Spectroscopic characterization of the interactions of bovine serum albumin with medicinally important metal ions: platinum (IV), iridium (III) and iron (II)

Hassan A. Alhazmi1,2, Mohammed Al Bratty1, Abdulkarim M. Meraya3, Asim Najmi1, Md Shamsher Alam1, Sadique A. Javed1 and Waquar Ahsan1

1Department of Pharmaceutical Chemistry, College of Pharmacy, Jazan University, PO Box 114, Jazan, Saudi Arabia; 2Substance Abuse and Toxicology Research Center, Jazan University, PO Box 114, Jazan, Saudi Arabia; 3Department of Clinical Pharmacy, College of Pharmacy, Jazan University, PO Box 114, Jazan, Saudi Arabia

Serum albumin protein plays a key role in the transportation and distribution of bioactive species including metal ions and metal-based drugs and, therefore, the nature of their binding could provide important insight for the development of new drugs. In the present investigation, binding interactions of bovine serum albumin (BSA) with three biologically important metal ions: Pt4+, Ir3+ and Fe2+ were screened using easy-to-use and cost-effective Fourier-Transform Infrared (FT-IR) and Ultraviolet-Visible (UV-Vis) spectroscopic techniques. Prior to the screening, the protein and metal ions were allowed to interact at physiological pH (7.4) and the spectral changes were monitored upon interaction. In FT-IR spectrum, the position of amide I band (C=O stretching) was shifted from 1652 cm−1 in case of free BSA to 1659, 1657 and 1656 cm−1 in BSA-Pt4+, BSA-Ir3+ and BSA-Fe2+ complexes, respectively. This spectral shifting was due to the binding of metal ions to N and O atoms of BSA peptide bonds. The interaction was further demonstrated by a remarkable reduction in spectral intensities of amide I and II bands. Secondary protein structure analysis revealed conformational changes characterized by a substantial decrease in α-helix (11.29–27.41%) accompanied by an increase in β-sheet and β-antiparallel contents. The absorption of BSA at a constant concentration at 280 nm was successively reduced as the concentration of Pt4+ and Ir3+ ions increased. On the other hand, the absorption of BSA-Fe2+ complex successively increased with the increase in the concentration of Fe2+ in the test solution. The binding constants for BSA-Pt4+, BSA-Ir3+ and BSA-Fe2+ complexes were calculated to be 1.55×104, 5.67×103 and 3.78×102 M−1, respectively. The results revealed that the three metal ions showed binding affinities with the BSA protein in the order: Ir3+>Fe2+>Pt4+

Keywords: bovine serum albumin, binding interaction, metal ions, FT-IR spectroscopy, UV-Vis spectroscopy

Received: 25 August, 2020; revised: 09 November, 2020; accepted: 01 December, 2020; available on-line: 17 February, 2021

Acknowledgements of Financial Support: This work was funded by Deanship of Scientific Research, Jazan University, Jazan, Saudi Arabia (grant no. Waed-41-26).

Abbreviations: ACE, Affinity Capillary Electrophoresis; BSA, Bovine Serum Albumin; DNA, Deoxyribonucleic Acid; FT-IR, Fourier-Transform Infrared; HSA, Human Serum Albumin; NMR, Nuclear Magnetic Resonance; NO, Nitric oxide; Trp, Tryptophan; UV-Vis, Ultraviolet-Visible.

INTRODUCTION

Serum albumin is the most abundant and one of the most extensively investigated circulatory plasma protein owing to its multifunctional characteristics and remarkable capacity to bind a variety of ligands. In the biological system, it is responsible for transportation and distribution of a large number of metal ions, therapeutic agents, metabolites, nutrients and other compounds and, therefore, displays several biochemical and pharmaceutical applications (de Wolf & Brett, 2000). Among different serum albumins, bovine serum albumin (BSA) shows the highest resemblance to human serum albumin (HSA) as the only differences concern the surface of the molecules. The binding characteristics of both albumins are similar; therefore, BSA is widely chosen as an appropriate model protein in most of the drug-protein and metal ion-protein interaction studies (Majorek et al., 2012; Urquiza et al., 2012; Xiang et al., 2010). BSA is a relatively large heart-shaped globular protein (molecular weight 66.4 kDa), consisting of 583 amino acid residues in a single chain cross-linked with seventeen cysteine residues (Fig. 1). The binding sites for endogenous and exogenous ligands including drugs are generally present in subdomains IA and IIA of BSA molecule; these sites are also known as Sudlow’s site I and II, respectively (Tayeh et al., 2009).

Some metal ions are important because of their crucial role in maintaining the normal physiological functions of the biological system, and they are also used to treat and

Figure 1. Bovine serum albumin (3D structure) (Downloaded from protein data bank; ID: 4F5S (www.rcsb.org))
diagnose a variety of diseases. Binding of the metal ions to biomolecules including proteins, serves various physiological and medicinal purposes, such as transportation of bioactive metal ions as well as metal-based drugs to their sites of action through metal ion-serum albumin interaction (Alhazmi, 2019). Metal ions are complexed to develop metal-based drugs, which have been extensively studied as potential therapeutic agents for several diseases. Currently, a large number of metal-based therapeutic agents are used for the treatment and diagnosis of various diseases. In addition, a range of metal complexes is in the clinical trial phase of drug development demonstrating a significant therapeutic potential for the management of several health issues (Boros et al., 2020; Bruijnincx & Sadler, 2008).

It is evident that the studies of serum protein-metal ion interactions are important to understand the pharmacology and pharmacokinetic profile of bioactive metal ions as well as metal-based drugs. Moreover, binding to metal ions changes the conformation of the protein molecule and may lead to protein unfolding and even aggregation if exposed to higher concentrations and can cause neurodegenerative diseases. The extent and type of binding of metal ions to albumin and other important body proteins are important to characterize in order to minimize the exposure and therefore the untoward effects of metal-based compounds on the body. The protein-metal ion interactions have been characterized using a number of modern analytical techniques including fluorescence spectroscopy, FT-IR spectroscopy, UV-Vis spectrophotometry, circular dichroism spectroscopy, X-ray crystallography, NMR spectroscopy, mass spectrometry, capillary electrophoresis and affinity chromatography (Alhazmi et al., 2017; Samari et al., 2012; Xu et al., 2008; Belatik et al., 2012; Grasso and Spoto 2013). FT-IR spectroscopy and UV-Vis spectrophotometry have several advantages over the other techniques because they offer simple, fast, accurate and cost-effective methods for studying protein-metal ion interactions. In UV-Vis spectrophotometry, measurement of UV-absorption of protein before and after the interaction with the metal ion provides an insight to their complexation behavior. Moreover, the absorption spectrum is also helpful to understand the structural changes in protein after ligand interaction (Kragh-Hansen, 1981; Xu et al., 2013). FT-IR spectroscopy provides information about the structural dynamics and ligand interactions of biomolecules such as proteins, nucleic acids, enzymes etc. Characteristic functional groups present in the structures of biomolecules possess vibrational fingerprints at specific frequencies of IR light allowing the determination of structure and composition of these functional groups by examining the position and intensity of the spectral bands in FT-IR. Amide I (C=O stretching) and amide II (NH bending) are the characteristic bands present in the FT-IR spectrum of BSA protein (Korkmaz et al., 2012; D’Souza et al., 2008; Jackson and Mantsch 1996) (Fig. 2), the position and intensities of which can be measured before and after interaction with metal ions to assess the nature, type and extent of binding.

Herein, the present study was focused on the investigation of binding interactions of three medicinally and physiologically important metal ions (Pt(IV), Ir(III) and Fe(III)) with BSA at physiological pH using FT-IR spectroscopy and UV-Vis spectrophotometry. With the help of UV-Vis spectrophotometry, conformational changes in the BSA molecule were identified and measured. The binding constant was determined using the absorption spectrum of the metal ion-BSA complex by taking fixed concentration of protein and increasing amounts of metal ions. FT-IR spectrum was used to evaluate the alteration of characteristic amide bands of BSA upon interaction. Moreover, a possible modification in the secondary structure of BSA (amide I band) was studied using the curve-fitting method.

**MATERIALS AND METHODS**

**Chemicals and instruments**

Bovine Serum Albumin (BSA, 99%), platinum (IV) chloride (PtCl₄), iridium (III) chloride hydrate (H₂Cl₃IrO), iron (II) chloride (FeCl₂), Tris powder, CH₃COOH were purchased from Sigma Aldrich, Steinheim, Germany and were used without further purification. The double-distilled ultrapure water was produced in our lab. FT-IR spectrometer (Nicolet iS10, Thermo Scientific, USA) and double beam UV-Visible Spectrophotometer (Shimadzu, Japan) were used to perform FT-IR and UV-Vis spectroscopic measurements, respectively.

**Preparation of the solutions**

Tris buffer (20 mM) solution was prepared by dissolving 2.42 g of Tris base in 200 mL ultrapure water and adjusting the pH to 7.4 using acetic acid. The final volume was adjusted to 1000 mL using ultrapure water. Protein (BSA) solution (0.5 mM) was prepared by dissolving 1.65 g of BSA powder in 50 mL of the described Tris buffer. Stock solutions of metal ions (1 mM) were prepared by dissolving appropriate quantities of metal salts in 50 mL Tris buffer.

**FT-IR spectroscopic measurements**

**Methodology.** The protein (BSA) and metal ions (Ir(III), Fe(II) and Pt(IV)) solutions were appropriately mixed to obtain target protein (0.25 mM) and metal ion (0.25 and 0.5 mM) concentrations. The complexes of metal ions and the protein were prepared separately by incubating the above mixtures at room temperature for 2 h. FT-IR spectra of hydrated film samples of BSA protein alone and metal ion-protein complexes were obtained in the range of 4000–400 cm⁻¹ at 4 cm⁻¹ resolution and 100 scans. Difference spectrum was generated by subtracting the spectrum of pure BSA from the spectrum of metal ion-protein complexes (Dousseau et al., 1989).

![Figure 2. Amide I and Amide II band vibrations in the infrared spectrum of BSA protein](image)
Protein conformation analysis. Upon metal ion-protein complex formation, spectral shifting and intensity variations of amide I (1700–1600 cm⁻¹), amide II (1550 cm⁻¹) and amide A bands (3500 cm⁻¹) were analyzed. Alterations in the secondary structure of the protein were also studied using the previously reported method (Byler et al., 1986). Six significant peaks of amide I band, associated with the BSA secondary structure were deconvoluted by the curve fitting method using OriginPro 2019b software (Byler et al., 1986). Using Gaussian functions, the characteristic peaks of α-helix, random coil, β-sheet, β-turn and β-antiparallel at 1660–1650, 1648–1638, 1637–1614, 1678–1670 and 1691–1680 cm⁻¹, respectively, were set and their corresponding areas were calculated. These areas were summed up to get the total area of amide I band. Percentages of amide I components were calculated by dividing the corresponding peak area by the total area (Ahmed et al., 1995).

UV-Vis spectroscopy measurements

Methodology. The UV-Vis spectroscopic analyses were performed to investigate the complexation of BSA with tested metal ions using the method described previously (Zhong et al., 2004; Stephanos et al., 1996) with a slight modification. The 0.5 mM BSA and 1 mM metal ion solutions (Ir⁴⁺, Fe⁷⁺ and Pt⁴⁺) were prepared separately by dissolving accurately measured quantity of BSA and metal salts in 20 mM Tris buffer solution pH 7.4. The metal stock solutions were further diluted using the same Tris buffer to achieve working concentrations of 8, 16, 24, 32, 40, 48, 56 and 64 µM, whereas, the BSA solution was diluted to a concentration of 24 µM. The protein and metal ion solutions were mixed in equal proportions (1:1) by stirring at room temperature in order to obtain different metal ion concentrations of 0, 4, 8, 12, 16, 20, 24, 28, 32 µM and the BSA concentration of 12 µM in the final solution. The above mixtures were vortexed and incubated at 25±2°C for 2 h. After the incubation, absorption spectra were recorded for the BSA-protein (0.25 mM), a notable reduction in intensities of the characteristic peaks of α-helix, random coil, β-sheet, β-turn and β-antiparallel at 1660–1650, 1648–1638, 1637–1614, 1678–1670 and 1691–1680 cm⁻¹, respectively by dissolving accurately measured quantity of BSA and metal salts in 20 mM Tris buffer solution pH 7.4.

Determination of Binding Constants. Binding constant (K) was determined using absorbance data for BSA before and post-complexation with metal ions using a previously reported method (Zhong et al., 2004; Stephanos et al., 1996). If we assume only one type of interaction exists between metal ions and BSA in the aqueous solution, equations 1 and 2 can be established:

\[
BSA + Metal \rightleftharpoons BSA:Metal
\]

\[
K = \frac{[BSA: Metal]}{[BSA][Metal]}
\]

where: \(K\) is the binding equilibrium constant for metal ion:BSA complexes.

Considering \([BSA:Metal] = C_B\)

\[
K = \frac{C_B}{(C_{BSA}\cdot C_{Metal}) - C_B}
\]

Where: \(C_{BSA}\) and \(C_{Metal}\) are the analytical concentrations of BSA and metal ions in aqueous solutions, respectively. As per the Beer-Lambert law:

\[
C_{BSA} = \frac{A_B - A}{\varepsilon_{BSA}}\lambda
\]

\[
C_{Metal} = \frac{A - A_B}{\varepsilon_{Metal} \cdot \lambda}
\]

Where: \(A_B\) and \(A\) are the absorbances of BSA in the absence and presence of metal ions, respectively, at 280 nm wavelength, \(\varepsilon_{BSA}\) and \(\varepsilon_{Metal}\) are the molar extinction coefficients of BSA and the bound metal ions, respectively, whereas, \(\lambda\) is the path length and was assumed to be 1 cm.

Now, if we substitute the values of \(C_{BSA}\) and \(C_{Metal}\) from equations (4) and (5) to equation (3), it can be deduced to:

\[
A_B = \frac{A_B}{\varepsilon_{BSA} \cdot \lambda} + \frac{A - A_B}{\varepsilon_{Metal} \cdot \lambda} \cdot K \cdot C_{Metal}
\]

Using this equation, a linear double reciprocal plot was obtained with \(\frac{1}{A_B}\) on X-axis, and \(\frac{1}{A - A_B}\) on Y-axis. The binding constant (K) was calculated as the ratio of the intercept to the slope.

RESULTS AND DISCUSSION

FT-IR analysis

Upon interaction of metal ions (0.5 mM) with BSA protein (0.25 mM), a notable reduction in intensities of...
amide I and II bands of \( \text{Ir}^{3+} \)-BSA and \( \text{Fe}^{2+} \)-BSA complex was observed, whereas a minor change was found in case of \( \text{Pt}^{4+} \)-BSA complex (Fig. 3B, 3D, 3F). In the difference spectra of \( \text{Pt}^{4+} \)-BSA complex, amide I (1659 cm\(^{-1}\); negative) and amide II (1535 cm\(^{-1}\); negative) bands were present (Fig. 3C). In \( \text{Ir}^{3+} \)-BSA and \( \text{Fe}^{2+} \)-BSA complexes, amide I bands were observed at 1657 cm\(^{-1}\) (positive) and 1656 cm\(^{-1}\) (positive) while amide II bands were present at 1539 cm\(^{-1}\) (positive) and 1538 cm\(^{-1}\) (positive) (Fig. 3E, 3G). The loss of intensity might be due to the reduction in α-helix component in the protein structure. Additionally, amide I band shifted from 1652 cm\(^{-1}\) in case of free BSA to 1659, 1656 and 1657 cm\(^{-1}\) for \( \text{Pt}^{4+} \)-BSA, \( \text{Fe}^{2+} \)-BSA and \( \text{Ir}^{3+} \)-BSA complexes, respectively. Shifting of the amide bands might have happened due to the binding of metal ions to C-N and C-O groups of BSA protein. All the selected metal ions showed a considerable effect on carbonyl stretching vibration. However, C-N\(_{\text{amide}}\) and C-N\(_{\text{backbone}}\) vibrations were affected weakly by \( \text{Ir}^{3+} \) and \( \text{Fe}^{2+} \) ions.

The secondary structure quantitative analysis was also performed using the curve fitting method (Belatik et al., 2012) and the amide I band of free BSA was deconvoluted using the Origin software. It showed the presence of α-helix (1652 cm\(^{-1}\); 62%), β-turn (1671 cm\(^{-1}\); 16%), β-sheet (1617, 1630 cm\(^{-1}\); 14%), random coil (1639 cm\(^{-1}\); 5%) and β-antiparallel (1684 cm\(^{-1}\); 3%) as main components of the protein structure. In secondary structure analysis of complexes, a marked reduction in the α-helix content (62% in case of free BSA to 55, 48 and 45% for \( \text{Pt}^{4+} \)-BSA, \( \text{Fe}^{2+} \)-BSA and \( \text{Ir}^{3+} \)-BSA complexes, respectively) was observed with a rise in β-sheet component (14% in case of free BSA to 16, 18 and 19% for \( \text{Pt}^{4+} \)-BSA, \( \text{Fe}^{2+} \)-BSA and \( \text{Ir}^{3+} \)-BSA complexes, respectively) (Table 1; Fig. 4A–D). The percentage change in the α-helical content was also calculated for the three complexes and was found to be −27.41, −22.58 and −11.29% for \( \text{Ir}^{3+} \), \( \text{Fe}^{2+} \) and \( \text{Pt}^{4+} \) metal ion complexes, respectively (Table 2) indicating that the interaction of \( \text{Ir}^{3+} \) ion with BSA was the strongest followed by \( \text{Fe}^{2+} \) and \( \text{Pt}^{4+} \). These findings support the “hard and soft acids and bases (HSAB) theory” of metal-ligands interaction since a strong interaction of hard metal ion (\( \text{Ir}^{3+} \)) with \( \text{C}=\text{O} \) ligand of BSA protein was observed. The decrease in intensity of the bands was because of the reduction in α-helix portion of amide I band of the protein at the tested metal ions concentration.

### UV-Vis Spectroscopy

The effect of increasing concentration of metal ions (\( \text{Pt}^{4+} \), \( \text{Ir}^{3+} \), and \( \text{Fe}^{2+} \)) on the structural variations of BSA protein was studied (Fig. 5A–C) using UV-Vis spectroscopy. The absorption spectra for \( \text{Ir}^{3+} \) and \( \text{Pt}^{4+} \) complexes with BSA (Fig. 5A, 5B) showed that the peak intensities of BSA at 280 nm decreased upon complexation with \( \text{Pt}^{4+} \) and \( \text{Ir}^{3+} \) ions as the concentrations of metal ions increased from 4 to 32 µM. The observed hypochromism effect with a minor shift in the wavelength indicated the interaction of \( \text{Pt}^{4+} \) and \( \text{Ir}^{3+} \) metal ions with the hydrophobic region of the protein. It thus revealed the conformational change in BSA (Buranaprapuk et al., 2000) upon interaction. The hypochromism effect also indicated the increased polarity and decreased hydrophobicity around the tryptophan residue (Varlan et al., 2010). The binding constants for BSA-\( \text{Pt}^{4+} \) and BSA-\( \text{Ir}^{3+} \) adducts were calculated to be \( 1.55\times10^7 \) M\(^{-1}\) and \( 5.67\times10^{04} \) M\(^{-1}\), respectively (Fig. 6A, 6C). On the other hand, the intensities of absorption peaks of BSA increased upon the addition of increasing concentration of \( \text{Fe}^{2+} \) ion from 4 to 32 µM (Fig. 5B). The hyperchromic effect was observed in this case which indicated that the \( \text{Fe}^{2+} \) ion interacted with the exposed tryptophan residue on the protein surface through electrostatic forces (Maciej et al., 2013a). The binding constant for BSA-\( \text{Fe}^{2+} \) complex was determined to be \( 3.78\times10^{10} \) M\(^{-1}\) (Fig. 6B).

### Table 1. Secondary structure analysis of BSA protein and its \( \text{Pt}^{4+} \), \( \text{Ir}^{3+} \), and \( \text{Fe}^{2+} \) complexes at physiological pH

| Amide I Components (cm\(^{-1}\)) | Free BSA (%)* | \( \text{Ir}^{3+} \)-BSA complex (%)** | \( \text{Fe}^{2+} \)-BSA complex (%)** | \( \text{Pt}^{4+} \)-BSA complex (%)** |
|-----------------------------------|-------------|----------------|----------------|----------------|
| α-helix (± 4) 1660–1650           | 62          | 45             | 48             | 55             |
| β-sheet (± 2) 1637–1614           | 14          | 19             | 18             | 16             |
| Random coil (± 2) 1648–1638       | 5           | 14             | 11             | 11             |
| β-turn (± 2) 1678–1670            | 16          | 10             | 12             | 13             |
| β-antiparallel (±1) 1691–1680     | 3           | 11             | 13             | 6              |

*Percentage secondary structure of free BSA (0.25 mM); **Percentage secondary structure of \( \text{Ir}^{3+} \), \( \text{Fe}^{2+} \) and \( \text{Pt}^{4+} \) complexes (0.5 mM)

### Table 2. Comparative evaluation of FT-IR and UV-Vis spectroscopy results and the findings of ACE for metal ions-protein interaction.

| Metal Ions | \( \Delta R / R_c \pm cnf \) values in ACE* (Alhazmi, 2015) | % change in α-helix (Present study) | Binding constant (M\(^{-1}\)) (Present study) |
|------------|-------------------------------------------------------------|------------------------------------|---------------------------------------------|
| \( \text{Ir}^{3+} \) | −0.2511±0.0132                                               | −27.41                             | 5.67\times10^{04}                           |
| \( \text{Fe}^{2+} \) | −0.1290±0.0100                                               | −22.58                             | 7.8×10^{10}                                |
| \( \text{Pt}^{4+} \) | −0.0335±0.0105                                               | −11.29                             | 2.8\times10^{10}                           |

*\( \Delta R \), Difference in mobility ratios; \( R_c \), mobility ratio of BSA alone; cnf, confidence interval
Previously, we studied the interactions of several biologically and medicinally important metal ions with important biological proteins using affinity capillary electrophoresis (ACE) (Alhazmi et al., 2015). The results obtained in the present study were compared to the results of our previous study on these metal ions in order to establish the accuracy of the employed spectroscopic techniques. The percentage variation in the α-helical component obtained from FT-IR spectroscopic characterization of the interactions of bovine serum albumin

Figure 4. Curve-fitted spectra of amide I band at 1700–1600 cm⁻¹ ($R^2=0.99$) for (A) free BSA (B) Ir⁺³-BSA (C) Pt⁺⁴-BSA (D) Fe⁺²-BSA complexes

Figure 5. Spectral overlay of increasing concentration of (A) Ir⁺³ ion (B) Fe⁺² ion, and (C) Pt⁺⁴ ion on UV absorption of BSA; $C_{BSA}=12$ µM; $C_{metal}=0, 4, 8, 12, 16, 20, 24, 28$ and 32 µM; Tris buffer (pH 7.4)
troscopy and the binding constants of metal ions calculated using UV-Vis spectroscopy were compared to the mobility ratio of these metal ions measured using ACE (Table 2). Interestingly, similar findings were obtained using all the techniques, and Ir$^{3+}$ ions showed the strongest binding to BSA followed by Fe$^{2+}$ and Pt$^{4+}$ ions. The comparable results revealed that all three methods could successfully be used to analyze metal ions-protein interactions with adequate accuracy.

Studying protein-metal ion interaction is of prime importance in the development of metal-based drugs as the affinity of these drugs towards numerous biomolecules such as proteins and nucleic acids would determine their potential to be used as therapeutic agents against various diseases. Metal-based agents, especially the coordination compounds, exhibit adjustable ligand kinetics, flexible geometries and promising redox activities to be used against microbial infections (Alyar et al., 2012; Asadi et al., 2014; Bellu et al., 2005), tumors (Xin Zhang & Lippard, 2003; Bakhtri & Ochiai, 1999), and as radiopharmaceuticals (Guo & Sadler, 1999). Previously, a number of mononuclear and polynuclear divalent metal ion complexes such as Cu$^{2+}$, Zn$^{2+}$, Ni$^{2+}$, Pt$^{2+}$ and Co$^{2+}$ with BSA protein were studied (Sathyadevi et al., 2012; Gharagozlou & Boghaini, 2008; Krishnamoorthy et al., 2011; Sathyadevi et al., 2011; Samari et al., 2012). The specificity of the binding interactions between metal ions as ligands and proteins is affected by the planarity of the ligands (Sathyadevi et al., 2011) and it was suggested that the metal ions bind to the Trp134 residue present on the protein surface which is the most accessible one (Xue et al., 2012). The displacement studies revealed that metal ions preferentially bind to the subdomain IIA present in the site-I of the BSA protein (Asadi et al., 2014; Samari et al., 2012).

Extensive ligand exchange is one of the key features of the metal-based drugs which are responsible for the interaction with essential biomolecules in the body system including proteins, enzymes and nucleic acids. Examples of such drugs are Pt$^{2+}$-based anticancer drugs: cisplatin, carboplatin and oxaliplatin, Au$^{-}$-compound auranofin, an anti-arthritic agent and several others. Metal ions covalently bind to the biomolecules such as DNA, proteins and enzymes which might inhibit their function leading to cell death through apoptosis, necrosis etc. The serious adverse effects encountered by patients receiving metal-based chemotherapeutic agents are due to lack of selectivity, as the metal-based agents not only bind to their target present in the cancerous cells, they also bind covalently to the biomolecules of the normal cells. For instance, DNA in the cancerous cells is the main target for cisplatin, however, it can interact with proteins as well (Han Ang & Dyson, 2006; Messori & Merlino, 2016).

It was observed that the interaction between metal ions and BSA results in the disruption of disulfide bonds of the protein leading to perturbation of its secondary structure. The α-helix conformation is partially lost leading to unfolding of the protein (Samari et al., 2012) and there is also a change in polarity around the tryptophan (Trp) residue (Ehteshami et al., 2013) due to the molecular interactions including rearrangements, energy transfer, collision quenching processes etc. (Samari et al., 2012; Ehteshami et al., 2013; Jalali et al., 2014). Due to the partial unfolding or rearrangement of the protein’s structure, the distance between the amino acid residues (Trp) changes leading to the reduction in the energy transfer and collision quenching between the neighboring amino acid residues. This would change the polarity of the microenvironment surrounding the amino acid residues to which they are exposed. The ligand exchange kinetics of the metal ion is greatly affected by its oxidation state and, therefore, it might be more active (or more reactive) in one oxidation state, while less active (less reactive, even may be inactive) in another state. Due to this difference, there exists an intrinsic mechanism of activation, permitting the administration of less active (hence less toxic) species, which upon activation by oxidation or reduction elicit their activity. This approach has been used to administer Pt(IV)- and Ru(II)-based compounds, which are bioactivated via reduction to Pt(II) and Ru(II) species, respectively, possessing enhanced cytotoxic properties (Graf & Lippard, 2012; Gibson,
to measure the extent of their interaction. Shifting of amide A, amide I and amide II bands in FT-IR spectra were also observed supporting the interaction of these metal ions with the binding sites of BSA protein. Significant reduction in the α-helical content and its conversion to β-antiparallel and β-sheet proved the alteration and partial unfolding of the secondary structure of BSA protein. As the subdomain I of BSA protein containing the Trp134 residue is more resistant to denaturation than the subdomain II, this partial unfolding would result in the exposure of subdomain IIA in the site I, where the Trp213 residue is present, making it more exposed to the polar environment. The UV-Vis spectroscopy results revealed that the metal ions interacted with the protein surface through electrostatic forces and bound to the hydrophobic aromatic amino acid residues of the protein changing its conformation. The calculated binding constants showed that the selected metal ions bind efficiently to BSA protein. The experimental results of UV-Vis and FT-IR spectroscopic studies showed that the tested metal ions interacted with the BSA protein in the order: \( \text{Ie}^{3+} > \text{Fe}^{2+} > \text{Pt}^{4+} \). These results could be helpful to understand the binding dynamics of the selected metal ions with BSA in vivo and the transportation and biotransformation of BSA-metal ion complexes inside the body.

Acknowledgement

The authors are thankful to the Deanship of Scientific Research, Jazan University for providing financial assistance to carry out the research work.

Declarations of interest

The authors report no disclosures of interest.

Authors’ contribution

All authors contributed equally to this work.

REFERENCES

Ahmed A, Tajmir-Riahi HA, Carpenter R (1995) A quantitative secondary structure analysis of the 33 kDa extrinsic polypeptide of photosystem II by FTIR spectroscopy. FEBS Lett. 363: 65–68. https://doi.org/10.1016/0014-5793(95)00282-E.

Alhazmi HA (2019) FT-IR Spectroscopy for the identification of binding sites and measurements of the binding interactions of important metal ions with bovine serum albumin. Sci. Pharm. 87: 5. https://doi.org/10.3390/sciphar8700005.

Alhazmi HA, Al Beatty M, Juwel SA, Lalitha KG (2017) Investigation of transferrin interaction with medicinally important noble metal ions using affinity capillary electrophoresis. Pharmazie 72: 243–248. https://doi.org/10.1691/ph.2017.6170.

Alhazmi HA, Nachbar M, Alibshir HM, El-Hady DA, Redweik S, El-Deeb S, Watrig H (2015) A comprehensive platform to investigate protein–metal ion interactions by affinity capillary electrophoresis. J. Pharm. Biomed. Anal. 107: 311–317. https://doi.org/10.1016/j.jpba.2015.01.017.

Alyar H, Alyar S, Unal A, Ozbek N, Şahin E, Karacan N (2012) Synthesis characterization and antimicrobial activity of m-toluenesulfonamide N,N’-1,2-ethanediylbis (mtenso) and [Cu(II)(phenanthroline)2]mtenso complex. J. Mol. Struct. 1028: 116–125. https://doi.org/10.1016/j.molstruc.2012.06.046.

Asadi M, Asadi Z, Zarei L, Sadi SB, AmiriDorsani Z (2014) Affinity to bovine serum albumin and anticancer activity of some new water-soluble metal Schiff base complexes. Spectrochim. Acta A Mol. Biomol. Spectrosc. 133: 697–706. https://doi.org/10.1016/j.saa.2014.05.031.

Bakhtiar R, Ochiai EI (1999) Pharmacological applications of inorganic complexes. Gen. Pharmacol. 32: 525–540. https://doi.org/10.1016/S0306-3623(98)00237-7.

Bilelak A, Hothandani S, Carpenter R, Tajmir-Riahi HA (2012) Locating the binding sites of Pb(II) ion with human and bovine serum albumins. PLas ONE 7: e36723. https://doi.org/10.1371/journal.pone.0036723.
cytotoxic activities of bivalent transition metal hy...
Xiang Y, Wu F (2010) Study of the interaction between a new Schiff-base complex and bovine serum albumin by fluorescence spectroscopy. *Spectrochim. Acta. A Mol. Biomol. Spectrosc.* 77: 430–436. https://doi.org/10.1016/j.saa.2010.06.030

Xin-Zhang C, Lippard SJ (2003) New metal complexes as potential therapeutics. *Curr. Opin. Chem. Biol.* 7: 481–489. https://doi.org/10.1016/S1367-5931(03)00081-4

Xu H, Yao N, Xu H, Wang T, Li G, Li Z (2013) Characterization of the interaction between eupatorin and bovine serum albumin by spectroscopic and molecular modeling methods. *Int. J. Mol. Sci.* 14: 14185–14203. https://doi.org/10.3390/ijms140714185

Xu X, Zhang L, Shen D, Wu H, Liu Q (2008) Oxygen-dependent Oxidation of Fe(II) to Fe(III) and Interaction of Fe(III) with Bovine serum albumin leading to a hysteretic effect on the fluorescence of bovine serum albumin. *J. Fluor.* 18: 193–201. https://doi.org/10.1007/s10895-007-0263-4

Xue F, Xie C-Z, Zhang Y-W, Qiao Z, Qiao X, Xu J-Y, Yan S-P (2012) Two new dicopper(II) complexes with oxamido-bridged ligand: Synthesis crystal structures DNA binding/cleavage and BSA binding activity. *J. Inorg. Biochem.* 115: 78–86. https://doi.org/10.1016/j.jinorgbio.2012.05.018

Zhong W, Wang Y, Yu JS, Liang Y, Ni K, Tu S (2004) The interaction of human serum albumin with a novel antidiabetic agent-SU-118. *J. Pharm. Sci.* 93: 1039–1046. https://doi.org/10.1002/jps.20005