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Probing the binding of trypsin to glutathione-stabilized gold nanoparticles in aqueous solution

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ABSTRACT

We investigate the interaction of trypsin with glutathione-stabilized Au nanoparticles (NPs) using fluorescence, synchronous fluorescence and ultraviolet (UV) absorption spectroscopy. We find that trypsin binds strongly to the Au NPs with a static quenching mechanism, and that the interaction is characteristic of positive cooperative binding. Furthermore, we determine the binding constants and the thermodynamic parameters, which suggest that the main binding forces between the glutathione-stabilized Au NPs and trypsin are electrostatic interactions and hydrogen bonding. Analysis of UV–vis absorption spectra suggests that aggregation of the Au NPs occurs in the trypsin/Au NPs system, which significantly alters the conformation of the protein.

1. Introduction

Gold nanoparticles (Au NPs) have received much recent research attention for a diverse range of biomedical applications, including photothermal and targeted drug delivery treatments for cancer [1], and as probes for many biodiagnostic systems [2]. With these potential applications, it is important to study the biological effects of Au NPs. There have several studies into the binding association of proteins with Au NPs, which have typically shown that proteins undergo significant conformational changes upon association with Au NPs [3–7]. It has been suggested that a dispersion of Au NPs in a biological medium will result in the formation of a biological corona on the surface of the nanoparticles, which can potentially lead to conformational changes of the biomolecules [8].

Over the past five years, attention increasingly focused on interactions between Au NPs and common plasma proteins [4–6,9,10], which are related to the various housekeeping functions of blood plasma. However, the limited studies on common plasma proteins has not been sufficient for enabling an understanding of the nature of the interaction between Au NPs and proteins, as well as the biological effects of Au NPs. So, it is important to investigate the effects of Au NPs on a wide range of proteins. It is known that the serine proteinase family is critical in digestion, blood coagulation and the complement system [11]. An understanding of the interactions between serine proteinase and Au NPs is expected to provide important systematic knowledge of the effects of Au NPs in organisms, and to contribute to the development of safe and biocompatible applications of Au NPs. Trypsin has a small molecular weight and five disulfide bonds, and is an important member of the serine proteinase family. Interestingly, trypsin can dissolve denatured proteins, but has no effect on native proteins. In addition, its relatively small size and well-characterized three-dimensional structure make it a good candidate for an analysis of the interaction with Au NPs. Previous studies on the conjugation of trypsin with Au NPs have already presented some useful information about the binding mechanism, surface chemistry of nanoparticles and enzymatic activity [12–14]. We have previously reported a method of accurately determining the concentration of glutathione-stabilized Au NPs colloidal suspension [15], which enables us to obtain quantitative information on the interaction between Au NPs and trypsin.

Here, we investigate the binding of Au NPs with trypsin in aqueous solutions. The main goals are to characterize the binding mechanism and the conformational changes of the protein that occur in response to the interaction with Au NPs. The cooperative binding mechanism, the thermodynamic parameters, the binding constants, the major forces and the changes in the protein conformation are investigated in detail using a variety of physical and spectroscopic techniques. We construct a binding model for Au NPs and trypsin, which helps us to gain insights into the distribution and

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transport of NPs in a biological system, as well as the metabolism of Au NPs. This deep understanding of the NP–protein complex is expected to be beneficial to the future development of functional and safe NPs.

2. Experimental

2.1. Materials

The glutathione-stabilized Au NPs used in the experiments were identical to those reported by us previously [15], and the concentration of the stock solution was 4.14×10^{-5} M. Trypsin and fluorescein isothiocyanate (FITC) were purchased from Sigma and used without further purification. The 1.0×10^{-4} M trypsin stock solution was prepared by dissolving trypsin in 10 mL of 0.01 M phosphate buffered saline (PBS, pH 7.4). Dialysis cellulose membranes (cutoff 10 kDa; Medicell International, London, United Kingdom) were prepared according to standard methods and immediately used. Deionized water was used throughout to form the aqueous solutions, and the other chemicals were of analytical grade and used as received.

2.2. Methods

2.2.1. Fluorescence spectroscopy

The fluorescence spectra were recorded using a Cary Eclipse fluorescence spectrophotometer (Varian, America) operating at an excitation wavelength of 278 nm. Both the excitation and emission slit widths were 5 nm. Two-hundred and fifty microliters of the stock solution of trypsin was titrated via successive addition of the stock solution of Au NPs, to give final concentrations in the range 0–1.02×10^{-6} M. To evaluate the effect of temperature on the interaction between trypsin and the Au NPs, fluorescence spectra were measured at temperatures of 293, 303 and 310 K. The temperature was maintained using an SHPDC-0515 thermostatic bath.

2.2.2. Synchronous fluorescence spectroscopy

Synchronous fluorescence spectra were obtained by scanning simultaneously the excitation and emission wavelengths using a monochromator. When the wavelength difference $\Delta\lambda = \lambda_{em} - \lambda_{ex}$ was fixed at either 15 or 60 nm, the spectra provide characteristic information of tyrosine or tryptophan residues, respectively [16].

2.2.3. The measurement of fluorescence lifetime

Fluorescence lifetime measurements were performed on a FLS 980 fluorometer (Edinburgh Instruments Ltd.) equipped with an integrating sphere in room temperature. The integrating sphere consists of a 120 mm inside diameter spherical cavity. The concentration of trypsin and Au NPs is, respectively, 0.5×10^{-6} M and 1.0×10^{-6} M. The fluorescence lifetime data for protein in the absence and presence of Au NPs were fitted to a multi-exponential function.

2.2.4. Labeling of protein with FITC

Trypsin was dissolved in carbonate-bicarbonate buffer (pH 9.0), and labeled with FITC in a darkened lab. The FITC was first dissolved in a few drops of dimethylformamide (DMF), then made up to volume in pH 9.0 carbonate–bicarbonate buffer. The mole ratio of FITC to trypsin was varied over the range 0.1–20 by changing the amount of FITC added while keeping BSA constant. Samples were left on a shaker to react for 8 h at room temperature in the dark. The FITC–BSA solution was dialysed with cellulose membranes against 0.01 M PBS (pH 7.4) four times for 1 day each in the dark at 8 °C to remove any uncoupled FITC. The light absorption at 495 nm was finally below 0.005 for the supernatant. The dialysis membranes were washed prior to use in distilled water for at least 4 h with extensive rinsing once every hour. The extent of FITC conjugation with the protein in each reaction was monitored by absorbance in the regions corresponding to protein and FITC (278 nm for trypsin, 495 nm for FITC).

2.2.5. UV absorption spectrum

The UV absorption spectra of trypsin and trypsin/Au NPs complexes were recorded at room temperature using a TU-1810 spectrophotometer (Puxi Analytic Instrument Ltd., China) equipped with 1.0 cm quartz cells.

3. Results and discussion

3.1. Mechanism of fluorescence quenching and binding mode

Fluorescence quenching may occur via various mechanisms, but is usually classified as either static or dynamic. Static quenching arises from the formation of a ground complex, whereas dynamic quenching mainly results from collisional diffusion or intermolecular rearrangements. Dynamic and static quenching can be distinguished in several ways, including the different dependence on temperature, the determination of values of the bimolecular quenching rate constant (K_q) and comparison of the absorption spectra of protein with and without a quenching agent.

An effective method of distinguishing between dynamic and static quenching is ultraviolet–visible (UV–vis) differential spectroscopy [17]. Collisional quenching influences only the excited states of the fluorophore, and the absorption spectrum of the biomacromolecule remains unchanged. In contrast, the formation of a ground state complex alters the absorption spectrum of the fluorophore [18]. Fig. 1 shows UV–vis spectral data for the trypsin–NP system, as well as trypsin and the Au NPs only. The UV–vis absorption spectrum of trypsin, the spectrum of the Au NPs, and the differential spectrum are not superposed. This indicates that the quenching mechanism of trypsin by Au NPs results from the formation of a ground state complex.

The sensitivity, reproducibility and convenience of fluorescence spectroscopy have led to its widespread application in the study of the structure and conformation of proteins [19–22]. Specifically, when proteins interact with small molecules [19,20] or NPs [21,22], the emission characteristics of tryptophan, tyrosine and phenylalanine residues can provide useful information for investigating the binding mechanisms. This work is based on the fact that Au

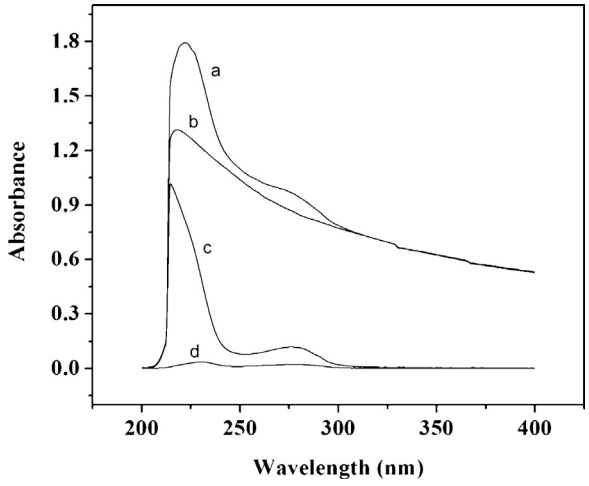

Fig. 1. UV–vis absorption spectra of (a) the trypsin–Au-NP system, (b) the Au NPs only, (c) trypsin only, and (d) the UV–vis differential absorption spectrum between the trypsin–Au-NP system and the Au NPs. The concentration of trypsin was 1.0×10^{-6} mol L^{-1}, and the concentration of Au NPs was 5×10^{-7} mol L^{-1}.