nanoparticles. The samples were carefully degassed using pure nitrogen gas for 15 min. Quartz cells (4 x 1 x 1 cm) with high vacuum Teflon stopcocks were used for degassing.

The fluorescence quenching measurements were carried out in a JASCO FP-6500 spectrofluorimeter. The slit width (each 5 nm) and scan rate (500 nm/min) were constantly maintained for all measurements. Absorption spectral measurements were recorded using a Cary 300 UV–Vis spectrophotometer. Fluorescence lifetime measurements were carried out in a picosecond time correlated single photon counting (TCSPC) spectrometer. The excitation source was the tunable Ti-sapphire laser (TSUNAMI, Spectra Physics, USA). The fluorescence decay was analyzed by using the software provided by IBH (DAS-6). The particle size of the prepared AgTiO₂ nanoparticles were analyzed by transmission electron microscopy (recorded using TECNAI G² model).

3. Results and discussion

3.1. Characterization of the AgTiO₂ nanoparticles

Transmission electron microscope pictures have been taken for the prepared Ag–TiO₂ nanoparticles (Fig. 1) to give an idea about the particle size and surface modifications effected during doping of the metal on TiO₂ nanoparticles. The TEM pictures permit easy differentiation of metal nanoparticles (small dark areas) and TiO₂ (large bright areas), that is, an Ag nanoparticle is seen on the surface of a TiO₂ particle as a dark dot. Also, there is the possibility of metal particles to be incorporated into the interstitial positions of the semiconductor particles. Further, it is observed that the average size of silver in the Ag–TiO₂ particles is in the range 10–20 nm.

3.2. XRD characterization of AgTiO₂ nanoparticles

Fig. 2 shows the XRD spectrum of AgTiO₂ nanoparticles. Diffractions that are attributable to the anatase phase of TiO₂ crystals (1 0 1) are clearly detectable at 2θ = 25° (JCPDS 21-1272) in Fig. 2. Peaks at 2θ = 38.4°, 44.5° and 64.6° in AgTiO₂ are assigned to (1 1 1), (2 0 0) and (2 2 0) planes of silver (JCPDS), which proves that the TiO₂ surfaces are covered with metal particles.

3.3. Absorption characteristics of BSA–AgTiO₂ nanoparticles

Fig. 3 shows the absorption spectra of BSA in the presence and absence of colloidal AgTiO₂ nanoparticles at different concentrations. From this study we observed that upon increasing the concentration of colloidal AgTiO₂ the absorption of BSA increases regularly with the peak shift of around 5 nm. It is due to the
adsorption of BSA on the surface of colloidal AgTiO2 nanoparticles, supported by similar observations reported earlier [24]. The equilibrium for the formation of complex between BSA and colloidal AgTiO2 nanoparticles can be given by Eq. (1), where $K_{\text{app}}$ represents the apparent association constant:

$$
\text{BSA} + \text{AgTiO}_2 \rightleftharpoons \text{BSA} \cdot \text{AgTiO}_2
$$

$$
K_{\text{app}} = \frac{[\text{BSA} \cdot \text{AgTiO}_2]}{[\text{BSA}] \cdot [\text{AgTiO}_2]}
$$

The change in intensity of the absorption peak (280 nm) as a result of the formation of the surface complex were utilized to obtain $K_{\text{app}}$ according to Benesi and Hildebrand [25]:

$$
\text{A}_{\text{obs}} = (1 - \alpha)C_0 + \alpha C_0 C_c
$$

where $\text{A}_{\text{obs}}$ is the observed absorbance of the solution containing different concentrations of colloidal AgTiO2 at 280 nm, $\alpha$ is the degree of association between BSA and AgTiO2, $C_{\text{BSA}}$ and $C_c$ are the molar extinction coefficients at the defined wavelength ($\lambda = 280$ nm) of BSA and the formed complex, respectively, in water. Eq. (2) can be expressed as Eq. (3), where $A_0$ and $A_c$ are the absorbances of BSA and the complex at 280 nm, respectively, with a concentration of $C_0$:

$$
\text{A}_{\text{obs}} = (1 - \alpha)A_0 + \alpha A_c
$$

At relatively high AgTiO2 concentrations, $\alpha$ can be equated to $(K_{\text{app}}[\text{AgTiO}_2])/(1 + K_{\text{app}}[\text{AgTiO}_2])$. In this case, Eq. (3) can be changed to Eq. (4):

$$
\frac{1}{\text{A}_{\text{obs}} - A_0} = \frac{1}{A_c - A_0} + \frac{1}{K_{\text{app}}(A_c - A_0)[\text{AgTiO}_2]}
$$

The enhancement of absorbance at 280 nm was due to absorption of the surface complex, based on the linear relationship between $1/(\text{A}_{\text{obs}} - A_0)$ vs reciprocal concentration of colloidal AgTiO2 with a slope equal to $1/K_{\text{app}}(A_c - A_0)$ and an intercept equal to $1/(A_c - A_0)$ (Fig. 3, insert). The value of the apparent association constant ($K_{\text{app}}$) determined from this plot is $5.58 \times 10^5$ M$^{-1}$. Further, the reason for the higher association constant of AgTiO2 compared to that reported for TiO2 [15] may be due to the larger surface area of the AgTiO2 nanoparticles.

### 3.4. Fluorescence quenching of BSA by colloidal AgTiO2 nanoparticles

The fluorescence quenching is described by the Stern–Volmer relation:

$$
I_0/I = 1 + K_{\text{sv}}[Q] = 1 + K_q \tau_0[Q]
$$

where $I_0$ and $I$ are the fluorescence intensities of BSA in the absence and presence of quencher, $K_{\text{sv}}$ is Stern–Volmer constant, $k_q$ is the bimolecular quenching rate constant, and $\tau_0$ is the average lifetime of BSA, $10^{-8}$ s [26]. $[Q]$ is the concentration of the quencher. Fig. 4 shows the effect of increasing the concentration of colloidal AgTiO2 on the fluorescence emission spectrum of BSA. Addition of colloidal AgTiO2 resulted in the quenching of BSA fluorescence emission and there is no peak shift and no new peak was observed. It is noted that a complex formed between colloidal AgTiO2 and BSA is responsible for the quenching of BSA. According to Eq. (5), a linear plot [Inset, Fig. 4] between $I_0/I$ against $[\text{AgTiO}_2]$ was obtained and from the slope we calculated the quenching rate constant ($k_q$) of various kinds of quenchers to biopolymers is $2.0 \times 10^{10}$ M$^{-1}$s$^{-1}$ [26], but for the BSA–AgTiO2 system a higher quenching rate constant ($1.64 \times 10^{15}$ M$^{-1}$s$^{-1}$) was obtained. This shows that the quenching is not dynamic in nature, it depends on the formation of a complex between AgTiO2 and BSA (Scheme 2). Further the type of interaction between BSA and colloidal AgTiO2 was also confirmed by time resolved spectroscopy.

#### 3.5. Fluorescence lifetime measurements

Fig. 5 shows the fluorescence decay of BSA in the absence and presence of colloidal AgTiO2 nanoparticles. BSA exhibits single exponential decay not only in dilute solutions but also in the pres-
ence of colloidal AgTiO2 nanoparticles. On increasing the concentration of colloidal AgTiO2 there is no change in the lifetime of BSA. This observation shows that the quenching follows a static mechanism. It also supports the formation of a ground state surface complex. (In Fig. 5 although the decay traces of BSA in the absence and presence of colloidal AgTiO2 were actually plotted, the lifetime of BSA remained the same under both conditions, hence merging of the kinetic traces was observed, and the plot looks like a single decay curve.) For static quenching, we can deduce the binding constant (K) resulting from the formation of a ground state complex between fluorophore and the quencher.

3.6. Binding constant and number of binding sites

The relationship between the fluorescence intensity and quenching medium can be deduced from the following formula:

\[ nQ + B \rightarrow \frac{K}{Q} \cdot B \]

where B is the biomolecule with the fluorophore, Q is the quencher molecule, \( Q_{eq} \cdot B \) is the quenched biomolecules and the resultant constant K is given by

\[ K = \frac{Q_{eq} \cdot B}{[B]} \]

If the overall amount of biomolecules (bound or unbound with the quencher) is \( B_0 \), then \( [Q] = [Q_{eq} \cdot B] + [B] \), here [B] is the concentration of unbound biomolecules. Thus the relationship between fluorescence intensity and the unbound biomolecule is \( [B]/[B_0] = F/F_0 \), that is

\[ \log \left( \frac{F_0 - F}{F} \right) = n \log [Q] + \log K \]

where K is the binding constant of AgTiO2 with BSA, which can be determined from the plot of \( \log([F_0 - F]/F) \) versus \( \log([Q]) \) as shown in Fig. 6. Thus we obtained the binding constant “K” as \( 3.71 \times 10^5 \text{ M}^{-1} \) and binding sites “n” (1.06) for AgTiO2 with BSA from the intercept and slope of Fig. 5. The value of K obtained from the data of fluorescence quenching matches well with that determined from the absorption spectral changes. The good agreement between these values of K highlights the validity of assumption proposed for the association between BSA and colloidal AgTiO2 nanoparticles.

3.7. Characteristics of synchronous fluorescence spectra

To explore the structural changes of BSA by the addition of colloidal AgTiO2, we measured the synchronous fluorescence spectra of BSA with the concentrations of colloidal AgTiO2 used for the fluorescence quenching study. Synchronous fluorescence spectra provide information on the molecular microenvironment, particularly in the vicinity of fluorophore functional groups [27]. The fluorescence of BSA is due to the presence of tyrosine, tryptophan and phenylalanine residues. Hence spectroscopic methods are usually applied to study the conformation of the serum protein. In synchronous fluorescence spectroscopy, according to Miller [28], the difference between the excitation and emission wavelength (\( \Delta \lambda = \lambda_{\text{exc}} - \lambda_{\text{em}} \)) reflects the spectra of chromophores with the different natures. With larger \( \Delta \lambda \) values, such as 60 nm, the synchronous fluorescence of BSA is characteristic of the tryptophan residue, and smaller \( \Delta \lambda \) values, such as 15 nm, are characteristic of tyrosine [29]. The synchronous fluorescence spectra of BSA with various concentrations of colloidal AgTiO2 were recorded at \( \Delta \lambda = 15 \text{ nm} \) (Fig. 7) and \( \Delta \lambda = 60 \text{ nm} \) (spectra not shown here). With an increasing concentration of colloidal AgTiO2 the intensity of tyrosine decreased, and a red-shift of the emission wavelength (Fig. 7) was observed. At the same time, the tryptophan fluorescence emission decreased regularly, but no significant change in wavelength was observed. It suggests that the interaction of colloidal AgTiO2 with BSA affects the conformation of the tyrosine region, but not the tryptophan micro-region. The tyrosine fluorescence spectrum may represent that the conformation of BSA has changed, leading to a strengthening of the polarity around the tyrosine residues and a weakening of the hydrophobicity [30]. It is important to note that colloidal AgTiO2 affects only the tyrosine residues in the BSA moiety. This is because tyrosine contains one aromatic hydroxyl group, unlike tryptophan.

In our previous study, riboflavin was used as a sensitizer for colloidal TiO2 [31] and it was observed that riboflavin interacted through the hydroxyl group of colloidal TiO2. In addition, tyrosine can undergo an excited state ionization, resulting in the loss of a proton from the aromatic hydroxyl group. Hence it is clear that the presence of a hydroxyl group in the tyrosine residues may be
responsible for the interaction of BSA with AgTiO$_2$, similar to 2,3-diazabicyclo [2.2.2] oct-2-ene (DBO) interacting with the aromatic hydroxyl group of tyrosine in BSA [32], as reported by Anbazhagan and Renganathan (Scheme 3).

4. Summary

The interaction between colloidal AgTiO$_2$ nanoparticles and BSA has been studied by various spectroscopic measurements. The results presented clearly indicate that colloidal AgTiO$_2$ nanoparticles quench the fluorescence emission of BSA through a static mechanism, which is further confirmed by the unaltered lifetime of BSA by time resolved measurements. The quenching rate constant, binding constant and number of binding sites were calculated according to the relevant fluorescence quenching data. From the synchronous fluorescence spectra, it is shown that the conformational change of BSA is induced by the interaction of colloidal AgTiO$_2$ nanoparticles with the tyrosine micro-region of the BSA molecules. The binding study of drugs with nanoparticles is of great importance in pharmacy, pharmacology and biochemistry. This study is expected to provide important insight into the interactions of the physiologically important protein BSA with metal nanoparticles. Information is also obtained about the effect of the environment on the BSA structure, which may be correlated to its physiological activity.

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