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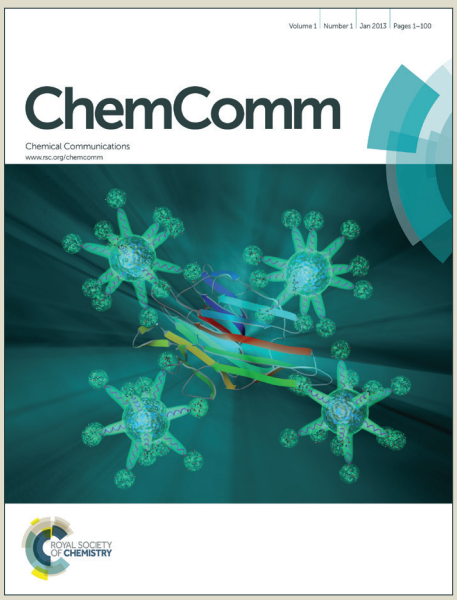

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ARTICLE TYPE

DNA photocleavage in anaerobic conditions by a Ru(II) complex: a new mechanism

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$[Ru(bpy)_2(py\text{-}SO_3)]^+$ (bpy = 2,2'-bipyridine and py-SO_3 = pyridine-2-sulfonate) was found to undergo py-SO_3 dissociation upon visible light irradiation (≥ 470 nm) via Ru-O homolysis, producing reactive free radical species and therefore being able to not only photobind but also photocleave DNA in hypoxic conditions.

DNA cleaving agents have found wide applications in DNA function modulation and structure detection and received extensive exploration as promising drug candidates in tumor treatment.[1] Of them, DNA photocleavers are of particular interest due to their capability to modify DNA functions in a spatially and temporally controlled manner, conferring tumor treatment high selectivity.[2] By virtue of their tunable photophysical and photochemical properties, Ru(II) complexes have drawn great attention in developing photoactivated drugs and diagnostic agents, including DNA photocleavers.[3] Many Ru(II) complexes, *e.g.* $[Ru(bpy)_3]^{2+}$ (bpy = 2,2'-bipyridine), photocleave DNA via singlet oxygen ($^1O_2$), which is generated by energy transfer from the triplet metal-to-ligand charge transfer (^{3}MLCT) state to O_2.[2,4] The O_2-dependent mechanism excludes the use of these Ru(II) complexes against hypoxic tumor cells. The hypoxic tumor cells can be the most resistant to radiotherapy and chemotherapy and susceptible toward metastasis,[5] as a result, much recent emphasis has been placed on pursuing O_2-independent DNA photocleavers, a challenging and rewarding task. However, few Ru(II) complexes exhibit DNA photocleaving activities in anaerobic conditions so far, and the strategies behind these examples are limited. One successful strategy is taking advantage of the potently oxidizing ^{3}MLCT states of the tap/hat/bpz-based Ru(II) complexes to damage DNA, where tap = 1,4,5,8-tetraazaphenanthrene, bpz = 2,2'-bipyrazine, and hat = 1,4,5,8,9,12-hexaazatriphenylene.[6] Recently, MacDonnell and coworkers reported DNA cleavage by the ^{3}MLCT state of $[Ru(II)(bpy)_2(1,10\text{-phenanthroline-5,6-dione})]^{2+}$ in its hydrated form.[7] An alternative strategy makes use of the strongly oxidizing Ru(III) states, generated upon photoinduced electron transfer either intermolecularly[8] or intramolecularly[9,10] from ^{3}MLCT to an electron acceptor. For example, Turro et al. appended two sequentially linked viologen groups to a Ru(II) polypyridine complex to achieve DNA photocleavage under N_2 atmosphere.[9b] Brewer et al. constructed Ru(II)/Os(II)-Rh(III) mixed-metal supramolecular complexes, in which Rh(III) serves as electron trap to achieve Ru(III)/Os(III) state, by which photoinduced DNA scission was realized in an O_2-independent manner.[10] Here we reported on a new Ru(II) complex, $[Ru(bpy)_2(py\text{-}SO_3)]^+$ (py-SO_3 = pyridine-2-sulfonate), which can photocleave DNA in hypoxic conditions by a novel reactive radical mechanism.

As a matter of fact, the original purpose of the synthesis of $[Ru(bpy)_2(py\text{-}SO_3)]^+$ is to explore its photo-binding toward DNA. It has been well established that Ru(II) complexes bearing photolabile ligands are particularly useful in developing O_2-independent photoactivated anticancer drugs since the resultant Ru fragments may covalently bind DNA in a manner similar to cisplatin.[11] Despite intensive investigations on the photoinduced ligand dissociation reactions of Ru(II) complexes, examples of photolabile bidentate ligands are rare.[12] Photolabile bidentate ligands may be more beneficial than their monodentate counterparts because their higher coordination stability in the dark corresponds to lower dark toxicity to normal cells. Moreover, photolabile bidentate ligands may provide two reactive sites at the same time on Ru center, enabling crosslinking of biological components, *e.g.* DNA intrastrand/interstrand crosslinking, and thus presenting varied spectrum of antitumor activity. Glazer et al. recently rendered bipyridine-based ligands photolabile by introducing methyl substituents at 6- and 6'-positions to distort the coordination geometry of the ligands.[13] We proposed that bidentate ligands with weakened coordination strength of one dentate might be photolabile and $[Ru(bpy)_2(py\text{-}SO_3)]^+$ was prepared to examine this hypothesis. Organosulfonates (RSO_3^-) are generally regarded as poor metal ligands. Until recently, increasing attention was focused on them, particularly in the field of MOF (metal-organic framework), owing to their flexible coordination modes as the result of their weak interaction with metal ions.[14] No coordination of RSO_3^- to Ru center was reported so far. Our experimental results reveal that $[Ru(bpy)_2(py\text{-}SO_3)]^+$ undergoes py-SO_3 dissociation upon visible light irradiation (≥ 470 nm). Surprisingly, reactive free radicals are generated during py-SO_3 dissociation. As a result, $[Ru(bpy)_2(py\text{-}SO_3)]^+$ can photobind and photocleave DNA

simultaneously in hypoxic environments. This bimodal DNA damage may better restrict DNA repair, a common drug resistance mechanism. More notably, the DNA scission in our cases lies in neither ^{3}MLCT nor Ru(III) state, opening a new avenue for the development of O_2-independent Ru(II) based DNA photocleavers.

As shown in Figure S1, $[Ru(bpy)_2(py\text{-}SO_3)]^+$ adopts an octahedral coordination geometry, where py-SO_3 chelates to Ru center by both N and O atoms. The bond length of Ru-O (2.135Å) is much longer than that of Ru-N (2.013-2.064 Å) (Table S1 and S2), indicative of its labile feature.

$[Ru(bpy)_2(py\text{-}SO_3)]^+$ shows a good stability in the dark (Figure S2 and S3). Upon visible light irradiation (≥ 470 nm), however, the absorption spectrum underwent remarkable changes, as shown in Figure S4. An isosbestic point was found at 478 nm, indicating one new species formed. After 90 min of irradiation, the absorption maximum moved to 490 nm, in line with that of $[Ru(bpy)_2(H_2O)_2]^{2+}$,[12b, 15] suggesting the dissociation of py-SO_3. The photoinduced ligand dissociation proceeded more rapidly in CH_3CN (Figure S5). In this case, three isosbestic points were observed and the final spectrum can overlap perfectly with that of $[Ru(bpy)_2(CH_3CN)_2]^{2+}$, confirming further the dissociation of py-SO_3. ^{1}H NMR and high-resolution ESI-MS experiments provide more evidence for the photodissociation of py-SO_3 (Figure S6-S9).

We then examined the photobinding capability of $[Ru(bpy)_2(py\text{-}SO_3)]^+$ to pBR322 DNA using gel electrophoresis technique. The combination of $[Ru(bpy)_2(py\text{-}SO_3)]^+$ and irradiation under Ar atmosphere decreased the migration rate of supercoiled (Form I) DNA dramatically (Lane 2-4, Figure 1), a typical feature for the covalent binding of Ru complexes and in line with the photoinduced ligand substitution behavior of $[Ru(bpy)_2(py\text{-}SO_3)]^+$. Surprisingly, the intensity of nicked circular (Form II) DNA increased gradually with increasing the concentration of $[Ru(bpy)_2(py\text{-}SO_3)]^+$ (Lane 2-5), suggesting that DNA single strand scission occurred. Under the same condition, $[Ru(bpy)_2(CH_3CN)_2]^{2+}$ can only retard the DNA mobility (Lane 4, Figure S10), while $[Ru(bpy)_3]^{2+}$ and py-SO_3H are totally inert (Lane 3 and 5, Figure S10), though $[Ru(bpy)_3]^{2+}$ photocleaved plasmid DNA from Form I to Form II by $^1O_2$ mechanism[2] in aerobic environment (Lane 6, Figure 1). Additionally, the presence of O_2 has negligible effect on the activities of $[Ru(bpy)_2(py\text{-}SO_3)]^+$ (Lane 2, Figure S11). All results demonstrate that $[Ru(bpy)_2(py\text{-}SO_3)]^+$ are able to photobind and photocleave DNA simultaneously in hypoxic conditions.

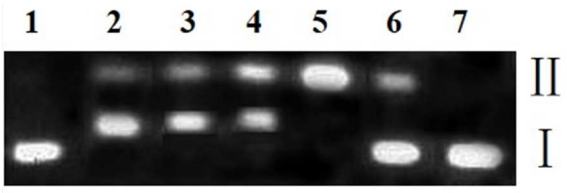

Figure 1. Agarose gel electrophoresis pattern of pBR322 DNA (100 μM in base pairs) in Ar-saturated Tris-EDTA (5 mM, pH = 7.5) upon irradiation (≥ 470 nm) for 15 min in the presence of varied concentrations of $[Ru(bpy)_2(py\text{-}SO_3)]^+$. Lane 1, DNA alone; lane 2, 4 μM; lane 3, 8 μM; lane 4, 12 μM; lane 5, 20 μM; lane 6, $[Ru(bpy)_3]^{2+}$ (50 μM), air-saturated; lane 7, $[Ru(bpy)_2(py\text{-}SO_3)]^+$ (50 μM), dark control. I and II denote supercoiled circular and nicked circular plasmid DNA, respectively.

The poor oxidizing ability of the ^{3}MLCT state of $[Ru(bpy)_2(py\text{-}SO_3)]^+$ (Figure S12) and the absence of any strongly oxidizing agents in the system rules out the role of the ^{3}MLCT and Ru(III) states. Thus, the most likely reason responsible for the O_2-independent DNA photocleavage of $[Ru(bpy)_2(py\text{-}SO_3)]^+$ may be certain reactive intermediates generated during the ligand dissociation.

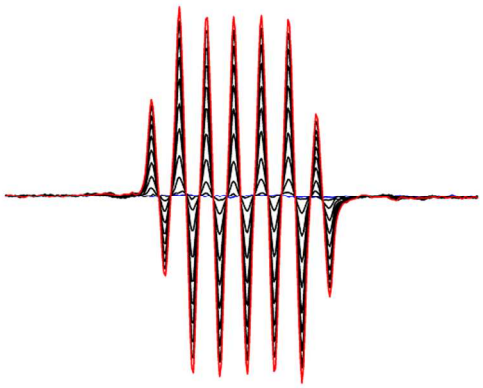

Figure 2. EPR signals obtained after laser irradiation (355 nm) of an Ar-saturated CH_3CN solution of $[Ru(bpy)_2(py\text{-}SO_3)]^+$ (1 mM) and DMPO (50 mM).

Using DMPO (5,5-dimethyl-1-pyrroline-*N*-oxide) as spin trapping agent, we obtained a seven-line EPR signal with intensity ratio of 1:2:2:2:2:2:1 and hyperfine coupling constants of a^N = 6.8 G, a_γ^{H1} = 3.5 G and a_γ^{H2} = 3.3 G, upon irradiation of $[Ru(bpy)_2(py\text{-}SO_3)]^+$ in Ar-saturated CH_3CN (Figure 2 and S13). The signal is in good agreement with the EPR signal of DMPOX (5,5-dimethyl-2-pyrrolidone-1-oxyl).[16] Control experiments (Figure S14 and S15) exclude the participation of $^1O_2$ in DMPOX formation. Rosen and Rauckman[16] proposed that spin trapping of hydroperoxyl radical by DMPO followed by rearrangement may lead to DMPOX. Based on this, we tentatively put forth a possible mechanism for the generation of DMPOX in our system. As shown in Figure 3, $[Ru(bpy)_2(py\text{-}SO_3)]^+$ molecules, at least partially if not all, undergo Ru-O homolysis upon irradiation to release py-$SO_3^{\cdot}$ free radicals, which are then trapped by DMPO and experience a series of rearrangements to form DMPOX.

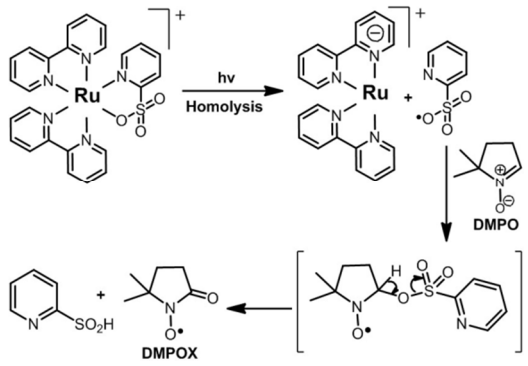

Figure 3. A possible mechanism for DMPOX formation in the case of $[Ru(bpy)_2(py\text{-}SO_3)]^+$.

We also carried out EPR experiments in Ar-saturated DMSO and aqueous solutions. In DMSO, the spin adduct of DMPO and

methyl radical ($\cdot CH_3$) was observed with a^N = 14.8 G and a^H = 21.4 G (Figure S16).[17] It is well known that hydroxyl radical ($\cdot OH$) can quantitatively react with DMSO to produce methanesulfinicacid (MSA) and methyl radical.[18] This reaction has been widely utilized to probe or quench $\cdot OH$. Our result suggests that py-$SO_3^\cdot$ radicals are reactive enough to generate secondary $\cdot CH_3$ radicals in DMSO. Similarly, in Ar-saturated aqueous solution of $[Ru(bpy)_2(py\text{-}SO_3)]^+$, irradiation led to the formation of $\cdot OH$ (Figure S17), demonstrating the potent reactivity of py-$SO_3^\cdot$ again. The exact mechanism of DNA cleavage is still unclear, and the role of bpy radial anion bound on Ru(II) center cannot be excluded. We found DMSO and TEMPO, effective scavengers of diffusible oxygen-based radicals and carbon radicals respectively,[5] quenched DNA scission efficiently (Figure S18, lane 4-6), supporting the involvement of free radicals in DNA cleavage. Moreover, we found that the photooxidation products of 9-EtG sensitized by $[Ru(bpy)_2(py\text{-}SO_3)]^+$ in anaerobic condition or $[Ru(bpy)_3]^{2+}$ in aerobic condition are dramatically different (Figure S19), indicative of a different DNA photocleavage sites and products from classical $^1O_2$ mechanism. We further examined the photoreaction products of $[Ru(bpy)_2(py\text{-}SO_3)]^+$ and CT-DNA in anaerobic conditions using reported method,[7] and furfural was detected (Figure S20), suggesting a H-atom abstraction reaction from the C5' position.[1c] Though $[Ru(bpy)_2(py\text{-}SO_3)]^+$ can photobind and photocleave DNA in both aerobic and anaerobic conditions, low phototoxicity was observed (Figure S21), presumably due to its poor cellular uptake and/or improper subcellular localization. Ligand modification is underway to tune these properties.

Homolytic cleavage of organo-metal bond has received extensive and intensive studies for better understanding the role of B_{12} coenzyme and for fruitful applications as catalysts in organic transformations and controlled radical polymerizations.[19] More efforts were paid on first line transition metals, particularly Co complexes. Our work adds a new example and a promising biological application for scanty organo-Ru bond homolysis.[20]

In summary, we found that $[Ru(bpy)_2(py\text{-}SO_3)]^+$ undergoes photoinduced dissociation of py-SO_3 at least partially by Ru-O homolysis, generating reactive radical species and accounting for its simultaneous photobinding and photocleavage toward DNA in anaerobic conditions. This novel mechanism opens a new avenue for constructing O_2-independent DNA photocleavers for selective inactivation of hypoxic tumor cells.

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Notes and references

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† Electronic supplementary information (ESI) available: Synthesis, crystallographic data (CCDC 1011182), UV-visible, ^1H NMR, MS and EPR spectra of $[Ru(bpy)_2(py\text{-}SO_3)]^+$, and gel electrophoresis of DNA. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c000000x/

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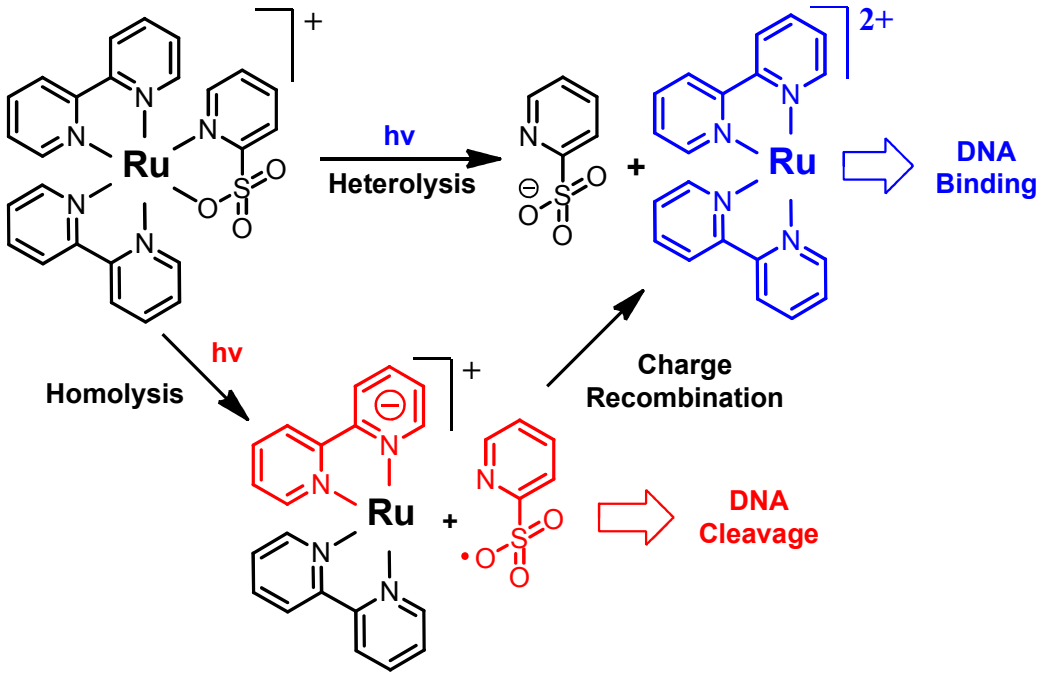

Photoinduced homolytic cleavage of Ru-O bond of a novel Ru(II) complex leads to formation of ligand-based reactive radicals capable of breaking DNA in an oxygen-dependent manner and Ru fragments capable of binding DNA covalently.