Journal of Inorganic Biochemistry 102 (2008) 2052–2059

Contents lists available at ScienceDirect

Journal of Inorganic Biochemistry

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

DNA binding and bending by dinuclear complexes comprising ruthenium polypyridyl centres linked by a bis(pyridylimine) ligand

Ursula McDonnell [a], Matthew R. Hicks [a], Michael J. Hannon [b,1], Alison Rodger [a,*]

[a] Department of Chemistry, University of Warwick, Coventry CV4 7AL, UK
[b] School of Chemistry, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK

ARTICLE INFO

Article history:
Received 9 March 2008
Received in revised form 3 June 2008
Accepted 3 June 2008
Available online 20 June 2008

Keywords:
Bimetallo complexes
Ruthenium metal complexes
DNA binding
Linear dichroism
DNA bending

ABSTRACT

The interaction of enantiomerically pure dinuclear complexes of the form $[Ru_2(L–L)_4L^1]^{4+}$ (where L–L = 2,2′-bipyridine (bpy) or 1,10-phenanthroline (phen) and L^1 = bis(pyridylimine) ligand ($(C_5H_4N)C{=}N(C_6H_4))_2CH_2$)) with ct-DNA have been investigated by absorbance, circular dichroism, fluorescence displacement assays, thermal analysis, linear dichroism and gel electrophoresis. The complexes all bind more strongly to DNA than ethidium bromide, stabilise DNA and have a significant bending effect on DNA. The data for Δ,Δ-$[Ru_2(bpy)_4L^1]^{4+}$ are consistent with it binding to DNA outside the grooves wrapping the DNA about it. By way of contrast the other complexes are groove-binders. The phen complexes provide a chemically and enantiomerically stable alternative to the DNA-coiling di-iron triple-helical cylinder previously studied. In contrast to the di-iron helicates, the phen complexes show DNA sequence effects with Δ,Δ-$[Ru_2(phen)_4L^1]^{4+}$ binding preferentially to GC and Λ,Λ-$[Ru_2(phen)_4L^1]^{4+}$ to AT.

1. Introduction

Ruthenium polypyridyl complexes have applications in the fields of biochemistry, photochemistry and photophysics [1–8]. The last few decades have seen an increased interest in ruthenium(II) polypyridyl complexes as building blocks in supramolecular devices due to their favourable excited state and redox properties as well as structural probes for DNA [9–14]. Ruthenium complexes are also showing promising results in anti-tumor activity and they target a broad spectrum of cancers [15–20].

The most investigated mononuclear polypyridyl ruthenium complexes are the ruthenium tris(chelates), $[Ru(bpy)_3]^{2+}$ (bpy = 2,2′-bipyridyl) and $[Ru(phen)_3]^{2+}$(phen = 1,10-phenanthroline). The bipyridine and phenanthroline ligands are coordinated to the octahedral metal centre and are shaped like three-bladed propellers resulting in the enantiomers corresponding to right and left handed screws, denoted Δ or P and Λ or M, respectively. The binding modes of $[Ru(phen)_3]^{2+}$ to DNA have been disputed in literature for several years; however, there is general agreement that $[Ru(phen)_3]^{2+}$ displays enantiomeric selectivity in binding to DNA [11,12,21–25]. The mononuclear ruthenium(II) complexes span only 1–2 DNA base pairs and are easily displaced from DNA at low ionic strengths [26]. A number of dinuclear complexes have been reported in the literature and as they have increased in size, shape and charge they show a greater DNA binding affinity and are generating much interest as probes for DNA [27]. Dinuclear complexes possessing two $[Ru(phen)_2dppz]^{2+}$ (dppz = dipyrido[3,2-a:2′,3′-c]phenazine) units linked together using a bridging ligand have been shown to form a bis(intercalating) metallo complex [28]. Other dinuclear bis(intercalator)s include complexes of the form $[\{Ru(dpq)_2\}_2(phen\text{-}x\text{-}SOS\text{-}x\text{-}phen)]^{4+}$ which bind to DNA via the dpq ligand, (dpq = dipyrido[3,2-d:2′3′-f]quinoxaline; SOS = 2-mercaptoethyl ether; x = 3, 4 or 5) [13,26]. Different binding affinities and site sizes can be achieved by systematically varying the linker and its point of attachment. The bridged dinuclear ruthenium complex $[\{Ru(Me_2bpy)_2\}_2(\mu\text{-bpm})]^{4+}$ (bpm = 2,2′-bipyrimidine) binds strongly to open structures such as partially denatured DNA [29].

In contrast to the literature, our work on dinuclear ruthenium compounds has been targeted at groove-binders which could span ~5 base pairs and have increased charge compared with the mononuclear complexes. The starting point for our design was the di-iron triple helicate cylinders of Fig. 1. This parent compound is tetracationic and has demonstrated strong non-covalent binding to DNA with exciting possibilities for structural control of DNA. When added to genomic or other long DNA, it intramolecularly coils up DNA [30–33], and when added to short palindromic oligonucleotides with AT-rich central sequences [34] it recognises or creates a three-way junction from three DNA oligonucleotides. Because of these exciting and unprecedented DNA binding properties we were intrigued to explore the effects on DNA of compounds

* Corresponding author. Fax: +44 2476 524112/+44 2476 575795.
E-mail addresses: m.j.hannon@bham.ac.uk (M.J. Hannon), a.rodger@warwick.ac.uk (A. Rodger).
[1] Fax: +44 121 414 7871.

0162-0134/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.jinorgbio.2008.06.006

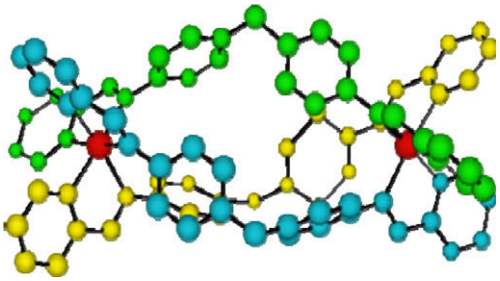

Fig. 1. Structure of the iron triple-helical cylinder, $[Fe_2L^1_3]^{4+}$, where $L^1 = C_{25}H_{20}N_4$ a bis(pyridylimine) ligand containing a diphenylmethane spacer.

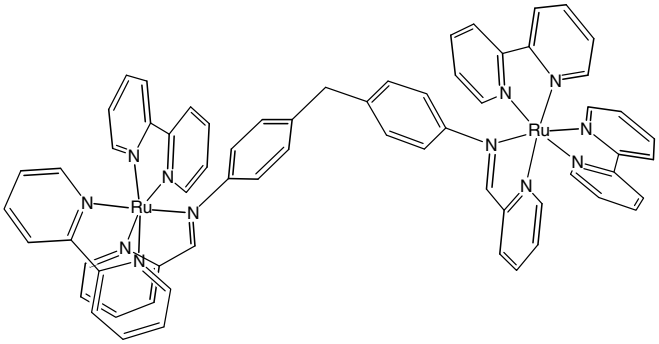

Fig. 2. $[Ru_2(bpy)_4L^1]^{4+}$.

with related structures. Complexes based on ruthenium are particularly attractive because of their photochemical properties and their high kinetic stability towards racemisation and ligand exchange. However, this stability towards ligand exchange reactions does complicate the synthesis: the ruthenium triple-stranded cylinder can be prepared [6] but not in a high yielding supramolecular self-assembly reaction [30,31,34,35,38] as used to make the di-iron cylinder. The focus herein was the design of alternate ruthenium(II) compounds of type illustrated in Fig. 2 where two ruthenium bis(chelates) have been linked into one unit with a single bis(pyridylimine) linker ligand common to both metals [38]. The synthesis and characterisation of the diruthenium bipyridine complex $[Ru_2(bpy)_4L^1]^{4+}$ and its phenanthroline analogue $[Ru_2(phen)_4L^1]^{4+}$ are reported in Ref. [38]. Enantiopure $[Ru(LL)Cl_2]$ starting materials afforded the homochiral ΔΔ and ΛΛ enantiopure products. In structure, these dinuclear metal complexes are each part way between being two stacked monoruthenium tris(chelates) (so might bind in the high loading modes of such complexes) [23] and a less symmetric version of the di-iron cylinder. In this paper, we report their DNA binding.

2. Materials and methods

2.1. Chemicals

All reagents and solvents were purchased commercially and used without further purification unless otherwise stated. Ultrapure water (18.2 Ω) was used in all experiments. Calf thymus DNA (ct-DNA, Type 1 highly polymerised sodium salt form) was purchased from Sigma-Aldrich Chemical Co. Ltd. The synthetic double-stranded DNA copolymers, poly[d(A–T)$_2$] and poly[d(G–C)$_2$], were purchased from Amersham Biosciences. Plasmid DNA pBR322 was purchased from New England Biolabs. All polynucleotides were dissolved in water (ct-DNA requiring overnight refrigeration to become solubilised) and frozen until the day of the experiment. The concentrations (bases per litre) of the DNA solutions were determined spectroscopically using the molar extinction coefficients: ct-DNA $\varepsilon_{258} = 6600\ M^{-1}\ cm^{-1}$; poly[d(A–T)$_2$] $\varepsilon_{262} = 6600\ M^{-1}\ cm^{-1}$; poly[d(G–C)$_2$] $\varepsilon_{254} = 8400\ M^{-1}\ cm^{-1}$. The concentration of pBR322 as supplied was 1000 μg/ml. Fifty millilitres of 0.2 M sodium cacodylate (4.28 g of Na $(CH_3)_2AsO_2 \cdot 3H_2O$ in 100 ml) was mixed with 9.3 ml; of 0.2 M hydrochloric acid and diluted to 200 ml with water to make 100 mM sodium cacodylate buffer, pH = 7.0. (Δ,Δ)-$[Ru_2(bpy)_4L^1](PF_6)_4$ (referred to as (P)-bpy herein) [36,37]; (Λ,Λ)-$[Ru_2(bpy)_4L^1](PF_6)_4$ (referred to as (M)-bpy); (Δ,Δ)-$[Ru_2(phen)_4L^1](PF_6)_4$ (referred to as (P)-phen); (Λ,Λ)-$[Ru_2(phen)_4L^1](PF_6)_4$ (referred to as (M)-phen) and their diastereomeric mixtures were prepared as described previously [38]. $[Fe_2L^1_3]^{4+}$ was prepared according to our previously described proceedures [30,31,36,40]. The ruthenium complex concentrations were determined by weight and the iron complexes concentrations were determined using UV/visible absorbance spectroscopy and the extinction coefficient $\varepsilon_{574\ nm} = 16{,}900\ M^{-1}\ mol^{-1}$[41].

2.2. Absorbance and circular dichroism spectroscopy

One solution containing: DNA (1500 μM), NaCl (50 mM), sodium cacodylate buffer (1 mM) and metal complex (15 μM) and a second solution containing NaCl (50 mM), sodium cacodylate buffer (1 mM) and metal complex (15 μM) were prepared. The first spectrum measured was of the DNA–metal complex solution. By adding increasing volumes of the second DNA-free solution to the cuvette, the concentration of DNA was decreased whilst the metal complex, NaCl and sodium cacodylate buffer concentrations remained constant. UV/visible absorbance spectra were collected on a Jasco V-550 and CD spectra were collected on a Jasco J-715 spectropolarimeter. A water baseline was subtracted from each data set.

2.3. Fluorescence competition binding assay

A solution of DNA (12 μM), NaCl (50 mM), buffer (1 mM) and ethidium bromide (EB, 15 μM) was prepared. The ruthenium complex concentration was incrementally increased from EB:metal complex ratios of 200:1 to 1:1 while keeping the concentrations of DNA and EB constant. Fluorescence spectra were collected at each ratio on a Perkin Elmier LS50B fluorimeter with excitation at 540 nm, excitation slit 10.0 and emission slit 15.0 nm.

2.4. Thermal analysis

The stability of DNA in the presence of the bimetallo complexes was monitored by measuring the absorbance at 260 nm (1 nm bandwidth, average time: 10 s, ramp rate 0.5 °C/min) as a function of temperature. The experiment was run simultaneously on six masked 1 cm pathlength cuvettes of 1.2 ml volume using a Peltier controlled 6-sample cell-changer in a Cary 1E spectrophotometer. One DNA, and five DNA–metal complex solutions of different DNA:complex ratios were prepared for each run. T_m was calculated by smoothing the data over 20 data points and numerically differentiating using Kaleidagraph.

2.5. Flow linear dichroism

A flow linear dichroism (LD) titration series was carried out using a Jasco J-715 spectropolarimeter adapted for LD spectroscopy whilst keeping the DNA concentration constant at 200 μM. A large volume Couette flow LD cell built by Crystal Precision Optics, Rugby (now available from Dioptica Scientific Ltd.) based on the design described in [42] was used in all experiments. One solution containing: DNA (200 μM), NaCl (50 mM) and sodium cacodylate buffer (1 mM) and a second solution containing: DNA (400 μM), metal