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Studies on the Interaction of Dinitratobis(phen) Cadmium Complex with DNA

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DNA-binding properties of the dinitratobis(phen) cadmium complex $[Cd(phen)_2(NO_3)_2]$ (where phen = 1,10-phenanthroline) have been investigated with absorption titration, fluorescence spectroscopy, viscosity measurement, molecular modeling and density functional theory (DFT) calculations. The results indictate DNA-binding mode of the complex to be weak groove binding rather than partial intercalative interaction expected of the extended planar aromatic phen ring. In addition, the DNA cleavage study was carried out by gel electrophoresis experiment. The results showed that the complex both hardly cleaves pBR322 DNA in the absence and present ascorbate. So it is suggested that the formation of cadmium complex can decrease cadmium toxicity to some extents.

Keywords cadmium complex, DNA interaction, density functional theory (DFT)

Introduction

Cadmium, as a by-product of zinc mining, fossil fuel, base metal smelting, combustion and atmospheric transport, is a highly toxic metal and a potent carcinogen. Even at very low concentrations cadmium ion also can cause extensive damage to kidney, liver, lungs, prostates and hematopoietic systems of human, so it has been accepted by the International Agency for Research on Cancer as a category carcinogen.[1,2] Recent studies suggest that cadmium-induced carcinogenicity involve direct or indirect interaction of cadmium with DNA because DNA offers many binding sites for cadmium,[3-6] although the exact molecular mechanism of cadmium-induced carcinogeniciy remains largely unclear. Indeed, it has been reported that Cd(II) ion can react with nucleobases, nucleic metallothionein and plasmid DNA causing extensive damage to these targets.[7] However, most studies on cadmium complex are focused on generating new materials up to now and there are few reports about the interaction of cadmium complexes with DNA.

In addition, chemistry of phenanthroline (phen) ligand and its complexes has been intensively investigated in recent years, owing to their coordination properties and diverse applications. Moreover, the ligand has been the subject of extensive investigation due to its versatile chelating behaviour for which it is widely used in analytical chemistry as a selective metal extracting agent as well as in spectroscopic determination of certain transition metals. Industrialization has resulted in an increase of heavy metals in the environment and as a consequence of their detrimental effects on health there is a corresponding interest in their detection and sequestration. Cadmium belongs to a category of heavy metal ions and is a non-essential metal. According to the literature,[8,9] one general mechanism for cadmium detoxification is the chelation of the metal. Notably, the chelation of Cd(II) ion with EDTA or citric acid decreases the binding affinity Cd(II) nucleobase.[10] It was therefore found worthwhile to carry out a systematic study of dinitratobis(phen) cadmium complex $[Cd(phen)_2(NO_3)_2]$ that includes the synthesis, and physicochemical and structural characterization, the DNA-binding properties of this compound by the method of experiment combined with theory.

Experimental

Materials and methods

All chemicals were of reagent grade and used without further purification unless otherwise noted. Triple distilled deionized water was used for preparing the Tris-HCl buffer. Calf thymus (CT) DNA and plasmid pBR322 DNA were purchased from Sino-American Biotechnology. The purity ($A_{260}/A_{280} > 1.9$) and concentration ($\varepsilon_{260} = 6600\ mol^{-1}\cdot cm^{-1}$) of CT DNA were checked by UV-vis spectroscopy.[11] For the gel electrophoresis experiments, supercoiled pBR322 DNA (0.7 μg) was treated with Cd(II) complex in Tris-HCl buffer (10 mmol•L^{-1} Tris, 6.2 mmol•L^{-1} NaCl, pH 7.26) and the solutions were incubated for 4 h at 37 ℃ in the

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dark. All other experiments involving CT DNA were carried out in aqueous 50 mmol•L^{-1} NaCl/5 mmol•L^{-1} Tris HCl [tris(hydroxymethyl) aminomethane hydrochloride] buffer at pH 7.12.

Microanalyses (C, H, N) were performed on a VARI-EL elemental analyzer. Molar conductivity at room temperature was measured in 10^{-4} mol•L^{-1} DMF solutions using a DDS-11A numeric conductivity instrument. ^{1}H NMR spectra were recorded on a Bruker DRX 300 MHz NMR spectrometer. The X-ray data were collected on a Bruker SMART APEX CCD diffractometer.[12] The structure was solved by direct methods (SHELXS-97).[13-15] Fluorescence measurements were made with a Perkin-Elmer LS50B fluorescence spectrometer. UV-Vis spectra were determined on a Shimadzu UV-365 spectrophotometer. Viscosity experiments were carried on an Ubbelodhe viscometer at a constant temperature at (20.0±0.1) ℃.

DFT structural optimizations at the B3LYP/LANL2DZ level[16-18] were performed on the complex. The imaginary frequencies and DFT wavefunction instabilities checked at the same theoretical level. All the calculations in this work were carried out by using the Gaussian 03 program.[19]

The complex-DNA interactions were also studied by molecular modeling. All calculations were performed in SGI workstation with Insight II software package. Because the CT DNA used in the experimental work was too large for current computational resources to model, the structure of the DNA d(CCGTCGACGG)$_2$ (a familiar sequence used in oligodeoxynucleotide study) was constructed in BIOPOLYMER Module of Insight II package to study the DNA binding characters of the complex. The DNA-complex interactions were examined by comparing the potential energy differences among different binding sites of both minor and major grooves.

Synthesis of the complex

For the preparation of the title compound, 1,10-phenanthroline (2.00 mmol, 0.396 g), iminodiacetic acid (1.00 mmol, 0.133 g) and $Cd(NO_3)_2$•$4H_2O$ (1.00 mmol, 0.308 g) were dissolved in a ethanol-water (1∶1, *V*∶*V*) solution (25 mL). The reaction mixture was refluxed for 30 min with stirring and cooled slowly, before being filtered and kept at room temperature. Red crystal is formed after a month. Anal. calcd for $C_{24}H_{16}N_6O_6Cd$: C 48.29, H 2.70, N 14.08; found C 48.10, H 2.77, N 14.01; ^{1}H NMR (DMSO-d_6) δ: 8.90 (d, J=5.67 Hz, 2H), 8.88 (d, J=6.39 Hz, 2H), 8.28 (s, 2H), 8.05 (dd, J=5.67, 6.39 Hz, 2H); molar conductivity (ohm^{-1}•cm^2•mol^{-1}): 4.96.

Results and discussion

Description of the crystal structure

The structure of the complex was revealed by a single crystal X-ray diffraction study. The mononuclear complex is a neutral molecule due to the two coordinated nitrate anions. Within the crystal structure, the Cd atom is surrounded by four pyridyl nitrogen atoms of two phen ligands and four oxygen atoms connected to two nitrate groups to complete a tetragonal antiprismatic coordinated geometry (Figure 1). The cadmium complex with the same coordinated condition has been reported.[20,21] Each nitrate ion in the title compound is acting as a bidentate coordinating agent. The four N atoms from two-phen are not co-planar, which has a significant departure from ideal planarity of the CdN_4 unit (the mean deviation from plane is 0.3070 Å). So the equatorial planar has much twist. The dihedral angle between the two phen rings is 24.0°. The four O atoms of two nitrates are also not co-planar and have much twist 56.4°. Cd atom lies in a distorted dodecahedron environment, in which the Cd—$N_{(phen)}$ bond distance of 2.341(4)—2.342(4) Å is within the normal range observed in polypyridyl Cd(II) complexes.[22] While the Cd—O_{NO_3} bond length of 2.582(5)—2.595(5) Å is a little longer than the value of reference.[23]

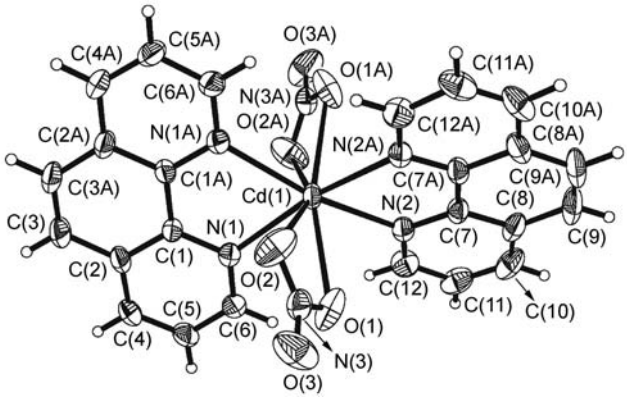

Figure 1 View of the structure of the complex.

Electronic absorption spectral studies

In order to investigate the mode of the cadmium complex binding to DNA, absorption titration experiment has been carried out. When DNA with different concentrations are added to 1×10^{-5} mol•L^{-1} cadmium complex in 5 mmol•L^{-1} Tris-HCl/50 mmol•L^{-1} NaCl buffer, the changes of the electronic absorption spectra are shown in Figure 2. On the incremental addition of DNA, the band shows very low hypochromism accompanied by a very small blue shift. On the other hand, some metal complexes with polypyridyl ligand exhibit classical hypochromism with a distinct red shift. They have been suggested to bind to DNA strongly through intercalation of the extended heterocyclic aromatic chromophores of the ligands between the DNA base pairs.[24] So it is obvious that the cadmium complex, which exhibits hypochromism much lower than these metalointercalators, is involved in DNA surface binding rather than intercalative interaction.

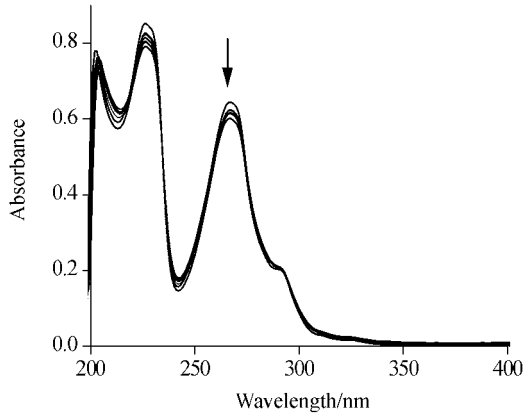

Figure 2 Absorption spectra of complex in the presence of different molar ratio of $c_{DNA}/c_{complex}(R_t)$. R_t=0, 2.0, 4.0, 6.0, 8.0, 12.0. Arrow shows the absorbance changing upon the increase of DNA concentration.

Emission studies

In the absence of DNA, when excitation wavelength is 275 nm, the complex can emit luminescence in 5 mmol•L^{-1} Tris-HCl/50 mmol•L^{-1} NaCl buffer at ambient temperature, with a maximum appearing at 371 nm. Upon addition of CT DNA, the fluorescence intensity of the complex slightly increases with the emission maximum almost unaffected. The results of the emission titration for cadmium complex are illustrated in Figure 3. This effect arises because the complex is bound in a relatively nonpolar enviroment compared to water in the presence of DNA. However, the increase in fluorescence intensity is much less than that for the intercalators containing polypyridyl ligand,[25,26] implying a very weak association to DNA of cadmium complex. The much weak association of the complex may be explained by visualizing the complex in the molecular modeling to be shallow bound in the DNA groove.

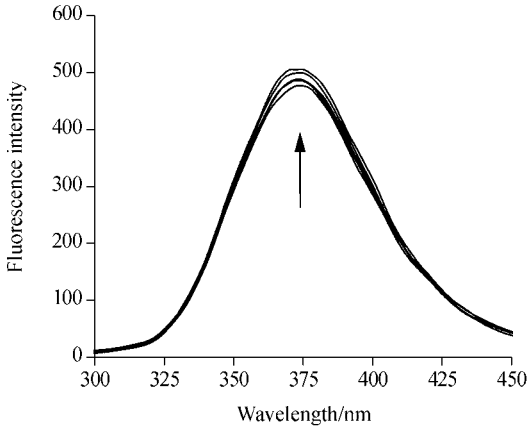

Figure 3 Fluorescence spectra of complex in the presence of different molar ratio of $c_{DNA}/c_{complex}(R_t)$. R_t=0, 4.0, 10.0, 12.0, 20.0, 40.0. Arrow shows the intensity changing upon the increase of DNA concentration.

Viscosity measurements

Photophysical probes generally provide necessary but insufficient clues to support the DNA-binding model. Therefore, hydrodynamic method, which is sensitive to DNA length change, offers the most definitive method of inferring the binding mode of DNA binding agents.[27] Intercalating agents are expected to destack the base pairs causing lengthening of the double helix resulting in an increase in the viscosity of DNA. In contrast, non-classical intercalation or groove binding of the complex could bend or kink the DNA helix, reduce its effective length and concomitantly its viscosity.[28] The effect of the cadmium complex and the classical intercalator ethidium bromide on the viscosity of DNA is depicted in Figure 4. In contrast to ethidium bromide, the relative viscosity of DNA slightly decreases on binding to cadmium complex. It is suggested the effective intercalation of the phen ring of cadmium complex be discouraged by several factors and the complex prefers to engage in DNA groove binding with phen ring, rather than in intercalative DNA interaction.

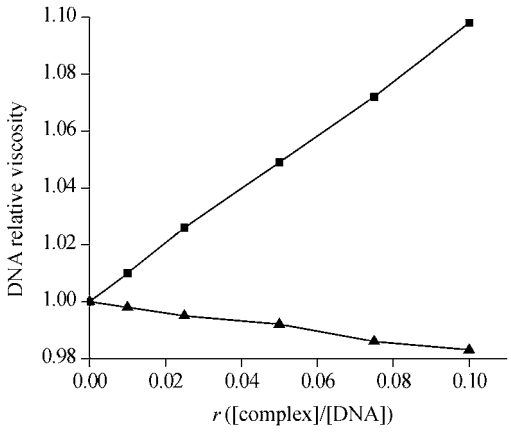

Figure 4 The effect of ethidium bromide (■) and cadmium complex (▲) on the relative viscosity of CT DNA.

Molecular modeling

To obtain further support for the DNA binding mode, the binding energies of the complex were calculated with the oligodeoxynucleotide.[29] The modeling results are tabulated in Table 1. As the table shows, the values of total energy indicate that the intercalations of phen ligand into T_4C_5 and C_5G_6 region DNA minor groove are the most preferential binding model of complex-DNA interactions. The model of interaction of complex with DNA minor groove T_4C_5 region is shown in Figure 5. As the Figure shows, the phen ligand of complex inserts into the minor groove, while the main part of the complex locates out of the DNA double helix. It can be seen from the model that the interaction of the complex and DNA is relatively weak, which closely corresponds with the experimental results mentioned above. The results can be explained from two aspects. Firstly, due to the electroneutral property of the cad-

Table 1 The energy (in kcal/mol) distribution of the complex-DNA interactions

Groove[a]	BS	Inter	Bond	Angle	Torsion	Out	Non-B	VDW	Elect	Total
Major	C_2G_3	815.2	30.7	576.9	203.0	4.7	1940.3	37.9	1902.4	2755.5
	G_3T_4	820.7	32.3	583.5	201.2	3.6	1916.6	41.3	1875.3	2737.3
	T_4C_5	819.9	31.5	582.3	202.2	3.8	1893.8	39.1	1854.7	2713.7
	C_5G_6	818.9	31.1	577.9	205.1	4.9	1889.9	42.1	1847.8	2708.9
Minor	C_2G_3	802.5	29.2	571.2	198.4	3.8	1941.9	34.7	1907.1	2744.4
	G_3T_4	793.5	29.3	565.0	195.0	4.2	1906.5	23.4	1883.1	2700.0
	T_4C_5	802.1	28.8	571.2	197.7	4.4	1842.2	30.8	1811.5	**2644.3**
	C_5G_6	813.6	30.5	575.3	203.4	4.4	1838.9	19.3	1819.5	**2652.5**

[a] Groove means which groove the complex interacts; BS＝Bind site; Inter＝internal energy, it's the sum of bond stretch (Bond), variety of bond angle (Angle), bond torsion (Torsion) and out-of-plane bend of bonds (Out); Non-B＝non-bond interactions, it's the sum of Van de Waals energy (VDW) and electrostatic energy (Elect); Total＝total energy, it's the sum of Inter and Non-B.

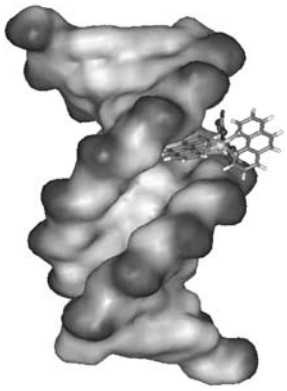

Figure 5 The binding model between the cadmium complex and oligodeoxynucleotide.

mium complex, the classical electrostatic interaction between the complex and DNA should be very weak. Secondly, the spatial collision between the NO_3^- ligands of the complex, the classical electrostatic interaction between complex with DNA framework not only affects the insertion of phen ligands to DNA base pairs, but also makes the complex leave from DNA minor groove easily.

Cleavage studies of plasmid pBR322 DNA

It has been suggested that cadmium-induced carcinogenicity involve DNA damage by cadmium to a great extent. Recent studies on DNA cleavage with cadmium ion suggested that Cd^{2+} bind covalently to nucleobases in DNA and cause DNA damage.[2-6] Moreover, damage to plasmid DNA due to Cd^{2+} is found to be greatly potentiated by the presence of ascorbate. The results show that, in presence of Cd, biological antioxidants ascorbate which normally play a protective role, can play a more damaging role due to the production of reactive oxygen species.[4] We investigated the cadmium complex interaction with pBR322 DNA by Agarose gel electrophoresis. The results indicate that the stable complex hardly cleaves DNA in the absence and presence of ascorbate, while Cd^{2+} ion can effectively cleave DNA in the same condition (Figure 6). According to the suggested mechanism of DNA cleavage by Cd^{2+} ion, we think that it is very difficult for center cadmium to bind to the other ligand groups synchronously because of the strong stability and crowded ligand situation in the cadmium complex. Consequently the formation of the complex may reduce cadmium- induced carcinogenicity to some extent.

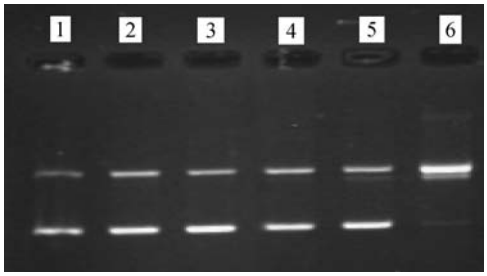

Figure 6 Agarose gel electrophoresis patterns for the cleavage of DNA under various conditions. lane 1, DNA; lane 2, DNA＋1 mmol•L^{-1} ascorbic acid; lane 3, DNA＋10^{-4} mol•L^{-1} complex; lane 4, DNA＋10^{-4} mol•L^{-1} complex＋1 mmol•L^{-1} ascorbic acid; lane 5, DNA＋10^{-4} mol•L^{-1} Cd^{2+}; lane 6, DNA＋10^{-4} mol•L^{-1} Cd^{2+}＋1 mmol•L^{-1} ascorbic acid.

DFT studies

Aiming to more easily understand the properties of the complex observed in the experiments, we have carried out DFT calculations. The results from calculations not only are consistent with those of the experimental studies on solid structural distances, IR spectroscopy and NMR chemical shift, but also offer us further explanation of the weak binding affinity of the complex with DNA.

The frontier molecular orbitals, in particular, the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) are very important because they relate to not only the spectral properties but also the DNA-binding affinities of the complexes. The interaction between the complex and DNA can be analyzed by applying the frontier molecular orbital theory established by Fukui.[30-32] According to this theory, for a reaction controlled by the

this theory, for a reaction controlled by the orbital interactions, if the HOMO energy of one reactant is higher than LUMO energy of the other reactant, they are easier to take reaction. Kruita[33] reported recently a perfect simplified approximation model by the DFT method for stacked base pairs with backbones, which are feasible for discussion on the trends of complex-DNA interactions. The calculated energies of the HOMO and NHOMO (next HOMO) for the CG/GC stacking are −1.27 and −1.33 eV. On the other hand, for the cadmium complex, the DFT results showed that the energies of its LUMO and NLUMO (next LUMO) are −2.27 and −2.26 eV. Energy gap between the HOMO of the stacked base pairs and the LUMO of the complex is about 1 eV, indicating the weak DNA-binding affinity. We consider, as also indicated by above-mentioned experimental and molecular modeling studies, that the electroneutral and crowed spatial structure of the complex affect its interaction with DNA.

Conclusion

We report here the synthesis and characterization of cadmium (II) complex and its interaction with DNA. The results show that the complex weakly binds to the minor groove of DNA, although the complex has planer aromatic phen ligand. This observation is supported by molecular modeling studies, which reveal that binding energy of the complex to the TC region of DNA minor groove is lower than to any other region of DNA. The results from DFT calculations also offer us further explanation of the complex weak binding affinity with DNA. And the complex both hardly cleaves pBR322 DNA in absence and present ascorbate by gel electrophoresis experiment. So we think that the formation of cadmium complex can decrease cadmium toxicity to some extents.

Supplementary material

Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with Cambridge Crystallographic Data Center as supplementary publication nos. CCDC 259489. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK, (fax: 0044-1233-336033 or e-mail: deposit@accdc.cam.ac.uk).

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