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Multispectroscopic DNA-Binding studies and antimicrobial evaluation of new mixed-ligand Silver(I) complex and nanocomplex: A comparative study

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ABSTRACT

A novel mixed-ligand Ag(I) complex, , has been synthesized and characterized by the elemental analysis, IR spectroscopy and 1HNMR. In the formula, dian and phen are N-(4,5-diazafluoren-9-ylidene)aniline and 1,10-phenanthroline, respectively. This complex also has been prepared at nano size by sonochemical technique and characterized by the FTIR and scanning electron microscopy (SEM). To evaluate the biological preferences of the Ag(I) complex and nanocomplex and verify the relationships between the structure and biological function, in vitro DNA binding and antibacterial experiments have been carried out. DNA-complex interaction has been pursued by electronic absorption titration, luminescence titration, competitive binding experiment, effect of ionic strength, thermodynamic studies, viscometric evaluation and circular dichroism spectroscopy in the physiological pH. Each compound displays significant binding trend to the CT-DNA. The mode of binding to the CT-DNA probably is a moderate intercalation mode with the partial insertion of the planar ligands between the base stacks of double-stranded DNA. The relative viscosities and circular dichroism spectra of the CT-DNA with the complex solutions, confirm the intense interactions of the Ag(I) complex and nanocomplex with DNA. An in vitro antibacterial test of the complex and nanocomplex on a series of the Gram-positive bacteria (Staphylococcus aureus, Enterococcus faecalis) and the Gram-negative bacteria (Escherichia coli, Pseudomonas aeruginosa) shows a remarkable antibacterial feature of the Ag(I) complex. The MIC values (minimum inhibitory concentration) of the compounds compare with silver nitrate and silver sulfadiazine. The bacterial inhibitions of the Ag(I) complex and nanocomplex are agreed to their DNA binding affinities.

1. Introduction

Nowadays, inorganic chemists are interested in the medicinal inorganic chemistry which is a multidisciplinary field combining chemistry, pharmacology, biochemistry and toxicology [1]. Their researches offer opportunities for the design of therapeutic drugs not achievable by organic compounds [2]. In this area of research, chemists can benefit from the diversity of coordination numbers and geometries, redox states, thermodynamic and kinetic characteristics, and intrinsic properties of the metal ion and ligands which cause a large kinds of reactivities [3]. In the inorganic complexes, several parameters such as the size, structure and orientation of the ligands in the coordination sphere of the central metal atom influence on their DNA binding ability [4]. The application of the mixed-ligand complexes permits variation in the size, geometry, planarity, hydrophobicity, charge, and hydrogen-bonding ability of the complexes, it may lead to less or more changes in the binding modes, affinity, location, and even to a different cleavage effects [5,6].

To design more proper chemotherapeutic agents, it seems essential to know about the nature of the interactions of metal complexes with DNA. Binding of small inorganic or organic molecules to DNA can affect multiple biological processes like transcription and replication, in which DNA participates. There are variant types of sites in the DNA molecule where noncovalent binding of metal complexes can take place: (1) between two base pairs along the helix (intercalation), (2) in the major and minor grooves, and (3) on the outside of the helix (electrostatic interaction) [7].

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List of abbreviations

Dian	N-(4, 5-diazafluoren-9-ylidene) aniline
Dafone	4,5-diazafluoren-9-one
Phen	1,10-phenanthroline
CD	Circular dichroism
CT-DNA	Calf-thymus deoxyribonucleic acid
DMSO	Dimethylsulfoxide
EB	Ethidium bromide
UV	Ultra-violet
MIC	Minimum Inhibitory Concentration
AgSD	Silver sulfadiazine
SEM	Scanning Electron Microscopy

As we know, Schiff bases are important class of compounds in medicinal inorganic chemistry. They show biological applications such as antitumor [8], antibacterial [9] and antifungal activity [10]. Also 1,10-Phenanthroline and its organic derivatives, have been shown to disturb the functioning of many biological systems, both in the metal-free state or as the metal-coordinated ligand. These N,N′-chelating heterocycles complexes have been used as anticancer [11] and antimicrobial agents [12], DNA intercalators [13], and as nucleoside constituents for incorporation into the backbone of DNA [14]. The quest to design novel appropriate phenanthrolines is ongoing, and methods include synthesizing them from elementary building blocks [15]. These efforts performed in such a way to tune the chemical and physical properties of the 1,10-phenanthroline π system through chemical modification [16,17]. With this in mind, we sought to modify biological activity and compatibility by attempting to prepare a Schiff base, phen-type ligand by reacting dafone with aniline, in which dafone is the phenanthroline derivate itself and can be prepared by an oxidizing method [18].

Among the metal complexes, many silver(I) complexes are widely used for antibacterial purposes [19,20]. One example can be silver(I) sulfadiazine (AgSD) which has been applied clinically as an antibacterial and antifungal agent in cases of severe burns [21]. Despite the fact that the mechanism of antimicrobial nature of silver(I) complexes has not been well established, it is accepted that the silver(I) ions interfere with cell growth. Besides, silver(I) complexes of N/O donor ligands are proposed to target some bacterial proteins containing thiol groups in their active sites, for the reason that the soft sulfur coordinates readily to the soft silver(I) center [22]. Simple Ag(I) salts, such as $AgNO_3$ and $AgClO_4$, exhibit good anti-Candida activity too [23]. Ag^+ ion almost certainly is the active agent [24].

Many silver(I) complexes with various kind of the donating atoms in their ligands have also been studied for their antitumor activity [25,26]. They show selectivity against various types of cancer cells in regard to the kind of the ligands coordinated to silver(I) ions [27] and in some cases they have shown larger DNA-binding constants (more stable DNA–Ag complex) and comparable or higher in vitro anti-proliferative actions than that have been observed for cisplatin, the famous chemotherapy drug [28].

Recently, Medical science researches have taken advantage of nanosized particles which offer special different characteristics in comparison with macro particles such as better biological properties [29,30]. Advances in nanomaterials technologies develop new biomedicines in diagnosis and therapies steps and nanocarriers for drugs and genes delivery [31,32]. Over decades, development of nanoscale constructs with different components, sizes and shapes have been performed. Until now 30 clinically used nanomedicine and more than 300 under clinical trial candidates have been produced [29]. Especially in case of cancer therapy, using nanodrugs can eliminate several limitations of common chemotherapy such as poor water solubility of most drugs (Cisplatin, Paclitaxel, and Doxetaxel) [33,34], so this field remains a field of high activity. Newly the nanosized silver containing compounds have attracted more interests in the modern medicine [35,36].

The mentioned characteristics can make the complexes of silver(I) and phen-type ligands attractive molecules for bioinorganic chemists. In our laboratory, we have synthesized new complex and nanocomplex with these components and carried out studies on their DNA binding properties and antibacterial activities. The Ag(I) complex and nanosized one are of such importance that they show good behaviors. Additionally, the comparison of different size particles has shown that better biological activities can be predicted for the nanosized materials as it has been experienced before with other researchers [29].

2. Materials and methods

2.1. Main reagents and apparatus

All chemicals were of reagent grade quality and used without further purification. Most of them were commercially available and purchased from Merck Company.4,5-diazafluoren-9-one (dafone) was prepared following the Henderson method [18]. Calf thymus DNA (CT-DNA) type I, was procured from Sigma Chemical Company in solid Na^+ salt form and used as received. It was stored at .

Carbon, hydrogen and nitrogen contents (CHN Microanalysis) were determined on Eager 300 Summarize elemental analyzer. Molar conductance (in $\Omega^{-1}cm^2mol^{-1}$) was measured at room temperature on Systronics Conductivity Bridge 305, using a conductivity cell of 1.0 cell constant. IR spectrum was recorded on a FT-IR JASCO 680-PLUS spectrometer using KBr pellets from 4000 to 400 cm^{-1}. The ^1H NMR spectra were obtained on a Bruker 300 MHz Avance III spectrometer operating at room temperature. Electronic spectrum was recorded on UV-JASCO-570 spectrometer using corvettes of 1 cm path length. The mass spectra were recorded on an Agilent Technology (HP) mass spectrometer, operating at an ionization potential of 70 eV. The viscosity measurements were carried out using Ubbelodhe viscometer, soaked in a thermostated water bath at 26 °C. Emission spectra were measured on a Varian spectrofluorimeter model Cary Eclips in a thermostated cuvette holder. CD measurements were recorded on an Aviv Spectropolarimeter model 215 at 25 °C with a quartz cell of 0.1 cm path length. All the experiments involving interaction of the complexes with CT-DNA were carried out in TRIS buffer (containing 5 mM tris(hydroxymethyl)aminomethane and 50 mM sodium chloride that adjusted to pH 7.2 with HCl). The CT-DNA was dissolved in TRIS buffer over two nights. The CT-DNA solution was stored at 4 °C and discarded after no more than 4 days. Solutions of DNA gave ratios of UV absorbance at 260 and 280 nm above 1.8, showing that the DNA was sufficiently pure and free of protein [37]. DNA concentration per nucleotide was determined by absorption spectroscopy at 260 nm using the molar absorption coefficient 6600 $dm^3\,mol^{-1}cm^{-1}$ [38]. Concentrated stock solution of the Ag(I) complex was prepared by dissolving in 5% DMSO and diluting with TRIS buffer to the required concentrations for all the experiments. Using this method, the amount of DMSO in working solutions became less than 0.1% v/v. The stock solution of the Ag(I) nanocomplex was dispersed in the double distilled H_2O.