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Synthesis, DNA-binding and spectral properties of novel complexes $[RuL_2(idpq)]^{2+}$ (L = bpy, phen) with embedded C=O

Xue-Wen Liu [a,b], Lian-Cai Xu [a,c], Hong Li [c], Hui Chao [a], Kang-Cheng Zheng [a,*], Liang-Nian Ji [a,*]

[a] The Key Laboratory of Bioinorganic and Synthetic Chemistry of Ministry of Education, School of Chemistry and Chemical Engineering, Sun Yat-Sen University, Xin-Gang Street, Guangzhou 510275, PR China
[b] Department of Chemistry and Chemical Engineering, Hunan University of Arts and Science, ChangDe 415000, PR China
[c] Department of Chemistry, Southern China Normal University, Guangzhou 510000, PR China

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ABSTRACT

A novel ligand idpq with embedded C=O and its two complexes, $[Ru(bpy)_2(idpq)]^{2+}$ **1** and $[Ru(phen)_2(idpq)]^{2+}$ **2** (bpy = 2,2′-bipyridine; phen = 1,10-phenanthroline; idpq = indeno[1,2-*b*]dipyrido [3,2-*f*:2′,3′-*h*]-quinoxaline-6-one), have been synthesized and characterized by elemental analysis, ES-MS, ^1H NMR, UV–vis and CV. The DNA-binding behaviors of both complexes were studied by spectroscopic methods and viscosity measurements. The results indicate that the two complexes can all bind to CT-DNA in an intercalative mode, and they have rather high DNA-binding constants, which are $(1.7 \pm 0.4) \times 10^6\ M^{-1}$ and $(4.0 \pm 0.6) \times 10^6\ M^{-1}$, respectively. The results also show that these two Ru(II) complexes can promote photocleavage of pBR322 DNA. Their DNA-binding and electronic absorption-spectral properties were further studied by the DFT/TDDFT methods. The DNA-binding behaviors and difference of these complexes were reasonably explained, and the simulated absorption spectra were in good agreement with the experimental ones.

1. Introduction

During the past decades, Ru(II) polypyridyl complexes have been widely studied due to their potential applications in stereoselective probes of nucleic acid structures, molecular "light switches", DNA-photocleavage, optical reagents and solar energy utilizations [1–12]. It is well-established that these Ru(II) complexes can bind to DNA in a non-covalent interaction such as electrostatic binding, groove binding, or intercalation mode, in which the intercalation mode is the most interesting one because there is strong π–π stacking interaction between the intercalative ligand of the complex and DNA base-pairs. The intercalation interaction not only closely relates to their above-mentioned wide applications, but also offers a wide and interesting research field for experimental and theoretical works [3,13–18].

The Ru(II) polypyridyl complexes with great potential applications have inspired vigorous interests of scientists to continuously design novel species. Many novel polypyridyl-type ligands (L) used as intercalative ligand and their corresponding Ru(II) complexes $[Ru(phen)_2L]^{2+}$, $[Ru(bpy)_2L]^{2+}$ and so on were designed, synthesized and characterized. Meanwhile, their DNA-binding, DNA-photocleavage and spectral properties were deeply investigated. A great number of reports have shown that via improving the structure of ligand, in particular, intercalative ligand, most of resulting Ru(II) polypyridyl-type complexes were found to have special DNA-binding, DNA-photocleavage and spectral properties so that their applications in biochemistry and photochemistry were further developed [3,8,18–27]. So far many designs or structural modifications of the intercalative ligands mainly come down to three aspects as follows: (1) Introducing electron-withdrawing or electron-pushing substituents on some typical intercalative ligand, e.g., dpq, pip and dppz and so on [13,24,28]. (2) Extending the conjugative aromatic ligand area on the basis of bpy, phen, dpq, pip and dppz, including symmetrical (e.g., phehat) and asymmetrical ones (e.g., ptda; ptdp) [29,30] (3) Embedding some N

Abbreviations: bpy, 2,2-bipyridine; phen, 1,10-phenanthroline; idpq, indeno[1,2-*b*]dipyrido[3,2-*f*:2′,3′-*h*]-quinoxaline-6-one; dpq, dipyrido[3,2-d:2′,3′-*f*]-quinoxaline; pip, 2-phenylimidazo[4,5-*f*][1,10]-phenanthrolin; dppz, dipyrido [3,2-*a*:2′,3′-*c*]phenazine; phehat, 1,10-phenanthrolino[5,6-*b*]1,4,5,8,9,12-hexaaza-triphenylene; ptda, 3-(pyridine-2-yl)-*as*-triazino[5,6-*f*]acenaphthylene; ptdp, 3-(pyridin-2-yl)-*as*-triazino-[5,6-*f*]phenanthrene; ppd, pteridino[7,6-*f*][1,10]-phenanthroline-1,13(10H,12H)–dione; dmppd, 10,12-dimethyl-ppd; taptp, 4,5,9,18-tetraazaphenanthreno[9,10-*b*]triphenylene; CT-DNA, calf thymus DNA; FAB-MS, fast atom bombardment mass spectra; ESI-MS, electrospray ionisation mass spectrometry; CV, cyclic voltammetry; DFT, density functional theory; TDDFT, time-dependent density functional theory; Tris, tris(hydroxymethyl)aminomethane hydrochloride; EDTA, ethylenediaminetetraacetic acid; EB, ethidium bromide = 3,8-diamino-5-ethyl-6-phenylphenanthridinium bromide; MLCT, metal-to-ligand charge transfer; CD, circular dichroism.

* Corresponding authors. Tel.: +86 20 84110696; fax: +86 20 84112245.
E-mail addresses: ceszkc@mail.sysu.edu.cn (K.-C. Zheng), cesjln@mail.sysu.edu.cn (L.-N. Ji).

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atoms with greater electronegativity into conjugative skeleton of ligand (e.g., taptp). [31] In fact, there are still many methods on design or structural modification of intercalative ligand in order to obtain novel Ru(II) complexes with an excellent bioactivity. Recently, some novel Ru(II) polypyridyl-type complexes in which a carbonyl C=O group is introduced into intercalate ligand, i.e., $[Ru(bpy)_2(ppd)]^{2+}$ and $[Ru(bpy)_2(dmppd)]^{2+}$ have been reported.[32,33] Since introducing a carbonyl C=O into intercalative ligand not only enlarges the conjugative area of the intercalative ligand but also makes the LUMO energy of corresponding Ru(II) complex reduce [13], it can be expected the DNA-binding and photocleavage and related properties of the resulting Ru(II) complex will be improved. However, so far the reports on this aspect remain quite unfrequent. Therefore, it is a very significant work to design and synthesize novel Ru(II) polypyridyl complexes with C=O group embedded into their intercalative ligand as well as reveal their biochemical properties.

In this work, we report the synthesis and characterization of a novel ligand (idpq = indeno[1,2-*b*]dipyrido[3,2-*f*:2′,3′-*h*]-quinoxaline-6-one) with embedded C=O and without any symmetry as well as its two Ru(II) complexes, $[Ru(bpy)_2(idpq)]^{2+}$ **1** and $[Ru(phen)_2(idpq)]^{2+}$ **2**. We expect these novel Ru(II) complexes have some improved properties in DNA-binding, DNA-photocleavage and spectral behaviors. Therefore, in this paper, the DNA-binding, DNA-photocleavage and spectral properties of these novel Ru(II) complexes are carefully studied. In addition to experimental works, the theoretical study of these complexes using DFT/TDTFT methods is also carried out in order to reveal the trend in their DNA-binding affinities as well as explain their spectral properties.

2. Experimental

All reagents and solvents were purchased commercially and used without further purification unless otherwise noted. The dialysis membrane was purchased from Union Carbide Co. and treated by means of general procedure before use [19]. Solutions of CT-DNA in 50 mM NaCl, 5 mM Tris–HCl (pH 7.2) gave a ratio of UV–vis absorbance of 1.8–1.9:1 at 260 and 280 nm, indicating that the DNA was sufficiently free of protein [34]. The concentration of DNA was determined spectrophotometrically using a molar absorptivity of 6600 $M^{-1}\,cm^{-1}$ (260 nm) [35]. Double-distilled water was used to prepare buffers. The complexes *cis*-$[Ru(bpy)_2Cl_2]\cdot 2H_2O$, *cis*-$[Ru(phen)_2Cl_2]\cdot 2H_2O$ [36], 5-nitro-1,10-phenanthroline [37,38], 6-amino-5-nitro-1,10-phenanthroline [39], 5,6-diamino-1,10-phenanthroline [40], $[Ru(bpy)_2(5,6\text{-}(NH_2)_2\text{-}phen)]^{2+}$ and $[Ru(phen)_2(5,6\text{-}(NH_2)_2\text{-}phen)]^{2+}$ [41] were prepared by the literature methods.

2.1. Physical measurement

Microanalyses (C, H, and N) were carried out with a Perkin-Elmer 240Q elemental analyser. Fast atom bombardment mass spectra were measured on a VG ZAB-HS mass spectrometer with 3-nitrobenzyl alcohol as matrix and electrospray ionisation mass spectra were recorded on a LQC system (Finngan MAT, USA) using CH_3CN as mobile phase. The spray voltage, tube lens offset, capillary voltage and capillary temperature were set to 4.50 kV, 30.00 V, 23.00 V and 200 °C, respectively, and the *m/z* values were quoted for the major peaks in the isotope distribution. ^1H NMR spectra were recorded on a Bruker ARX-500 spectrometer with $(CD_3)_2SO$ for the complexes at 500 MHz at room temperature. All chemical shifts relative to TMS (tetramethylsilane) were given. Absorption spectra were recorded on a Perkin-Elmer Lambda850 spectrophotometer and emission spectra were recorded on a Perkin-Elmer Ls55 spectrofluorophotometer at room temperature. Cyclic voltammetry was performed on an Autolab PGSTAT-30 electrochemical analysis system and by using GPES 4.8 software package (Eco Chemie, the Netherlands). The supporting electrolyte was 0.1 M tetrabutylammonium hexafluorophosphate in acetonitrile freshly distilled from phosphorus pentaoxide. All samples were purged with nitrogen prior to measurements. A standard three-electrode system comprising a platinum microcylinder working electrode and a platinum-wire auxiliary electrode and a saturated calomel reference electrode (SCE) was used. CD spectra were recorded on a JASCO J-810 spectrometer in a quartz cuvette with 10 mm path-lengths at 25 °C. Ten acquisitions per spectrum were run from 200 nm to 400 nm in 50 nm/min.

The absorption titrations of Ru(II) complexes in buffer (5 mM Tris–HCl, 50 mM NaCl, pH 7.2) were performed by using a fixed ruthenium concentration to which increments of the DNA stock solution were added. Ruthenium solutions employed were 20 μM in concentration and calf thymus DNA was added to a ratio of 6:1 [DNA]/[Ru]. Ruthenium–DNA solutions were allowed to incubate for 10 min before the absorption spectra were recorded.

Viscosity measurements were carried out using an Ubbelohde viscometer maintained at a constant temperature of 30.0 ± 0.1 °C (in a thermostatic bath). Flow time was measured with a digital stopwatch and every sample was measured three times and an average flow time was calculated. Data were presented as $(\eta/\eta^0)^{1/3}$ vs. binding ratio [41], where η is the viscosity of DNA in the presence of complex and η^0 is the viscosity of DNA alone.

Equilibrium dialysis measurements were conducted at room temperature with 5 cm^3 of CT-DNA (0.5 mM) sealed in a dialysis bag (cellulose membrane) and 10 cm^3 of the complexes (20 M) outside the bag with the solution stirred for 12 h. In the control experiments, 5 cm^3 Tris–HCl buffer was used instead of CT-DNA. Before use, the dialysis membranes were boiled for *ca.* 1 h in a 1% EDTA and 3% $NaHCO_3$ solution, and then rinsed in doubly deionised water.

For the gel electrophoresis experiments, supercoiled pBR322 DNA (0.1 μg) was treated with Ru(II) complexes in 50 mM Tris, 18 mM NaCl buffer, pH 7.8, and the solutions were incubated for 1 h in the dark, then irradiated at room temperature with an UV lamp (365 nm, 10 W). The samples were analyzed by electrophoresis for 1 h at 75 V in TBE buffer (89 mM Tris, 89 mM boron hydroxide, 2 mM EDTA) containing 1% agarose gel. The gel was stained with 0.5 μg/ml ethidium bromide and then photographed under UV light.

2.2. Synthesis

2.2.1. idpq (indeno[1,2-b]dipyrido[3,2-f:2′,3′-h]-quinoxaline-6-one)

A solution of 5,6-diamino-1,10-phenanthroline (0.063 g, 0.3 mmol) and ninhydrin (0.054 g, 0.3 mmol) in 5% dilute acetic acid (60 ml) was stirred for 30 min at room temperature. The yellow precipitate was collected by filtration, washed with water, and vacuum-dried. Yield: 0.088 g, 85.6% Anal. (%): (Found: C, 75.35; H, 3.09; N, 16.81. Calcd for $C_{21}H_{10}N_4O$: C, 75.44; H, 3.01; N, 16.76); FAB-MS: *m/z* = 335 (M+1).

2.2.2. $[Ru(bpy)_2(idpq)](ClO_4)_2\cdot H_2O$ (**1**)

A solution of ninhydrin (0.054 g, 0.3 mmol) in 5% dilute acetic acid (40 ml) was added to a solution of $[Ru(bpy)_2(5,6\text{-}(NH_2)_2\text{-}phen)](ClO_4)_2$ (0.165 g, 0.2 mmol) in 1 ml CH_3CN, and the mixture was refluxed under argon for 2 h. Upon cooling, the resulting clear red solution was treated with a saturated aqueous solution of $NaClO_4$, and a red precipitate was obtained. The crude product was purified by column chromatography on alumina with acetonitrile–toluene (2:1, v/v) as an eluent. The main red band was collected. The solvent was removed under reduced pressure and red microcrystals were obtained. Yield: 0.136 g, 72%; Anal. (%): (Found: