DNA/BSA interactions of new gold(III) complexes with some pyridine-containing ligands

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Abstract: Pyridine-containing ligands, 4,5-diazafluoren-9-one (dafone) and pyrazino[2,3-*f*][1,10]phenanthroline (ppz), were used for the synthesis of two novel gold(III) complexes, [$AuCl_3$(dafone)] (**Au1**) and [$AuCl_2$(ppz)][$AuCl_4$] (**Au2**). In this study, we investigated their interactions with bovine serum albumin (BSA) and calf thymus DNA (ct-DNA) using fluorescence emission spectroscopy to assess their binding affinity towards these biomolecules. Considering that serum albumin consists of three domains (I–III), each subdivided into two subdomains (A and B), fluorescence competition experiments were also performed using site-specific markers to locate the binding sites of the complexes. Eosin Y was used as a marker for Site I (subdomain IIA), ibuprofen for Site II (subdomain IIIA), and digitoxin for Site III (subdomain IB). The results, including the calculated binding constants (K_A), revealed that both **Au1** and **Au2** interact with BSA and ct-DNA, with **Au1** exhibiting a higher affinity toward ct-DNA. Furthermore, the K_A values of both **Au1** and **Au2** decreased in the presence of eosin Y, indicating that their primary binding site is likely located within Site I of BSA.

Keywords: gold(III) complexes, pyridine-containing ligands, protein interactions, DNA interactions

1. Introduction

Gold(III) complexes are promising agents in medicinal inorganic chemistry, and are explored for possible anticancer, antimicrobial, and antiparasitic applications [1]. With this in mind, we synthesized two gold(III) complexes, [$AuCl_3$(dafone)] (**Au1**) and [$AuCl_2$(ppz)][$AuCl_4$] (**Au2**), incorporating pyridine-containing ligands, 4,5-diazafluoren-9-one (dafone) and pyrazino[2,3-*f*][1,10]phenanthroline (ppz), respectively (Figure 1). These complexes were characterized using spectroscopic tehniques (IR, ^{1}H and ^{13}C NMR) and X-ray diffraction analysis. As a continuation of our work, we examined the interactions of the **Au1** and **Au2** complexes with bovine serum albumin (BSA) and calf

thymus DNA (ct-DNA) using fluorescence emission spectroscopy, aiming to evaluate their binding affinities toward these biomolecules.

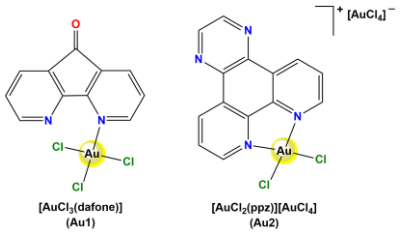

Figure 1. Structural formulas of the investigated gold(III) complexes **Au1** and **Au2**

2. Methodology

2.1 Chemicals

Hydrogen tetrachloridoaurate(III) trihydrate ($H[AuCl_4]\cdot 3H_2O$), 4,5-diazafluoren-9-one (dafone), pyrazino[2,3-*f*][1,10]phenanthroline (ppz), ethanol, dichloromethane (CH_2Cl_2), dimethyl sulfoxide (DMSO), phosphate-buffered saline (PBS), bovine serum albumin (BSA), calf thymus DNA (ct-DNA), ethidium bromide (EthBr), eosin Y (eos Y), ibuprofen (ibu), and digitoxin (dig) were obtained from Sigma-Aldrich Chemical Co (Steinheim, Germany).

2.2 BSA and DNA Binding Study

Stock solutions of **Au1** and **Au2** complexes were prepared in DMSO (1.0×10^{-2} M). Their interactions with BSA and ct-DNA were studied using fluorescence emission spectroscopy on a Shimadzu RF-6000 spectrofluorometer. BSA binding study was performed by tryptophan fluorescence quenching experiments in PBS (pH = 7.4) at room temperature, in the range of 295 – 500 nm, with an excitation wavelength of 290 nm. The concentration of BSA was constant (4.45 μM), while the concentrations of **Au1** and **Au2** were gradually increased (0 – 19.6 μM). Competitive binding experiments were performed using the site markers, eosin Y (eos Y), ibuprofen (ibu), and digitoxin (dig). BSA and each marker were mixed in equimolar amounts, followed by titration with increasing concentrations of **Au1** and **Au2**. The excitation and emission parameters remained as described above. DNA binding experiments were also carried out in PBS, by keeping [ct-DNA]/[EthBr] = 10, while increasing the concentrations of **Au1** and **Au2** complexes (0 – 32.9 μM). Measurements were performed in the wavelength range of 525 – 750 nm, with an excitation wavelength of 520 nm.

3. Results and Discussion

3.1. BSA Binding Study

Serum albumin is one of the most abundant proteins in blood plasma and plays a role in the transport of variuos drugs. Fluorescence emission spectra of BSA of constant concentration were recorded in the presence of an increasing concentrations of **Au1** and **Au2**. As shown in Figure 2, BSA fluorescence intensity decreases by increasing the

concentration of the complex, indicating its interactions with this protein. The values of binding constants (K_A) for **Au1** and **Au2** (Table 1) are high enough to indicate their binding to BSA, which can transport them to the corresponding biological targets [2].

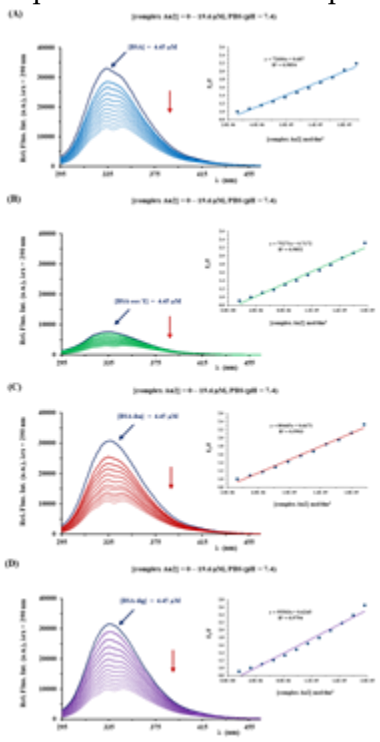

Figure 2. The BSA emission spectra in the presence of an increasing concentration of **Au2** complex (A) and in presence of the site markers (B – D). The red arrow shows the changes of the intensity after the addition of the complex. Inserted graph: Stern-Volmer plot of F_0/F *vs*. [**Au2**]

Table 1. Values of the BSA binding data for the gold(III) complexes **Au1** and **Au2** in the absence and presence of eosin Y (eos Y), ibuprofen (ibu) and digitoxin (dig) as the site markers

	K_{sv} (M^{-1})	Hypochromism (%)	K_q ($M^{-1}s^{-1}$)	K_A (M^{-1})	*n*
complex **Au1**-BSA	$(8.60 \pm 0.03) \times 10^4$	49.1	8.60×10^{12}	3.91×10^6	1.40
complex **Au1**-BSA-eos Y	$(3.94 \pm 0.03) \times 10^4$	43.2	3.94×10^{12}	8.28×10^5	1.30
complex **Au1**-BSA-ibu	$(6.74 \pm 0.03) \times 10^4$	50.3	6.74×10^{12}	2.23×10^6	1.35
complex **Au1**-BSA-dig	$(5.83 \pm 0.02) \times 10^4$	49.1	5.83×10^{12}	9.97×10^5	1.28
complex **Au2**-BSA	$(1.06 \pm 0.04) \times 10^5$	60.5	1.06×10^{13}	1.69×10^6	1.29
complex **Au2**-BSA-eos Y	$(1.25 \pm 0.03) \times 10^5$	60.6	1.05×10^{13}	4.95×10^5	1.17
complex **Au2**-BSA-ibu	$(1.21 \pm 0.03) \times 10^5$	64.8	1.21×10^{13}	7.47×10^5	1.20
complex **Au2**-BSA-dig	$(1.53 \pm 0.05) \times 10^5$	68.5	1.53×10^{13}	2.41×10^6	1.29

Based on the results presented in Table 1, the greatest decrease in the BSA binding constants of the investigated complexes was observed in the presence of eosin Y, suggesting that they may compete with this site marker and bind to Site I of BSA.

3.2. DNA Binding Study

The emission spectra of the EthBr-ct-DNA system were recorded in the absence and presence of an increasing amount of the investigated **Au1** and **Au2** complexes. In both cases, the addition of the gold(III) complexes led to decrease in fluorescence intensity, indicating their ability to interact with ct-DNA. Among the two complexes, **Au1** exhibited a stronger binding affinity toward ct-DNA, with a calculated binding constant (K_A, Table 2) comparable to that of EthBr ($K_A = 2 \times 10^6$ M^{-1}), suggesting that **Au1** could bind to DNA through the intercalative mode [3].

Table 2. Values of the binding constants of **Au1** and **Au2** complexes with ct-DNA

	K_{sv} (M^{-1})	Hypochromism (%)	K_q ($M^{-1}s^{-1}$)	K_A (M^{-1})	n
Au1	$(2.56 \pm 0.03) \times 10^4$	41.9	2.56×10^{12}	1.62×10^6	1.43
Au2	$(1.30 \pm 0.01) \times 10^4$	28.9	1.30×10^{12}	3.36×10^4	1.11

4. Conclusions

The results obtained within this study indicate that the gold(III) complexes with pyridine-containing ligands can interact with both BSA and ct-DNA. Their ability to bind BSA suggests they could be efficiently transported to the target cells. Once inside the cell, these complexes may interact with DNA and exert their mode of action. Notably, **Au1** shows a higher affinity toward ct-DNA and presents a potential intercalator.

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References

[1] B.Đ. Glišić, M.I. Djuran., *Gold complexes as antimicrobial agents: an overview of different biological activities in relation to the oxidation state of the gold ion and the ligand structure*, Dalton Transactions, 43 (2014) 5950-5969.

[2] A. Tarushi, E. Potatoglou, J. Kljun, I. Turen, G. Psomas, D. P. Kessissoglou., *Interaction of Zn(II) with quinolone drugs: Structure and biological evaluation,* Dalton Transactions, 40 (2011) 9461-9473.

[3] Y. Shi, C. Guo, Y. Sun, Z. Liu, F. Xu, Y. Zhang, Z. Wen, Z. Li., *Interaction between DNA and microcystin-LR studied by spectra analysis and atomic force microscopy*, Biomacromolecules, 12 (2011) 797-803.