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Comparative serum albumin interactions and antitumor effects of Au(III) and Ga(III) ions

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ABSTRACT

In the present study, interactions of Au(III) and Ga(III) ions on human serum albumin (HSA) were studied comparatively *via* spectroscopic and thermal analysis methods: UV–vis absorbance spectroscopy, fluorescence spectroscopy, Fourier transform infrared (FT-IR) spectroscopy and isothermal titration calorimetry (ITC). The potential antitumor effects of these ions were studied on MCF-7 cells *via* Alamar blue assay. It was found that both Au(III) and Ga(III) ions can interact with HSA, however; Au(III) ions interact with HSA more favorably and with a higher affinity. FT-IR second derivative analysis results demonstrated that, high concentrations of both metal ions led to a considerable decrease in the α-helix content of HSA; while Au(III) led to around 5% of decrease in the α-helix content at 200 μM, it was around 1% for Ga(III) at the same concentration. Calorimetric analysis gave the binding kinetics of metal–HSA interactions; while the binding affinity (K_a) of Au(III)–HSA binding was around $3.87 \times 10^5\ M^{-1}$, it was around $9.68 \times 10^3\ M^{-1}$ for Ga(III)–HSA binding. Spectroscopy studies overall suggest that both metal ions have significant effects on the chemical structure of HSA, including the secondary structure alterations. Antitumor activity studies on MCF7 tumor cell line with both metal ions revealed that, Au(III) ions have a higher antiproliferative activity compared to Ga(III) ions.

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Introduction

Human serum albumin (HSA) is the most abundant and the major ligand binding serum protein, and one of the most important focal point in pharmaceutical industry, since it can bind with various drug molecules and modify their pharmacokinetic properties [1]. The chemical structure of HSA is composed of a single polypeptide chain of 585 amino acids that is divided into three homologous domains (I–III): I (1–195), II (196–383) and III (384–585); and each domain consists of two subdomains (A and B) [2]. Due to its chemical nature, HSA involves in various important physiological and pharmacological processes; therefore, small molecules (of HSA) interactions have been an essential field of research in biochemistry, life sciences, clinical medicine and toxicology for the last decades [2].

Different types of gold and gallium complexes have been synthesized and evaluated in terms of their antitumor potency [3–7]. It was reported that, while gold compounds can affect cancer cell proliferation by inhibiting telomerase and STAT3 [6] or enzyme thioredoxin reductase activity [7]; gallium complexes can exhibit antitumor activity in the presence of phenolate rings [4], amine and pyridine groups by inhibiting the activity of proteasome [8]. Previous studies reported that, *in vitro* interactions of Au(III) complexes with calf thymus DNA are generally weak, different from cisplatin, suggesting these complexes have a weaker binding profile on genomic DNA [9]. However, it is also known that, Au(III) complexes can bind strongly with model proteins, can overcome the cisplatin resistance, and some of the Au(III) compounds have shown high antitumor activity at *in vitro* conditions, indicating different mechanisms take place for Au(III) compounds' antitumor activity compared to cisplatin [9]. Ga(III) has shown antitumor activity against several cancer types and it shows antitumor activity and apoptosis mainly *via* iron deprivation [10]. On the other hand, it was also reported that, Ga(III) shows low toxicity on many tumor cell types and does not generally induce apoptosis at therapeutic conditions, whereas it shows apoptosis when concentrations of Ga(III) reaches especially high values [10]. Although separate studies of gold and gallium for their antitumor activity are numerous in the literature, studies on comparative antitumor activity of these

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metals are rare, so further studies are needed to discriminate between these two metals for their antiproliferative activity.

In this paper, the interactions of Au(III) and Ga(III) ions with HSA were examined by several spectroscopic and thermal analysis techniques, and differential antitumor activity of these two metal ions on a model tumor cell line were determined. We describe here the effects of Au(III) and Ga(III) bindings on HSA and their antiproliferative activity on tumor cells comparatively, and anticipate that our results will enhance further studies on the antineoplastic properties of Au(III) and Ga(III) ions.

Materials and methods

Materials and sample preparation

All chemical reagents and HSA in lyophilized powder form were purchased from Sigma–Aldrich (USA). Au(III) chloride hydrate, Ga(III) nitrate hydrate and HSA solutions were prepared in a 10 mM Tris (tris(hydroxymethyl)aminomethane)–HCl buffer (pH 7.40) to ensure that the pH of the solutions are stable in all experiments. Powdery HSA stock was stored at +4 °C. Fresh HSA solutions were prepared prior to each experiment.

UV–vis absorbance spectroscopy

HSA solutions were prepared in 10 mM Tris–HCl buffer. Different concentrations (10, 40, 80, 160, 320 μM) of Au(III) chloride hydrate or Ga(III) nitrate hydrate solutions were mixed with 10 μM HSA solutions and then incubated for 2 h. After 2 h incubation, UV–vis absorbance spectra of the samples were read by a NanoDrop 2000 Spectrophotometer (Thermo-Scientific, USA). The wavelength range was 220–420 nm.

Fluorescence spectroscopy

HSA solutions were prepared in 10 mM Tris–HCl buffer with a final concentration of 10 μM. Different concentrations (10, 20, 40, 80, 160, 320, 640, 1280 μM) of Au(III) chloride hydrate and Ga(III) nitrate hydrate solutions were mixed with HSA solutions and fluorescence readings were taken after 10 min of incubation in an unilluminated room. Excitation wavelength was 290 nm and emission wavelength range was 300–450 nm. Reading was conducted with a SpectraMax M5 Microplate Reader (Molecular Devices, USA) in a quartz cuvette with 1 cm path length.

Isothermal titration calorimetry (ITC)

Calorimetric analysis of metal–HSA bindings was carried out at 25 °C on an iTC_{200} microcalorimeter (Microcal®). The sample cell was filled with 200 μM of HSA and 4 mM of Au(III) chloride hydrate or Ga(III) nitrate hydrate solutions were injected to the reaction cell. The reaction cell was stirred continuously at 300 rpm to avoid protein foaming. Origin 7.0 software was used for data acquisition and manipulation. ΔG (Gibbs free energy) calculations were made by the following formula [11]:

$$\Delta G = -RT \ln K_a = \Delta H - T\Delta S \quad (1)$$

where T is the absolute temperature in Kelvin (298 K) and $R = 8.3151\ \mathrm{J\,mol^{-1}\,K^{-1}}$.

FT-IR spectroscopy

HSA solutions with a concentration of 250 μM, and Au(III) chloride hydrate or Ga(III) nitrate hydrate solutions at varying concentrations (100 and 200 μM at final) were prepared for FT-IR measurements. HSA and metal solutions were mixed at different ratios and incubated for 2 h. After 2 h incubation, 20 μL of final mixes were dropped and dried on a 96-well plate at 37 °C for 1 h. After drying, the 96-well plate was utilized in FT-IR transmittance analysis by using Nicolet 6700 FT-Raman Spectrometer (Thermo-Scientific, USA). FT-IR measurements, curve-fitting analysis and basic modifications (*e.g.*, baseline and background corrections) were done by OMNIC™ software. Background corrections for H_2O and CO_2 were carried out for each analysis. Duplicate samples were utilized in each analysis and experiments were repeated for at least two times.

Protein secondary structure analyses were made by using OMNIC™ software. Second derivative analysis of the amide I region ($1600–1700\ \mathrm{cm^{-1}}$) was performed to examine how amide I region was affected by exposure to different concentrations of metal solutions. Curve fitting analysis of the amide I region was performed to estimate approximate ratios of the three main subgroups of protein secondary structures with respect to the total protein content, which are adopted at the regions of: $1630\ \mathrm{cm^{-1}}$ for β-sheet, $1655\ \mathrm{cm^{-1}}$ and $1661\ \mathrm{cm^{-1}}$ for α-helix, and $1678\ \mathrm{cm^{-1}}$ for β-turns. All tests were done in duplicate.

Cell viability assay

MCF-7 cells (American Type Culture Collection) were cultured in 75 $\mathrm{cm^2}$ culture flasks using Dulbecco's Modified Medium (DMEM; Gibco) supplemented with 10% FBS and 1% penicillin/streptomycin (Gibco) at 37 °C and 5% CO_2. The viability of them were determined *via* Alamar blue assay. In 96 well-plate, cells were seeded at the density of 1×10^4 cells per well and cultured at standard conditions prior to metal salt solutions treatment. Varying concentrations of sterilized metal salt solutions (0–1000 μM) prepared in DMEM were added to the harvested cells, and the cells were then incubated for 24 h at 37 °C and 5% CO_2. Prior to the end of 24 h incubation period (after 21 h incubation under dark conditions), old medium was replaced with a serum free fresh culture medium containing 10% Alamar blue (AbD Serotec, USA). Fluorescence value of each group was measured (Ex = 530 nm, Em = 590 nm) with a M5 Microplate Reader (Molecular Devices, USA). Fluorescence value of each group was divided by the fluorescence value of untreated control and presented as a percentage of the control. Results were analyzed for statistical significance using ANOVA and Tukey tests. Changes were considered significant at $p < 0.05$. Each experiment was performed in quadruplicate ($n = 4$).

Results and discussion

UV–vis absorbance spectra

Absorption peaks of HSA were observed at 280 nm (Fig. 1). While presence of Au(III) at increasing concentrations causes changes in the actual spectrum of HSA with an increase in the total intensity of absorbance at 280 nm, Ga(III) did not exhibit a considerable difference on the HSA spectra in general. This result may indicate stronger binding characteristics and higher binding affinity for Au(III), since Au(III) has a higher impact on the spectra of HSA, probably by significantly altering the actual geometry of this protein. While the actual spectrum of HSA is highly altered at increasing concentrations of Au(III), the total intensity of absorption spectra increased gradually with the increase in Au(III) content. Such an increase in the absorption spectra may indicate the numbers of aromatic acid residues of HSA which are extended into aqueous environment are increased gradually [12]. This outcome may occur due to the exposure of a large hydrophopic pocket (*i.e.*, IIA subdomain of HSA), which contains a tryptophan (Trp-214), to

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