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Hydrolytic Cleavage of DNA by a Ruthenium(II) Polypyridyl Complex

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The complex $[Ru(bpy)_2(BPG)]Cl_2$ (**1**) containing hydrogen-bond donor (N–H atoms) and acceptor (O atoms) groups mediates hydrolytic cleavage of plasmid pBR322 DNA in an enzyme-like manner. The kinetic aspects of DNA cleavage under pseudo- and true-Michaelis–Menten conditions are detailed.

The design of DNA- and RNA-specific agents capable of controlled chemical cleavage are of paramount importance due to their potential use as drugs, regulators of gene expression, and tools for molecular biology.[1] Metal complexes are attractive reagents for the cleavage of nucleic acids due to their inherently diverse structure and reactivity. Several studies have been carried out on the complexes which cleave DNA through an oxidative pathway which requires a co-reactant such as an oxidizing or reducing agent, light, or redox-active metal center in addition to the principal cleavage agent.[2−7] The disadvantage of oxidative cleavage agents is that they produce diffusible radicals which give rise to multiple cleavage sites by modifying the deoxyribose moiety, which results in fragments that cannot be re-ligated. On the other hand, agents that promote the hydrolytic cleavage of the phosphodiester backbone of DNA do not suffer from these drawbacks. Therefore, there has been a substantial increase in the development of reagents suitable for cleaving DNA hydrolytically under physiological conditions which could be useful not only in molecular biology and drug design but also in elucidating the precise role of metal ions in enzyme catalysis.[8−18] Reagents which promote efficient hydrolytic cleavage of DNA are rhodium(III) intercalators attached to peptide moieties,[19] macrocyclic complexes of lanthanides,[20] and polyamine complexes of cobalt(III).[21] The most recent studies involving hydrolysis of DNA has been restricted to copper(II) complexes.[8,10,14,17,18] Of those, however, the most highly efficient hydrolytic cleavage agents are mononuclear copper(II) complexes[8c,14,17,22] in which a copper-bound hydroxyl group is the active species in the hydrolysis of the nucleic acid phosphate backbone.

The majority of ruthenium−polypyridyl complexes noncovalently bind to DNA in different modes, viz. electrostatic, surface binding or intercalation and initiate DNA cleavage reactions on photoirradiation either by electron transfer to base forming covalent photoadducts or by energy transfer to molecular oxygen generating $^1O_2$[23] or rarely by a hydrolytic mechanism.[15]

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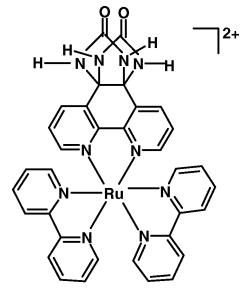

[Ru(bpy)$_2$(BPG)]Cl$_2$ (1)

Figure 1. Schematic representation of the ruthenium polypyridyl complex of bipyridine-glycoluril (BPG) used in this study.

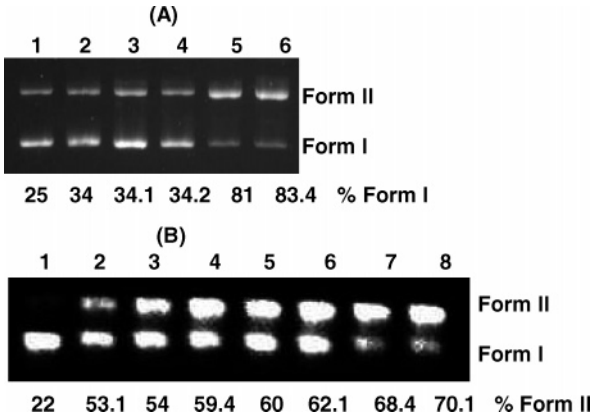

Figure 2. (A) Cleavage of plasmid pBR322 DNA (60 μM, in base pairs) in the presence of 20 μM **1** at various incubation times at 37 °C in Tris−boric acid−EDTA (TBE) buffer, pH 8.2, Lane 1: incubation times 10 h at 37 °C; lane 2 − DNA + 1 (5 h), lane 3 − DNA + 1 (10 h), lane 4 − DNA + 1 (12 h), lane 5 − DNA + 1 (16 h), lane 6 − DNA + 1 (18 h); (B) Cleavage of plasmid pBR322 DNA (90 μM, in base pairs) with varying concentration of **1** (incubation for 14 h at 37 °C); Lane 1, DNA control; lane 2, DNA + 10 μM **1**; lane 3, DNA + 20 μM **1**; lane 4, DNA + 30 μM **1**; lane 5, DNA + 40 μM **1**; lane 6, DNA + 50 μM **1**; lane 7, DNA + 60 μM **1**; lane 8, DNA + 70 μM **1**; Form I, supercoiled form; Form II, nicked circular form.

In the present work we report DNA cleavage by a ruthenium polypyridyl complex, [Ru(bpy)$_2$(BPG)]Cl$_2$ **(1)** (Figure 1), without any bound water molecule but with the peripheral urea moiety of the ancillary bipyridyl-glycoluril ligand which hydrolyzes the phosphodiester bond efficiently. The crystal structure of **1** indicates an intricate hydrogen-bonding network between urea groups.[24] On the basis of the structure of **1** (Figure 1), we hypothesize that these urea groups are involved in DNA binding. The hydrolytic degradative pathway is supported by several experimental protocols discussed below which were performed to distinguish different mechanisms through which **1** might damage DNA.

From the DNA-cleavage experiment[25] in a medium of Tris−boric acid−EDTA (TBE) buffer (pH 8.2) in dark conditions by gel electrophoresis (Figure 2A), it is observed that micromolar concentrations of **1** cleave DNA, as evidenced by the disappearance of form I (supercoiled form) of the plasmid and the appearance of the form II (nicked circular form). Significant DNA cleavage is observed in an argon atmosphere (Figure S1 in the Supporting Information), and cleavage is neither enhanced nor inhibited in the presence of D_2O by complex **1** (Figure S1 in the Supporting Information), suggesting an oxygen-independent cleavage pathway. The absorbance vs time profile using rhodamine B as a reporter molecule for the generation of hydroxyl radicals[8c,26] reveals that there is no change in absorbance on addition of **1** (Figure S2 in the Supporting Information), which rules out the possibility of the generation of hydroxyl radicals.

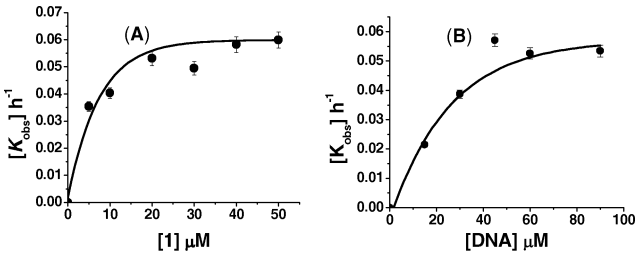

Figure 3. (A) Plot showing saturation kinetics for the cleavage of plasmid pBR322 DNA (60 μM, in base pairs) with different complex concentrations (5−50 μM) of **1** at 37 °C in TBE buffer (pH 8.2) (B) Saturation kinetics of the cleavage of plasmid pBR322 DNA using 20 μM complex **1** with different concentrations of the plasmid pBR322 DNA at 37 °C in TBE buffer (pH 8.2) (The fits to the data are according to the equations given in the Supporting Information).

In order to investigate the role of radicals in the DNA damage by this complex, reactions were performed under aerobic conditions by incubating the complexes with DNA for 18 h in the presence of hydroxyl radical scavengers (DMSO and mannitol), singlet oxygen scavengers (NaN_3, histidine and DABCO), and a superoxide scavenger (superoxide dismutase, SOD). The results show that DNA cleavage by **1** is not inhibited by any of the classical radical scavengers (Figure S3 in the Supporting Information).

To ascertain the mechanism of the DNA cleavage reaction by **1**, the form II (NC) obtained from the cleavage of the SC DNA has been isolated, treated with T4 ligase enzyme, and subjected to gel electrophoresis.[26] We have observed ~60% conversion of the form II to its original form I, indicating that a hydrolytic mechanism dominates over other mechanisms (Figure S4 in the Supporting Information).

The kinetic aspects of the hydrolytic DNA cleavage have been investigated in order to determine the hydrolytic cleavage rate (details in the Supporting Information). Reactions were carried out under pseudo-Michaelis−Menten conditions by using various concentrations of **1** (10−70 μM) and constant DNA concentration (60 μM), which results in formation of form II from the form I, as shown in Figure 3A. The decrease of form I fit well to a single-exponential decay curve and follow pseudo-first-order kinetics. Rates of cleavage were calculated by means of the Lineweaver−Burk method (Figure S5 in the Supporting Information) to give the kinetic parameters $V_{max'} = 0.06 \pm 0.003$ h^{-1}, $K_M = 3.80$ μM, $k_{cat}/K_M = 1.58 \times 10^4$ h^{-1} M^{-1} (Table 1). DNA cleavage

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Table 1. Pseudo-Michaelis−Menten Kinetic Analysis for Hydrolytic Cleavage of DNA

complex	K_M (M)	k_{cat}[a] (h^{-1})	k_{cat}/K_M[b] (h^{-1} M^{-1})	ref
$[Ru(bpy)_2BPG]^{2+}$	3.80×10^{-6}	0.06 ± 0.003	1.58×10^4	this work
$[Ru(bpy)_2BPG]^{2+*}$	57.14×10^{-6}	0.11 ± 0.002	1.92×10^3	this work
Cu−neamine	3.9×10^{-6}	1.86	4.8×10^5	8c
Cu−neamine*	41.6×10^{-6}	3.57	8.6×10^4	8c
Co^{3+}−cyclen	9.8×10^{-4}	0.79	8.1×10^2	16a
Eu^{3+}	3.9×10^{-5}	0.25	6.4×10^3	16d
(Eu^{3+}) ionophore	5.7×10^{-4}	2.10	3.7×10^3	16d
$Cu(9aneN_3)$	nd	~0.04	nd	10b
$[Cu(dpq)_2(H_2O)]^{2+}$	nd	4.7 ± 0.2	nd	17
$[Cu(dpq)_2(H_2O)]^{2+*}$	nd	5.58 ± 0.4	nd	17
$[Cu(L^1)_2(Br)](ClO_4)_5$	6.5×10^{-5}	4.15	nd	22
$[Cu(L^2)_2(Br)](ClO_4)_5$	7.5×10^{-5}	0.43	nd	22
$[Cu(bipy)(NO_3)_2]$	3.3×10^{-4}	0.61	nd	22

[a] These calculated values are k_{obs}, at saturation levels (i.e., V_{max}).Under true-Michaelis−Menten conditions (with constant complex concentration), $k_{cat} = V_{max}/[E]_0$. In all the above cases, only for $[Ru(bpy)_2BPG]^{2+}$*, Cu-neamine* and $[Cu(dpq)_2(H_2O)](ClO_4)_2$* (which were obtained under true-Michaelis−Menten conditions), the complex concentration was varied during kinetic characterization. [b] The specificity constants for individual complexes, and a measure of the efficiency of DNA hydrolysis; nd = not determined; L^1 = 5,5′-di(1-(triethylammonio)methyl)-2,2′-dipyridyl cation; L^2 = 5,5′-di(1-(tributylammonio)methyl)-2,2′-dipyridyl cation.

reactions were also monitored under true-Michaelis−Menten kinetic conditions using a constant complex concentration (20 μM) and varying the DNA concentration (15−90 μM) (Figure 3B). Under these conditions, rates of cleavage were calculated (Figure S5 in the Supporting Information) to give the kinetic parameters $V_{max} = 0.11 \pm 0.002$ h^{-1}, $K_M = 57.14$ μM, $k_{cat}/K_M = 1.92 \times 10^3$ h^{-1} M^{-1} (Table 1).

Under these conditions, **1** binds to the plasmid DNA in an enzyme-like manner. Rate constants of DNA cleavage hydrolyzed by Cu (II) complexes have been reported in the literature when using the pseudo-Michaelis−Menten kinetic equations. However, there are only a few reports on Cu(II) complexes by using the ligands neamine[8c] and the planar heterocyclic base dpq[17] for which rate constants of DNA cleavage under both pseudo- and true-Michaelis−Menten conditions were calculated. Here, in the present study, we have obtained the specificity constant of the order of 10^4 h^{-1} M^{-1} for complex **1** under hydrolytic conditions.

As far as we are aware of, the only other hydrolytic cleavage of DNA by ruthenium−polypyridyl complex was reported by Barton et al. for $[Ru(DIP)_2macro]^{n+}$, where $Ru(DIP)_2$ binds to DNA via intercalation and macro is a chelating ligand with two polyamine tridentate armlike segments which bind certain divalent metal cations so as to deliver its coordinated nucleophile to the phosphate backbone for hydrolysis of the anionic diester.[15] The hydrogen bonds between the DNA phosphate backbone oxygen atoms and the N−H groups of the ruthenium-bound bipyridine-glycoluril facilitate hydrolytic cleavage by **1**. Recently, Farrell et al. have reported a phosphate backbone binding mode for a polynuclear platinum(II) complex that has planar arrays of hydrogen-bond donors, leading to association with the DNA backbone.[27] A similar hydrogen-bonding network is critical in promoting DNA binding by **1** and explains why only a single strand break is observed in our experiments. Complex **1** hydrogen bonds at one site on the DNA backbone initiating cleavage but does not rearrange to initiate a second cleavage reaction, resulting in a single turnover.

In summary, we report a ruthenium−polypyridyl complex which cleaves DNA hydrolytically which is unprecedented in the vast ruthenium(II) polypyridyl−DNA chemistry. Further applications and molecular modification of the ruthenium(II) polypyridyl complexes for nucleic acid-related molecular devices are in progress.

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Supporting Information Available: Plasmid DNA cleavage experiment in the presence of argon atmosphere and D_2O (Figure S1), Rhodamine B assay for hydroxyl radical experimental details (Figure S2), DNA cleavage experiment in the presence of different radical scavengers (Figure S3), T4 ligase enzymatic assay experimental details (Figure S4), details of kinetic measurements, double reciprocal plot of pseudo-Michaelis−Menten kinetics for **1** (Figure S5), double reciprocal plot of true-Michaelis−Menten kinetics for **1** (Figure S5), synthesis and characterization of **1**, electronic absorption spectra of **1** in the presence of CT-DNA at various incubation times (Figure S6), electronic absorption spectra of **1** in the absence and presence of CT-DNA (Figure S7); cleavage of plasmid DNA at various concentrations of **1** (Figure S8). This material is available free of charge via the Internet at http://pubs.acs.org.

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