Interaction between keratin and silver nanoparticles: investigation by spectrofluorimetry

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In this study, the fluorescence quenching of keratin with silver nanoparticles was investigated by spectrofluorimetry. The interaction mechanism was analyzed by the Stern-Volmer equation. Moreover, the number of binding sites, the binding constant, and the thermodynamic functions of the reaction between keratin and silver nanoparticles at different temperatures were calculated separately. The results show that silver nanoparticles can quench the fluorescence of keratin, and the reaction between silver nanoparticles and keratin is completed in a few seconds. High pH value is beneficial for the reaction between silver nanoparticles and keratin. As to the quenching variety between silver nanoparticles and keratin, it is a static quenching, and the magnitude of the reaction constants is 10^{10}, much larger than that of small molecules with proteins. Also, the main type of the reaction between keratin and silver nanoparticles is a hydrophobic reaction with an endotherm and entropy increase in the process. This study could be helpful to understand the underlying interaction mechanisms between bio-macromolecules and silver nanoparticles.

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1. Introduction

Keratin is one of the most abundant proteins, being the main component of wool, nail, horns and other epithelial coverings; it plays an important role in the protective function of skin [1]. The feature of keratin is that the content of cysteine residues is much higher than other proteins. In wool, keratin occupies about 50 wt% of the cortical cells [2]. Because of the high content in the raw materials, keratin costs few expenses in extraction and purification. Nowadays, the development of keratin-based materials has received much attention for application in biomedical and biotechnological fields, due to their biodegradability, biocompatibility and their capability to support fibroblast cell growth [3, 4].

In recent years, silver nanoparticles has been in highlight with its unique properties, such as high electric conductivity [5], high catalytic effect [6] and high antibacterial activity [7, 8]. Also, it has already been used in keratin-based materials. Xiaowen Lü [9] et al reported a facile method to prepare stable silver nanoparticles using extracted wool keratin as a capping agent. Hee Yeon Ki [10] et al treated wool textiles with a sulfur nano-silver colloidal solution and demonstrated that the finished wool fabrics with the colloid had various functionalities, such as mothproofing, antibiotic, and antistatic property. Moreover, Wang Xuan et al [25] treated sheep fur with silver nanoparticles as an antibacterial agent, and obtained a good antibacterial effect of the treated sheep fur. Obviously, these studies have exhibited the potential applications of silver nanoparticles in the field related to keratin bio-materials. However, there are still some concerned problems about how to apply silver nanoparticles effectively into keratin-based systems. Such as the citrate capped silver nanoparticles shows little affinity to wool or cotton without the use of pre-treatments or toxic chemicals, which could often have a negative influence on the intrinsic properties of textile or keratin substrate [26]. One of the key issues is that the interaction mechanism between silver nanoparticles and bio-macromolecules has not been well understood. Though the interaction between silver nanoparticles and some proteins, e.g.: serum albumins [14], hemoglobin [21] and myoglobin [22] etc., has been investigated in detail, the studies about the interaction between keratin and silver nanoparticles have not been reported yet.

Herein, we report the study of the interaction mechanism between keratin and silver nanoparticles using spectrofluorimetry. It is a very useful technique in analyzing the molecular conformation of a protein under various circumstances [11, 12], which may be helpful to illuminate the interaction mechanisms between keratin and silver nanoparticles. C. Gaidau et al [13] have reported the study of collagen functionalization with metallic nanoparticles using this technique. In the present work, the fluorescence quenching of keratin with silver nanoparticles was investigated. And the interaction mechanism was analyzed by the Stern-Volmer equation. Moreover, the

number of binding sites, the binding constant, the thermodynamic functions of the reaction between keratin and silver nanoparticles at different temperatures were calculated separately.

2. Experimental

2.1. Preparation of silver nanoparticles

A 1.5×10^{-2}g of $AgNO_3$ was dissolved in a 100mL of distilled water and placed in water bath at 30℃ for 15min. A 100mL of benzalkonium bromide (2×10^{-2}g) solution was added dropwise to the $AgNO_3$ solution with intense stirring for 30min to form a mixed solution. Subsequently, a 200mL of solution containing $NaBH_4$ (7.4×10^{-3}g) and benzalkonium bromide (4×10^{-2}g) were added dropwise into the mixed solution for 1.5h to obtain silver nanoparticles. Then, the prepared silver nanoparticles were conserved at 30℃ [24]. Size distribution of the silver nanoparticles was performed using a nanoparticle size analyzer (Zetasizer NanoS90, Malvern company, England), with a result of average particles size of 24nm. For the silver colloid with [Ag]= 2.4×10^{-2}g·L^{-1}, it was calculated that the concentration of the silver nanoparticles was about 8.25×10^{-11}mol·L^{-1} [14].

2.2. Spectrofluorimetry of keratin with silver nanoparticles

A 20mL of 4.8×10^{-2}g·L^{-1} keratin solution (5 wt%, Molecular weight 40-50KDa, TGI Company, Japan) and a 20mL of 4.1×10^{-11} mol·L^{-1} silver nanoparticles were mixed together, and the reaction was kept for 30s, 6min, 15min, 30min, 60min and 120min, separately. The mixture of 20mL 4.8×10^{-2}g·L^{-1} keratin solution and 20mL distilled water was used as a control.

A 20mL of 5.0×10^{-2}g·L^{-1} keratin solution and a 20mL of 4.1×10^{-11} mol·L^{-1} silver nanoparticles were mixed together. And the pH values of the mixtures were adjusted to 5.5，6.5, 7.0, 7.5 and 8.5 separately, with 0.01mol/L NaOH solution or 0.01mol/L HCl solution as required, and the reaction was kept for 120min.

A 20mL of 5.0×10^{-2}g·L^{-1} keratin solution and a 20mL of 5×10^{-12} - 2.5×10^{-11} mol·L^{-1} silver nanoparticles were mixed together. And the pH values of the mixtures were adjusted to 7.5 with 0.01mol/L NaOH solution or 0.01mol/L HCl solution as required, and the reaction was kept for 120min. The mixture of 20mL 5.0×10^{-2}g·L^{-1} keratin solution and 20mL distilled water was used as a control.

The mixed solutions listed above were scanned with a fluorescence spectrometer (F-4010, Hitachi, Ltd. Tokyo, Japan) at temperature of 26□, 33℃ and 40℃ separately, and the fluorescence intensity was recorded at 296nm.

3. Results and discussion

3.1. Influence of the reaction time on the fluorescence quenching of keratin with silver nanoparticles

Fig. 1 shows the fluorescence spectra of 4.8×10^{-2}g·L^{-1} keratin induced by 4.1×10^{-11} mol·L^{-1} silver nanoparticles at pH 7.2 at different reaction time. It can be seen that with the addition of silver nanoparticles to the keratin solution, the fluorescence intensity decreases rapidly. This indicates that the silver nanoparticles can quench the fluorescence of keratin. The fluorescence intensity reduces significantly with increasing reaction time from 0s to 30s. But as the reaction time is more than 30s, there is no obvious variation of the fluorescence spectra, showing that the reaction between keratin and silver nanoparticles is very fast, completed in a few seconds.

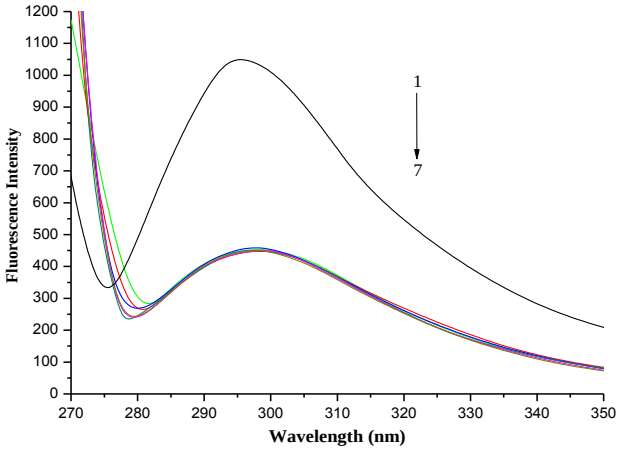

Fig. 1. The fluorescence spectra of 4.8×10^{-2}g·L^{-1} keratin induced by 4.1×10^{-11} mol·L^{-1} silver nanoparticles at pH 7.2 at different reaction time 1 - 0s, 2 - 30s, 3 - 6min, 4 – 15min, 5 - 30min, 6 - 60min, 7 - 120min

3.2. Influence of the pH values on the fluorescence quenching of keratin with silver nanoparticles

As shown in Fig. 2, the pH value of keratin influences the reaction, specifically, the higher the pH value is, the more evident the fluorescence quenching is. Because keratin is a zwitterionic polymer, which would show higher affinity with silver nanoparticles at the polyanion state than that at the polycation state [27]. Therefore, when the pH values is farther from the isoelectric point (pH 3.6 in the experiment) of the keratin, the molecule could be more easily interacted with silver nanoparticles, so the quenching is more obvious.

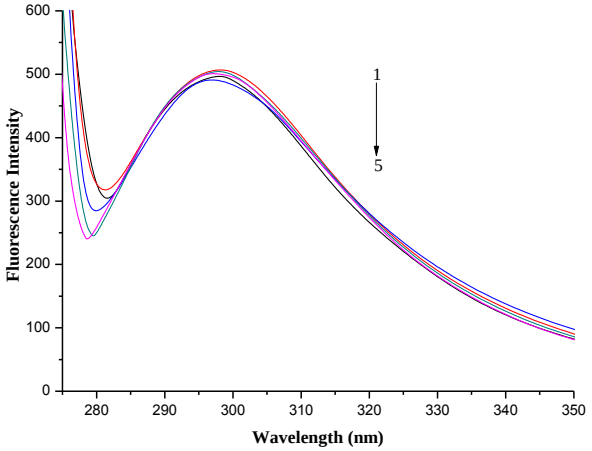

Fig. 2. The fluorescence spectra of 5.0×10^{-2}g·L^{-1} keratin induced by 4.1×10^{-11} mol·L^{-1} silver nanoparticles at different pH values 1 - 5.5, 2 - 6.5, 3 - 7.0, 4 - 7.5, 5 – 8.5.

3.3. Influence of the concentration of silver nanoparticles on the fluorescence quenching of keratin

Fig. 3 shows that with the addition of silver nanoparticles, the shape of the fluorescence spectrums keeps unchangeable, but the fluorescence intensity decreases regularly. This indicates that the silver nanoparticles is a fluorescence quenching material for keratin and the energy of keratin was transferred to silver nanoparticles.

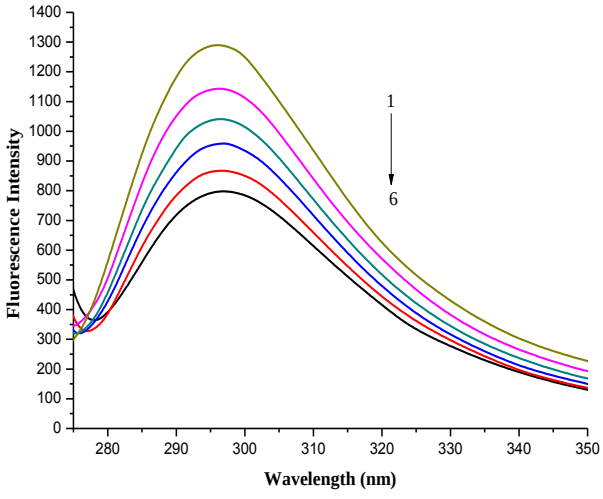

Fig. 3. The fluorescence spectra of 6.0×10^{-2}g·L^{-1} keratin induced by different concentration of silver nanoparticles at pH 7.5 1 - 0 mol·L^{-1}, 2 - 5×10^{-12} mol·L^{-1}, 3 - 1×10^{-11} mol·L^{-1}, 4 - 1.5×10^{-11} mol·L^{-1}, 5 - 2×10^{-11} mol·L^{-1}, 6 - 2.5×10^{-11} mol·L^{-1}.

3.4. Analysis of the fluorescence quenching mechanism

The fluorescence quenching means that the fluorescence intensity decreases with the physical or chemical interactions between the fluorescent substance and the solvent or solute. There are two types: dynamic quenching and static quenching. Static quenching occurs when the fluorescence quencher and the fluorescent substance react at a ground state and then complex non-fluorescing compounds are formed. Dynamic quenching is a mutual process occurring at an excited state such as in an energy or electron transfer process between the fluorescence quencher and the fluorescent substance.

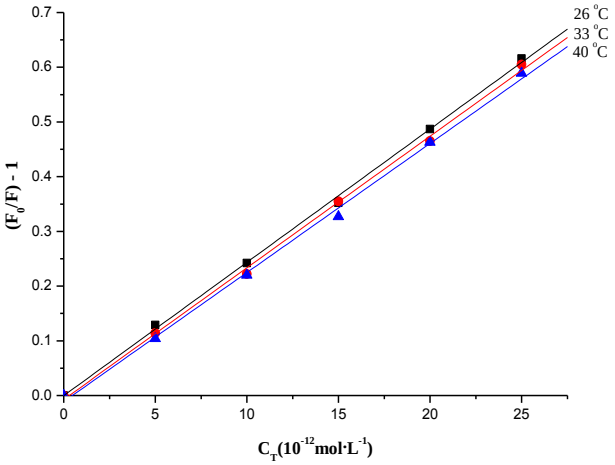

Fig. 4. The Stern-Volmer relationship between the concentration of silver nanoparticles and relative fluorescence intensity of keratin at different temperatures.

For both the dynamic and static quenching, the fluorescence intensity is proportional to the fluorescence quencher concentration. The linear Stern-Volmer equation is as follows [15]:

$$F_0/F = 1 + K_q\tau_0C_t = 1 + K_{sv}\,C_t \quad (1)$$

Where F_0 and F are the fluorescence intensities in the absence and presence of the fluorescence quencher of the keratin, separately; K_{sv} is the quenching constant equal to $K_q\tau_0$, in which K_q is the biomolecular quenching rate constant and τ_0 is the average lifetime of the fluorescent molecules without addition of a quencher; C_t is the concentration of the silver nanoparticles in the mixed solution.

Table 1. The quenching constant (K_{sv}) and quenching rate constant (K_q) at different temperatures

T (℃)	K_{sv} (10^{10} L·mol^{-1})	K_q (10^{18} L·mol^{-1}·s^{-1})	Correlation coefficient
26	2.44	2.44	0.9986
33	2.41	2.41	0.9981
40	2.36	2.36	0.9973

The Stern-Volmer relationship between the concentration of silver nanoparticles and relative fluorescence intensity of keratin at different temperatures was shown in Fig. 4. The slope coefficient of the straight line in Fig. 4 is the quenching constant K_{sv}. According to the fluorescent lifetime of biomolecular (τ_0)-10^{-8}s [16], the biomolecular quenching rate constant K_q can be calculated through the Stern-Volmer equation: $K_q\tau_0 = K_{sv}$. The results are shown in Table 1.

Since dynamic fluorescence depends on diffusion rate, and diffusion rate increases with rising temperature, which means that the constant of dynamic quenching (K_{sv}) rises with increasing temperature [17]. But as indicated in Fig. 4, the slope of the quenching curve reduces with the increase of the temperature. So the fluorescence quenching of keratin with silver nanoparticles is not dynamic quenching but static quenching. Additionally, because that the fluorescence quenching rate constants (K_q) of keratin with silver nanoparticles (see in Table 1) are all larger than the largest diffusion and collision quenching constant (2.0×10^{10} L·mol^{-1}·S^{-1}) [16], the fluorescence quenching is classified as a static quenching.

3.5. Analysis of the binding constant and the number of binding sites

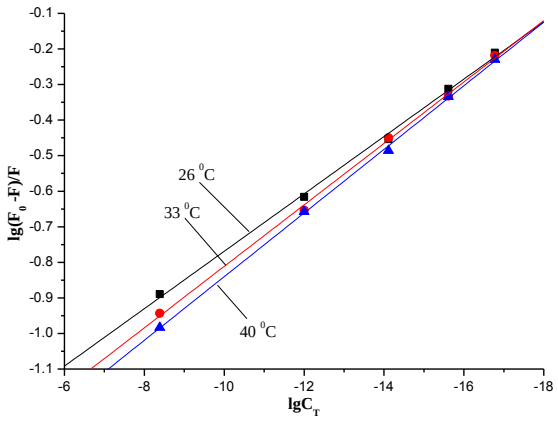

Fig. 5 .The relationship between the fluorescence intensity and the concentration of silver nanoparticles at pH 7.5.

If there are equal and independent binding sites (n) between keratin (K) and the fluorescence quenchers silver nanoparticles (S), the quenching reaction between keratin and silver nanoparticles is as follows:

$$nK+S=K_nS \quad (2)$$

The binding constant K_A is:

$$K_A = C_{KnS} / C_S^n \cdot C_K \quad (3)$$

Where C_S = concentration of fluorescence quencher (K);

C_K = concentration of keratin;

C_{KnS} = concentration of K_nS.

The fluorescent substance concentration (C_{K0}) is:

$$C_{K0} = C_{SnK} + C_K$$

Then,

$$K_A = C_{K0} - C_K / C_S^n \cdot C_K \quad (4)$$

During the static fluorescence quenching process, the fluorescence intensity is proportional to the concentration of the free fluorescent substance, and the equation is as follows:

$$C_K / C_{K0} = F/F_0 \quad (5)$$

According to (4) and (5),

$$\lg (F_0-F)/F = \lg K_A + n\lg C_S \quad (6)$$

The binding constant (K_A) and the number of reaction sites (n) in equation (6) can be calculated from the intercept ($\lg K_A$) and the slope in Fig. 5, and the results are shown in Table 2.

Table 2. The number of reaction sites (n) and reaction constants (K_A) at different temperatures

T (℃)	K_A (10^{10})	n
26	1.11	0.97
33	5.87	1.04
40	14.1	1.07

It can be seen the binding ratio of keratin and silver nanoparticles at different temperatures is about 1:1. The binding ratio and the reaction constants increase with the increasing temperature, showing high temperature is beneficial for the reaction between keratin and silver nanoparticles. It is reported that the magnitude of the reaction constants between metal ions, medicines, small molecules and proteins, separately, is $10^3 \sim 10^6$ [18, 19]. And the magnitude of reaction constants between keratin and silver nanoparticles is 10^{10}, showing that the reaction between keratin and silver nanoparticles is much stronger than that of small molecules with proteins.

3.6. Calculation of the thermodynamic functions

The ΔH_m^Θ, ΔG_m^Θ and ΔS_m^Θ values, which illustrate the main type of the reaction between silver nanoparticles and keratin, can be calculated through the following Van't Hoff equations.

$$\Delta G_m^\Theta = \Delta H_m^\Theta - T\Delta S_m^\Theta \quad (7)$$

$$\Delta G_m^\Theta = -RT\ln K \quad (8)$$

$$\ln[K_{T2}/K_{T1}] = \Delta H_m^\Theta (T_2-T_1)/RT_1T_2 \quad (9)$$

Where T_1, T_2 and T are the temperatures of the reaction.

ΔH_m^Θ is considered as a constant when the temperature slightly changes. Combining the reaction constants (K_A) in Table 2, ΔH_m^Θ, ΔG_m^Θ and ΔS_m^Θ can be calculated by equation (7), (8) and (9), separately. The results are shown in Table 3.

Table 3. The thermodynamic functions of keratin with silver nanoparticles at different temperatures.

T (K)	ΔH_m^Θ ($KJ \cdot mol^{-1}$)	ΔG_m^Θ ($KJ \cdot mol^{-1}$)	ΔS_m^Θ ($J \cdot mol^{-1} \cdot K^{-1}$)
299	181.0	-57.5	797.7
306	181.0	-63.1	797.7
313	181.0	-66.9	792.0

As can be seen in Table 3, the ΔG_m^Θ at the three temperatures are all below zero, showing that the reaction between silver nanoparticles and keratin is spontaneous. The ΔH_m^Θ and the ΔS_m^Θ are all above zero in the experiment, according to the thermodynamic regulation of reaction types [20], the main type of reaction between keratin and silver nanoparticles is a hydrophobic reaction with an endotherm and entropy increase in the process.

3.7. Pictorial description of the reaction between keratin and silver nanoparticles

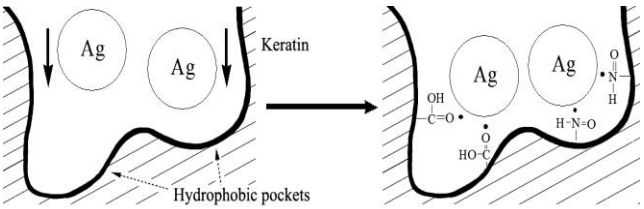

Fig. 6. The pictorial description of the reaction between keratin and silver nanoparticles.

From the researches above, the reaction between keratin and silver nanoparticles could be speculated with a pictorial description (Fig. 6). "Hydrophobic regions" or "hydrophobic pockets" shown in Fig. 6 are formed at the positions where there are amino acid residues with aromatic or aliphatic side chains of keratin polypeptides in aqueous solution [23]. At the beginning, silver nanoparticles with hydrophobic surface enter into the "hydrophobic pockets" through hydrophobic effect. Then, electrostatic bonds might be formed between the surface of silver nanoparticles and the sulphur atoms of cysteine residues, the carboxyl oxygen, and the nitrogen atoms of peptide or residues. Consequently, the microenvironments around tryptophan and tyrosine residues are disturbed by the silver nanoparticles, making the residues buried inside the hydrophobic pockets [21]. Since the fluorescence of keratin is mainly attributed by tryptophan and tyrosine residues [17], so it is then quenched by reaction with the silver nanoparticles.

4. Conclusion

This study shows silver nanoparticles can quench the fluorescence of keratin, and the reaction between silver nanoparticles and keratin is completed in a few seconds. High pH value is beneficial for the reaction between silver nanoparticles and keratin. As to the quenching variety between silver nanoparticles and keratin, it is a static quenching, and the magnitude of the reaction constants is 10^{10}, much larger than that of small molecules with proteins. Also, the main type of the reaction between keratin and silver nanoparticles is a hydrophobic reaction with an endotherm and entropy increase in the process.

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