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Light induced DNA scission by a luminescent mixed-ligand uranyl complex

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Abstract

Efficient cleavage of supercoiled pBR322 DNA by X-ray crystallographically characterized complex, $[UO_2(phen)(aba)(OC_2H_5)]$ (phen = 1,10-phenanthroline; aba = 4-dimethylamino benzoate) has been observed on irradiation with UV (350 nm) or visible light without any external additives through a mechanistic pathway involving singlet oxygen. This complex having 1,10-phenanthroline as an intercalator/binder to supercoiled DNA and *N*,*N*-(dimethylamino)benzoate as a chromophore.

Keywords: Uranyl complex; Plasmid DNA; Photocleavage; Physiological pH; Homogeneous catalyst

1. Introduction

Development of photonucleases under mild conditions and without adding any external reagent is a powerful complementary method to the more widely used hydrolytic or oxidative pathways [1,2]. These materials also offer considerable potential in the post-genome era as reagents for nucleic acid sequencing, photodynamic therapy, anti-tumor and antiviral drugs [3]. In photodynamic therapy, use of a photosensitizer has emerged as a promising protocol in the treatment of cancer and allied diseases and in this regard, use of visible light can reduce photodamage of healthy tissues [4]. Several varieties of metal complexes containing 3d–5d metal ions have been used as synthetic photonucleases [5]. Uranyl ion (UO_2^{2+}) having favorable photophysical properties [6] and high binding affinity [7] towards phosphate backbone across the minor groove of DNA, is worth pursuing as a possible photonuclease. Besides, this ion was found to be potentially important in many biochemical applications such as probing local DNA structure and metal ion-binding sites in a DNA four-way junction as well as mapping the electronegative potential of DNA [8–10]. Photonucleases are also useful in photo-footprinting which is a better technique than thermal-footprinting [11] for monitoring biochemical processes [12]. In earlier investigations, $UO_2(CH_3COO)_2 \cdot 2H_2O$ and $UO_2(NO_3)_2 \cdot 6H_2O$ salts have been used in photoinduced DNA scission and other biochemical applications [13,7]. However, one of the major disadvantages of working with simple UO_2^{2+} salts for a wide range of biochemical applications is that, the pH must be maintained at neutral or highly acidic to prevent the uranyl ion from forming insoluble aggregates [14]. To circumvent this problem, an adenylated homopolymer with bound UO_2^{2+} group [15] has been reported as a heterogeneous photonuclease. In all these cases, however, the exact speciation remain doubtful. We report here that X-ray crystallographically characterized complex, $[UO_2(phen)(aba)(OC_2H_5)]$ (phen = 1,10-phenanthroline, aba = 4-dimethylamino benzoate) with 1,10-phenanthroline as an intercalator [16] exhibits facile DNA scission on irradiation with tungsten lamp under homogeneous condition within the physiological pH range (pH 6–8).

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2. Materials and experimental

2.1. Chemicals and reagents

Supercoiled plasmid DNA (pBR322) was from New England Biolabs. Ethidium bromide, catalase and superoxide dismutage (SOD) were from Sigma Aldrich Ltd. and sodium cacodylate buffer was from SRL, Mumbai. Ethylenediaminetetraacetic acid (EDTA) and D-manitol were from S.D. Fine Chemicals, Mumbai. All these chemicals were used as received while all buffer solutions were prepared using Millipore® water.

2.2. Synthesis of $[UO_2(phen)(aba)(OC_2H_5)]$ (1)

The complex **1** was prepared by adding an aqueous solution of uranyl acetate dihydrate (424 mg, 1.0 mmol) to a mixture of 1,10-phenanthroline (198 mg, 1.0 mmol) and 4-dimethylamino benzoic acid (165 mg, 1.0 mmol) in EtOH (10 ml). Upon stirring for 5 h at room temperature, the desired product precipitated as a light yellow solid that was collected by filtration, washed thoroughly with EtOH and dried in vacuo. Yield 80%. Single crystals suitable for X-ray crystallography could be grown by slow evaporation of a dilute solution of the compound in mixed dichloromethane:ethanol solvent (1:1, v/v) in 50% yield. *Anal.* Calc. for $C_{23}H_{23}U_1N_3O_5$: C, 42.22; H, 3.6; N, 6.4. Found: C, 42.34; H, 3.51; N, 6.37%. IR (KBr phase): 2919vw, 2793vw, 1602vs, 1566m, 1517m, 1478s, 1415vs, 1365s, 1194vs, 1072vs, 917vs cm^{-1} (vs, very strong; s, strong; m, medium; vw, very week; w, week).

2.3. X-ray data collection and crystal structure determination

Single crystal X-ray data on **1** was collected at 100 K on a Bruker SMART APEX CCD diffractometer using graphite monochromated Mo Kα radiation ($\lambda = 0.71073$ Å). The linear absorption coefficients, scattering factors for the atoms, and the anomalous dispersion corrections were taken from International Tables for X-ray Crystallography. The data integration and reduction were processed with SAINT [17] software. An empirical absorption correction was applied to the collected reflections with SADABS [18] using XPREP [19]. The structure was solved by the direct method using SHELXTL [20] and was refined on F^2 by full-matrix least-squares technique using the SHELXL-97 [21] program package. All non-hydrogen atoms were refined anisotropically while the hydrogens were treated as riding atoms using SHELXL default parameters. The crystal data for **1** is given in Table 1 while selected bond distances and angles are collected in Table 2.

2.4. Plasmid photocleavage assay

Plasmid DNA (pBR322) cleavage reactions were performed in sodium cacodylate buffer (pH 7.5, 32 °C). To ensure complete solubility of the complex, 1 mM stock solution in DMF was prepared. From this stock solution 2 μL was used for every cleavage reactions. Total reaction volume was kept at 20 μL and the final concentration of the complex was 100 μM. Weight of supercoiled plasmid pBR322 DNA was 7–8 ng/μL. The reaction mixture was irradiated with a tungsten lamp (200 W) and constant temperature (32 °C) was maintained with a Julabo circulation bath in 1.5 mL eppendorff tubes. The tungsten lamp was kept 3 cm away from the sample container. For scavenger experiments, concentration of the scavengers were 100 mM and final activity of SOD and catalase were 24 u/μL. The inhibition reactions were done by adding the reagent prior to complex addition. All cleavage reactions were quenched with 5 μL of loading buffer containing 100 mM of EDTA, 50% glycerol in Tris–HCl (pH 8.0) and the samples were loaded onto 0.7% agarose gel (Biozym) containing ethidium bromide (1 μg/1 mL). Electrophoresis was done for 1 h at constant current (80 mA) in 0.5X TBE buffer. Gels were imaged with a PC-interfaced Bio-Rad Gel Documentation System 2000.

Table 1
Crystal data and refinement parameters for **1**

Compound	**1**
Empirical formula	$C_{23}H_{23}N_3O_5U$
Formula weight	659.47
Crystal system	monoclinic
Space group	$P2_1/n$
a (Å)	10.098(5)
b (Å)	17.327(5)
c (Å)	12.569(5)
β (°)	103.727(5)
V (Å^{-3})	2136.4(15)
Z	4
$F(000)$	1256
D_{calc} (g cm^{-3})	2.050
Crystal size (mm^3)	$0.10 \times 0.09 \times 0.06$
T (K)	100 (2)
μ (Mo Kα) (mm^{-1})	7.639
Reflections measured	5221
Unique reflections used [$(I \geqslant 2\sigma(I)$]	4080
Parameters (N)	289
Goodness-of-fit	0.990
Final R indices [$(I \geqslant 2\sigma(I)$]	$R_1 = 0.0532$, $wR_2 = 0.1264$
R indices (all data)	$R_1 = 0.0701$, $wR_2 = 0.1359$
Maximum/minimum residual (e Å^{-3})	5.920/−4.039
Refinement method	full-matrix least-squares on F^2

Table 2
Selected bond lengths (Å) and angles (°) in compound **1**

U1–N1	2.596(6)	N1–U1–N2	63.3(2)	O1–U1–O2	53.67(16)
U1–N2	2.597(7)	N1–U1–O2	76.56(18)	O1–U1–O5	85.87(19)
U1–O1	2.419(6)	N2–U1–O5	80.7(2)	O3–U1–O4	175.5(2)
U1–O2	2.458(5)	O4–U1–N1	84.4(2)		
U1–O3	1.788(5)	O3–U1–N1	91.2(2)		
U1–O4	1.788(5)	O3–U1–N2	89.6(2)		
U1–O5	2.123(5)	O4–U1–N2	89.5(2)		