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Effects of selected two noble metal ions in medicine on the structure and activity of bovine serum albumin: A multi-spectral studies

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ABSTRACT

In the present study, the toxic influence of two metallic ions on serum albumin was studied by using fluorescence, UV/vis, circular dichroism spectroscopy, and molecular docking, with the aim of delineating the effects of Pt(II) and Au(III) ions on the secondary and tertiary structure, the transportation function, the thermal stability, and the esterase-like activity of bovine serum albumin (BSA). The conformational change occurring in protein structure was monitored by CD and fluorescence spectroscopy. Pt(II) and Au(III) could react with some functional groups of BSA, making the secondary structure change and increasing the microenvironment around Tyr residues of BSA. The content of α-helix decreased remarkably and the contents of β-sheet, and random coil increased with increasing concentration of Pt(II) or Au(III) ion. Compared with Pt(II) ion, Au(III) ion is more likely to damage the tertiary structure of BSA and a higher number of Tyr residues are affected in Au(III)-BSA system. The fluorescence and UV/vis spectral results indicated the formation of Pt(II)-BSA and Au(III)-BSA complexes. In addition, metal ion binding decreased the ANS binding ability, the reversibility of the thermal unfolding process and the esterase-like activity of BSA. Therefore, this paper is expected to improve the toxicity evaluation mechanism of platinum and gold ions.

1. Introduction

The potential of metal ions as pharmaceuticals has importantly contributed to the research of new drugs for various diseases [1]. In recent years, a lot of noble metal-based complexes have been designed as anticancer agents and applied in potential chemotherapeutics [2]. For example, some platinum metal-based anticancer drugs, cisplatin, carboplatin, oxaliplatin, and other platinum metal-based anticancer drugs are often used in different chemotherapeutic regimes [3–5]. Gold complexes are another important kind of metal-based drugs. For example, various gold thiolate drugs and gold phosphine-based complexes were found to be highly cytotoxic to tumour cells in vitro and have been used in the clinic to treat mainly rheumatic diseases [6,7]. Overall, platinum and gold metal-based drugs have been demonstrated to possess unique properties and showed important candidates for bio-medicine applications. Since, cancer chemotherapy is a long process, the metabolism of platinum and gold metal ions in vivo take a long time.

Human intake of platinum and gold ions occurs during wound routes. When these metal ions add in human blood, they are most likely to bond with nucleic acids and/or proteins having binding sites with metal ions during the accumulation in the target organs (liver, bone and kidney). Especially the protein properties including charge, moment, and electrons are the major factors for their binding with metal ions [8]. The influences of metal ions on the folding/unfolding of proteins and the effects of metal ions on the protein conformational and functional changes are illustrated by their relevance in numerous neurodegenerative diseases such as Wilson's, Alzheimer's, and Parkinson's diseases [9]. If the metal-protein complex is formed, the secondary structure of protein, the micro-environments of some residues can be affected by metal binding [10]. R. Guzzia et al. have used multi-spectral methods to investigate the heat induced aggregation of human serum albumin(HSA) with and without an equimolar amount of Cu(II) and Zn (II). The results showed that the presence of Cu(II) and Zn(II) ions does not affect the thermally induced aggregation process and the morphology of HSA aggregates [11]. Actinides and Ce(IV) can alter the secondary conformation of HSA with a significant decrease of a-helix [12]. As for platinum and gold, their toxicity can be ameliorated with chelation therapy with anticancer agents. Hence, studies on the

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interactions of Pt or Au ion with proteins are important in understanding their transport, distribution, toxicity mechanisms, side-effects at molecular level. The analysis of metal ion–protein binding interactions is not only to understand the role of metal ions in living systems, but also to elucidate the structure-function relationships and biological properties of metal-proteins complexes [9].

Serum albumin, the most abundant protein in the circulatory system, accounts for 52–60% of the total plasma protein of a wide variety of vertebrates [13]. It has many important physiological functions, such as to maintain the blood pH and osmotic pressure, to transport different kind of bioactive small molecules [14–16]. As one of serum albumin, BSA is often selected as protein model about the binding interaction of biomolecule with metal ions [17–20]. Chromium (III) was found to be induce the fluorescence quenching of BSA by a dynamic process in the beginning, and to turn static at later stages. In addition, both dichromate and chromate bind in similar electrostatic fashion to BSA [19]. Compared with Ca^{2+}, Al^{3+}, Ni^{2+}, and Co^{2+}, both Cu^{2+} and Zn^{2+} are easy to cause the precipitation of BSA [17]. W. Wang et al. have also using fluorescence and CD spectroscopy to study the influence of metal ions including Zn^{2+}, Ca^{2+}, Cd^{2+} and Co^{2+} on folding pathway and conformational stability of bovine serum albumin (BSA), indicating the evidence that the metal ions alter the urea-induced unfolding pathway of protein [20]. However, there are little research works about the binding interactions of Pt or Au ion with BSA and the effects of their binding on the c conformational stabilities and changes of protein.

Herein, the thermal-induced structural stability by Pt(II) or Au(III) binding is characterized by far-UV circular dichroism (CD), fluorescence, and UV/vis spectroscopy. It is found that Pt(II) and Au(III) behave effects on the structural stability and unfolding of BSA. The Pt(II) and Au(III) binding does great damage to the secondary and tertiary level structures of BSA simultaneously. This work has important implications not only to interpret the mechanism and give insight of the binding interactions of Pt(II) or Au(III) ion with serum albumin, but also to create a new way to elucidate the toxicity of heavy metal ions targeting to proteins.

2. Materials and methods

2.1. Materials

Both $HAuCl_4{\cdot}3H_2O$ (Au% ≥ 48%) and K_2PtCl_4(≥98%) was purchased from Aladdin Industrial Corporation(Kaplan Ave, City of Industry). BSA (A1933, lyophilized powder, ≥98%) was purchased from Sigma (St, Louis, MO, USA). Analytical standards of p-nitrophenyl acetate (p-NPA) (≥ 98%) and 8-Anilino-1-naphthalenesulfonic acid (ANS) (≥ 98%) were obtained from Aladdin Industrial Corporation (Kaplan Ave, City of Industry). All other chemicals used were of analytical reagent or higher. Experiments were carried out in 0.02 mol/L potassium phosphate buffer (pH 7.40).

2.2. Methods

2.2.1. Circular dichroism measurements

CD spectra were obtained on a Chirascan spectrometer (Applied Photophysics Ltd., Leatherhead, Surrey, UK). BSA secondary structure was followed in the far-UV range from 200 to 260 nm. For each BSA solution, the scan rate and response time were set at 30 nm/min and 0.5 s with three scans. Hence, CD samples contained 2.0×10^{-6} mol/L BSA, the concentrations of Pt(II) or Au(III) ion were increase from 0 to 20.0×10^{-6} mol/L in metal ion-protein system. In order to obtain the effects of Pt(II) or Au(III) ion binding on the thermal stability of BSA, the reaction temperature was varied from 20 to 90 °C, taking CD scan at 5 °C intervals, with 300 s increments. The different secondary structures of protein were analyzed by using the program CD spectra deconvolution program (CDNN) [21].

2.2.2. Fluorescence measurements

A LS–50B Spectrofluorimeter (Waltham, Massachusetts, USA) was used to record fluorescence spectra of BSA. The excitation wavelength was excited at 280 nm, and emission wavelength was scanned from 280 to 500 nm and the slit widths of excitation and emission were set at 5 nm. Synchronous fluorescence spectra (SFS) of BSA were recorded at $\Delta\lambda(\lambda_{em}-\lambda_{ex}) = 15$ or 60 nm. During ANS binding assay experiment, BSA (2.0×10^{-6} mol/L) was firstly prepared with pH 7.40 0.02 mol/L potassium phosphate buffer, and then Pt(II) and Au(III) ions were added to BSA-ANS solution and allowed to react for 24 h. The λ_{ex} was set at 370 nm in order to obtain the fluorescence spectra of ANS. The Time-resolved intensity decays were carried out using a FLS920 Combined Fluorescence Lifetime and Steady State Spectrometer (Edinburgh, UK). During the time- resolved fluorescence measurement, BSA in the absence and presence of Pt(II) or Au(III) ions solution were excited at 280 nm and the decay was measured through a 50 ns time scale at a time resolution of 0.0122 s/channel.

2.2.3. UV/vis measurements

A Specord S 600 spectrophotometer (Analytik jena, Thuringia, Germany) was used to obtain the UV/vis absorption spectra of BSA solution. Pt(II) or Au(III) ion was added into each 2.5 mL of 2.0×10^{-6} mol/L BSA solution with potassium phosphate buffer and allowed to react for 24 h before measurement. In addition, the effect of Pt(II) or Au(III) ion on the esterase activity of BSA were also recorded by using UV/vis spectral method. The p-nitrophenyl acetate (p-NPA) was selected as substrate to measure the esterase activity of BSA, because p-NPA is decomposed into p-nitrophenyl at 405 nm (ε = 17,700 L/mol/cm) [22,23].

2.2.4. Molecular modeling

The docking software AutoDock 4.2 and AutoDock Tools 1.5.6 were also used to calculate the binding interactions of Pt(II) and Au(III) ion with BSA. The native structure of BSA was taken from Protein Data Bank having PDB ID 3V03 [24]. The three-dimensional structures of $[PtCl_4]^{2-}$ and $[AuCl_4]^-$ were generated and were optimized at DFT/B3LYP/ LanL2DZ by Gaussian 09 [25]. All water molecules and compounds in the original structure of BSA were removed and the polar hydrogen and the Gasteiger charges were added before the molecular modeling calculation. In addition, a big grid box of 126×126×126 points was used to provide enough space for the translational and rotational walk of $[PtCl_4]^{2-}$ and $[AuCl_4]^-$. All other default parameters were used in the blind docking calculation. When molecular calculation added, the Molegro Molecular Viewer software was used to analysis the most favorable docked structure of Pt(II) and Au(III) [26].

3. Results and discussion

3.1. The secondary structural changes of BSA induced by Pt(II) or Au(III) ion binding

CD comprises a very helpful method to provide more detailed information about the secondary structure a globular protein [19,27–31]. Therefore, CD was used to provide the secondary structural changes of BSA induced by Pt(II) or Au(III) ion binding. The CD spectrum of BSA alone and BSA- Pt(II)/Au(III) system at room temperature in the far-UV region were shown in Fig. 1, A1, B1, two strong negative ellipticity at 208 nm ($\pi \rightarrow \pi^*$) and 222 nm ($n \rightarrow \pi^*$) and an intense positive peak around 190 nm ($\pi \rightarrow \pi^*$) showed that it is predominately α-helical of BSA. Obvious changes in the spectra of BSA were observed with increaing concentration of Pt(II) or Au(III) ion, indicating that the α-helical structure of BSA was disordered. CD spectra were evaluated by CDNN software and the percentages of protein different structure were given in Fig. 1 (A2, B2). It is found that the relative contents (in %) of the α-helix, β-sheet, and random coil changed obviously. The metal ions-induced alterations in the proportion of BSA secondary structures