BOVINE SERUM ALBUMIN INTERACTIONS WITH METAL COMPLEXES

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Abstract

The continuous search for new molecules with therapeutic abilities has led to the synthesis and characterization of a large number of metal complexes, proven to exhibit potential as pharmacological agents through their antibacterial, antiviral, antifungal and antineoplastic properties. As serum albumins play a key role in drug pharmacokinetics and pharmacodynamics, the study of coordination compounds affinity towards this class of proteins, as well as understanding the mechanism through which they interact is crucial. The aim of this review is to focus on the structure and biological functions of bovine serum albumin, the design of metal complexes that are able to bind to the biomolecule, as well as the experimental techniques employed in the study and evaluation of these interactions.

Keywords: drug-protein interaction, coordination complex, fluorescence spectroscopy, UV-Vis absorption spectroscopy.

Introduction

Of all the molecules encountered in living organisms, proteins are the most abundant and diverse from a functional point of view. From the hormones and enzymes that control metabolism, the framework forming collagen in bones, the contractile proteins in muscles, to the haemoglobin and albumin in the bloodstream and immunoglobulins fighting infections, almost every life process relies on this class of molecules.

Albumin is the most abundant protein in the vertebrates' organisms (up to 40 mg/ml) and the most prominent plasma protein (about 60% of the total protein content of plasma). It is one of the first discovered and most intensely studied proteins [1].

The investigation of the binding amplitude and mechanism of interaction of small molecules with serum albumins is crucial for the understanding of drug pharmacodynamics and pharmacokinetics, as the nature and strength of that interaction has a great influence on drug absorption, distribution, metabolism and excretion [2,3].

When approaching the evaluation of small molecules affinity for albumins, bovine serum albumin (BSA) is usually selected as a relevant model, due to its structural similarity with human serum albumin (76%) [4,5], its low cost and wide availability.

Bovine serum albumin structure and biological functions

The studies conducted by Brown and co-workers regarding bovine serum albumin materialized in solving its complete chemical sequence, while the crystal structure of the protein was published in 2012 by Majorek *et al.* [6].

The BSA molecule consists of 583 amino acids, bound in a single chain cross-linked with 17 cystine residues (eight disulfide bonds and one free thiol group), and has a molecular mass of 66400 Da [1]. The amino acid chain is made up of three homologous but structurally distinct domains (I, II and III), divided into nine loops by the disulfide bonds and arranged in a heart-shaped molecule.

Manuscript received: 05.10.2014
Accepted: 20.10.2014
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Each domain consists of two sub-domains, A and B. The secondary structure of the protein is mainly α-helical (74%), with the remaining polypeptide chain occurring in turns and in extended or flexible regions between sub-domains [1,6,7].

One of the characteristic structural features of BSA is its low tryptophan, methionine, glycine and isoleucine content, while it is abundant in ionic amino acids, such as glutamic acid and lysine. The ionized residues confer the protein a high total charge, of 185 ions per molecule at neutral pH, contributing to its solubility [1].

BSA is a globular non-glycosylated protein, one of the few plasma proteins lacking carbohydrate groups, as it is synthesized in the liver without prosthetic groups or other additives.

Many endogenous and exogenous compounds (including drugs, hormones, xenobiotics and fatty acids [8]), once entered the blood stream, are transported and disposed of as a consequence of forming a complex with serum albumins. This class of proteins also contribute to the colloid blood osmotic pressure and the maintenance of blood pH [9,10], but one of the most important property of albumins is that they bind to different compounds in a reversible manner. The protein often increases the apparent solubility of hydrophobic drugs in the plasma and influences the circulation, metabolism and efficacy of drugs [1,4]. Serum albumins are also involved in controlling the ionized or biologically active concentrations of Ca^{2+} and Mg^{2+} in mammals [6].

It is known that the drug affinity for plasma proteins directly influence the drug concentration in the bloodstream and its biological effect. Generally, the effect of weak protein binding is short lifetime or poor distribution, while strong binding leads to a decrease in drug concentration, as it is the unbound fraction of the drug that shows pharmacological activity.

BSA shows discrete binding sites with different specificities, the most important ones being referred to as site-I and site-II, located in hydrophobic cavities of sub-domains IIA and IIIA, respectively [1]. Site markers are small molecules that have specific binding locations in the albumin structure and are often used in studying the interaction of different ligands with the protein. Site-I markers include warfarin, phenylbutazone, dansylamide and iodipamide, while ibuprofen, flufenamic acid and diazepam are site-II markers [11].

Metal complexes interacting with BSA

In the recent years, Bioinorganic chemistry has provided numerous examples of structures with high affinity towards biomolecules, including nucleic acids and proteins, and showing real potential to be developed into therapeutic agents, fighting bacterial, viral and/or fungal infections [12-16], targeting different types of tumors [17,18], radiopharmaceuticals and superoxide dismutase and insulin mimics [19]. Coordination compounds exhibit unique properties offering interesting opportunities in designing new pharmacologically active molecules, such as adjustable ligand kinetics and redox activity and a large variety of geometries and coordination numbers offered by metal ions, resulting in high structural diversity [20].

The BSA interaction and binding ability of a large variety of mononuclear and polynuclear Cu^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}, Pt^{2+} complexes with aromatic ligands (some of them bearing known pharmacologically active moieties) has been investigated [2,3,9,10,21,22].

As in the case of the interaction of metal complexes with other classes of biomolecules, it has been suggested that the planarity of the ligands coordinating the metal centre plays an important role in enhancing protein binding ability [21].

A certain degree of binding specificity was observed for metal complexes, due to weak interactions between the ligand sphere and the protein binding site, as some studies suggested that metal complexes most likely affect the Trp134 residue, found on the surface of the protein, and thus more accessible [23]. Other research groups, after performing displacement experiments, concluded that complexes preferentially bind to subdomain IIA (site-I) [13,22].

An interaction between BSA and metal complexes often leads to a perturbation of the secondary structure of the protein, by disrupting the disulfide bonds and leading to a partial loss of α-helix conformation with the subsequent unfolding of the protein [22], or a change in the polarity of the environment to which the tryptophan residues are exposed [24], as a result of molecular interactions, such as excited-state reactions, molecular rearrangements, energy transfer, ground-state complex formation or collision quenching [7,22,25].

Experimental techniques employed in the study of the interaction between BSA and metal complexes

Fluorescence spectroscopy studies

An efficient approach in evaluating the interaction between metal complexes and BSA is the use of fluorescence spectroscopy.

BSA has fluorescent properties and emits intensely upon excitation. Responsible for its intrinsic fluorescence are three amino acid residues found in its structure: tryptophan, tyrosine and phenylalanine [1], but as the relative ratio of fluorescence intensity for these amino acids is 100: 9: 0.5, it seems probable that the fluorescence of BSA arises mainly from its two tryptophan residues: Trp-134 located on the surface of sub-domain IB and Trp-212 located within a hydrophobic binding pocket in sub-domain IIA [24,26].

The fluorescence spectra are recorded in the absence

and in the presence of increasing concentrations of metal complex. In the case of an interaction with the coordination compound, the fluorescence intensity of the protein at around 345 nm decreases regularly as the concentration of the probe increases. Moreover, a red or blue shift of the emission maximum in the fluorescence spectrum of the albumin is indicative of an increase in the hydrophobicity of the microenvironment around the tryptophan residues [27], while no change in the position of the emission maximum suggests no alteration in the local dielectric environment of BSA [28]

Fluorescence quenching can occur by different mechanisms, usually classified as static quenching (a non-fluorescent ground-state complex is formed between the fluorophore and the quencher), dynamic quenching (a collisional process, the fluorophore and the quencher interact during the transient existence of the excited state), or a simultaneous static and dynamic quenching [5,25].

A series of parameters are used to define and evaluate the interaction between BSA and the probe: the Stern-Volmer quenching constant, the quenching rate constant, the binding constant and the number of binding sites.

The fluorescence quenching is described by the Stern-Volmer equation [25,29]:

$$I_0/I = 1 + K_{SV}[Q] = 1 + k_q\tau_0[Q]$$

where I_0 and I are the fluorescence intensities of BSA in the absence and in the presence of the quencher (i.e. the metal complex), respectively, K_{SV} is the Stern-Volmer quenching constant, [Q] is the concentration of the quencher, k_q is the quenching rate constant of the biomolecule and τ_0 is the average lifetime of the molecule in the absence of the quencher.

A linear I_0/I *vs.* [Q] plot indicates that a single type of quenching mechanism is involved, either static or dynamic, while a deviation from linearity suggests a mixed quenching mechanism [30].

The K_{SV} value is obtained from the plot I_0/I *vs.* [Q]. Values with a magnitude order of 10^5 M^{-1} for K_{SV} are considered to be indicative of a relatively strong interaction between BSA and metal complexes [2,5,8,9,22].

Considering the well known connection between the K_{SV} quenching constant and the k_q quenching rate constant

$$k_q = K_{SV}/\tau_0$$

and taking into account that the fluorescence lifetime of the biopolymer is 10^{-8} s [22,28], the k_q values may be calculated, also providing information referring to the type of quenching occurring in the presence of a certain quencher [16].

Dynamic and static quenching show certain characteristics, such as their different dependence on temperature reflected in the changes in the fluorescence spectrum of the fluorophore. In the case of static quenching, an increase in temperature leads to a lower stability of the complex and a decrease in the quenching constant. In the case of dynamic quenching, a faster diffusion is a consequence of an increase in temperature and, thus, the quenching rate increases [5,22].

When small molecules bind independently to a set of equivalent sites on a molecule, the equilibrium between free and bound molecules is represented by the Scatchard equation [9,10]:

$$\log[(I_0-I)/I] = \log[K_b] + n \log[Q]$$

where I_0 and I are the fluorescence intensities of the protein in the absence and in the presence of the quencher (i.e. the metal complex) respectively, [Q] is the concentration of the quencher, K_b is the binding constant and n is the number of binding sites.

The K_b values are obtained from the plot $\log[(I_0-I)/I]$ *vs.* $\log[Q]$. Values with a magnitude order in the range of 10^3-10^6 M^{-1} for K_b are reported as being indicative of an efficient interaction with the protein [3,5,10]. Generally, the binding constant of a compound to serum albumin should be high enough to ensure that a significant amount gets transported and distributed through the organism, but, at the same time, low enough so that the compound can be released once it reaches its target. Such an optimum range is considered to be $10^4 – 10^6$ M^{-1} [31,32].

The number of binding sites indicates the number of independent class of binding sites for the complexes on BSA and are generally approximately $n \approx 1$ [21].

UV-Vis absorption spectroscopy studies

A simple method used to distinguish between the two possible quenching mechanisms is by UV-Vis absorption spectroscopy. The absorption spectra are recorded in the absence and in the presence of increasing concentrations of metal complex. The absorbance spectrum of BSA shows two characteristic bands, at around 220 nm and 280 nm, due to the α-helix structure of the protein and the aromatic amino acid residues respectively [22]. Usually, a marked decrease in the 220 nm absorbance peak is reported, due to the perturbation of the secondary structure of the protein [22, 33], while the changes observed in the 280 nm band are more subtle, indicating that, to some extent, the environment in the close proximity of the aromatic amino acid residues is altered [34].

A static quenching, consequence of a new species formed between the quencher and the ground-state of the fluorophore, leads to considerable changes in the absorption spectra at 280 nm (either a red or a blue shift, bathochromism or hypsochromism respectively). In the case of dynamic quenching, the absorption spectra suffer no modifications, as only the excited-state fluorescent molecule interacts with the quencher [3,5,10].

Conclusions

In the context of the increasing incidence of drug resistance, the search for new therapeutic agents represents a continuous struggle, and metal complexes are among the promising types of molecules showing antibacterial, antiviral and antineoplastic potential. One of the crucial steps in developing a new and effective drug is studying and understanding its ability to bind to albumins, as they play a key role in its transport, distribution, metabolism and excretion. With this in mind, we highlighted the essential notions related to bovine serum albumin from the point of view of its structure and functions, as well as the elements to be taken into consideration when designing metal complexes with protein binding abilities. Moreover, the most important instrumental techniques employed to study the type and strength of the metal complexes – BSA interactions were discussed, as well as the type of information they provide: fluorescence spectroscopy and UV-Vis absorption spectroscopy.

Acknowledgements

The authors are grateful to Professor Gloria Alzuet-Piña (Faculty of Pharmacy, Universidad de València) for the useful discussions and for the help provided in writing this paper.

The present work was supported financially by research Project POSDRU 107/1.5/S/78702.

References

1. Peters TJ. All about Albumin: Biochemistry, Genetics, and Medical Applications. San Diego: Academic Press; 1995.
2. Liu H, Shi X, Xu M, Li Z, Huang J, Bai D, et al. Transition metal complexes of 2, 6-di ((phenazonyl-4-imino)methyl)-4-methylphenol structure and biological evaluation. Eur J Med Chem. 2011;46:1638-1647.
3. Sathyadevi P, Krishnamoorthy P, Jayanthi E, Butorac RR, Cowley AH, Dharmaraj N. Studies on the effect of metal ions of hydrazone complexes on interaction with nucleic acids, bovine serum albumin and antioxidant properties. Inorganica Chimica Acta. 2012;384:83-96.
4. Urquiza NM, Naso LG, Manca SG, Lezama L, Rojo T, Williams PAM, Ferrer EG. Antioxidant activity of methimazole–copper(II) bioactive species and spectroscopic investigations on the mechanism of its interaction with Bovine Serum Albumin. Polyhedron. 2012;31(1):530-538.
5. Xiang Y, Wu F. Study of the interaction between a new Schiff-base complex and bovine serum albumin by fluorescence spectroscopy. Spectrochim Acta A Mol Biomol Spectrosc. 2010;77(2):430-436.
6. Majorek KA, Porebski PJ, Dayal A, Zimmerman MD, Jablonska K, Stewart AJ, Chruszcz M, Minor W. Structural and immunologic characterization of bovine, horse, and rabbit serum albumins. Mol Immunol. 2012;52(3-4):174-182.
7. Jalali F, Dorraji PS, Mahdiuni H. Binding of the neuroleptic drug, gabapentin, to bovine serum albumin: Insights from experimental and computational studies. Journal of Luminescence. 2014;148:347-352.
8. Bal W, Christodoulou J, Sadler PJ, Tucker A. Multi-metal binding site of serum albumin. J Inorg Biochem. 1998;70:33-39.
9. Gharagozlou M, Boghaei DM. Interaction of water-soluble amino acid Schiff base complexes with bovine serum albumin: fluorescence and circular dichroism studies. Spectrochim Acta A Mol Biomol Spectrosc. 2008;71(4):1617-1622.
10. Krishnamoorthy P, Sathyadevi P, Cowley AH, Butorac RR, Dharmaraj N. Evaluation of DNA binding, DNA cleavage, protein binding and in vitro cytotoxic activities of bivalent transition metal hydrazone complexes. Eur J Med Chem. 2011;46(8):3376-3387.
11. Ni Y, Su S, Kokot S. Spectrofluorimetric studies on the binding of salicylic acid to bovine serum albumin using warfarin and ibuprofen as site markers with the aid of parallel factor analysis. Anal Chim Acta. 2006;580(2):206-215.
12. Alyar H, Alyar S, Ünal A, Özbek N, Şahin E, Karacan N. Synthesis, characterization and antimicrobial activity of *m*-toluenesulfonamide, *N,N′*-1,2-ethanediylbis (mtsen) and [Cu(II)(phenanthroline)$_2$]mtsen complex. Journal of Molecular Structure. 2012;1028:116-125.
13. Asadi M, Asadi Z, Zarei L, Sadi SB, Amirghofran Z. Affinity to bovine serum albumin and anticancer activity of some new water-soluble metal Schiff base complexes. Spectrochim Acta A Mol Biomol Spectrosc. 2014;133(0):697-706.
14. Bellú S, Hure E, Trapé M, Trossero C, Molina G, Drogo C, et al. Synthesis, structure and antifungal properties of Co(II)–sulfathiazolate complexes. Polyhedron. 2005;24(4):501-509.
15. Blasco F, Perello L, Borras J, Garcia-Granda S. Cobalt(II), nickel(II), and copper(II) complexes of sulfonamide derivatives: synthesis, spectroscopic studies and antibacterial activity. Crystal structure of [Co(sulfacetamide)$_2$(NCS)$_2$]. J Inorg Biochem. 1996;61:143-154.
16. Gao C-Y, Qiao X, Ma Z-Y, Wang Z-G, Lu J, Tian J-L, Xu J-Y, Yan S-P. Synthesis, characterization, DNA binding and cleavage, BSA interaction and anticancer activity of dinuclear zinc complexes. Dalton Trans. 2012;41(39):12220-12232.
17. Xin Zhang C, Lippard SJ. New metal complexes as potential therapeutics. Curr Opin Chem Biol. 2003;7:481-489.
18. Bakhtiar R, Ochiai EI. Pharmacological applications of inorganic complexes. Gen Pharmacol. 1999;32:525-540.
19. Guo Z, Sadler PJ. Metals in medicine. Angewandte Chemie International Edition. 1999;38:1512-1531.
20. Meggers E. Targeting proteins with metal complexes. Chem Commun. 2009(9):1001-1010.
21. Sathyadevi P, Krishnamoorthy P, Butorac RR, Cowley AH, Bhuvanesh NSP, Dharmaraj N. Effect of substitution and planarity of the ligand on DNA/BSA interaction, free radical scavenging and cytotoxicity of diamagnetic Ni(II) complexes: a systematic investigation. Dalton Trans. 2011;40(38):9690-9702.
22. Samari F, Hemmateenejad B, Shamsipur M, Rashidi M, Samouei H. Affinity of two novel five-coordinated anticancer Pt(II) complexes to human and bovine serum albumins: a spectroscopic approach. Inorg Chem. 2012;51(6):3454-3464.
23. Xue F, Xie C-Z, Zhang Y-W, Qiao Z, Qiao X, Xu J-Y, Yan S-P. Two new dicopper(II) complexes with oxamido-bridged ligand: Synthesis, crystal structures, DNA binding/cleavage and BSA binding activity. J Inorg Biochem. 2012;115(0):78-86.
24. Ehteshami M, Rasoulzadeh F, Mahboob S, Rashidi M-R. Characterization of 6-mercaptopurine binding to bovine serum albumin and its displacement from the binding sites by quercetin and rutin. Journal of Luminescence. 2013;135:164-169.
25. Lakowicz JR. Principles of Fluorescence Spectroscopy.

Second Edition ed. New York: Kluwer Academic/Plenum Publishers; 1999.
26. Vignesh G, Arunachalam S, Vignesh S, James RA. BSA binding and antimicrobial studies of branched polyethyleneimine–copper(II)bipyridine/phenanthroline complexes. Spectrochim Acta A Mol Biomol Spectrosc. 2012;96(0):108-116.
27. Ray A, Koley Seth B, Pal U, Basu S. Nickel(II)-Schiff base complex recognizing domain II of bovine and human serum albumin: Spectroscopic and docking studies. Spectrochim Acta A Mol Biomol Spectrosc. 2012;92(0):164-174.
28. Jayabharathi J, Thanikachalam V, Perumal MV. A study on the binding interaction between the imidazole derivative and bovine serum albumin by fluorescence spectroscopy. Journal of Luminescence. 2012;132:707-712.
29. Valeur B. Molecular Fluorescence. Principles and Applications. Weinheim: Wiley; 2001.
30. Eftink MR, Ghiron CA. Fluorescence quenching studies with proteins. Anal Biochem. 1981;114:199-227.
31. Rajendiran V, Karthik R, Palaniandavar M, Periasamy VS, Akbarsha MA, Srinag BS, et al. Mixed-Ligand Copper(II)-phenolate Complexes: Effect of Coligand on Enhanced DNA and Protein Binding, DNA Cleavage, and Anticancer Activity. Inorg Chem. 2007;46(20):8208-8221.
32. Tarushi A, Totta X, Papadopoulos A, Kljun J, Turel I, Kessissoglou DP, et al. Antioxidant activity and interaction with DNA and albumins of zinc-tolfenamato complexes. Crystal structure of [Zn(tolfenamato)(2)(2,2'-dipyridylketoneoxime)(2)]. Eur J Med Chem. 2014;74:187-198.
33. He Y, Wang Y, Tang L, Liu H, Chen W, Zheng Z, et al. Binding of Puerarin to Human Serum Albumin: A Spectroscopic Analysis and Molecular Docking. J Fluoresc. 2008;18(2):433-442.
34. Shen L, Yang Z, Tang R. Synthesis, luminescence properties of Eu(III) and Tb(III) complexes with a novel aromatic carboxylic acid and their interactions with bovine serum albumin. Spectrochim Acta A Mol Biomol Spectrosc. 2012;98:170-177.