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Photoinduced DNA Cleavage Promoted by Two Copper(II) Complexes of Tetracyclines and 1,10-Phenanthroline

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Supporting Information

ABSTRACT: In this report, we demonstrate how UV-light exposure can enhance DNA cleavage promoted by two copper(II) complexes of tetracyclines and 1,10-phenanthroline about 40 times in comparison to nonirradiated conditions. In addition, new aspects regarding their DNA binding properties, as well as the mechanism of the cleavage reaction, were also investigated.

Photocleavage of nucleic acids by transition-metal complexes emerges as a field of great interest to improve the development of new biotechnological and therapeutic approaches.[1−4] The basis of photoinduced DNA cleavage is the photogeneration of reactive oxygen species (ROS), which abstract hydrogen atoms from the DNA sugar moiety or oxidize nucleobases, especially guanine.[1,5,6] Such compounds can be particularly useful in photodynamic therapy (PDT).

PDT is a promising anticancer treatment that involves the selective retention of a photosensitizer, such as an organic compound or a metal complex, in malignant tumors followed by exposure to light irradiation in the presence of oxygen. The ROS produced by the photoactivated drug initiate a series of events, including DNA fragmentation, resulting in cell death.[7−9] Several metal complexes, mainly those containing copper(II), exhibit photoinduced DNA cleavage under UV or visible light, attracting attention to the design of new transition-metal complexes.[2,3,10−14] Tetracyclines compose a family of quinolone antibiotics whose main mechanism of antimicrobial activity is the inhibition of protein synthesis.[15] Furthermore, the self-cleaving activity of the hammerhead ribozyme[16] and the ribozyme from the human hepatitis delta virus[17] is also inhibited by tetracyclines, as well as nuclear pre-mRNA splicing in vitro.[18] This class of molecules shows affinity for RNA as well as DNA,[19] mainly in the presence of divalent ions such as copper(II). In addition, the presence of copper(II) is essential to induce single- and double-strand breaks in plasmid DNA.[20]

Therefore, because of the fact that tetracyclines present high photosensitivity,[21−23] there have been reports about their ability to photoinduce damage to plasmid DNA in the presence[19] or absence[24] of soluble Cu^{II} ions. Indeed, tetracycline/Cu^{II} also exhibits photoinduced degradation of bovine serum albumin.[25] The ability of tetracycline/Cu^{II} to photoinduce damage to biomolecules is intrinsically linked to the generation of ROS, mainly hydroxyl ($OH^{\bullet}$) and superoxide anion ($O_2^{\bullet-}$) radicals.[19,20,25,27] We have previosuly reported the DNA cleavage ability of two ternary copper(II) complexes of tetracyclines and 1,10-phenantroline: $[Cu(dox)(phen)]^{2+}$ (**1**) and $[Cu(tc)(phen)]^{2+}$ (**2**) (where dox = doxycycline, tc = tetracycline, and phen = 1,10-phenanthroline; Figure S1 in the Supporting Information, SI).[26] Both complexes showed oxidative DNA cleavage ability in the dark and even in the absence of a coreactant. Taken together, this evidence and the redox properties of the Cu^{II} center suggest that complexes **1** and **2** may exhibit DNA photocleavage activity when exposed to an adequate illumination source. This report describes that UV-light exposure can indeed enhance the cleavage of DNA by these complexes and also modify their mechanism of action, in comparison to our previous results in dark conditions.[26] In addition, new interesting aspects regarding the DNA binding mechanisms of **1** and **2** were also investigated.

Herein, the thermal denaturation profile of the DNA molecule was analyzed in the absence or presence of the complexes to confirm and measure their DNA binding ability (Figure S2 in the SI). The DNA melting temperature (T_m) in the absence of the complexes was estimated to be 55.4 °C. In contrast, in the presence of **1**, T_m of DNA increases 2.6 °C, while complex **2** promoted a T_m rise of 2.3 °C. These results suggest that the complexes bind to DNA, hindering the conversion of the double- to single-stranded structure of the nucleic acid.

The effect of the complexes on the secondary structure of DNA was verified by circular dichroism (CD) spectroscopy. Both complexes induce a remarkable hyperchroic effect on the 275 nm band of DNA, associated with base-pair stacking, suggesting stabilization of this secondary structure (Figure 1). The band at 245 nm, relative to the right helicity of B-DNA,[28] was partially affected after addition of the complexes. In both cases, a minor red shift (5−8 nm) was also observed for the 275 nm band. DNA intercalators, such as ethidium bromide, induce a strong hyperchroism on the 275 nm band as well as a significant hypochroism on the 245 nm band,[29] while DNA groove binders promotes the opposite effect.[30] The single hyperchroism of the 275 nm band of DNA induced by **1** and **2** seems to be related to intercalation into DNA; however, this evidence cannot alone be used to classify these complexes as DNA intercalators.

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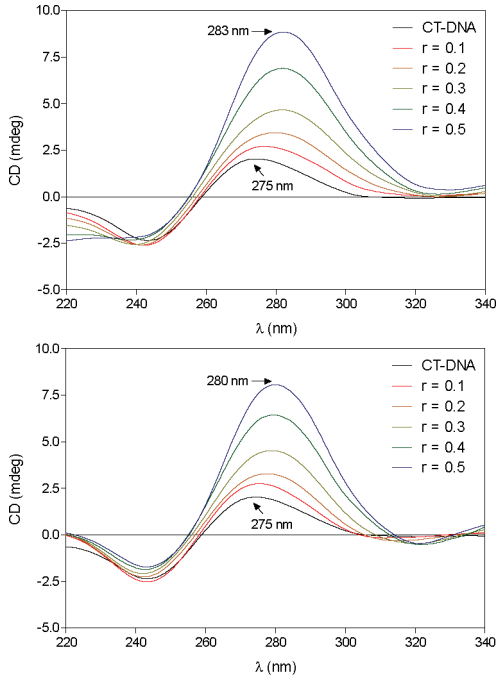

Figure 1. CD spectra of CT-DNA in the absence or presence of increasing amounts of **1** (A) and **2**. (B) Experimental conditions: [CT-DNA] = 100 μM base pairs, [buffer] = 10 mM Tris/HCl (pH 7.4); [complex] = 0−50 μM, temperature = 37 °C.

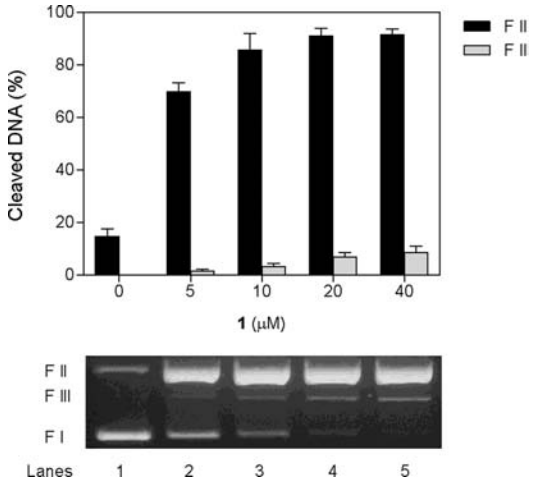

Figure 2. Cleavage of supercoiled DNA by **1**. Reaction conditions: [DNA] = 400 ng, ∼30 μM in base pairs; [buffer] = 10 mM Tris/HCl (pH 7.4); [complex] = 0−40 μM; incubation = 5 min under UV light (λ = 365 nm, 12 W). Representative data from two independent experiments.

DNA UV photocleavage promoted by **1** and **2** was examined following the conversion of pBSK II supercoiled DNA (F I) to the open-circular (F II), and linear (F III) DNA forms using agarose gel electrophoresis to separate the cleavage products. Both complexes exhibit extensive plasmid DNA cleavage after only 5 min of photoirradiation with UV light (λ = 365 nm, 12 W). The presence of **1** even at a low concentration range (5−40 μM) promotes depletion of the supercoiled DNA form, with the consequent formation of open-circular and linear DNA as a result of single- and double-stranded breaks, respectively (Figure 2).

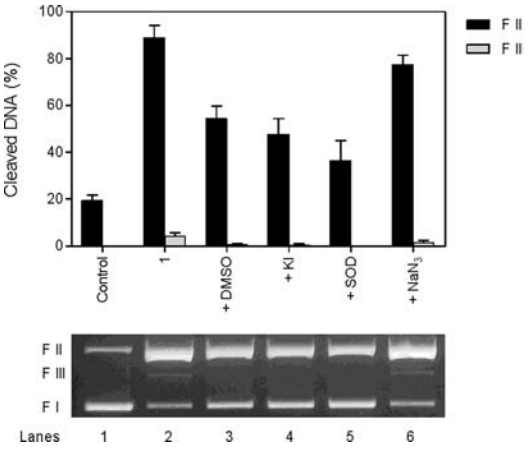

Figure 3. Effect of ROS scavengers on the cleavage of supercoiled DNA by **1**. Reaction conditions: [DNA] = 400 ng, ∼30 μM in base pairs; [buffer] = 10 mM Tris/HCl (pH 7.4); [complex] = 20 μM; [DMSO] = 10% (v/v); [SOD] = 15 units; [KI] = 10 mM; [NaN3] = 10 mM; time = 5 min under UV light (λ = 365 nm, 12 W). Representative data from two independent experiments expressed as mean ± standard deviation.

Similar results were obtained with DNA treated in the presence of **2** (Figure S3 in the SI). In contrast, cleavage of supercoiled DNA by **1** or **2** at 20 μM in the dark reached ∼80% after a longer incubation time (4 h),[26] while under UV light a similar amount of cleaved DNA is obtained in only 5 min at the same complex concentration. The UV-induced rate enhancement was determined by kinetic assays. To serve as controls, a series of DNA cleavage reactions were conducted to evaluate the cleavage ability of each complex component, i.e., the free ligands and copper chloride alone, and no considerable cleavage was observed (Figure S4 in the SI).

To assess the participation of electrostatic interactions on DNA photocleavage, the addition of increasing amounts of NaCl ranging from 50 to 300 mM to the reaction medium was performed (Figure S5 in the SI for **1** and Figure S6 in the SI for **2**). For both complexes, as the ionic strength was increased, a proportional decrease in the cleavage of DNA was observed. These findings suggest that electrostatic interactions between complexes and DNA have a strong effect over the cleavage activity once the presence of high salt concentrations (i.e., NaCl) neutralizes the negative charges in DNA, preventing the binding of complexes.

To verify the participation of ROS in the DNA scission event promoted by **1** and **2** under UV light, different ROS scavengers [dimethyl sulfoxide (DMSO) for $OH^{\bullet}$; superoxide dismutase (SOD) for $O_2^{\bullet -}$; KI for peroxide-type species; NaN_3 for $^1O_2$] were added to the reaction mixtures prior to the complexes (Figure 3 for **1** and Figure S7 in the SI for **2**). DMSO, SOD, and KI partially inhibited DNA cleavage, while NaN_3 promoted a slight inhibition profile. These results indicate the presence of $OH^{\bullet}$ and $O_2^{\bullet -}$ radicals in the DNA cleavage process, as was previously reported in dark conditions[26] but now with the participation of peroxide-type species and $^1O_2$. Thus, UV-light irradiation seems not only to enhance DNA cleavage by the complexes but also to alter the cleavage mechanism at least in terms of ROS involved because $^1O_2$ was not previously found in dark conditions.

To properly demonstrate how photoexposure enhances the activity of **1** and **2** toward DNA, kinetic analysis of DNA cleavage was performed under UV light and compared to assays in dark conditions (Figure 4).

All of the kinetic assays were performed using an excess of catalyst (complex) over the substrate (DNA) to obtain a

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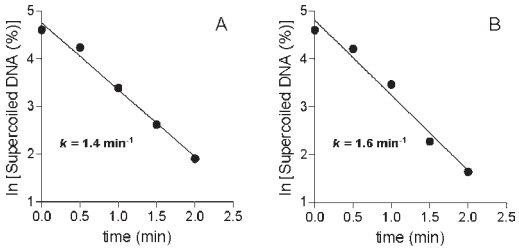

Figure 4. Pseudo-first-order kinetics of DNA cleavage by **1** (A) and **2** (B). Reaction conditions: [DNA] = 400 ng, ~ 30 μM; [buffer] = 10 mM Tris/HCl (pH 7.4); [complex] = 100 μM; time = 0−2 min under UV light.

pseudo-first-order kinetic scenario. In dark conditions, the rates of DNA cleavage (k_{dark}) by the complexes (at 100 μM) were estimated to be 0.036 and 0.043 min^{-1} for **1** and **2**, which correspond to half-lives for supercoiled DNA of approximately 19 and 16 min, respectively.[26] Under UV light, however, the DNA cleavage reaction proceeds with rates (k_{UV}) of about 1.4 and 1.6 min^{-1} in the presence of **1** and **2** (at 100 μM), respectively, which correspond to a half-life of supercoiled DNA lower than 30 s (Figure 4). The k_{UV}/k_{dark} relationship is around 37 for **1** and 39 for **2**, demonstrating that UV light enhances the complex activity ~40-fold. There are few examples in the literature regarding the kinetic analysis of DNA photocleavage by metal complexes.[31] The complex $[Fe^{II}(tdzp)_3]$ presents an impressive $k_{cat.}$ (i.e., k_{UV} at complex saturation) of 0.31 min^{-1} under conditions similar to those used in this work. The title complexes seem to be at least 4−5 times more active than $[Fe^{II}(tdzp)_3]$. To prove the stability of **1** and **2** after UV-A-light irradiation, electrospray ionization mass spectrometry (MS) measurements were performed with the two complexes (Figure S8 in the SI). These results strongly suggest that the complexes remain unaltered after UV-A-light exposure, suggesting their stability under the DNA photocleavage assay conditions.

Furthermore, UV-light exposure increases the cytotoxic activities of both complexes. The IC_{50} values obtained in the dark for complexes **1** and **2** are 3.23 and 6.65 μM and, after irradiation, 0.35 and 1.0 μM, respectively. Then, upon irradiation, there is an increase in the cytotoxicity of approximately 9 times for **1** and 7 times for **2** (Figure S9 in the SI).

In summary, the results presented here successfully show new aspects of the DNA binding properties of two ternary copper(II) complexes and also that UV-light irradiation enhances the cleavage ability of these complexes toward DNA by about ~40-fold and the cytotoxic activity by about 8-fold. The effect of UV light extends to the DNA cleavage mechanism because $^1O_2$ seems to be involved in this process under UV light but not in dark conditions. The use of UV light appears to be an attractive alternative to increase the cleavage of DNA and the cytotoxic activity by synthetic agents.

■ ASSOCIATED CONTENT

Supporting Information. Experimental procedures for DNA binding and photocleavage assays and Figures S1−S9. This material is available free of charge via the Internet at http://pubs.acs.org.

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