Singlet Oxygen-Mediated DNA Photocleavage with Ru(II) Polypyridyl Complexes

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Received: September 17, 2001; In Final Form: December 12, 2001

The efficiency of photosensitized DNA cleavage and quantum yields of singlet oxygen production (Φ_Δ) have been determined for the metal complexes $[Ru(bpy)_2(ddz)]^{2+}$, $[Ru(phen)_2(ddz)]^{2+}$, $[Ru(phen)_3]^{2+}$, and $[Ru(dsdp)_3]^{4-}$ (bpy = 2,2′-bipyridine, ddz = dibenzo[*h*,*j*]dipyrido[3,2-*a*:2′,3′-*c*]phenazine, phen = 1,10-phenanthroline, and dsdp = 1,10-phenanthroline-4,7-diphenylsulfonate). Φ_Δ values (0.19−0.52) have been measured in O_2-saturated D_2O solution, both in the absence and presence of DNA. Protection of the photoexcited Ru(II) complexes from O_2 quenching imparted by the polynucleotide was evaluated from their emission lifetimes (0.13−3.7 μs) measured at three different O_2 concentrations. Our results show the highest photocleavage efficiency for those sensitizers that display strong affinity for DNA (binding constant $K_b > 10^6$ M^{-1}), despite their lower Φ_Δ values (0.08−0.10 for the three cationic complexes vs 0.40 for the tris-dsdp, in H_2O/air/DNA). Kinetic analysis (agarose gel electrophoresis) of the photocleavage reaction of plasmid DNA reveals that *single*-strand breaks are produced with the four photosensitizers, as expected for a singlet oxygen-mediated mechanism. Moreover, an intercalative binding of the metal complex, compared to groove-bound or unbound sensitizers of the same family, is shown to accelerate double-strand break that occurs by accumulation of single-strand breaks.

I. Introduction

Enzymes involved in the cleavage of phosphodiester bonds in polynucleotides (nucleases) have been extremely useful in many applications, but their large size or limited range of sequence-recognition capabilities prevent their general use. These shortcomings of natural nucleic acid cleavage agents have led to the design and development of synthetic cleavers as molecular tools for biotechnology.[1,2] A particular class of nucleic acid cleavage agents includes those activated photochemically.[3] One appealing characteristic of these photoreagents is the fact that all components of the system can be mixed together without initiating the chemical reaction until the sample is irradiated. Potential advantages of DNA photocleavers as therapeutical species also result from the capability to control irradiation, in both a spatial and temporal sense, and the possibility to activate them in vivo with light delivered through optical fibers. A variety of compounds (ketones, organometallic complexes, polycyclic heteroaromatic drugs, etc.) have been shown to photoactivate cleavage of the DNA sugar−phosphate backbone, by way of two possible mechanisms. One of them (type I) involves *direct* reaction with the nucleic acid of an excited state of the photosensitizer, while other reaction pathways (type II) require an *intermediate reactive species* such as the superoxide anion or singlet molecular oxygen.[4,5]

Electronically excited compounds that intersystem cross to their triplet state and have a proper triplet energy can generate singlet molecular dioxygen ($^1O_2$) by energy transfer to ground-state oxygen ($^3O_2$).[6] Singlet oxygen photosensitizers have important applications in both natural and artificial photosystems, as this reactive oxygen species is a key intermediate in several chemical and biological processes such as photooxidation reactions,[7] photosensitized cytotoxicity,[8] and photodynamic therapy.[9,10] Since the lowest electronically excited state of molecular oxygen ($^1\Delta_g$) is relatively long-lived, with lifetimes in the range of a few microseconds in aqueous media,[11] reaction of $^1O_2$ may occur at some distance from its generation point. Both physical and chemical processes involved in $^1O_2$ deactivation are of great importance. The radiative decay of $^1O_2$ is frequently used for its detection. In fact, monitoring the characteristic $^1O_2$ luminescence at 1270 nm has proven extremely useful for investigating $^1O_2$ deactivation mechanisms as well as for determining quenching rate constants and quantum yields of $^1O_2$ production.[6,12−15] Photooxidation reactions mediated by singlet oxygen are manifold: 1,2-, 1,3-, and 1,4-additions to olefins and aromatic compounds to yield dioxetanes, allylic hydroperoxides and endoperoxides; oxidation of sulfides, phenols, etc.[7] There is some debate on whether $^1O_2$ produces frank strand breaks or not and to what extent. In fact, there is no evidence of direct strand cleavage by either type I or type II mechanisms. Reactions of singlet oxygen with nucleosides and DNA occur selectively on the guanine moiety to yield 8-oxo-7,8-dihydroguanine (8-oxoGua) as the (initial) oxidation main product.[3,5,16−18] It has been shown recently that 8-oxoGua is not an alkali-labile site; however, secondary oxidation products of 8-oxoGua in the polynucleotide may lead to strand break upon piperidine treatment.[18] Although to a lesser extent, singlet

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10.1021/jp013542r CCC: $22.00
Published on Web 03/21/2002

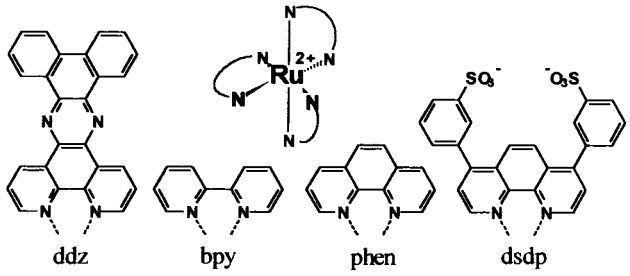

Figure 1. Chemical structure of the bidentate ligands contained in the complexes $[Ru(bpy)_2(ddz)]^{2+}$, $[Ru(phen)_2(ddz)]^{2+}$, $[Ru(phen)_3]^{2+}$, and $[Ru(dsdp)_3]^{4-}$. The abbreviations used stand for dibenzo[*h*,*j*]-dipyrido[3,2-*a*:2′,3′-*c*]phenazine (ddz), 2,2′-bipyridine (bpy), 1,10-phenanthroline (phen), and 1,10-phenanthroline-4,7-diphenylsulfonate (dsdp).

oxygen has been also shown to produce frank strand breaks at guanine sites in the DNA.[5,19]

The interaction and photoreactivity of Ru(II) polypyridyl complexes with DNA and polynucleotides have been the focus of intense research work in the last 15 years.[20−24] These coordination compounds display a unique combination of photophysical, photochemical, redox, and structural characteristics that make them suitable candidates to develop spectroscopic probes and photoreagents for nucleic acids. Depending on the size, shape, hydrophobicity, and charge of the octahedral Ru(II) complex, it can bind noncovalently to DNA in different ways, namely electrostatic interaction with the negatively charged phosphate backbone, surface binding in the minor or major groove of the double helix, or intercalation of one of the ligands between the stacked base pairs. The ^{3}MLCT (metal-to-ligand charge transfer) excited state of Ru(II) polypyridyl complexes can initiate DNA cleavage reactions either by electron transfer to a base (type I) or by energy transfer to molecular oxygen (type II). The former mechanism occurs with highly oxidizing photoexcited Ru(II) complexes (like those containing tetraazaphenanthrene or hexaazatriphenylene ligands), for which both covalent photoadducts with DNA and photoproduction of alkaline labile sites on the biopolymer have been identified.[25] DNA photocleavage via singlet oxygen generation has been described for the trisbipyridyl and trisphenanthroline Ru(II) complexes or their substituted analogues.[24,26−29] Although the quantum yields of $^1O_2$ production (Φ_Δ) have been determined for some Ru(II) polypyridyl complexes in various solvents,[30−32] no data are available for this parameter when the photosensitizer is bound to DNA.

In the present study, we discuss the $^1O_2$-mediated DNA photocleavage efficiency in relation to both the DNA binding features of the different Ru(II) complexes investigated and the changes on their Φ_Δ upon binding to the polynucleotide. To that aim we have measured and analyzed the photocleavage kinetics of plasmid DNA for the complexes $[Ru(bpy)_2(ddz)]^{2+}$, $[Ru(phen)_2(ddz)]^{2+}$, $[Ru(phen)_3]^{2+}$, and $[Ru(dsdp)_3]^{4-}$ (bpy = 2,2′-bipyridine, ddz = dibenzo[*h*,*j*]dipyrido[3,2-*a*:2′,3′-*c*]phenazine, phen = 1,10-phenanthroline, and dsdp = 1,10-phenanthroline-4,7-diphenylsulfonate). The structures of all these ligands are depicted in Figure 1. We have also determined the Φ_Δ values and rate constants of ^{3}MLCT excited-state quenching by O_2 (k_q), both in the absence and presence of DNA for all these metal compounds. The heteroleptic complexes $[Ru(bpy/phen)_2(ddz)]^{2+}$ incorporate one chelating ligand with a large aromatic structure so that a high-affinity intercalative association to DNA ($K_b > 10^6$ M^{-1}) is observed.[33] A moderate (nonintercalative) affinity for DNA has been reported for $[Ru(phen)_3]^{2+}$ ($K_b \approx 10^4$ M^{-1}, see more details in the Discussion section),[34] while an electrostatic repulsion with the anionic polynucleotide is expected for $[Ru(dsdp)_3]^{4-}$. Values of Φ_Δ as high as 0.24 and 0.43 in air-equilibrated D_2O have been determined for the tris-phen and tris-dsdp complexes, respectively, while $^1O_2$ photoproduction becomes quantitative when the latter complex is dissolved in organic solvents such as perdeuterated methanol.[31] Such data show the potential of those photosensitizers if the main factors that govern the type II DNA cleavage induced by those reagents are uncovered.

II. Materials and Methods

Materials. The complexes $[Ru(bpy)_2(ddz)]Cl_2$ and $[Ru(phen)_2(ddz)]Cl_2$ were obtained according to the method described elsewhere.[33] The synthesis and purification of $Na_4[Ru(dsdp)_3]$ from commercial dsdp ligand have been reported.[31] $[Ru(bpy)_3]Cl_2$ and $[Ru(phen)_3]Cl_2$ (Aldrich) were used as received. All experiments have been conducted in tris buffer (3 mM, pH 7.0 tris(hydroxymethyl)aminomethane) or in D_2O (+99.90% D, CEA CE Saclay or SDS, France). Water was obtained from a Millipore Milli-Q purification system. The concentration of the complexes in the aqueous solutions was determined spectrophotometrically using the following values for the absorption coefficients (ϵ/L mol^{-1} cm^{-1}): $\epsilon_{452} = 14000$ ($[Ru(bpy)_3]^{2+}$), $\epsilon_{447} = 19000$ ($[Ru(phen)_3]^{2+}$), $\epsilon_{462} = 29300$ ($[Ru(dsdp)_3]^{4-}$), $\epsilon_{400} = 16000$ ($[Ru(bpy)_2(ddz)]^{2+}$), and $\epsilon_{399} = 17700$ ($[Ru(phen)_2(ddz)]^{2+}$). Absorption spectra were recorded with a Varian Cary-3Bio spectrophotometer (Australia) interfaced to a Compaq Deskpro 2000 computer for data acquisition and instrument control.

Sonicated calf-thymus DNA (CT-DNA), with an average size of 1500 base pairs (bp), was from Pharmacia Biotech (Sweden). The lyophilized material (170 AU) was dissolved in 4.0 mL and extensively dialyzed against tris buffer. The final stock concentration has been calculated to be approximately 3.3 mM-(bp) from the absorption spectrum ($\epsilon_{258} = 12800$ L mol^{-1} cm^{-1}). DNA solutions in D_2O were obtained after three cycles of lyophilization, redissolution in D_2O, and equilibration in this solvent for 60 min. Plasmid pBR322 DNA (5 AU/ml) and molecular weight marker X were from Boehringer-Mannheim (Germany).

Binding of the Photosensitizer to DNA. A gentle magnetic stirring was applied to all samples containing Ru(II) complex and CT-DNA for at least 30 min before the measurement. The percentage of photosensitizer bound to the polynucleotide at the different concentrations employed in these experiments has been calculated according to the McGhee−von Hippel noncooperative model.[35] The binding constant (K_b) and the site size (n) values used in each case were $K_b = 4 \times 10^4$ M^{-1}, $n = 3.5$ bp for $[Ru(phen)_3]^{2+}$, and $K_b \geq 10^6$ M^{-1}, $n = 3.0$ bp for $[Ru(bpy)_2(ddz)]^{2+}$ and $[Ru(phen)_2(ddz)]^{2+}$.[34,36]

Photocleavage of pB*R*322 Plasmid DNA. Each 45 μL sample containing pB*R*322 (40 μM(bp)) and Ru(II) complex (0.40−0.47 μM) in tris buffer was placed in every other well of a 96-well white microtiter plate (FluoroNunc PolySorp from Nunc, Denmark). After 20 min incubation, irradiation was performed at 442 nm using a He−Cd laser (Melles-Griot Omnichrome 4056R-40M, CA) via a 1 m long 3 mm diameter liquid-core optical fiber (Lumatec, Germany). The light guide end was attached consecutively to the different wells containing the samples using a custom-made poly(tetrafluoroethylene) drilled lid. The laser power measured at the exit of the fiber (Ophir PD200, Ophir Optronics Ltd., Israel) was 33.5 ± 0.5 mW and the absorbance at 442 nm of the different Ru(II) samples was calculated to be 9.0×10^{-4} for 0.47 μM [Ru-

$(bpy)_2(ddz)]^{2+}$, 8.5×10^{-4} for 0.47 μM $[Ru(phen)_2(ddz)]^{2+}$, 9.8×10^{-4} for 0.40 μM $[Ru(phen)_3]^{2+}$, and 1.4×10^{-3} for 0.40 μM $[Ru(dsdp)_3]^{4-}$.

After irradiation for a selected time, 15 μL of each sample were transferred to an Eppendorf tube and mixed with 3 μL of loading buffer (0.25% bromophenol blue and 40% sucrose). All the samples were analyzed by 1% agarose gel electrophoresis in Tris-acetate (TAE) buffer (pH 7.5−8.0), followed by 10 min of ethidium bromide staining (0.5 μg mL^{-1}). Images of the stained DNA bands in the gels were obtained using an UVI-Tec photodocumentation system and the fluorescence intensities were quantified with the UVI-Band Windows Application V97.04 software. The lower affinity of ethidium for intercalation into supercoiled DNA (form I) was taken into account by dividing the corresponding intensity by 0.6.[37,38] The percentage of each plasmid form in a lane was calculated by dividing the intensity of the corresponding band by the sum of the intensities of all bands in the same lane.

The kinetic study of the DNA photocleavage reaction was performed by analyzing the evolution of the plasmid forms as a function of the irradiation time. Both the single-strand and double-strand break models (abbreviated ssb and dsb, respectively) have been used for this purpose.[39] The ssb model (eq 1) considers that each cleavage event converts the supercoiled DNA (form I) to nicked circular (or relaxed) DNA, form II, with a rate constant k_1. Subsequent nicking turns form II into linear DNA (form III) with a rate constant k_2. Further cleavage of form III converts it to smaller linear fragments with a rate constant k_3. On the other hand, the dsb model (eq 2) assumes that the cleavage agent can convert form I directly to the linear form III, which can also be produced from form II.

$$\mathrm{I} \xrightarrow[k_1]{} \mathrm{II} \xrightarrow[k_2]{} \mathrm{III} \xrightarrow[k_3]{} \ldots \quad (1)$$

$$\mathrm{I} \xrightarrow[k_4]{} \mathrm{III} \xrightarrow[k_3]{} \ldots; \qquad \mathrm{II} \xrightarrow[k_2]{} \mathrm{III} \xrightarrow[k_3]{} \ldots \quad (2)$$

Kinetic equations that predict the evolution of plasmid forms I, II, and III as a function of the irradiation time are obtained by integrating the rate laws of the processes considered by these two models. The values for the corresponding second-order rate constants for each Ru(II) complex were obtained by fitting the experimental data to each integrated rate law.[39]

Quenching of Ru(II) Complex Excited State by Molecular Oxygen. Emission lifetime measurements were performed for O_2-, air-, and N_2-saturated solutions of the Ru(II) complexes (10 μM in tris buffer), both in the absence and in the presence of 0.50 mM(bp) CT-DNA (r = [DNA](bp)/[Ru] = 50). For $[Ru(bpy)_2(ddz)]^{2+}$ and $[Ru(phen)_2(ddz)]^{2+}$, measurements were also carried out using 6-μM solutions in D_2O, free of DNA or containing 0.16-mM(bp) CT-DNA (r = 27). Measurements were carried out after purging with the corresponding gas for at least 30 min.

Emission decays were recorded using an Edinburgh Instruments (UK) FL-900 time-correlated single-photon counting (TCSPC) spectrometer, equipped with a red-sensitive Hamamatsu R-955 photomultiplier tube, thermoelectrically cooled at −24 °C. Excitation with a nitrogen discharge lamp pulsed at 20−40 kHz was performed at 337 or 358 nm, and the emission monochromator was set at 600 nm. The luminescence decay profiles at 25.0 ± 0.5 °C were collected by accumulation of 10,000 counts at the peak channel. The experimental data were analyzed using a nonlinear least-squares Marquardt algorithm contained in the original manufacturer software, and fitted to the equation

$$I(t) = A + \sum_i B_i \exp(-t/\tau_i) \quad (3)$$

where i = 1, 2, or 3, B_i is the preexponential factor associated to the emission lifetime τ_i, and A is a constant that accounts for the signal background. The reduced χ^2 parameter and the weighed residuals were employed to judge the goodness of the fits. For samples with multiexponential emission decay (i > 1), the so-called preexponential weighted emission lifetime (τ_M) was calculated according to eq 4,[40]

$$\tau_M = \sum_i B_i \tau_i / \sum_i B_i \quad (4)$$

The oxygen concentration in air-equilibrated and oxygen-saturated solutions (2.5×10^{-4} and 1.14×10^{-3} M, respectively) was determined from the barometric pressure (typically 710 Torr) and temperature.[41] The rate constant of the Ru(II) complex excited-state quenching by molecular oxygen (k_q) was calculated from the slope of the linear Stern−Volmer plot of the corresponding emission lifetime data.[42]

Singlet Oxygen Detection under Continuous Irradiation. The weak phosphorescence from $^1O_2$ at 1270 nm was used to determine Φ_Δ values in oxygen-saturated D_2O solution for $[Ru(bpy)_2(ddz)]^{2+}$ and $[Ru(phen)_2(ddz)]^{2+}$. The concentration of the Ru(II) complexes was approximately 30, 40, 50, or 80 μM in the absence of DNA and 44 μM in the presence of 0.55 mM(bp) DNA (r = 13). Rose bengal solutions in air-equilibrated D_2O were used as reference ($\Phi_\Delta^R = 0.75$).[43,44] The equipment based on steady-state irradiation of the sensitizer solution has been described previously.[12,13] The Ru(II) complexes were excited at 436 nm and rose bengal at 547 nm using a Xe/Hg lamp (1 KW, Osram, Müller), focusing optics and a monochromator (ISA Jobin-Yvon B204, 6-nm bandwidth). The absorbance of the sample and reference solutions at the corresponding excitation wavelengths were identical for each experiment in matched 1 cm fluorescence cells (Hellma). A series of measurements alternating between reference and sample were carried out, the irradiation time being 3 min for each sample. The incident radiant power (F_0, in mW) on the sample cell was measured with a thermopile (Laser Instrumentation, model 154) and used to calculate relative incident photonic rates ($P_0/P_0^R = (F_0\lambda_{exc})/(F_0^R\lambda_{exc}^R)$, where λ_{exc} and λ_{exc}^R are the excitation wavelengths for the sample and reference solutions, respectively). Measured F_0 was approximately 3.4 mW at 547 nm and 4.3 mW at 436 nm. The Φ_Δ values for the various complexes have been calculated according to eq 5

$$\Phi_\Delta = \Phi_\Delta^R \frac{S_\Delta F_0^R \lambda_{exc}^R}{S_\Delta^R F_0 \lambda_{exc}} \quad (5)$$

where S_Δ and S_Δ^R stand for the signals corresponding to the $^1O_2$ luminescence at 1270 nm measured for the sample and reference, respectively. Equation 5 is valid under conditions where $^1O_2$ quenching by the sensitizer may be neglected when compared with $^1O_2$ quenching by the solvent.

The luminescence from the Ru(II) complex extends to the near-infrared.[31] Therefore, the S_Δ values in O_2-saturated solution (S_{Δ,O_2}) were calculated using eq 6, where the contribution of the emission from the Ru(II) complex (S_{Ru,O_2}) has been subtracted from the luminescence signal measured at 1270 nm in O_2-saturated solution (S_{m,O_2}). The S_{Ru,O_2} value was calculated from the luminescence signal measured after purging the sample with argon ($S_{m,Ar}$) and the emission lifetimes of the Ru(II)

sensitizer in O_2- and Ar-saturated solutions (τ_{O_2} and τ_{Ar}, respectively).

$$S_{\Delta,O_2} = S_{m,O_2} - S_{Ru,O_2} = S_{m,O_2} - S_{m,Ar}(\tau_{O_2}/\tau_{Ar}) \quad (6)$$

Singlet Oxygen Detection Using Pulsed Excitation. Time-resolved detection of the $^1O_2$ phosphorescence at 1270 nm was used to measure $^1O_2$ lifetimes (τ_Δ) and to determine Φ_Δ for the investigated Ru(II) complexes in oxygen-saturated D_2O solutions, with and without DNA. To obtain the *same* absorbance values at the excitation wavelength for all the solutions in a series of measurements (approximately 0.42 without DNA and 0.38 in the presence of 2.5 mM(bp) DNA), the sensitizer concentrations typically used were approximately 0.1−0.6 mM. $[Ru(dsdp)_3]^{4-}$ was used as a reference sensitizer (Φ_Δ (D_2O/O_2) = 0.52).[31] Measurements were performed using an Edinburgh Instruments (UK) LP-900 laser flash photolysis system equipped with a Nd:YAG laser (Minilite II, Continuum, CA) for excitation at 532 nm and an EI-P fast Ge photodiode for near-infrared detection (at 1270 nm). An Ophir AN/2 energy meter with a thermopile head (Ophir Optronics Ltd., Israel) was employed to monitor the energy of the laser pulse (E), which was varied from 400 to 1000 μJ pulse^{-1}. Emission from the sample was monitored at 90° angle with respect to the excitation pathway, and detected after passing through an interference filter centered at 1270 nm (77-nm FWMH). Typically, 100−200 laser shots were averaged for each signal. All the $^1O_2$ luminescence decay profiles have been fitted to an exponential function after excluding the fast decay due to the Ru(II) sensitizer emission. After extrapolating the intensities of the $^1O_2$ signal at zero time for the sample and reference solutions (B_0 and B_0^R, respectively), Φ_Δ values were calculated from eq 7:

$$\Phi_\Delta = \Phi_\Delta^R \frac{B_0 E^R}{B_0^R E} \quad (7)$$

III. Results

Photocleavage Detection Using Electrophoresis. DNA photocleavage was sensitized by the four Ru(II) complexes used in this study (Figure 2), albeit to a different extent. Changes in pBR322 plasmid DNA are not detected, either when irradiated in the absence of ruthenium complex (data not shown), or when incubated with the sensitizer in the dark.

The photocleavage efficiency depends on the structure of the metal complex. After irradiating plasmid DNA in the presence of $[Ru(bpy)_2(ddz)]^{2+}$, the supercoiled native form (I) completely disappears, while the concentration of its linear (form III) and circular relaxed (form II) species increases. Moreover, some small linear DNA fragments appear (Figure 2). Running the same experiment after deoxygenation of the sample with argon for 20 min did not lead to any noticeable cleavage. DNA degradation is less important when the light-absorbing sensitizer $[Ru(phen)_2(ddz)]^{2+}$ is used: small linear fragments of the polynucleotide are not observed, although changes in the plasmid forms are similar to those found with the bpy analogue. Even after 10 min of illumination in the presence of $[Ru(phen)_3]^{2+}$, the plasmid form I still remains. $[Ru(dsdp)_3]^{4-}$ appears to be the least efficient of the four complexes.

Kinetic Analysis of Photocleavage. To study the photocleavage kinetics, samples with the same r (r = [DNA](bp)/[Ru]) ratio have been irradiated during various periods of time and analyzed by agarose gel electrophoresis. Data obtained thereof were fitted to the single-strand break (ssb) model as shown in Figure 3, and the rate constants k_1, k_2, and k_3 (see Experimental Section) were calculated (Table 1). The rate constants are in agreement with the photocleavage efficiency order observed in the electrophoresis experiments. The percentage of sensitizer bound to DNA under the corresponding experimental conditions are also listed in Table 1, for assessing a potential relationship between photocleavage efficiency and the extent of binding. The ddz complexes show the highest affinity for DNA, while the anionic $[Ru(dsdp)_3]^{4-}$ sensitizer does not bind at all to the double-stranded polynucleotide. It is possible that the slight basicity of the electrophoresis buffer (pH 7.5−8.0) could reveal some alkali-labile sites. In this case, the comparison between the DNA damage produced by the four investigated sensitizers would still be valid, since all the analyses have been performed exactly under the same conditions.

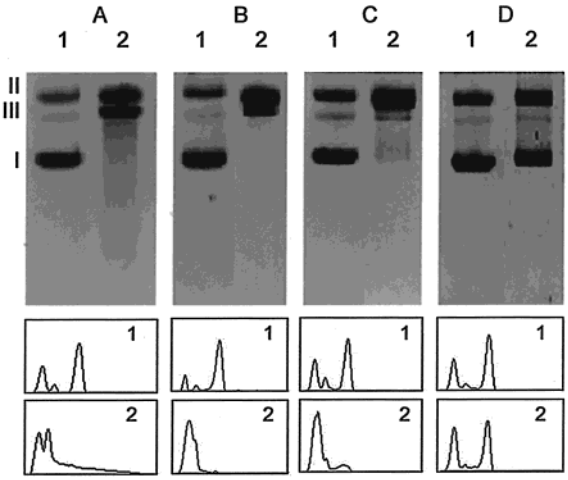

Figure 2. Electrophoresis of pBR322 DNA after illumination in the presence of the different Ru(II) complexes. Plasmid forms I, II, and III are, respectively, supercoiled, relaxed, and linear DNA. Control samples kept in the dark appear on lanes number 1; lanes number 2 correspond to samples irradiated for 10 min at 442 nm. A: $[Ru(bpy)_2(ddz)]^{2+}$ (r = 85). B: $[Ru(phen)_2(ddz)]^{2+}$ (r = 85). C: $[Ru(phen)_3]^{2+}$ (r = 100). D: $[Ru(dsdp)_3]^{4-}$ (r = 100). The densitometry of each lane is shown under the corresponding gel.

Experimental data could not be fitted to the dsb model in any case. If double-strand breaks are occurring, the concentration of DNA form II should decrease continuously as a function of irradiation time. However, the initial behavior observed in every case (an increase up to 80% of the overall plasmid DNA) is the opposite to that predicted by the dsb model.

Emission Lifetimes and Quenching of Ru(II) Complexes by O_2. Molecular oxygen is an efficient quencher of photoexcited Ru(II) polypyridyl complexes.[45] The emission lifetimes (τ) of the investigated sensitizers as a function of the O_2 concentration, both in the presence and in the absence of DNA, are collected in Table 2. Multiexponential decay profiles have been observed in several cases. Even without added polynucleotide, three components contribute to the luminescence of the ddz sensitizers, a phenomenon that has been attributed to self-aggregation and will be discussed elsewhere (Jiménez-Hernández, unpublished experiments). In the presence of DNA, the emission decay kinetics become biexponential for the two ddz complexes, as it has been observed already for the extensively studied $[Ru(phen)_3]^{2+}$ sensitizer and many other Ru(II) polypyridyls.[22,23,46] In case of multiexponential kinetics, a preexponential weighed emission lifetime (τ_M) has been calculated (Table 2). The mean lifetime τ_M is a reliable parameter for evaluating and comparing excited-state lifetimes in the presence of DNA.[47] In contrast to the ddz and phen complexes, the emission decay of $[Ru(dsdp)_3]^{4-}$ could be fitted to a single-

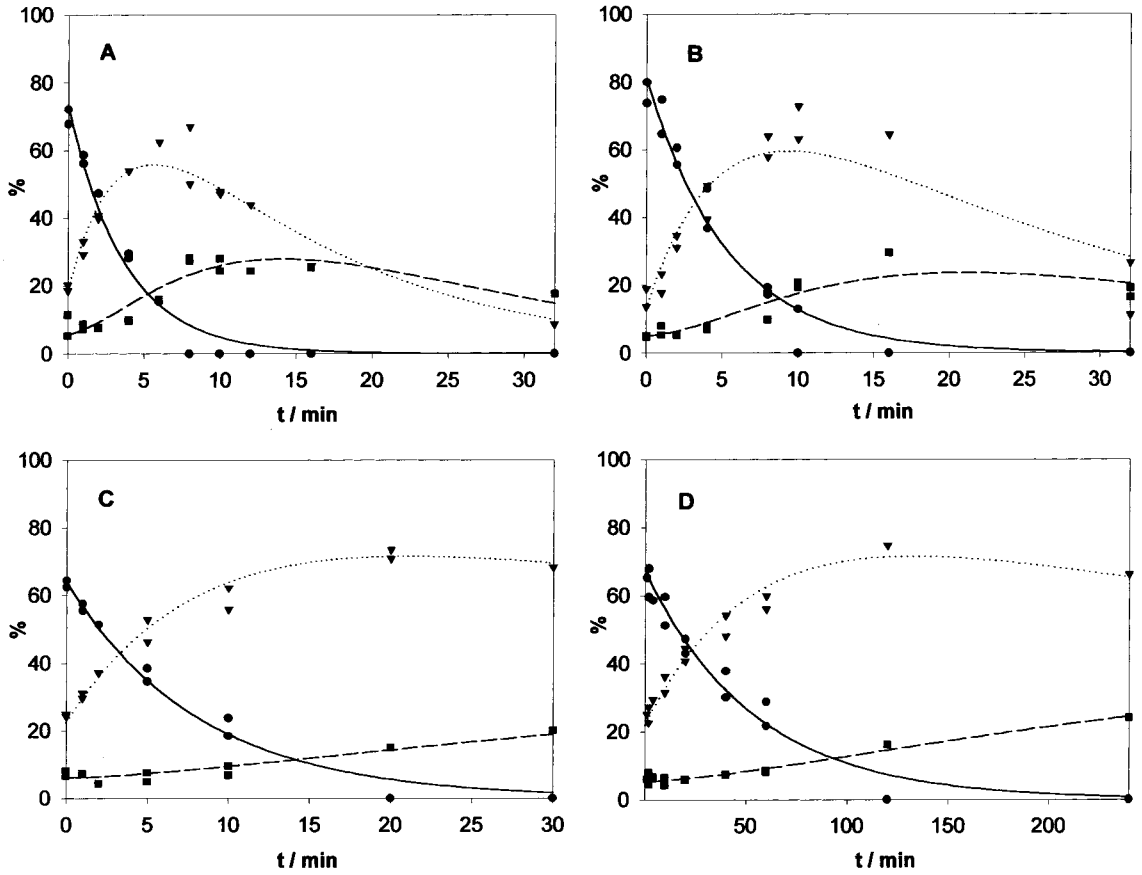

Figure 3. Percentages of pB*R*322 plasmid DNA forms I (●), II (▼), and III (■) as a function of irradiation time, in the presence of a constant ruthenium complex concentration. Curve fits to the single-strand break model are also shown. A: $[Ru(bpy)_2(ddz)]^{2+}$ ($r = 85$). B: $[Ru(phen)_2(ddz)]^{2+}$ ($r = 85$). C: $[Ru(phen)_3]^{2+}$ ($r = 100$). D: $[Ru(dsdp)]^{4-}$ ($r = 100$).

TABLE 1: Rate Constants for Photosensitized Formation of Frank Breaks on PBR322 Plasmid DNA Calculated from the Single-Strand-Break Model[39]

	r^a	% $Ru_b{}^b$	$k_1{}^c$/ $\mu M^{-1} min^{-1}$	$k_2{}^c$/ $\mu M^{-1} min^{-1}$	$k_3{}^c$/ $\mu M^{-1} min^{-1}$	k_1/k_2
$[Ru(bpy)_2(ddz)]^{2+}$	85	>98	0.6 ± 0.1	0.16 ± 0.03	0.22 ± 0.05	3
$[Ru(phen)_2(ddz)]^{2+}$	85	>98	0.38 ± 0.08	0.09 ± 0.03	0.17 ± 0.04	4
$[Ru(phen)_3]^{2+}$	100	60	0.33 ± 0.05	0.020 ± 0.007	0.02 ± 0.02	15
$[Ru(dsdp)_3]^{4-}$	100	0	0.046 ± 0.009	0.004 ± 0.001	0.003 ± 0.002	12

[a] r = [DNA](bp)/[Ru]. [b] Percentages of bound complex were calculated from the McGhee−von Hippel interaction model (see Experimental Section). [c] Rate constant values and corresponding 95% confidence interval.

exponential function both in the presence and in the absence of polynucleotide.

Rate constants of ^{3}MLCT excited-state quenching by O_2 (k_q) in aqueous buffer solution were in the order of 10^9 M^{-1} s^{-1} (Table 2). For the three cationic Ru(II) complexes studied in this work, k_q decreases between 3 and 10 times in the presence of the DNA; for $[Ru(dsdp)_3]^{4-}$, no change was observed after adding the biopolymer (Table 2). Values of k_q have been determined also in D_2O for the ddz complexes and are within the experimental error from those obtained in H_2O.

Quantum Yields of Singlet Oxygen Production. Sensitized $^1O_2$ production quantum yields (Φ_Δ) have been determined in O_2-saturated D_2O solution. Results obtained from the two different experimental techniques used, namely, steady-state and time-resolved NIR emission from $^1O_2$, show an excellent agreement. These data, together with $^1O_2$ lifetime τ_Δ and other parameters calculated from them, are collected in Table 3. In the absence of DNA the most efficient $^1O_2$ generator is $[Ru(dsdp)_3]^{4-}$, followed by $[Ru(phen)_3]^{2+}$, $[Ru(phen)_2(ddz)]^{2+}$, and $[Ru(bpy)_2(ddz)]^{2+}$. Addition of DNA to the aqueous solution modifies Φ_Δ only for the cationic Ru(II) sensitizers.

From the experimental data in D_2O, the value of Φ_Δ in air-equilibrated H_2O solutions (conditions in which the photocleavage assays have been developed) can be calculated. The calculation can be accomplished using eqs 8 and 9.[31]

$$P^T_{O_2} = \tau k_q [O_2] \tag{8}$$

$$\Phi_\Delta = \Phi_T P^T_{O_2} f^T_\Delta \tag{9}$$

where $P^T_{O_2}$ is the proportion of triplet excited states quenched by O_2, f^T_Δ is the fraction of excited triplet sates quenched by O_2 yielding $^1O_2$ and Φ_T is the quantum yield of triplet excited state formation (intersystem crossing). The latter is considered to be equal to one for the complexes studied,[31] and it is assumed that f^T_Δ does not change significantly under the different conditions used in this work (O_2-saturated or air-equilibrated H_2O or D_2O solutions).

TABLE 2: Emission Lifetimes (τ_i), Relative Pre-exponential Factors (%α_i) and Mean Lifetimes (τ_M) Measured in Oxygen-, Air-, and Nitrogen-Saturated Tris Buffer (3 mM, pH = 7.0) Solutions for the Investigated Ru(II) Complexes, Both in the Absence and in the Presence of DNA ($r = 50$)

		τ_{O_2} /μs		τ_{air} /μs		τ_{N_2} /μs		$k_q/10^9$ [d]
	%Rub[a]	τ_i (%α_i)[b]	τ_M[c]	τ_i (%α_i)[b]	τ_M[c]	τ_i (%α_i)[b]	τ_M[c]	$M^{-1}\,s^{-1}$
$[Ru(bpy)_2(ddz)]^{2+}$	no DNA	0.05 (31)	0.13	0.05 (30)	0.17	0.05 (30)	0.18	1.6
		0.14 (60)		0.19 (60)		0.20 (60)		2.0
		0.31 (9)		0.41(10)		0.47 (10)		1.0
	>99	0.55 (60)	0.65	0.96 (60)	1.1	1.2 (60)	1.3	0.8
		0.81 (40)		1.3 (40)		1.6 (40)		0.5
$[Ru(phen)_2(ddz)]^{2+}$	no DNA	0.03 (20)	0.14	0.04 (20)	0.19	0.04 (20)	0.21	3.0
		0.14 (70)		0.20 (70)		0.21 (70)		2.0
		0.33 (10)		0.47 (10)		0.55 (10)		1.0
	>99	0.74 (65)	1.0	1.3 (65)	1.6	1.6 (65)	1.8	0.7
		1.6 (35)		2.0 (35)		2.3 (35)		0.2
$[Ru(phen)_3]^{2+}$	no DNA	0.18		0.48		1.0		4.0
	95	0.54 (40)	0.79	0.82 (40)	1.3	1.0 (40)	1.5	0.8
		1.0 (60)		1.6 (60)		1.9 (60)		0.5
$[Ru(dsdp)_3]^{4-}$	no DNA	0.27		0.95		3.6		3.0
	0	0.27		0.97		3.4		3.0

[a] Percentage of bound complex calculated from the McGhee-von Hippel interaction model (see Experimental Section). [b] $\%\alpha_i = (B_i/\sum B_i)100$ [c] Preexponential weighted emission lifetime (mean lifetime), $\tau_M = \sum(B_i\tau_i)/\sum B_i$; estimated error ±5%. [d] O_2 quenching rate constant measured from the emission lifetimes (see Experimental Section).

TABLE 3: Quantum Yields of Singlet Oxygen Production (Φ_Δ) Determined by Steady-State (S. S.) and Time-Resolved (T. R.) $^1O_2$ Emission, $^1O_2$ Lifetimes (τ_Δ), Proportion of Excited Triplet States Quenched by O_2 ($P^T_{O_2}$), and Fraction of Triplet Excited States Quenched by O_2 to Yield $^1O_2$ (f^T_Δ), Determined for O_2-Saturated D_2O unless Otherwise Stated

		Φ_Δ					
	%Rub[a]	S. S.	T. R.	τ_Δ /μs	f^T_Δ	$P^T_{O_2}$ (H_2O/air)	F_Δ (H_2O/air)
$[Ru(bpy)2(ddz)]^{2+}$	no DNA	0.21 ± 0.01	0.19 ± 0.01	65 ± 8	0.39 ± 0.04	0.088	0.034 ± 0.003
	>99	0.26 ± 0.03	0.30 ± 0.01	58 ± 7	0.51 ± 0.05	0.19	0.097 ± 0.010
$[Ru(phen)_2(ddz)]^{2+}$	no DNA	0.24 ± 0.01	0.23 ± 0.02	63 ± 7	0.41 ± 0.04	0.10	0.041 ± 0.004
	>99	0.25 ± 0.03	0.29 ± 0.05	49 ± 4	0.56 ± 0.10	0.15	0.084 ± 0.015
$[Ru(phen)_3]^{2+}$	no DNA	0.38[b]	0.39 ± 0.03	62 ± 4	0.46 ± 0.04	0.50	0.23 ± 0.02
	95	*c*	0.26 ± 0.03	64 ± 8	0.52 ± 0.06	0.17	0.088 ± 0.010
$[Ru(dsdp)_3]^{4-}$	no DNA	0.52[b]	0.52	63 ± 4	0.55	0.73	0.40
	0	*c*	0.52 ± 0.05	54 ± 3	0.55 ± 0.06	0.72	0.40

[a] Percentage of bound complex calculated from the McGhee−von Hippel interaction model (see Experimental Section). [b] Reference 31. [c] Not determined.

Using eq 8 and the experimental values for τ and k_q in D_2O (data not shown) and H_2O, $P^T_{O_2}$ has been calculated for both O_2-saturated/D_2O and air-equilibrated/H_2O solutions (Table 3). Knowing $P^T_{O_2}$, values of f^T_Δ have been obtained from experimental data in D_2O using eq 9. Then, this same equation was employed for evaluating Φ_Δ values in air-equilibrated H_2O solutions (Table 3).

In the presence of DNA, $P^T_{O_2}$ increases for the *ddz* complexes and decreases substantially for $[Ru(phen)_3]^{2+}$. However, f^T_Δ increases to the same value (approximately 0.52) for the three cationic complexes upon addition of the polynucleotide (Table 3). As a consequence of the combined effects, binding to DNA provokes a 2- to 3-fold increase in Φ_Δ for the heteroleptic ddz complexes and a 3-fold decrease for the homoleptic phen complex. In any case, Φ_Δ for the cationic sensitizers is significantly lower than the value obtained for the anionic dsdp complex. It should be noted that differences between Φ_Δ values in the absence and in the presence of DNA are much larger for air-equilibrated H_2O solutions than those obtained for O_2-saturated D_2O solutions (Table 3).

Within experimental error, the singlet oxygen lifetime (τ_Δ) in D_2O in the absence of polynucleotide is in agreement with literature values (approximately 65 μs).[11,48] However, in the presence of DNA, a small decrease in τ_Δ was observed (Table 3). From these data, the quenching rate constant of $^1O_2$ by DNA was evaluated to be about 1×10^6 $M(bp)^{-1}\,s^{-1}$, a value that matches those reported previously using rose bengal[16] or aluminum trisulfonatophthalocyanine[49] as sensitizer dyes.

IV. Discussion

Molecular oxygen is required to photocleave double-stranded DNA in the presence of Ru(II) polypyridyl complexes that do not undergo direct photochemistry with the polynucleotide. Emission lifetime measurements and control experiments with argon-saturated solutions show indeed no evidence that $[Ru(bpy)_2(ddz)]^{2+}$, $[Ru(phen)_2(ddz)]^{2+}$, $[Ru(phen)_3]^{2+}$, or $[Ru(dsdp)_3]^{4-}$ photoreact directly with DNA regardless their affinity for the nucleic acid. Moreover, the excited-state reduction potentials of the investigated complexes (0.79−0.99 V vs ENH)[33] preclude a thermodynamically allowed photoinduced electron transfer with the DNA guanines (1.10−1.24 V vs ENH).[5] Only those complexes that are capable of oxidizing the nucleobases (at least guanine) upon photoexcitation, have been shown to form *directly* photoadducts, alkali-labile sites, or frank strand breaks.[25] The intermediacy of singlet molecular oxygen in the photosensitized DNA cleavage with Ru(II) complexes has been put forward in many previous studies,[3] and our results are in agreement with such a mechanism. This study demonstrates, for the oxygen-mediated DNA cleavage, that the kinetic rate constant for conversion of DNA form I to form II (k_1) is higher for the Ru(II) sensitizers that display affinity for the polynucleotide regardless their quantum yield for singlet oxygen production

(Φ_Δ) in the absence of the biopolymer. The value of Φ_Δ of the dsdp-containing sensitizer (which does not bind to DNA) is 5 times that of the bpy/phen/ddz analogues, yet its k_1 is 1 order of magnitude smaller (Table 1). This fact indicates that a *close* Ru(II) sensitizer-DNA interaction is essential in order to produce a significant amount of $^1O_2$-mediated DNA photocleavage. The quantum yield of single-strand break formation (Φ_{ssb}) can be estimated from the k_1 values according to eq 10,

$$\Phi_{ssb} = k_1[\mathrm{Ru}][\mathrm{DNA}](\mathrm{bp}) \times V/P_a \qquad (10)$$

where [DNA] must be expressed in terms of plasmid DNA molecules, P_a (mol photons s^{-1}) is the absorption rate of the blue photons by the sensitizer (evaluated from the laser power at the illuminated solution and the solution absorbance) and V (L) is the sample volume.

The Φ_{ssb} values for the $[Ru(bpy)_2(ddz)]^{2+}$, $[Ru(phen)_2(ddz)]^{2+}$, $[Ru(phen)_3]^{2+}$, and $[Ru(dsdp)_3]^{4-}$ complexes (7×10^{-6}, 5×10^{-6}, 3×10^{-6}, and 3×10^{-7}, respectively) are in agreement with the few data reported in the literature (6.6×10^{-6} for the $[Ru(bpy)_3]^{2+}$ sensitizer, as evaluated by agarose gel electrophoresis,[50] which has shown similar efficiency than its $[Ru(phen)_3]^{2+}$ analogue.[28] The low Φ_{ssb} values measured for the investigated complexes are in agreement with the slow reaction rate of $^1O_2$ with DNA ($k_q = (1.4 \pm 0.6) \times 10^6$ $M(pb)^{-1}$ s^{-1}), due to the electrophilic character of $^1O_2$ and to the electron-deficient features and low nucleophilicity of the nucleic acid components (nitrogen bases, sugar, and phosphate units). Actually, the primary main reaction pathway of $^1O_2$ with DNA is guanine oxidation to generate 8-oxoGua (see Introduction).[3−5,18] From the similarity of the Φ_{ssb} values obtained for the three cationic Ru(II) sensitizers, it can be inferred that interaction with the polynucleotide is a major requirement for efficient $^1O_2$-mediated DNA photocleavage (see below).

As a matter of fact, DNA-bound $[Ru(phen)_3]^{2+}$ has been shown to lay into the major groove of the double-stranded polynucleotide but does not intercalate,[51] while Ru(II) dipyridophenazine complexes, such as those containing the ddz ligand, insert this ligand deeply into the nucleotide stacking.[52] If we compare the Φ_Δ values for the two $[Ru(phen)_2(L)]^{2+}$ (L = phen or ddz) complexes that display similar photophysical (τ) and photochemical (k_q) features when bound to DNA (Table 2), we found that *intercalation of the sensitizer* into the DNA base pair ladder *has little effect on* Φ_Δ, provided the *extent of binding* to the polynucleotide is the same. This finding should be attributed to a similar degree of exposure to molecular oxygen of both metal complexes when bound to ds-DNA and would support the recent proposal of "pseudo-intercalation" of the $[Ru(phen)_3]^{2+}$ complex (i.e. the phen ligand does not enter a fully opened intercalation pocket).[53]

The quenching rate constant of the photoexcited Ru(II) complex by dioxygen (k_q) is lower when the sensitizer is protected from the O_2 approach, regardless of the shielding mode. Protection may be due to self-aggregation of the Ru(II) complex (ddz complexes in water compared to homoleptic phen or dsdp complexes, Table 2) or imparted by the polynucleotide (free vs DNA-bound sensitizers, Table 2). Particularly remarkable is the high degree of shielding against collision with O_2 that intercalation into DNA provides to $[Ru(phen)_2(ddz)]^{2+}$, one of its k_q value being approximately 20 times lower than the typical one for Ru(II) complexes with phen ligands (4×10^9 dm^3 mol^{-1} s^{-1}).[45]

To analyze the effect of DNA on Φ_Δ of the Ru(II) sensitizers, two opposite effects on $P^T_{O_2}$ (eq 8) must be considered: (a) the increase of the *emission lifetime* of the metal complex (τ) upon binding to the nucleic acid due to a diminution in the ^{3}MLCT excited-state nonradiative deactivation, and (b) the decrease of the *quenching rate by* O_2 (k_q) due to protection of the sensitizer interacting closely with the polynucleotide.[46] Additionally, the effect of DNA on f^T_Δ (eq 9) must be considered. However, our results (Table 3) demonstrate that the latter has only a small contribution. Although a higher f^T_Δ should be expected for Ru(II) sensitizers in going from aqueous to less polar media such as the environment around the intercalated species, as observed for D_2O vs CD_3OD (f^T_Δ of approximately 0.5 and 1.0, respectively, see ref 31), the actual increase of f^T_Δ for the DNA-bound complexes is about 25%. Therefore, it has to be concluded that intercalation does not shield entirely the bulky Ru(II) sensitizers from water, even for the extended ddz complexes. The combined effects mentioned above induce the 2- to 3-fold rise in Φ_Δ of the ddz complexes as a consequence of intercalation into DNA, as well as the 3-fold decrease observed for $[Ru(phen)_3]^{2+}$. While the strong increase of their emission lifetime predominates in the former sensitizers, significant protection from O_2 quenching is the major effect for the latter.

The differential behavior of the intercalating complexes is also reflected in their photocleaving properties compared to the rest of Ru(II) sensitizers investigated in this work (Table 1). In fact, the ratio of rate constants for single-strand and double-strand breaks (k_1/k_2), the latter occurring due to proximate single-strand breaks in opposite chains of the polynucleotide,[54] is 3−5 times smaller for the ddz intercalators compared to either $[Ru(phen)_3]^{2+}$ or $[Ru(dsdp)_3]^{4-}$. This fact is probably due to the relatively long residence time on the DNA of the former complexes. The higher mobility of the nonintercalated sensitizers, either bound to DNA or free in solution, diminishes the probability of double-strand breaks resulting from the accumulation of single-strand cleavages promoted by $^1O_2$.

In conclusion, our studies demonstrate the importance of both a proper structural control of the Ru(II) sensitizer and the metal complex to DNA binding in order to achieve an effective photocleavage of the polynucleotide mediated by $^1O_2$. In contrast, the ability of the photosensitizer to produce such an active oxygen species in solution plays only a secondary role. Further work is currently in progress to investigate $^1O_2$ production of Ru(II) polypyridyls bound to microheterogeneous systems (micelles and microemulsions) that mimic biological media.

Acknowledgment. This work has been funded by the Spanish Ministry of Education and Culture (projects no. PPQ2000-0778-C02 and BIO99-0411) and the Madrid Autonomous Community (CAM, 07M/0082/2000). The authors thank the Spanish-German "Acciones Integradas" Program (HA98-128) for researchers' exchange grants. M. E. J.-H. and A.H.-B. thank Complutense University and CAM for a doctoral and training grant, respectively. Dr. M. C. Gutiérrez-Alonso (Madrid) and Dipl. Eng. C. G. Martínez (Karlsruhe) are gratefully acknowledged for preparing the ddz complexes and technical assistance with the $^1O_2$ spectrometer, respectively.

References and Notes

(1) Nielsen, P. E. *J. Mol. Recognit.* **1990**, *3*, 1−25.
(2) Sigman, D. S.; Mazumder A.; Perrin D. M. *Chem. Rev.* **1993**, *93*, 2295−2316.
(3) Armitage, B. *Chem. Rev.* **1998**, *98*, 1171−1200.
(4) (a) Cadet, J.; Vigny, P. In *Bioorganic Photochemistry*; Morrison, H., Ed.; Wiley: New York, 1990; Vol. 1, pp 1−272. (b) Kochevar, I. E.; Dunn, D. A. In *Bioorganic Photochemistry*; Morrison, H., Ed.; Wiley: New York, 1990; Vol. 1, pp 273−315.
(5) Burrows, C. J.; Muller, J. G. *Chem. Rev.* **1998**, *98*, 1109−1151.

(6) Wilkinson, F.; Helman, W. P.; Ross, A. B. *J. Phys. Chem. Ref. Data* **1993**, *22*, 113−131.
(7) Braun, A. M.; Maurette, M. T.; Oliveros, E. *Photochemical Technology*; Wiley: Chichester, U.K. 1991; pp 445−499.
(8) Cadenas, E. *Annu. Rev. Biochem.* **1989**, *58*, 79−110.
(9) Dougherty, T. J.; Gomer, C. J.; Henderson, B. W.; Jori, G.; Kessel, D.; Korbelik, M.; Moan, J.; Peng, Q. *J. Nat. Cancer Inst.* **1998**, *90*, 889−905.
(10) Ali, H.; van Lier, J. E. *Chem. Rev.* **1999**, *9*, 2379−2450.
(11) Wilkinson, F.; Hellman, H. P.; Ross, A. B. *J. Phys. Chem. Ref. Data* **1995**, *24*, 663−1021.
(12) Aminian-Saghafi, T.; Nasini, G.; Caronna, T.; Braun, A. M.; Oliveros, E. *Helv. Chim. Acta* **1992**, *75*, 531−538.
(13) Oliveros, E.; Besancon, F.; Boneva, M.; Kräutler, B.; Braun, A. M. *J. Photochem. Photobiol. B: Biol.* **1995**, *29*, 37−44.
(14) Tanielian, C.; Wolff, C. *J. Phys. Chem.* **1995**, *99*, 9825−9830.
(15) Scurlock, R. D.; Nonell, S.; Braslavsky, S. E.; Ogilby, P. R. *J. Phys. Chem.* **1995**, *99*, 3521−3526.
(16) Lee, P. C. C.; Rodgers, M. A. J. *Photochem. Photobiol.* **1987**, *45*, 79−86.
(17) Devasagayam, T. P. A.; Steenken, S.; Obendorf, M. S. W.; Schulz, W. A.; Sies, H. *Biochemistry* **1991**, *30*, 6283−6289.
(18) (a) Raoul, S.; Cadet, J. *J. Am. Chem. Soc*. **1996**, *118*, 1892−1898. (b) Duarte, V.; Gasparutto, D.; Yamaguchi, L. F.; Ravanat, J. L.; Martínez, G. R.; Medeiros, M. H. G.; Di Mascio, P.; Cadet, J. *J. Am. Chem. Soc*. **2000**, *122*, 12622−12628. (c) Ravanat, J. L.; Di Mascio, P.; Martínez, G. R.; Medeiros, M. H. G.; Cadet, J. *J. Biol. Chem.* **2000**, *275*, 40601−40604.
(19) Sies, H.; Menck, C. F. M. *Mutat. Res.* **1992**, *275*, 367−375.
(20) Erkkila, K. E.; Odom, D. T.; Barton, J. K. *Chem. Rev.* **1999**, *99*, 2777−2795.
(21) Moucheron, C.; Kirsch-De Mesmaeker, A.; Kelly, J. M. *Struct. Bonding* **1998**, *92*, 163-216.
(22) Nordén, B.; Lincoln, P.; Akerman, B.; Tuite, E. In *Metal Ions in Biological Systems*; Sigel, A., Sigel, H., Eds.; Dekker: New York, 1996; pp 177−252.
(23) Lecomte, J.-P.; Kirsch-De Mesmaeker, A.; Orellana, G. *J. Phys. Chem.* **1994**, *98*, 5382−5388.
(24) Chow, C. S.; Barton, J. K. *Methods Enzymol.* **1992**, *212*, 219−242.
(25) Moucheron, C.; Kirsch-De Mesmaeker, A.; Kelly, J. M. *J. Photochem. Photobiol., B: Biol.* **1997**, *40*, 91−106.
(26) Sentagne, C.; Chambron, J. C.; Sauvage, J. P.; Paillous, N. *J. Photochem. Photobiol., B: Biol.* **1994**, *26*, 165−174.
(27) Kelly, J. M.; Feeney, M. M.; Tossi, A. B.; Lecomte, J.-P.; Kirsch-De Mesmaeker, A. *Anti-Cancer Drug Des.* **1990**, *5*, 69−75.
(28) Tossi, A., B.; Kelly, J. M. *Photochem. Photobiol.* **1989**, *49*, 545.-556.
(29) Mei, H. Y.; Barton, J. K. *Proc. Nat. Acad. Sci. U.S.A.* **1988**, *85*, 1339−1343.
(30) Abdel-Shafi, A. A.; Beer, P. D.; Mortimer, R. J.; Wilkinson, F. *J. Phys. Chem. A* **2000**, *104*, 192−202.
(31) García-Fresnadillo, D.; Georgiadou, Y.; Orellana, G.; Braun, A. M.; Oliveros, E. *Helv. Chim. Acta* **1996**, *79*, 1222−1238.
(32) Mulazzani, Q. G.; Sun, H.; Hoffman, M. Z.; Ford, W. E.; Rodgers, M. A. J. *J. Phys. Chem.* **1994**, *98*, 1145−1150.
(33) Gutiérrez-Alonso, M. C. Ph.D. Thesis, Universidad Complutense de Madrid, Spain, 1996.
(34) Satyanarayana, S.; Dabrowiak, J. C.; Chaires, J. B. *Biochemistry* **1993**, *32*, 2573−2584.
(35) McGhee, J. D.; von Hippel, P. H. *J. Mol. Biol.* **1974**, *86*, 469−489.
(36) Jiménez-Hernández, M. E.; Ph.D. Thesis, Universidad Complutense de Madrid, Spain, 2001.
(37) Bauer, W.; Vinogard, J. *J. Mol. Biol*. **1968**, *33*, 141−171.
(38) Ciulla, T. A.; Van Camp, J. R.; Rosenfeld, E.; Kochevar, I. E. *Photochem. Photobiol.* **1989**, *49*, 293−298.
(39) Kishikawa, H.; Jiang, Y. P.; Goodisman, J.; Dabrowiak, J. C. *J. Am. Chem. Soc.* **1991**, *113*, 5434−5440.
(40) Carraway, E. R.; Demas, J. N.; DeGraff, B. A. *Anal. Chem*. **1991**, *63*, 332−336.
(41) Hitchman, M. L. *Measurement of Dissolved Oxygen*; Wiley-Interscience: New York, 1978.
(42) Demas, J. N. *Excited-State Liefetime Measurements*; Academic Press: New York, 1983.
(43) Gandin, E.; Lion, Y.; van der Vorst, A. *Photochem. Photobiol.* **1983**, *31*, 271−278.
(44) Murasecco-Suardi, P.; Gassmann, E.; Braun, A. M.; Oliveros, E. *Helv. Chim. Acta* **1987**, *70*, 1760−1773.
(45) Hoffman, M. Z.; Bolletta, F.; Moggi, L.; Hug, G. L. *J. Phys. Chem. Ref. Data* **1989**, *18*, 219−543.
(46) Orellana, G.; Kirsch-De Mesmaeker, A.; Barton, J. K.; Turro, N. J. *Photochem. Photobiol.* **1991**, *54*, 499−509.
(47) Jiménez-Hernández, M. E.; Orellana, G.; Montero, F. Portolés, M. T. *Photochem. Photobiol.* **2000**, *72*, 28−34.
(48) Martínez, L. A.; Martínez, C. G.; Klopotek, B. B.; Lang, J.; Neuner, A.; Braun, A. M.; Oliveros, E. *J. Photochem. Photobiol., B: Biol.* **2000**, *58*, 94−107.
(49) Dávila, J.; Harriman, A. *Photochem. Photobiol.* **1989**, *50*, 29−35.
(50) Aboul-Enein, A.; Shulte-Frohlinde, D. *Photochem. Photobiol.* **1988**, *48*, 27−34.
(51) Coury, J. E.; Anderson, J. R.; McFail-Isom, L.; Williams, L. D.; Bottomley, L. A. *J. Am. Chem. Soc.* **1997**, *119*, 3792−3796.
(52) Hiort, C.; Lincon, P.; Nordén, B. *J. Am. Chem. Soc.* **1993**, *115*, 3448−3454.
(53) Lincoln, P.; Nordén, B. *J. Phys. Chem. B* **1998**, *102*, 9583−9594.
(54) Freifelder, D.; Trumbo, B. *Biopolymers* **1969**, *7*, 681−693.