Inorganica Chimica Acta 381 (2012) 95–103

Contents lists available at SciVerse ScienceDirect

Inorganica Chimica Acta

journal homepage: www.elsevier.com/locate/ica

Synthesis, characterization, photophysical studies and interaction with DNA of a new family of Ru(II) furyl- and thienyl-imidazo-phenanthroline polypyridyl complexes

Bruno Pedras [a,b,*], Rosa M.F. Batista [c], Laura Tormo [d], Susana P.G. Costa [c], M. Manuela M. Raposo [c,*], Guillermo Orellana [d,*], José Luís Capelo [a,b], Carlos Lodeiro [a]

[a] Grupo BIOSCOPE, Departamento de Química-Física, Facultad de Ciencias de Ourense, Universidad de Vigo, Campus de Ourense, E-32004 Vigo, Spain
[b] REQUIMTE, Departamento de Química, FCT-UNL, 2829-516 Caparica, Portugal
[c] Centro de Química, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal
[d] Departamento de Química Orgánica I, Facultad de Química, Universidad Complutense de Madrid, E-28040 Madrid, Spain

ARTICLE INFO

Article history:
Available online 23 July 2011

Fluorescence Spectroscopy: from Single Chemosensors to Nanoparticles Science – Special Issue

Keywords:
Ru(II) complexes
DNA
Phenanthroline
Thiophene
Furan

ABSTRACT

A new family of Ru(II) polypyridyl complexes (**C1** to **C6**) containing furyl- or thienyl-imidazo-phenanthroline ligands (**4**–**6**) were synthesized using microwave irradiation and characterized by elemental analysis, ^{1}H NMR, UV–Vis absorption and fluorescence spectroscopy, FAB, ESI-MS and MALDI-TOF-MS spectrometry. On the other hand, the novel furyl- or thienyl-imidazo-phenanthroline derivatives (**5**–**6**) were synthesized through the Radziszewski reaction and completely characterized by the usual spectroscopic techniques. The interaction of the complexes with calf thymus DNA in the absence and in the presence of different quenchers (ethidium bromide, potassium hexacyanoferrate(II) and methyl viologen) has been studied by absorption spectroscopy, steady-state and single-photon timing luminescence measurements. Their electronic spectra show visible absorption peaks at 457–463 nm, with red luminescence at 603–613 nm. The emission quantum yields of these complexes are between 0.006 and 0.016 in air-equilibrated DMSO solution. Luminescence lifetimes in water lie within the 0.4–1.0 μs range, with a non-exponential behavior due to aggregation of the probe. Ru(II) complexes **C3**, **C4**, **C5** and **C6** show intrinsic dsDNA-binding constants of 2.74×10^5, 3.02×10^5, 1.32×10^5 and 1.63×10^5 M^{-1}, respectively. The planar extended structure of the imidazo-phenanthroline ligands and the collected spectroscopic data suggest a partial intercalative binding mode of the novel metal probes to double-stranded DNA.

1. Introduction

Among the multiple application fields of ruthenium(II) polypyridyl complexes [1], its interaction with nucleic acids has been one of the most thoroughly studied over more than two decades [2]. Featuring a unique set of chemical properties such as stability, excited-state reactivity, redox potentials [3], luminescence and excited state lifetimes, these complexes have attracted considerable attention from a great number of researchers, finding applications in areas such as photophysics, photochemistry, supramolecular chemistry, bioinorganic chemistry and catalysis.

Concerning their interaction with biological structures, Ru(II) polyazaheteroaromatic compounds have been used as probes of the biopolymer tertiary structure, photocleavage agents and, in recent times, as inhibitors of biological functions [4,1a]. One of the most extensively studied metal complexes used as luminescent probe is $[Ru(bpy)_2(dppz)]^{2+}$ (bpy = 2,2′-bipyridine, dppz = dipyrido[3,2-*a*:2′,3′-*c*]phenazine) [5]. This complex functions as a molecular "light switch" for DNA because of the dramatic emission enhancement experienced by this probe and related phenazine complexes in the presence of double-stranded nucleic acids, being otherwise weakly emissive in aqueous solution. The reason for this behavior is the peculiar electronic nature of the phenazine ligand and the lowest-lying excited state swap that occurs in protic solvents [6], together with the intercalative binding mode to double-stranded DNA of Ru(II)-dppz and related complexes [7]. For all ruthenium(II) polypyridyl complexes, non-radiative vibrational deactivation with the water molecules can be minimized by a close interaction with a hydrophobic negatively charged surface [8], and the intimate contact (e.g. DNA intercalation) with the biopolymer protects the triplet excited state of the probe from the O_2 quenching [9], overall leading to a substantial increase in the ^{3}MLCT excited state lifetime.

* Corresponding authors at: REQUIMTE, Departamento de Química, FCT-UNL, 2829-516 Caparica, Portugal. Fax: +351 212 948 385 (B. Pedras), fax: +351 253 604382 (M.M.M. Raposo), fax: +34 91 394 4103 (G. Orellana).
E-mail addresses: b.pedras@dq.fct.unl.pt (B. Pedras), mfox@quimica.uminho.pt (M.M.M. Raposo), orellana@quim.ucm.es (G. Orellana).

0020-1693/$ - see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.ica.2011.07.020

Very recently these complexes have been applied as multifunctional biological agents for direct imaging of DNA in living cells [10]. By varying the ligands that constitute the complexes, it is possible to modify the nature and strength of their binding to nucleic acids. As mentioned above, all positively charged complexes are expected to be attracted to the anionic DNA, and those containing at least one extended heteroaromatic ligand in the coordination sphere may insert such ligand between adjacent base-pairs of double-stranded DNA (i.e., binding by intercalation) [2a,7,9a,11]. Indeed, it has been observed that while $[Ru(bpy)_3]^{2+}$ binds in a relatively weak manner to DNA (mostly through electrostatic interaction in one of the grooves), the interaction of $[Ru(phen)_3]^{2+}$ is stronger [12].

Therefore, in order to study the factors that influence the DNA-binding mode of Ru(II) complexes with extended and ancillary ligands, the structural diversity of the metal chelating structures must be taken into account. Moreover, a further tuning of the probe photophysical and DNA binding properties might be achieved when new extended conjugation systems are composed by different heterocyclic nuclei (molecular *meccano* concept).

Following our ongoing projects on fluorescent reporters and their sensing applications [13], we have tackled the synthesis and characterization of novel (oligo)thienyl- [14] and arylthienyl-imidazo-phenanthrolines [15] due to their interesting emissive properties (see below). Taking also into account that furans exhibit high fluorescence quantum yields [16] and the recent studies concerning the DNA binding and photocleavage of Ru(II) complexes of a 2-(5-methyl-furan-2-yl)imidazo[4,5-*f*][1,10]phenanthroline ligand [17], suggesting that these complexes bind to DNA through intercalation and, when irradiated at 400 nm, promote the photocleavage of the DNA, we set out to synthesize Ru(II) furyl-imidazo-phenanthroline complexes that were hitherto unknown.

In this way, four new ligands and six Ru(II) polypyridyl complexes, **C1** to **C6**, are photophysically characterized in this work, and the interaction of the latter with calf-thymus DNA has been followed by steady-state and single-photon timing luminescence measurements. The effect of three different quenchers on the emission properties of the DNA-bound complexes has also been studied, in an attempt to reveal the possible binding modes of the complexes to the nucleic acid [18].

2. Experimental

2.1. Materials

All reagents used in the present work were commercially available and used without further purification, unless otherwise stated. Progress of the reactions was monitored by thin layer chromatography (0.25 mm thick pre-coated Merck Fertigplatten Kieselgel $60F_{254}$ silica plates), while purification was carried out by silica gel column chromatography (Merck Kieselgel 60; 230–400 mesh). Calf thymus DNA was obtained from Pharmacia GE Healthcare and purified by extensive dialysis against Tris buffer. The concentration of stock solutions was determined spectrophotometrically using the molar absorption coefficient per base pair ($12\,800\ M^{-1}\ cm^{-1}$) at 258 nm [9a] and found to be 2.82 mM (for the stock solution used with **C1** to **C3**) and 2.02 mM (for the stock solution used with **C4** to **C6**) in base pairs. All the experiments with DNA were carried out in pH 7.0 3-mM Tris buffer.

2.2. Instrumentation

NMR spectra of the ligands were obtained on a Varian Unity Plus spectrometer at 300 MHz for ^1H NMR and 75.4 MHz for ^{13}C NMR or a Bruker Avance III 400 at 400 MHz for ^1H NMR and 100 MHz for ^{13}C NMR, using the solvent residual peak as internal reference. The solvents are indicated in parenthesis before the chemical shift values (δ in ppm relative to TMS). NMR spectra of the complexes were recorded on a Bruker AVANCE II at 400 MHz for ^1H NMR, and processed with the TOPSPIN 2.0 software (Bruker). Melting points were determined on a Gallenkamp apparatus and are uncorrected. Infrared spectra were recorded on a BOMEM MB 104 spectrophotometer. UV–Vis absorption spectra of the ligands (200–800 nm) were measured with a Shimadzu UV/2501PC apparatus and those of the complexes on a Varian Cary 3Bio spectrophotometer.

Mass spectrometry analyses of the ligands were performed at the C.A.C.T.I. – Unidad de Espectrometria de Masas of the University of Vigo, Spain. MALDI-TOF-MS spectra have been performed in a MALDI-TOF-MS model Voyager DE-PRO Biospectrometry Workstation equipped with a nitrogen laser radiating at 337 nm from Applied Biosystems (Foster City, United States) from the MALDI-TOF-MS Service of the REQUIMTE, Chemistry Department, Universidade Nova de Lisboa and in the MALDI-TOF-MS–MS model 4700 Applied Biosystems at the Faculty of Science of Ourense, University of Vigo. The acceleration voltage was 2.0×10^4 kV with a delayed extraction (DE) time of 200 ns. The spectra represent accumulations of 5×100 laser shots. The reflection mode was used. The ion source and flight tube pressures were less than 1.80×10^{-7} and 5.60×10^{-8} Torr, respectively.

The MALDI mass spectra of the soluble compounds (1 or 2 μg/μL) were recorded using the conventional sample preparation method for MALDI-MS without other MALDI matrix.

Elemental analyses were performed at the Analytical Services of the Laboratory of REQUIMTE-Departamento de Química, Universidade Nova de Lisboa, on a Thermo Finnigan-CE Flash-EA 1112-CHNS Instrument.

The steady-state luminescence measurements were carried out with a Perkin-Elmer LS-5 spectrofluorometer. Luminescence lifetimes were determined at 25 ± 1 °C using ca. 10^{-5} M solutions of the Ru(II) complexes by the single-photon timing (SPT) technique with an Edinburgh Analytical Instruments LP900 kinetic spectrometer. Excitation of the samples was carried out with a Horiba NanoLED-07N 405-nm pulsed laser diode (<700 ps). A wide band-pass 405-nm interference filter (Edmund Scientific) was placed in front of the laser source and cut-off filters (590 nm, Lambda Research Optics) were used in the emission path to avoid distortions from the laser light scattering. The luminescence decay profiles were fit either to a single exponential function or to a sum of 2–3 exponential functions with the original Marquardt algorithm-based EAI decay analysis software. Satisfactory fits were obtained in all cases, as judged from the weighted residuals, the goodness-of-fit χ^2 parameter and the autocorrelation function. No oxygen outgassing was performed. The accuracy in the measured lifetimes for the multi-exponential decay fits is estimated to be ±3% (1% for the single-exponential decays) and 10% for the relative weights. The pre-exponential weighted emission lifetime (τ_m) is defined according to Eq. (1), where τ_i is the emission lifetime for each component of the multi-exponential fit whose relative weight is $(\%)_i$.

$$\tau_m = \sum_i \frac{(\%)_i}{100}\tau_i \quad (1)$$

For the preliminary studies of complexes **C1** to **C3** in the presence of DNA, a solution of the metal complex in Tris buffer with a concentration of ca. 10^{-5} M was obtained by dilution of a concentrated DMSO stock solution of each complex. Each solution was mixed with DNA in different proportions, and their spectra recorded both in the presence and in the absence of the biopolymer. The mixtures were allowed to equilibrate for at least half an hour between each addition and the respective measurement.