28-29 September 2023,
Kragujevac, Serbia

www.iccbikg2023.kg.ac.rs

2nd International Conference on Chemo and Bioinformatics
ICCBIKG_2023

ICCBIKG
International Conference on Chemo and BioInformatics

BOOK OF PROCEEDINGS

2nd International Conference on Chemo and BioInformatics

ICCBIKG 2023

BOOK OF PROCEEDINGS

September 28-29, 2023

Kragujevac, Serbia

Sponsored by

2nd International Conference on Chemo and BioInformatics, Kragujevac, September 28-29, 2023, Serbia.

Editors:

Professor Dr. Zoran Marković

Professor Dr. Nenad Filipović

Technical Editors:

Jelena Živković

Marko Antonijević

Dr. Žiko Milanović

Dr. Vladimir Simić

Proofreading:

Marijana Dimović

Publisher:

Institute for Information Technologies, University of Kragujevac, Serbia, Jovana Cvijića bb, 2023

Press:

„Grafo Ink“, Kragujevac

Impression:

120 copies

CIP - Каталогизација у публикацији - Народна библиотека Србије, Београд

54:004(048)(0.034.2)
57+61]:004(082)(0.034.2)

INTERNATIONAL Conference on Chemo and BioInformatics (2 ; 2023 ;
Kragujevac) Book of Proceedings [Elektronski izvor] / 2nd International Conference on
Chemo and BioInformatics, ICCBIKG 2023, September 28-29, 2023 Kragujevac,
Serbia ; [editors Zoran Marković, Nenad Filipović]. - Kragujevac : University,
Institute for Information Technologies, 2023 (Kragujevac : Grafo Ink). - 1 USB
fleš memorija ; 1 x 2 x 6 cm

Sistemski zahtevi: Nisu navedeni. - Nasl. sa naslovne strane dokumenta. - Tiraž
120. - Bibliografija uz svaki rad.

ISBN 978-86-82172-02-4

a) Хемија -- Информациона технологија -- Зборници b) Биомедицина --
Информациона технологија -- Зборници

COBISS.SR-ID 125908489

The interaction studies of novel diaminophenazine gold(III) complex and Bovine Serum Albumin (BSA-ibuprofen and BSA-Eozine Y)

Ana S. Kesić[1,*], Snežana R. Radisavljević[2], Jovana V. Bogojeski[2], Biljana V. Petrović[2]

[1] University of Kragujevac, Institute for Information Technologies, Jovana Cvijica bb, 34000 Kragujevac, Serbia, e-mail: akesic@uni.ac.kg.rs
[2] University of Kragujevac, Faculty of Science, R. Domanovica 12, 34000 Kragujevac, Serbia

DOI: 10.46793/ICCBI23.407K

Abstract: It is well known that gold(III) complexes have found applications in medicinal inorganic chemistry. Considering this, the right choice of inert ligands in the structure of Au(III) complexes is crucial for predicting their properties and reactivity, especially towards biomolecules. Here are presented the results of the study of the interactions between the new gold(III) complex [Au(DAP)Cl_3], with 2,3-diaminophenazine (DAP) as an inert ligand, and BSA. Specifically, serum albumin is the main soluble protein in the circulatory system of humans. The metabolism of drugs, their distribution, free concentration, and effectiveness strongly depend on the drug-albumin interaction. Investigation of the interactions of the [Au(DAP)Cl_3] complex with bovine serum albumin (BSA) under physiological conditions was performed by fluorescence spectroscopy. This method was also used to identify the binding site on the BSA molecule, with eosin Y as a marker for site I (subdomain IIA), and ibuprofen as a marker for site II (subdomain IIIA). The results have shown that the complex moderately reacts with the BSA molecule with just one binding site for the complex on the protein. Additionally, based on the results with site markers, especially with eosin Y, it can be concluded that the studied complex binds to site I of the BSA molecule.

Keywords: gold(III), complex, Bovine Serum albumin, interactions, Ibuprofen, Eosin Y

1. Introduction

Many studies have shown that the stability of Au(III) complexes can be achieved by proper choice of ligands during complexation [1]. Published results also confirmed that the proper choice of ligands is crucial for the good biological properties and reactivity of Au(III) complexes [2]. In the human bloodstream, the most abundant protein is serum albumin. The most significant role of serum albumin in the human organism is to transport various matters (hormones, fatty acids, ions, drugs, etc.) through the blood plasma to the organs and tissues. Metal ion complexes, with antitumor activity, often interact with proteins, both as part of their mechanism of action as well as for their transport and metabolism. Bovine serum albumin (BSA) is the most studied albumin due to its structural similarity to human serum albumin (HSA) [3]. Therefore, it is

important to examine the interactions between serum albumin protein, BSA, and transition metal complexes as potential anticancer drugs. Bovine serum albumins are heart-shaped proteins divided into three (I–III) domains, with each domain containing two subdomains (A and B) [4]. In the albumin molecule, transition metal complexes can bind at two sites within the BSA molecule, the site in the subdomain IIA and/or the site in the subdomain IIIA [5]. The fluorescence titration methods were used to identify the binding location of complexes on the BSA molecule, with eosin Y as a marker for site I (subdomain IIA) and ibuprofen as a marker for site II (subdomain IIIA). In the context of this study, we looked at how bovine serum albumin (BSA) interacted with the newly discovered complex, [Au(DAP)Cl_3] (where DAP =2,3-diaminophenazine) (Figure 1).

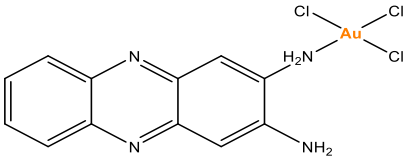

Figure 1. The structural formula of the investigated gold(III) complex.

2. Material and Methods

2.1 Chemicals and Apparatus

The initial potassium-tetrachloridoaurate(III) complex, K[AuCl4], was purchased from ABCR GmbH & Co. KG, 98%. Ligand 2,3-diaminophenazine (DAP), bovine serum albumin (BSA) and phosphate-buffered saline (PBS) were purchased from Sigma–Aldrich Chemical. Eosin Y and ibuprofen were commercially available and used as received. Doubly distilled water was used throughout the experiments. The stock solutions of BSA were prepared by dissolving the corresponding solid in PBS buffer at pH =7.4. All stock solutions were stored at 277 K and used within 3–5 days. Fluorescence spectra were collected on an RF-6000 spectrofluorometer (Shimadzu, Japan). The widths of the excitation and emission slits were both fixed at 10 nm.

2.2 Albumin binding studies

The quenching of the emission intensity of the solution with a fixed concentration of BSA (2 μM) and increasing concentration of the complex up to 9.09×10^{-6} M as a quencher was monitored at 365 nm. Fluorescence spectra were recorded at an excitation wavelength of 295 nm in the range between 300 - 500 nm. The Stern–Volmer and Scatchard equations [6.7] and graphs have been used to determine the strength of the interactions of the complex with BSA and to calculate the corresponding constants. The values of the dynamic quenching constant (K_{SV}) and the quenching rate constant (K_q) were calculated using the Stern-Volmer quenching equation. The association binding constant (K_b) and the number of the binding sites per albumin (n) were calculated using the Scatchard equation.

In addition, the competitive interactions of site markers (eosin Y, as a marker for site I of subdomain IIA, and ibuprofen, as a marker for site II of subdomain IIIA) and

complex towards BSA were studied using fluorescence emission spectroscopy. The excitation wavelength was set at 295 nm, with the fluorescence emission range between 300 and 500 nm. The solutions of BSA and markers were added in equimolar concentrations (2.0×10^{-6} M). Gold complex is added with increasing concentrations up to 9.09×10^{-6} M.

3. Results and discussion

Fluorescence spectroscopy measurements for the investigation of the interactions of the studied complex and bovine serum albumin, BSA, were performed. By adding the gold(III) complex solution to the BSA solution, a decrease in fluorescence emission appears at about 365 nm. The decrease in fluorescence (Figure 2a) can be attributed to the changes in the tertiary structure of the albumin (changes in the environment of the tryptophan) because of the binding of the complex to BSA. The Stern-Volmer constant (K_{sv}), bimolecular quenching constant (K_q) and other parameters (K_b and n) for the interaction of complex [Au(DAP)Cl$_3$] with BSA are given in Table 1.

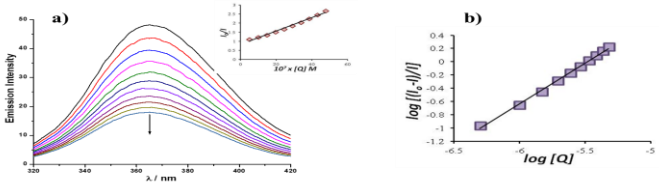

Figure 2. a) Emission spectra observed for the titration of BSA with [Au(DAP)Cl$_3$], insert plots of I_0/I *vs.* [Q] (b) Scatchard plots for the [Au(DAP)Cl$_3$] complex and BSA.

Table 1. Stern–Volmer constants (K_{sv}), bimolecular quenching constants (kq) and the other parameters (K_b and n) for the interactions of complex [Au(DAP)Cl$_3$] with BSA in the absence and presence of the site markers (ibuprofen and eosin Y).

System	K_{sv} (M^{-1})	K_q (M-1 s^{-1})	K_b (M^{-1} s^{-1})	n
Complex-BSA	$(3.70 \pm 0.08) \times 10^5$	$(3.70 \pm 0.08) \times 10^{13}$	$(5.61 \pm 0.06) \times 10^6$	1.23
Complex-BSA- ibuprofen	$(13.46 \pm 0.04) \times 10^5$	$(13.46 \pm 0.04) \times 10^{13}$	$(2.11 \pm 0.01) \times 10^6$	1.16
Complex-BSA- eosin-Y	$(3.14 \pm 0.03) \times 10^5$	$(3.14 \pm 0.03) \times 10^{13}$	$(1.49 \pm 0.09) \times 10^6$	1.13

Competitive experiments with BSA and site markers: The excitation wavelength for the fluorescence titration was 295 nm, and the emission was followed in the range of 300–500 nm. Changes in the emission of BSA solutions with the addition of the increasing concentration of the tested complex were monitored in the presence of the markers eosin Y and ibuprofen (Figures 3 and 4). According to the obtained values of constants ($2.11 \pm 0.01 \times 10^6$ and $1.49 \pm 0.09 \times 10^6$), obviously complex may bind both sites, but preferentially to site I/sub-domain IIA of BSA.

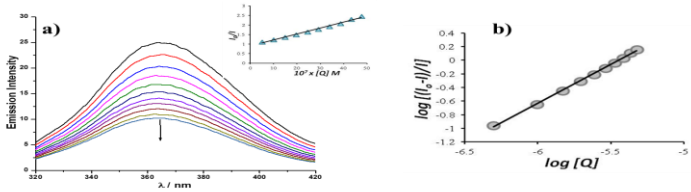

Figure 3. (a) Emission spectra of the titration of BSA in the presence of site marker eosin Y and complex [Au(DAP)Cl$_3$], insert plots of I_0/I *vs.* [Q] (b) Scatchard plot for [Au(DAP)Cl$_3$] complex

and BSA-eosin Y system.

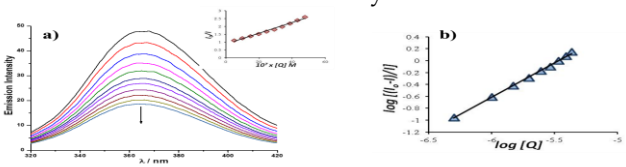

Figure 4. (a) Emission spectra of the titration of BSA in the presence of site marker ibuprofen and complex [Au(DAP)Cl$_3$], insert: plots of I_0/I *vs.* [Q]. (b) Scatchard plot for the [Au(DAP)Cl$_3$] complex and the BSA-ibuprofen system.

4. Conclusions

Based on the calculated values of the constant K_b can be confirmed that the studied complex moderately reacts with the BSA molecule. Also, the results of this study showed that Au(III) complex has a single binding site on the BSA molecule, which means that the complex can bind to either site I or site II of the protein, but not both. Additionally, based on the calculated values of the binding constants it can be concluded that the presence of the eosin Y site marker has a greater influence. However, in the presence of ibuprofen, only a slight change in the value of the constant was observed. These results indicate that the binding of the investigated complex should be located within site I of the BSA molecule.

Acknowledgment

This research is funded by the Ministry of Education and Ministry of Science, Technological Development and Innovation, Republic of Serbia, Grants No. 451-03-47/2023-01/200378 and 451-03-47/2023-01/200122.

References

[1] B. Bertrand, A. Casini., *A golden future in medicinal inorganic chemistry: the promise of anticancer gold organometallic compounds,* Dalton Trans, 43 (2014) 4209-4219.

[2] S. Radisavljević, A. Kesić, D. Ćoćić, R. Puchta, L. Senft, M. Milutinović, N. Milivojević, B. Petrović., *Studies of the stability, nucleophilic substitution reactions, DNA/BSA interactions, cytotoxic activity, DFT and molecular docking of some tetra- and penta-coordinated gold(iii) complexes*, New J Chem, 44 (2020) 11172-1187.

[3] B. Wu, C. Qu, Y. Wang, J. Zhao, H. Du,., *Comparison of the Quenching Effects of Two Main Components of Ziziphi Spinosae Semen on Serum Albumin Fluorescence*, J Fluoresc, 29 (2019) 1113-11.

[4] Y. Song, Y. Liu, W. Liu, F.A. Villamena, J.L. Zweier., *Characterization of the binding of the Finland trityl radical with bovine serum albumin,* RSC Adv, 4 (2014) 47649–47656.

[5] X.M. He, D.C. Carter., *Atomic structure and chemistry of human serum albumin*, Nature, 353 (1992) 209–2015.

[6] F. Dimiza, F. Perdih, V. Tangoulis, I. Turel, D.P. Kessissoglou, G. Psomas., J.*Interaction of copper(II) with the non-steroidal anti-inflammatory drugs naproxen and diclofenac: Synthesis, structure, DNA- and albumin-binding*, J Inorg Biochem, 105 (2011) 476–489.

[7] D.S. Raja, N.S. Bhuvanesh., *Effect of N(4)-Phenyl Substitution in 2-Oxo-1,2-dihydroquinoline-3-carbaldehyde Semicarbazones on the Structure, DNA/Protein Interaction, and Antioxidative and Cytotoxic Activity of Cu(II),* Natarajan Inorg Chem., 50 (2011) 12852–12866.