Photoelectron Transfer Processes with Ruthenium(II) Polypyridyl Complexes and Cu/Zn Superoxide Dismutase

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ABSTRACT: The processes that are photoinduced by $[Ru(bpz)_3]^{2+}$ (bpz = 2,2′-bipyrazyl) in the presence of Cu/Zn superoxide dismutase (Cu/Zn SOD) are investigated by laser flash photolysis and electron paramagnetic resonance (EPR) spectroscopy; they are compared to those of the system $[Ru(bpy)_3^{2+}$−Cu/Zn SOD]. Although the mechanism is complicated, primary and secondary reactions can be evidenced. First, the excited $[Ru(bpz)_3]^{2+}$ complex is quenched reductively by Cu/Zn SOD with the production of a reduced complex and an oxidized enzyme. The oxidation site of Cu/Zn SOD is proposed to correspond to amino acids located on the surface of the protein. Afterward and only when this reductive electron transfer to the excited complex has produced enough oxidized protein, another electron-transfer process can be evidenced. In this case, however, the charge-transfer process takes place in the other direction, i.e., from the excited complex to the Cu^{II} center of the SOD with the formation of Ru^{III} and Cu^{I} species. This proposed mechanism is supported by the fact that $[Ru(bpy)_3]^{2+}$, which is less photo-oxidizing than $[Ru(bpz)_3]^{2+}$, exhibits no photoreaction with Cu/Zn SOD. Because Ru^{III} species are generated as intermediates with $[Ru(bpz)_3]^{2+}$, they are proposed to be responsible for the enhancement of $[poly(dG-dC)]_2$ and $[poly(dA-dT)]_2$ oxidation observed when Cu/Zn SOD is added to the $[Ru(bpz)_3]^{2+}$−DNA system.

DNA regulates most aspects of cell life and constitutes an important drug target. The design of compounds able to bind and react with selective nucleotidic sequences is of great importance in probing biological processes and in developing therapeutic drugs. In this field, ruthenium complexes are very interesting compounds. According to their chirality and photophysical and photoredox properties, they can be used as DNA chiral (*1*−*3*) or luminescent probes (*4*−*6*), chemical photonucleases (*7*−*9*), and DNA photoreagents (*10*−*12*). Moreover, they are useful tools in the examination of charge transport through DNA (*13*, *14*). Ruthenium complexes can photosensitize DNA via mechanisms such as singlet oxygen production or photoelectron-transfer processes. Their photoreactivity toward the DNA double helix stems partly from the redox properties of their triplet metal-to-ligand charge transfer (^{3}MLCT) excited state, giving rise to photoredox processes with nucleobases (*12*, *15*, *16*).

Photoelectron transfer occurs (*16*−*18*) when the reduction potential of the complex in the excited state is more positive than the oxidation potential of the guanine [+1.35 V versus saturated calomel electrode (SCE)] (*19*), the most easily oxidizable nucleobase in DNA. However, in polynucleotides, this potential depends upon the stacking with the neighboring bases (*20*, *21*) and the possible coupling of the charge transfer with a proton transfer (*22*).

The photoelectron-transfer process generates (i) single-strand breaks of plasmid DNA (*12*, *16*, *23*), (ii) cation guanine radicals (*24*) that produce a family of oxidation products (*25*), and (iii) DNA photoadducts with specific Ru^{II} complexes (*10*−*12*, *26*). The photoreactivity of ruthenium complexes with DNA can be modulated by both their redox potential in the excited state and their interaction mode with the DNA double helix. However, their photochemical behavior with nucleic acids may also be affected by the presence of proteins. Stemp and Barton (*27*) showed that ferricytochrome *c*, a basic protein with a ring of lysine units surrounding the heme (*28*−*29*), may be associated electrostatically in vitro with DNA and may oxidatively quench the excited $[Ru(phen)_2dppz]^{2+}$. This reaction leads to the reduction of Fe^{3+}-cyt *c* to Fe^{2+}-cyt *c* and the formation of the oxidized Ru^{III} complex. In the presence of $\{poly(dG-dC)\}_2$, the Ru^{III} species of $[Ru(phen)_2dppz]^{2+}$, which is a powerful ground-state oxidant (1.36 V versus SCE) (*30*), produces guanine radicals.

In a previous work, it was shown that the photoreactivity of $[Ru(bpz)_3]^{2+}$ (bpz = 2,2′-bipyrazyl) with DNA is enhanced in the presence of Cu/Zn superoxide dismutase (Cu/Zn SOD) (*31*). This enzyme induces a drastic increase in DNA photodamage by electron-transfer such as single-strand breaks of plasmid DNA (*32*), oxidation of guanine in 5′ of a GG site, and DNA photoaddition (*31*). The Cu/Zn SOD from bovine erythrocyte contains one copper(II) cation and

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one zinc(II) cation (*33*) in each of its two identical subunits (*34*−*36*). This intracellular antioxidant enzyme catalyzes the disproportionation of superoxide anion into oxygen and hydrogen peroxide (*37*, *38*). The mechanism for this enzyme most likely involves the reduction of Cu^{II} by one superoxide followed by the oxidation of Cu^{I} back to Cu^{II} by a second superoxide (*39*−*41*). The participation of superoxide anions and hydroxyl radicals in the effect of Cu/Zn SOD on the photoprocesses of $[Ru(bpz)_3]^{2+}$ has been ruled out (*32*).

In this work, to obtain further mechanistic insight into the unexpected photoreactivity of $[Ru(bpz)_3]^{2+}$ in the presence of Cu/Zn SOD, we have studied the behavior of $[Ru(bpz)_3]^{2+}$ under irradiation with the metalloenzyme and in the presence and absence of guanine units of mono- or polynucleotides, using laser-induced transient absorption spectroscopy and electron paramagnetic resonance (EPR).

EXPERIMENTAL PROCEDURES

Materials. Tris(2,2′-bipyridyl)ruthenium(II), bis(2-pyrazinecarboxylato)copper(II), ruthenium(III) chloride hydrate, and potassium tetrafluoroborate were purchased from Aldrich (Saint Quentin en Yvelines, France) and used without any further purification. $[Ru(bpz)_3]^{2+}$ was prepared as described previously (*12*). TRIS buffer, phosphate buffer, Cu/Zn SOD, and *N-tert*-butyl-α-phenylnitrone (PBN) were from SIGMA (Saint Quentin Fallavier, France). $[Poly(dG\text{-}dC)]_2$ and $[poly(dA\text{-}dT)]_2$ were purchased from Amersham Pharmacia Biotech (Saclay, France) and were dialyzed versus a 10 mM TRIS/HCl (pH 7.2) solution.

Luminescence Spectroscopy. Luminescence measurements were carried out with a Photon Technology International fluorescence spectrophotometer. Samples were excited at 452 nm. Emission was observed between 500 and 800 nm; emission intensities were measured at 618 nm and corrected for the instrument response. Experiments with Cu/Zn SOD were carried out in 10 mM TRIS/HCl and 10 mM NaCl (pH 7.2). In the titration experiments, small aliquots of a concentrated protein solution were added to the sample containing either $[Ru(bpz)_3]^{2+}$ or $[Ru(bpy)_3]^{2+}$.

Laser Flash Photolysis. Laser flash photolysis experiments were performed in a crossbeam configuration using an Nd:YAG pulsed laser (Continuum NY 61-10) and a 300 W xenon lamp (ORC XM 300-5) as a monitoring source. Samples were pumped at 355 nm and probed at different wavelengths (370−650 nm). Absorption signals as a function of time were detected with an R-928 Hamamatsu photomultiplier tube whose ouput was applied to a digital oscilloscope (Hewlett−Packard HP 54200A) interfaced to a Hewlett−Packard HP 9816 S computer. Signals were averaged over 16 shots and corrected for baseline variations. Kinetic analyses of the decays were achieved by using exponential curve fittings. The transient absorption spectra start changing by repeating several times the measurements with the same solution in the beginning of the experiments. Therefore, when we write "after preirradiation" in laser flash photolysis experiments described in the Results and Discussion, we mean after we observed that the laser-induced transient absorption spectra have become reproducible. Because it is not possible to use the same irradiation conditions for the pulsed laser experiments and the EPR measurements, this is the only criteria that we could choose for the preirradiation conditions in the laser flash photolysis experiments.

UV Spectroscopy Analysis of a Photolyzed $[[Ru(bpz)_3]^{2+}$−Cu/Zn SOD] Solution. Photolysis experiments used a 250 W Oriel Hg lamp (Palaiseau, France) equipped with a cooling water filter and an interference filter Oriel 436 nm FS 10-50 (53% transmission, bandwidth of 11 nm). A solution containing 0.26 mM $[Ru(bpz)_3]^{2+}$ and 0.26 mM Cu/Zn SOD in an aqueous buffer of 10 mM TRIS/HCl and 10 mM NaCl (pH 7.2) was saturated with argon and irradiated at $\lambda = 436$ nm and 25 °C. Ultraviolet−visible absorption spectra were recorded on a Hewlett−Packard 8452 diode array spectrophotometer at various times of irradiation

EPR Spectroscopy: EPR Setup. EPR spectra were collected on a Bruker ESP 300E spectrometer equipped with a frequency meter and a Bruker PT 1000 cryostat system (spectral collection parameters are given in the captions of the figures). The light source was a 250 W Oriel Hg lamp (Palaiseau, France). The light was passed through a cooling water filter and an Oriel WG 335 UV filter (Palaiseau, France) at 3 mm ($\lambda < 340$ nm) and delivered via an optical fiber to the grid of the cavity. In these experimental conditions, samples were irradiated with a polychromatic light absorbed by the MLCT band of $[Ru(bpz)_3]^{2+}$ and which leads to a faster photoreactivity of $[Ru(bpz)_3]^{2+}$ with the Cu/Zn SOD.

EPR Detection of $[Ru(bpz)_3]^{1+}$ in the Presence of Cu/Zn SOD. Samples contained 10^{-3} M $[Ru(bpz)_3]^{2+}$ (or 10^{-3} M $[Ru(bpy)_3]^{2+}$) and 10^{-4} M Cu/Zn SOD in an aqueous buffer of 10 mM TRIS/HCl and 10 mM NaCl (pH 7.2). The solutions were saturated with argon, transferred into argon-flushed EPR tubes, and frozen at 110 K. All measurements were made under irradiation of frozen samples at 110 K and $\lambda > 340$ nm.

EPR Detection of Cu^{II} in Cu/Zn SOD after Preirradiation of a $[[Ru(bpz)_3]^{2+}$−Cu/Zn SOD] Solution. Irradiation conditions used in preirradiation experiments were identical to those described for the UV spectroscopy analysis of a photolyzed $[[Ru(bpz)_3]^{2+}$−Cu/Zn SOD] solution. Samples containing 10^{-4} M Cu/Zn SOD in an aqueous buffer of 10 mM TRIS/HCl and 10 mM NaCl (pH 7.2) were saturated with argon and irradiated for 10 min at $\lambda = 436$ nm and 25 °C, in the presence and absence of 10^{-3} M $[Ru(bpz)_3]^{2+}$. After irradiation, the samples were immediately transferred into argon-flushed EPR tubes, frozen at 110 K, and irradiated at $\lambda > 340$ nm. EPR spectra were recorded on the frozen solutions at 110 K upon irradiation.

Spin Trapping of Guanine and Adenine Radicals. Samples contained 10^{-4} M $[Ru(bpz)_3]^{2+}$ or 10^{-4} M $[Ru(bpy)_3]^{2+}$, 10^{-3} M base pairs of $[poly(dG\text{-}dC)]_2$ or $[poly(dA\text{-}dT)]_2$, 10^{-4} M Cu/Zn SOD, and 3 10^{-2} M PBN in an aqueous buffer of 10 mM TRIS/HCl and 10 mM NaCl (pH 7.2). The solutions were saturated with argon and transferred into argon-flushed quartz flat cells. EPR spectra were recorded in the dark at room temperature after 30 s or 1 min of irradiation of the samples at $\lambda > 340$ nm.

RESULTS AND DISCUSSION

Luminescence Quenchings of the bpz and bpy Complexes with Cu/Zn SOD. To investigate the effect of Cu/Zn SOD on the photochemistry of $[Ru(bpz)_3]^{2+}$, we examined the possibility of luminescence quenching of the complex by Cu/Zn SOD in comparison with $[Ru(bpy)_3]^{2+}$ in the same

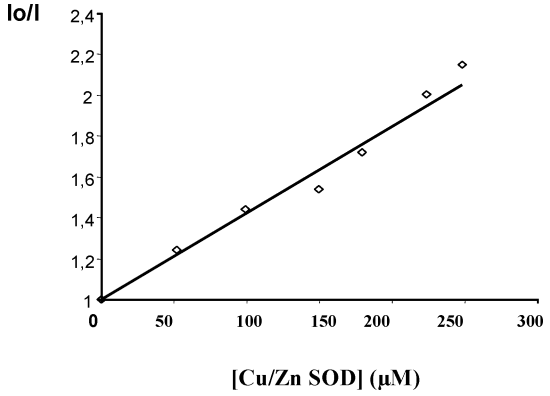

FIGURE 1: Stern−Volmer plot (I_0/I versus [Cu/Zn SOD]) for the quenching of luminescence of $[Ru(bpz)_3]^{2+}$ by Cu/Zn SOD. I_0 = intensity emission of $[Ru(bpz)_3]^{2+}$ in the absence of Cu/Zn SOD, and I = intensity of emission in the presence of Cu/Zn SOD (λ_{exc} = 452 nm; λ_{em} = 618 nm).

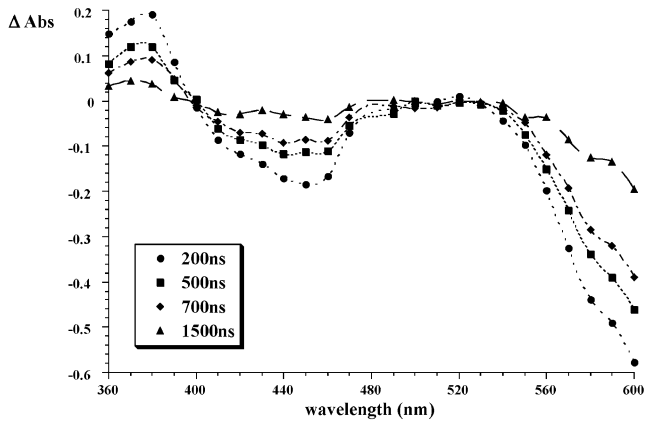

FIGURE 2: Difference transient absorption spectra for $[Ru(bpz)_3]^{2+}$ (3×10^{-5} M) in the absence of Cu/Zn SOD recorded 200, 500, 700, and 1500 ns after the laser pulse. All measurements were performed in Ar-saturated aqueous solutions, with pulsed irradiation at 355 nm.

experimental conditions. The Stern−Volmer plot of the I_0/I data (Figure 1) for $[Ru(bpz)_3]^{2+}$ with Cu/Zn SOD clearly shows a quenching of the excited metal complex by an increased concentration of the enzyme.

The corresponding quenching rate constant ($k_q = 4.7 \times 10^9$ M^{-1} s^{-1}) indicates efficient luminescence quenching, facilitated by electrostatic interaction between the positively charged Ru^{II} complex and the negatively charged Cu/Zn SOD at physiological pH (*42*, *43*). In contrast, the luminescence of $[Ru(bpy)_3]^{2+}$ is not quenched by the protein.

Because the reduction potential of the Cu^{2+} form of Cu/Zn SOD into the Cu^{1+} form is +160 mV versus SCE (*44*), electron transfer is thermodynamically possible from the excited bpz complex (estimated oxidation potential of −0.26 V versus SCE) (*45*) to the Cu^{II} enzyme. This process should be more favorable with the excited $[Ru(bpy)_3]^{2+}$ (E_{ox}* = −0.80 V versus SCE) (*45*). However, strangely enough, there is no quenching of the luminescence of $[Ru(bpy)_3]^{2+}$ by Cu/Zn SOD. This strongly suggests that the Cu^{2+} in the active site of the protein is protected from the excited $[Ru(bpy)_3]^{2+}$. Indeed, because the Cu^{2+}-binding site in each monomer of the protein is located at the bottom of a narrow, positively charged channel of approximately 12 Å (*43*, *46*), its accessibility to the Ru^{II} compound becomes rather problematic, which might reasonably explain the absence of oxidative luminescence quenching of excited $[Ru(bpy)_3]^{2+}$ by the enzyme. If this is true, the quenching of $[Ru(bpz)_3]^{2+}$* by Cu/Zn SOD should also be prevented. However, this is not the case. Therefore, the quenching must be attributed to another electron-transfer process. Actually, a reductive quenching of the excited state of the bpz complex by the polypeptide chain of the enzyme can also be considered. Indeed, it has been shown that the excited ^{3}MLCT state of oxidizing complexes such as $[Ru(TAP)_3]^{2+}$ and $[Ru(TAP)_2(phen)]^{2+}$ (TAP = 1,4,5,8-tetraazaphenanthrene) can be reduced by a few amino acids. Furthermore, it has been demonstrated that the photoinduced electron-transfer process toward this type of excited complex is accompanied by the formation of an adduct between the reduced Ru^{II} species and the oxidized amino acid (*47*). Because $[Ru(TAP)_3]^{2+}$ and $[Ru(bpz)_3]^{2+}$ exhibit almost the same oxidizing power in the excited state and because the enzyme contains at least one of the most reducing amino acid residues (tryptophan/tyrosine/histidine) on the protein surface (*48*), one might reasonably think that the quenching of luminescence of the bpz complex originates from an electron transfer from an amino acid residue of the enzyme surface toward the excited $[Ru(bpz)_3]^{2+}$.

Transients with $[Ru(bpz)_3]^{2+}$ and $[Ru(bpy)_3]^{2+}$ Formed in the Presence of Cu/Zn SOD by Laser Flash Photolysis. The existence of a photoinduced electron transfer from Cu/Zn SOD to the excited $[Ru(bpz)_3]^{2+}$ can be demonstrated by laser flash photolysis experiments. In the absence of Cu/Zn SOD, the difference transient absorption spectrum of $[Ru(bpz)_3]^{2+}$ in water (Figure 2) shows a depletion in the 410−470 nm region and a simultaneous growth of the absorption below 400 nm.

This corresponds to the disappearance of the ground-state complex because of the formation of the ^{3}MLCT excited state, which absorbs at 370−380 nm and emits at around 600 nm. Both the depletion and the absorption decay according to monomolecular processes, with the same rate constant of 1.33×10^6 s^{-1}. The corresponding lifetime is 753 ns, in agreement with the value previously reported for the emission decay in aqueous solution (760 ns) (*16*).

In contrast, in the presence of Cu/Zn SOD (6.3×10^{-4} M, pH 7, argon-purged solution), two different types of behavior are observed, depending upon the presence or absence of a preirradiation of the system "bpz complex + SOD" (the criteria of preirradiation are given in the *Laser Flash Photolysis* section in the Experimental Procedures).

$Ru(bpz)_3^{2+}$ + Cu/Zn SOD without Preirradiation of the Solution. In experiments performed without preirradiation of the solution, the difference transient absorption recorded after the laser pulse in the presence of Cu/Zn SOD shows a positive absorption at 500 nm, which decays in a few hundred microseconds (Figure 3).

This behavior is completely different from that observed in the absence of Cu/Zn SOD and demonstrates the existence of a long-lived species. Unfortunately, because of the relatively weak transient absorption, the whole transient spectrum cannot be precisely recorded and a good kinetic analysis of the data is difficult to obtain. However, the plot of the inverse of the change in transient absorbance ($1/\Delta Abs$) versus time gives a reasonably good linear relation. Therefore, the transient species might decay according to a bimolecular equimolecular process (inset in Figure 3) and

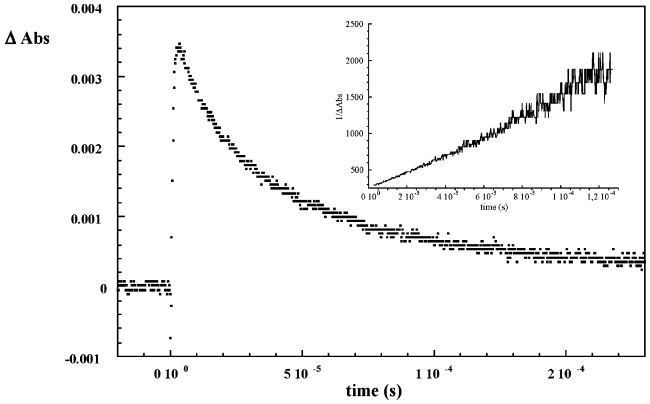

FIGURE 3: Difference transient absorption decay (500 nm) for $[Ru(bpz)_3]^{2+}$ (1×10^{-5} M) in the presence of Cu/Zn SOD (6.3×10^{-4} M) without preirradiation, under pulsed excitation (355 nm) in Ar-saturated aqueous solutions. (Inset) Kinetic analysis, plot of [1/ΔAbs versus time (μs)] from transient absorption decay at 500 nm.

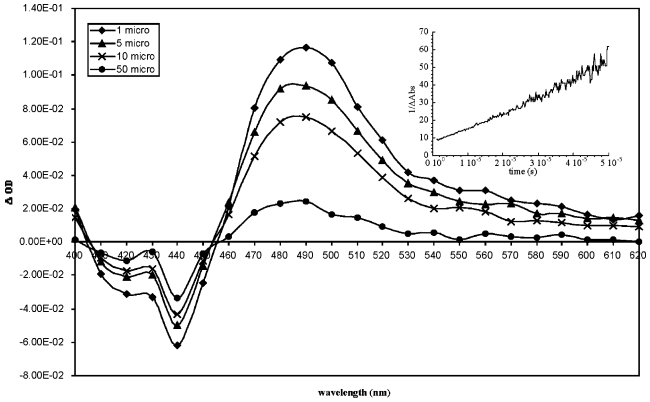

FIGURE 4: Difference transient absorption spectra for $[Ru(bpz)_3]^{2+}$ (1×10^{-5} M) in the presence of GMP (1×10^{-2} M) measured 1, 5, 10, and 50 μs after the laser pulse (355 nm) in Ar-saturated aqueous solutions. (Inset) Kinetic analysis, plot of [1/Δabs versus time (μs)] from transient absorption decay at 500 nm.

could be attributed to the presence of reduced $[Ru(bpz)_3]^{2+}$. Indeed, when another experiment is performed in the presence of guanosine 5′-monophosphate (GMP), which efficiently reduces the ^{3}MLCT state of $[Ru(bpz)_3]^{2+}$ (*16*), a transient absorption in the 460−580 nm region with a maximum of around 490 nm is also detected (Figure 4).

This absorption is similar to the transient absorption reported for other oxidizing complexes in the presence of GMP (*22*) and is characteristic of the monoreduced complex produced by process 1.

$$[Ru(bpz)_3{}^*]^{2+} + GMP \rightarrow [Ru(bpz)_3]^{1+} + GMP^{\cdot+} \quad (1)$$

In addition, studies in the literature have shown that the monoreduced $[Ru(bpz)_3]^{1+}$ complex can exhibit a long lifetime when it is not reoxidized by oxygen or by the oxidized reductant (*49*, *50*). In the present case, the transient absorption recorded at 500 nm decays in a few hundred microseconds according to a bimolecular equimolecular process (inset in Figure 4), confirming the disappearance of the monoreduced complex according to reaction 2.

$$[Ru(bpz)_3]^{1+} + GMP^{\cdot+} \rightarrow [Ru(bpz)_3]^{2+} + GMP \quad (2)$$

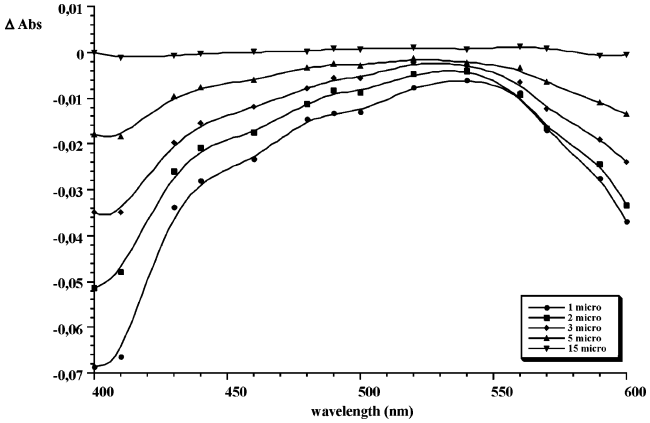

FIGURE 5: Difference transient absorption spectra after preirradiation of $[Ru(bpz)_3]^{2+}$ (1×10^{-5} M) in the presence of Cu/Zn SOD (6.3×10^{-4} M) recorded 1, 2, 3, 5, 10, and 15 μs after the laser pulse (355 nm). All measurements were performed in Ar-saturated aqueous solutions.

In conclusion, with $[Ru(bpz)_3]^{2+}$ in the presence of Cu/Zn SOD, the transients could correspond to the reduced complex and an oxidized SOD species. These results are in agreement with the occurrence of a reductive quenching of the excited $[Ru(bpz)_3]^{2+}$ by an amino acid of the protein chain of the enzyme in the Cu^{II} form (reaction 3). Moreover, the disappearance according to a bimolecular equimolecular process is in agreement with the back electron-transfer reaction 4.

$$[Ru(bpz)_3{}^*]^{2+} + Cu^{II}/Zn\ SOD \rightarrow [Ru(bpz)_3]^{1+} + Cu^{II}/Zn\ SOD^{\cdot+} \quad (3)$$

$$[Ru(bpz)_3]^{1+} + Cu^{II}/Zn\ SOD^{\cdot+} \rightarrow [Ru(bpz)_3]^{2+} + Cu^{II}/Zn\ SOD \quad (4)$$

$[Ru(bpz)_3]^{2+}$ + Cu/Zn SOD after Preirradiation of the Solution. The overall process described above for $[Ru(bpz)_3]^{2+}$ with the enzyme cannot easily explain the great enhancement of the photonuclease activity of $[Ru(bpz)_3]^{2+}$ under illumination when Cu/Zn SOD is added to the medium, as previously published (*31*, *41*).

Actually, a further investigation by laser flash photolysis experiments points to a more precise scheme for the photoinduced processes that are taking place. When the transient absorption spectra are recorded in the same conditions but with preirradiated solutions (see criteria of preirradiation in the Experimental Procedures), a dramatic change in transient behavior is observed, whereas there is no change in the absence of Cu/Zn SOD. Two depletion bands are clearly detected after the laser pulse: one around 400 nm and the other above 600 nm (Figure 5).

Both depletion processes have recovered in a time scale of the order of 15 μs. St. Clair et al., who have studied the spectroelectrochemistry of Cu/Zn SOD in its native Cu^{2+} form (*44*), have clearly shown that the Cu^{2+} enzyme absorbs around 675 nm. Therefore, the depletion in the transient spectrum in this wavelength region would correspond to the disappearance of Cu^{2+} and the formation of Cu^{1+} (which does not absorb at that wavelength) (*44*) at the active site of the protein. The negative absorption around 400 nm should be attributed not only to the depletion of the starting complex

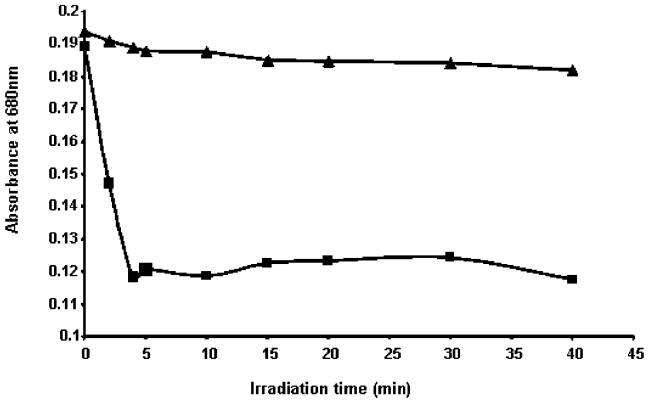

FIGURE 6: Absorbance at 680 nm of Cu/Zn SOD (0.26 mM) after different irradiation times at 436 nm in a deaerated condition, (▲) without $[Ru(bpz)_3]^{2+}$ and (■) with $[Ru(bpz)_3]^{2+}$ at 0.26 mM. Conditions of irradiation at 436 nm are described in the Experimental Procedures.

but also to the concomitant formation of oxidized species, possibly the oxidized complex $[Ru(bpz)_3]^{3+}$. Ru^{III} species are indeed known to absorb at around 390−450 nm, thus approximately at the same wavelength as the starting complex but with a smaller molar absorption coefficient ($\epsilon_{420\ nm} \approx 3600\ M^{-1}\ cm^{-1}$ for $[Ru(bpz)_3]^{3+}$) than that of the corresponding Ru^{II} complex ($\epsilon_{420\ nm} \approx 11\ 500\ M^{-1}\ cm^{-1}$ for $[Ru(bpz)_3]^{2+}$) (*51*).

As mentioned above, this photoredox process, which is a thermodynamically favorable electron transfer from the bpz excited complex to Cu^{II}, is not observed without preirradiation of the system "$[Ru(bpz)_3]^{2+}$−Cu/Zn SOD". Therefore, it is argued that, before the formation of Ru^{III}, the photoreduction of the complex by an amino acid of the protein chain would modify the protein (reaction 5) in such a way that the reduction of its Cu^{II} in the active site now becomes possible (reaction 6). Reaction 5 would thus correspond to a leak from the back electron transfer, i.e., reaction 4. Recent data on other Cu^{II} proteins have shown that the reduction potentials of their blue copper sites are modified either by a site-directed mutation or by a structural protein constraint (*52*). Unfortunately, on the basis of the present laser flash photolysis data, the different processes that follow the first redox reaction (reaction 3) cannot be more detailed.

$$Cu^{II}/Zn\ SOD^{\cdot +} \rightarrow \text{"modified" } Cu^{II}/Zn\ SOD \quad (5)$$

$$[Ru(bpz)_3{}^*]^{2+} + \text{"modified" } Cu^{II}/Zn\ SOD \rightarrow [Ru(bpz)_3]^{3+} + Cu^{I}/Zn\ SOD \quad (6)$$

On the other hand, there should be a leak to the back electron transfer from Cu^{I} to Ru^{III} formed by reaction 6, to explain the behavior under continuous illumination of $[Ru(bpz)_3]^{2+}$ with Cu/Zn SOD. Figure 6 shows indeed a clear decrease of absorption of the system complex/enzyme at 680 nm with the irradiation time, which could correspond to a permanent disappearance of Cu^{II}, i.e., not entirely regenerated by the reverse of reaction 6.

The fact that the Cu^{II} disappearance does not take place under irradiation of $[Ru(bpy)_3]^{2+}$ in the presence of Cu/Zn SOD (deoxygenated solution under argon) supports the need for a first oxidation of the enzyme (reaction 3) to observe the second electron transfer (reaction 6) by flash photolysis. The production of $[Ru(bpz)_3]^{3+}$, which escapes the back electron-transfer reaction of process 6, would explain the enhancement of the photonuclease activity of $[Ru(bpz)_3]^{2+}$ in the presence of Cu/Zn SOD, because Ru^{III} is an excellent oxidizing agent of DNA guanine and adenine units (vide infra).

$[Ru(bpy)_3]^{2+}$ + Cu/Zn SOD (with or without Preirradiation). In contrast to the above results, when the same laser flash photolysis experiments are performed under argon with $[Ru(bpy)_3]^{2+}$ in the presence of Cu/Zn SOD, the transient spectra recorded after the laser pulse do not show any spectral modifications, compared to the situation with the complex in the absence of Cu/Zn SOD. The transient absorption corresponds mainly to the depletion of the starting complex, with a recovery time of 724 ns, in accordance with the lifetime value measured for the luminescence lifetime of the complex in deoxygenated water, i.e., 630 ns (*45*). This confirms the need for the presence of a very oxidizing complex in the excited state, such as $[Ru(bpz)_3]^{2+}$, to generate a transient metallic reduced species. The absence of the transient reduced complex with $[Ru(bpy)_3]^{2+}$ and Cu/Zn SOD is, of course, in agreement with the absence of luminescence quenching of $[Ru(bpy)_3]^{2+}$ by Cu/Zn SOD.

Study by EPR of the Transients Formed under Irradiation of $[Ru(bpz)_3]^{2+}$ in the Presence of Cu/Zn SOD. To test the occurrence of a photoinduced electron transfer between $[Ru(bpz)_3]^{2+}$ and Cu/Zn SOD, EPR studies were carried out at low temperature (110 K) in deoxygenated conditions upon irradiation at $\lambda > 340$ nm. Before these experiments, there was not a preirradiation of the solution at room temperature. The EPR spectrum of the SOD in its Cu^{II} form and that of the reduced complex was characterized first.

The Cu/Zn SOD (10^{-4} M in 10 mM TRIS/HCl, 10 mM NaCl at pH 7.2, and an argon-purged solution) alone at 110 K produces an EPR signal (Figure 7a) typical of a type-II copper in an axially symmetric ligand environment (square planar or square pyramidal), attributed to the copper in the Cu/Zn SOD enzyme ($g_\perp = 2.087$, $g_\parallel = 2.274$, and $A_{\parallel Cu} = 137.3$ G) (*53*).

To identify the EPR signal corresponding to $[Ru(bpz)_3]^{1+}$, a reductive quenching of the excited $[Ru(bpz)_3]^{2+}$ (10^{-3} M; 10 mM TRIS/HCl, 10 mM NaCl at pH 7.2, and an argon-purged solution) by potassium ferrocyanide ($K_4Fe(CN)_6$, 10^{-1} M] was performed first. $Fe(CN)_6{}^{4-}$ should indeed efficiently quench the lowest ^{3}MLCT state of $[Ru(bpz)_3]^{2+}$ by electron transfer with the formation of $[Ru(bpz)_3]^{1+}$ (*45*). Figure 7b shows the EPR spectrum obtained after irradiation of the complex at 110 K with this reductant; it has a Gaussian constant $g = 2.0005$. In comparison with published spectra (*54*), this signal can clearly be assigned to $[Ru(bpz)_3]^{1+}$. No EPR signal can be detected in the absence of light or by omitting one of the two components, either the complex or the quencher.

By irradiation at 100 K of the bpz complex ($\lambda > 340$ nm) in the presence of SOD, two different EPR profiles can be observed, depending upon the presence or absence of a preirradiation at room temperature, as is the case in laser flash photolysis experiments.

$[Ru(bpz)_3]^{2+}$ + Cu/Zn SOD without Preirradiation of the Solution at Room Temperature. A mixture of $[Ru(bpz)_3]^{2+}$ (10^{-3} M) + Cu/Zn SOD (10^{-4} M) immediately frozen after preparation is irradiated for 10 min with a light source at λ

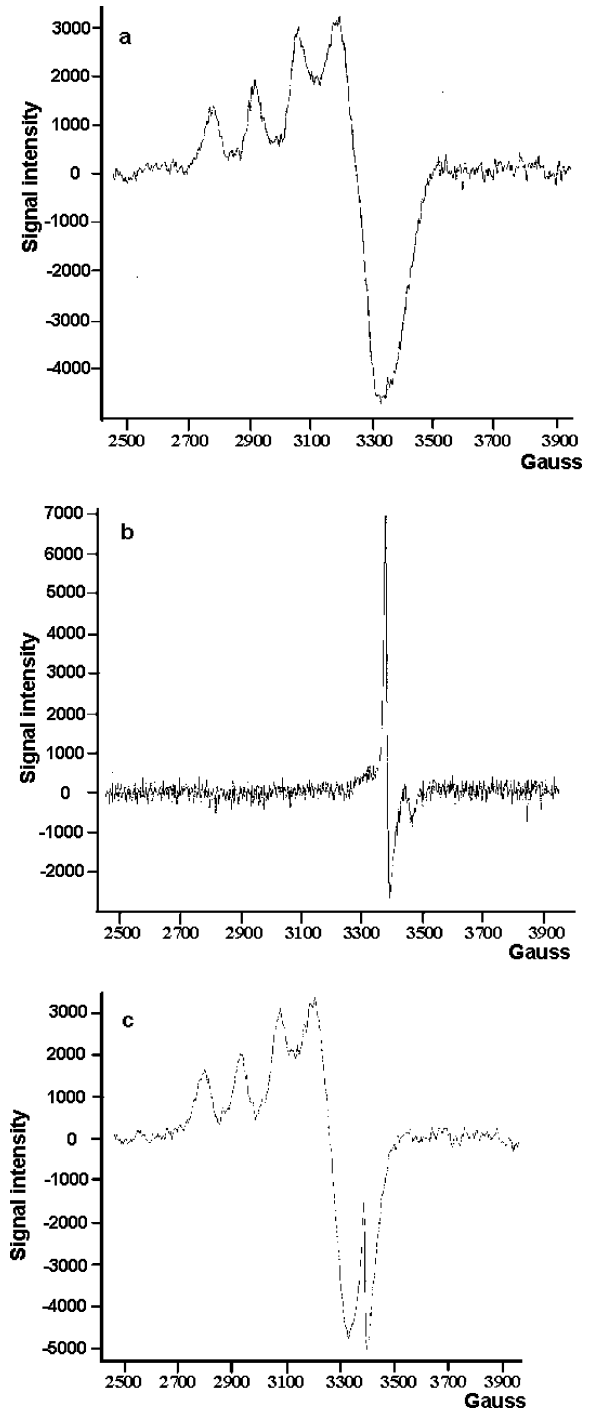

FIGURE 7: EPR spectra of a frozen solution of (a) Cu/Zn SOD (10^{-4} M), (b) $[Ru(bpz)_3]^{2+}$ (10^{-3} M) irradiated in the presence of $K_4Fe(CN)_6$ (10^{-1} M), and (c) $[Ru(bpz)_3]^{2+}$ (10^{-3} M) irradiated in the presence of Cu/Zn SOD (10^{-4} M). Irradiation conditions: argon-purged solutions frozen at 110 K and irradiated for $t = 10$ min at $\lambda > 340$ nm.

> 340 nm. The EPR spectrum of the irradiated system corresponds to the superposition of two signals (Figure 7c). The first signal with a Gaussian constant $g = 2.0004$ is assigned to the monoreduced $[Ru(bpz)_3]^{1+}$. This result supports the previous conclusion, i.e., the existence of a reductive quenching of the excited $[Ru(bpz)_3]^{2+}$ by Cu/Zn SOD (reaction 3). The second EPR signal can be attributed to SOD still in the Cu^{II} state ($g_\perp = 2.087$, $g_\parallel = 2.274$, and $A_{\parallel Cu} = 137.3$ G), because it is identical in line, shape, and intensity to that obtained for the enzyme in the absence of

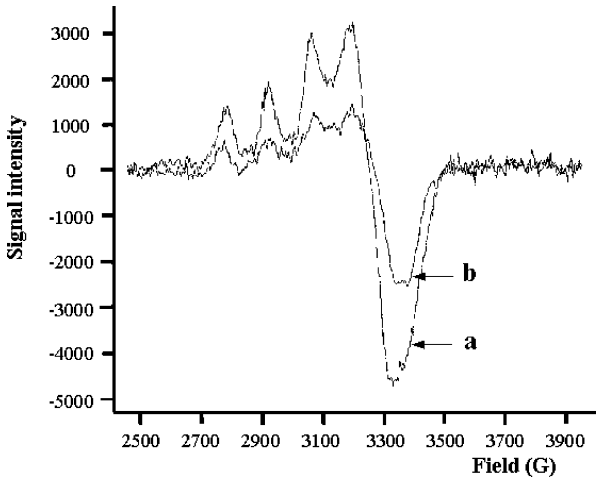

FIGURE 8: (a) Same as Figure 7a, i.e., EPR spectrum of a frozen solution at 110 K of Cu/Zn SOD (10^{-4} M). (b) EPR spectrum of $[Ru(bpz)_3]^{2+}$ (10^{-3} M) with Cu/Zn SOD (10^{-4} M) in a 10 mM TRIS/HCl buffer at pH 7.2 and 110 K after 10 min of irradiation at $\lambda > 340$ nm and after a 10 min preirradiation under argon at 436 nm at room temperature.

$[Ru(bpz)_3]^{2+}$ (Figure 7a). Thus, the Cu^{2+}/Zn SOD is not converted into a silent EPR form, as would be expected if Cu^{2+}/Zn SOD were reduced to Cu^{1+}/Zn SOD. Therefore, these data for the complex and for Cu/Zn SOD are in agreement with reaction 3 as described above.

When the same experiments are performed with $[Ru(bpy)_3]^{2+}$ and Cu/Zn SOD, no EPR signal corresponding to the monoreduced $[Ru(bpy)_3]^{1+}$ is detected, also in agreement with the previous conclusion, according to which no reductive quenching of the complex appears with the bpy compound.

$[Ru(bpz)_3]^{2+}$ + Cu/Zn SOD after a Preirradiation at Room Temperature. A solution containing $[Ru(bpz)_3]^{2+}$ (10^{-3} M) and Cu/Zn SOD (10^{-4} M) is preirradiated for 10 min at 436 nm and 25 °C (see conditions of preirradiation in the Experimental Procedures). After the solution was frozen at 110 K, the EPR spectrum under irradiation at $\lambda > 340$ nm (Figure 8b) shows the absence of the signal characteristic of $[Ru(bpz)_3]^{1+}$ and a sharp decrease in the intensity of the signal corresponding to Cu^{2+}/Zn SOD (Figure 8a), without a change in the line shape.

This can be attributed to the conversion of Cu^{2+}/Zn SOD into Cu^{1+}/Zn SOD, in accordance with the flash photolysis results (Figure 5) and the disappearance of Cu^{2+}/Zn SOD with the irradiation time of $[Ru(bpz)_3]^{2+}$ (Figure 6). As proposed above, the reduction of Cu^{2+}/Zn SOD to Cu^{1+}/Zn SOD could result from an oxidative quenching of the excited $[Ru(bpz)_3]^{2+}$ (after a preirradiation), leading to the formation of the Ru^{III} species. Because the complex in the Ru^{III} state is EPR-silent, these experiments cannot confirm its presence. However, as described below, indirect arguments in favor of its appearance can be obtained.

Spin Trapping of the Guanine and Adenine Radicals. In a previous work, we showed that the yield of alkali-labile damage generated by the excited $[Ru(bpz)_3]^{2+}$ selectively in guanine in 5′ of the 5′-GG-3′ site is increased by the addition of Cu/Zn SOD to the reaction medium (*31*). This sequence selectivity is the signature of an electron-transfer process with the formation of an oxidized guanine radical (*55*). Actually, the formation of the Ru^{III} species, as proposed above, might

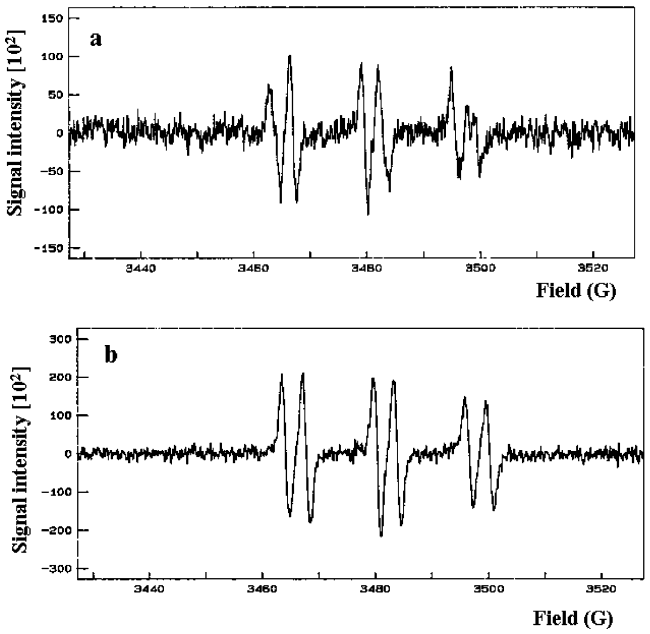

FIGURE 9: EPR spectra recorded in the dark for the spin-trapped guanine radicals produced by irradiation of $[Ru(bpz)_3]^{2+}$ with $[poly(dG\text{-}dC)]_2$ in the absence (a) and presence (b) of Cu/Zn SOD. An argon-purged solution containing $[Ru(bpz)_3]^{2+}$ (10^{-4} M), $[poly(dG\text{-}dC)]_2$ (10^{-3} M in base pairs), and PBN (3×10^{-2} M) in 10 mM TRIS/HCl buffer at pH 7.2 irradiated at $\lambda > 340$ nm for 1 min at room temperature. Cu/Zn SOD was used at the concentration of 10^{-4} M.

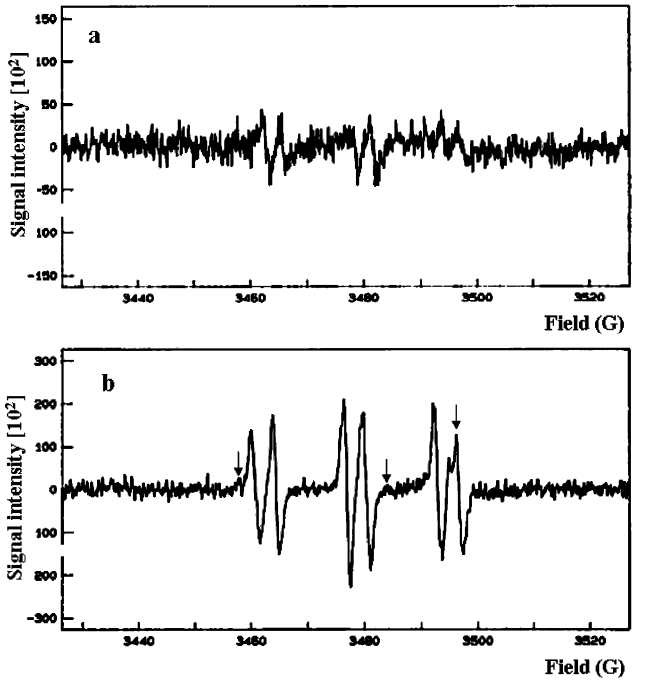

FIGURE 10: EPR spectra of the spin-trapped adenine radicals recorded in the dark and produced by irradiation of $[Ru(bpz)_3]^{2+}$ and $[poly(dA\text{-}dT)]_2$ in the absence (a) and presence (b) of Cu/Zn SOD (10^{-4} M). An argon-purged solution containing $[Ru(bpz)_3]^{2+}$ (10^{-4} M), $[poly(dA\text{-}dT)]_2$ (10^{-3} M in base pairs), and PBN (3×10^{-2} M) in 10 mM TRIS/HCl buffer at pH 7.2 irradiated at $\lambda > 340$ nm for 1 min at room temperature.

explain this increased oxidation, because in addition to the Ru^{II} excited species, Ru^{III} can also oxidize guanine. Therefore, spin-trapping experiments of the guanine radical were performed to confirm this hypothesis. However, because both the excited bpz Ru^{II} species and the ground-state bpz Ru^{III} are able to oxidize the guanine units, comparisons needed to be performed on the "complex + DNA" system with and without Cu/Zn SOD.

Spin-trapping experiments were carried out using PBN as a spin trap, which was shown to be appropriate for studying the formation of the guanine radicals in DNA under irradiation (*56*). This spin-trap compound is stable under the irradiation conditions of this work (data not shown). Thus, a solution of $[Ru(bpz)_3]^{2+}$ (10^{-4} M), containing $[poly(dG\text{-}dC)]_2$ (10^{-3} M in base pairs, in 10 mM TRIS/HCl, 10 mM NaCl at pH 7.2, and an argon-purged solution) and PBN (3 10^{-2} M), at first without Cu/Zn SOD, was irradiated at room temperature for 1 min at $\lambda > 340$ nm. The spectrum (Figure 9) recorded in the dark yielded the following parameters: $a_N = 16.2$, $a_H = 3.54$, and $g = 2.0058$ and corresponded closely to that assigned by Schiemann et al. to the trapping of the guanine radical with PBN. As expected for such species (*56*), the signal is stable for a few hours in the dark. The formation of the adduct PBN−guanine cation radical may result from the addition of the nitrone on the C_4 or C_5 sites of the guanine ring. These positions correspond to tautomeric carbon-centered radicals of the deprotonated guanine cation radical, which arise from the oxyl intermediate radical (*57*). In this case, the guanine radicals could stem from the one-electron oxidation of the guanine units by the excited bpz complex. Interestingly, the addition of Cu/Zn SOD (10^{-4} M) to the reaction mixture led to a 2-fold increase in the signal attributed to the spin trapping of the guanine radical by PBN, after 1 min of irradiation.

This clearly indicates an enhancement of the guanine oxidation by electron transfer in the presence of Cu/Zn SOD. This conclusion is compatible with the appearance of the Ru^{III} species (after a certain illumination time of the bpz Ru^{II} complex) able to oxidize guanine (reaction 7).

$$[Ru(bpz)_3]^{3+} + G \rightarrow [Ru(bpz)_3]^{2+} + G^{\cdot+} \qquad (7)$$

Moreover, because the Ru^{III} species [$E°(Ru^{3+}/Ru^{2+}) = +1.86$ V versus SCE in CH_3CN) (*45*) is not only capable of oxidizing the guanine but also the adenine units [$E°(A^{\cdot+}/A) = +1.79 \pm 0.02$ V versus SCE] (*19*) via an electron-transfer process, spin-trapping experiments were also performed with $[poly(dA\text{-}dT)]_2$ (10^{-3} M in base pairs; 10 mM TRIS/HCl, 10 mM NaCl at pH 7.2, and an argon-purged solution) in the presence of PBN (3×10^{-2} M) as a spin trap. In the absence of Cu/Zn SOD, no EPR signal was detected, and indeed, excited $[Ru(bpz)_3]^{2+}$ ($E°(Ru^{2+*}/Ru^{1+}) = +1.45$ V versus SCE in CH_3CN) (*45*) cannot oxidize adenine. However, after the addition of the Cu/Zn SOD and irradiation, an EPR signal (Figure 10) was detected with the following coupling constants: $g = 2.0057$, $a_N = 16.13$, and $a_H = 3.63$. Moreover, the arrows in Figure 10 identify the presence of additional lines, which are not observed on the EPR spectrum of the PBN−guanine radical adduct. These additional lines may be attributed to the presence of a nitrogen radical adduct as proposed in the literature (*58*). This result tends to show that the spin-trap agent PBN may bind on the N^6-centered, aniline-type adenine cation radical (*59*) which is the most stable tautomer of the adenine cation radical in aqueous solution (*60*).

This spectrum can be attributed to the trapping of the adenine cation radical by PBN and is stable for a few hours

in the dark. Thus, this one-electron oxidation of adenine is in agreement with the photoproduction of the Ru^{III} species.

When the same EPR experiments were carried out with $[Ru(bpy)_3]^{2+}$ and $[poly(dG\text{-}dC)]_2$ or $[poly(dA\text{-}dT)]_2$, no signal was detected in the absence or presence of Cu/Zn SOD. This means that no bpy Ru^{III} species was produced, because it would then have been able to thermodynamically oxidize the guanine and adenine units. This result supports the hypothesis of the need for prior reductive quenching of the excited Ru complex (possible with the bpz but not with the bpy compound), to have access (after modification of the enzyme) to the reducible Cu^{II} in the active site of the enzyme.

CONCLUSION

This work was motivated by the previous, intriguing observation in which the DNA photodamage and guanine oxidation induced by excited $[Ru(bpz)_3]^{2+}$ was increased in the presence of Cu/Zn SOD. The results of this work show that at least two successive electron-transfer processes could be involved and could account for this observation.

First, a reductive quenching of the excited $[Ru(bpz)_3]^{2+}$, probably by the external peptidic chain of Cu/Zn SOD, generates the monoreduced $[Ru(bpz)_3]^{1+}$ (reaction 3). Because of its high oxidizing power, the excited $[Ru(bpz)_3]^{2+}$ could indeed oxidize amino acids such as tyrosine and tryptophan (*61*). The resulting reduced $[Ru(bpz)_3]^{1+}$ gives rise to a back electron-transfer process (reaction 4). However, in competition with this process, other reactions could take place, as follows: (i) the photoadduct of the complex, for example, with tryptophan as recently shown for the compounds $[Ru(TAP)_3]^{2+}$ and $[Ru(TAP)_2phen]^{2+}$, could be formed on the SOD surface (*47*). These TAP complexes, which have approximately the same oxidizing power in the ^{3}MLCT as excited $[Ru(bpz)_3]^{2+}$, have indeed been shown to be able to oxidize tryptophan, with the formation of an adduct of the complex to the amino acid. (ii) Formation of protein dimers or protein aggregates via the production of intermediate radicals may also be possible (*62*). These reactions might induce sufficient structural changes in the enzyme to subsequently allow for the access of the complex to the redox site of the enzyme. Therefore, after the first photoprocess, the excited complex would be able to reduce the Cu^{II} in the active site of the protein (reaction 6). During this second step, Cu^{1+}/Zn SOD and mono-oxidized $[Ru(bpz)_3]^{3+}$ are generated. It is well-known that the $[Ru(bpz)_3]^{3+}$ species (escaped from the back electron transfer) can oxidize guanine and adenine units, which explains the increase in oxidized nucleotides in the presence of Cu/Zn SOD (reaction 7). Because the first step (reaction 3) is not possible with the bpy complex, no increase in nucleobase oxidation is observed by the addition of Cu/Zn SOD to the "$[Ru(bpy)_3]^{2+}$ and DNA" system under irradiation. The proposed mechanism for the photoredox process occurring when $[Ru(bpz)_3]^{2+}$ is irradiated in the presence of Cu/Zn SOD has important biological implications. Indeed, recent studies have shown that SOD can be a suitable target for the selective elimination of cancer cells (*63*). The survival of malignant cells strongly depends upon the presence of active SOD, because these cells produce many superoxide anions. Thus, inhibition of the SOD enzyme causes an accumulation of superoxide anions and subsequent cell death by apoptosis (*59*). The photoinduced structural alterations of the Cu/Zn SOD enzyme as proposed in this work could thus lead to its inactivation, accompanied by possible interesting effects in connection with cancer therapy.

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REFERENCES

1. Satyanarayana, S., Dabrowiak, J. C., and Chaires, J. B. (1993) Tris(phenanthroline)ruthenium(II) enantiomer interactions with DNA: Mode and specificity of binding, *Biochemistry 32*, 2573−2584.
2. Hiort, C., Lincoln, P., and Nordén, B. (1993) DNA binding of Δ- and Λ-$[Ru(phen)_2DPPZ]^{2+}$, *J. Am. Chem. Soc. 115*, 3448−3454.
3. Dupureur, C. M., and Barton, J. K. (1997) Structural studies of Λ- and Δ-$[Ru(phen)_2dppz]^{2+}$ bound to $d(GTCGAC)_2$: Characterization of enantioselective intercalation, *Inorg. Chem. 36*, 33−43.
4. Friedman, A. E., Chambron, J.-C., Sauvage, J.-P., Turro, N. J., and Barton, J. K. (1990) A molecular light switch for DNA: $Ru(bpy)_2(dppz)^{2+}$, *J. Am. Chem. Soc. 112*, 4960−4962.
5. Jenkins, Y., Friedman, A. E., Turro, N. J., and Barton, J. K. (1992) Characterization of dipyridophenazine complexes of ruthenium(II): The light switch effect as a function of nucleic acid sequence and conformation, *Biochemistry 31*, 10809−10816.
6. Olofsson, J., Onfelt, B., Lincoln, P., Norden, B., Matousek, P., and Parker, A. W., Tuite, E. J. (2002) Picosecond Kerr-gated time-resolved resonance Raman spectroscopy of the $[Ru(phen)_2dppz]^{2+}$ interaction with DNA, *Inorg. Biochem. 91*, 286−297.
7. Kelly, J. M., Tossi, A. B., McConnell, J. D., and OhUigin, C. (1985) A study of the interactions of some polypyridylruthenium(II) complexes with DNA using fluorescence spectroscopy, topoisomerisation and thermal denaturation, *Nucleic Acids Res. 13*, 6017−6034.
8. Fleisher, M. B., Waterman, K. C., Turro, N. J., and Barton, J. K. (1986) Light-induced cleavage of DNA by metal complexes, *Inorg. Chem. 25*, 3549−3551.
9. Tossi, A. B., and Kelly, J. M. (1989) A study of some polypyridylruthenium(II) complexes as DNA binders and photocleavage reagents, *Photochem. Photobiol. 49*, 545−556.
10. Jacquet, L., Kelly, J. M., and Kirsch-De Mesmaeker, A. (1995) Photoadduct between tris(1,4,5,8-tetraazaphenanthrene)ruthenium(II) and guanosine monophosphate-a model for a new mode of covalent binding of metal complexes to DNA, *J. Chem. Soc. Chem. Commun. 9*, 913−914.
11. Jacquet, L., Davies, R. J., Kirsch-De Mesmaeker, A., and Kelly, J. M. (1997) Photoaddition of $Ru(tap)_2(bpy)^{2+}$ to DNA: A new mode of covalent attachment of metal complexes to duplex DNA, *J. Am. Chem. Soc. 119*, 11763−11768.
12. Vicendo, P., Mouysset, S., and Paillous, N. (1997) Comparative study of $Ru(bpz)_3^{2+}$, $Ru(bipy)_3^{2+}$, and $Ru(bpz)_2Cl_2$ as photosensitizers of DNA cleavage and adduct formation, *Photochem. Photobiol. 65*, 647−655.
13. Stemp, E. D. A., Arkin, M. R, and Barton, J. K. (1997) Oxidation of guanine in DNA by $Ru(phen)_2(dppz)^{3+}$ using the flash-quench technique, *J. Am. Chem. Soc. 119*, 2921−2925.
14. Delaney, S., Yoo, J., Stemp, E. D., and Barton, J. K. (2004) Charge equilibration between two distinct sites in double helical DNA, *Proc. Natl. Acad. Sci. U.S.A. 101*, 10511−10516.
15. Sentagne, C., Chambron, J.-C, Sauvage, J.-P, and Paillous, N. (1994) Tuning the mechanism of DNA cleavage photosensitized by ruthenium dipyridophenazine complexes by varying the structure of the two non intercalating ligands, *J. Photochem., B 26*, 165−174.
16. Lecomte, J. P., Kirsch-De Mesmaeker, A., Feeney, M. M., and Kelly, J. M. (1995) Ruthenium(II) complexes with 1,4,5,8,9,12-hexaazatriphenylene and 1,4,5,8-tetraazaphenanthrene ligands: Key role played by the photoelectron transfer in DNA cleavage and adduct formation, *Inorg. Chem. 34*, 6481−6491.
17. Kirsch-De Mesmaeker, A., Orellana, G., Barton, J. K., and Turro, N. J. (1990) Ligand-dependent interaction of ruthenium(II) poly-

pyridyl complexes with DNA probed by emission spectroscopy, *Photochem. Photobiol. 52*, 461−472.
18. Lecomte, J. P., Kirsch-De Mesmaeker, A., Kelly, J. M., Tossi, A. B., and Görner, H. (1992) Photo-induced electron transfer from nucleotides to ruthenium-tris-1,4,5,8-tetraazaphenanthrene: Model for photosensitized DNA oxidation, *Photochem. Photobiol. 55*, 681−689.
19. Steenken, S., and Jovanic, S. (1997) How easily oxidizable is DNA? One-electron reduction potentials of adenosine and guanosine radicals in aqueous solution, *J. Am. Chem. Soc. 119*, 617−618.
20. Saito, I., Takayama, M., Sugiyama, H., Nakatani, K., Tsuchida, A., and Yamamoto, M. (1995) Photoinduced DNA cleavage via electron transfer: Demonstration that guanine residues located 5′ to guanine are the most electron-donating sites, *J. Am. Chem. Soc. 117*, 6406−6407.
21. Sugiyama, H., and Saito, I. (1996) Theoretical studies of GG-specific photocleavage of DNA via electron transfer: Significant lowering of ionization potential and 5′-localization of HOMO of stacked GG bases in B-form DNA, *J. Am. Chem. Soc. 118*, 7063−7068.
22. Ortmans, I., Elias, B., Kelly, J. M., Moucheron, C., and Kirsch-De Mesmaker, A. (2004) $[Ru(TAP)_2(dppz)]^{2+}$: A DNA intercalating complex, which luminesces strongly in water and undergoes photo-induced proton-coupled electron transfer with guanosine 5′-monophosphate, *J. Chem. Soc., Dalton Trans. 4*, 668−676.
23. Cadet, J., and Vigny, P. (1990) The photochemistry of nucleic acids, in *Bioorganic Photochemistry: Photochemistry and Nucleic Acids* (Morrison, H., Ed.), Vol. 1, pp 3−272, Wiley, New York.
24. Schulte-Frohlinde, D., Simic, M., and Görner, H. (1990) Laser-induced strand break formation in DNA and polynucleotides, *Photochem. Photobiol. 52*, 1137−1151.
25. Burrows, C. J., and Muller, J. G. (1998) Oxidative nucleobase modifications leading to strand scission, *Chem. Rev. 98*, 1109−1152.
26. Blasius, R., Nierengarten, H., Lhumer, M., Constant, J.-F, Defrancq, E., Dumy, P., van Dorsselaer, A., Moucheron, C., and Kirsch-De Mesmaeker, A. (2005) Photoreaction of $[Ru(hat)_2phen]^{2+}$ with guanosine 5′-monophosphate and DNA: Formation of new types of photoadducts, *Chem. Eur. J. 11*, 1507−1517.
27. Stemp, E. D. A., and Barton, J. K. (2000) The flash-quench technique in protein−DNA electron transfer: Reduction of the guanine radical by ferrocytochrome *c*, *Inorg Chem. 39*, 3868−3874.
28. Koppenol, W. H., and Margoliash, E. J. (1982) The asymmetric distribution of charges on the surface of horse cytochrome *c*. Functional implications, *J. Biol. Chem. 257*, 4426−4437.
29. Koppenol, W. H., Rusth, J. D., Mills, J. D., and Margoliash, E. J. (1991) The dipole moment of cytochrome *c*, *Mol. Biol. Evol. 8*, 545−558.
30. Murphy, C. J., Arkin, M. R., Ghatlia, N. D., Bossmann, S., Turro, N. J., and Barton, J. K. (1994) Fast photoinduced electron transfer through DNA intercalation, *Proc. Natl. Acad. Sci. U.S.A. 91*, 5315−5319.
31. Gicquel, E., Paillous, N., and Vicendo, P. (2000) Mechanism of DNA damage photosensitized by trisbipyrazyl ruthenium complex. Unusual role of Cu/Zn superoxide dismutase, *Photochem. Photobiol. 72*, 583−589.
32. Carrico, R. J., and Deutsch, H. F. (1970) The presence of zinc in human cytocuprein and some properties of the apoprotein, *J. Biol. Chem. 245*, 723−727.
33. Richardson, J. S., Thomas, K. A., and Richardson, D. C. (1975) α-Carbon coordinates for bovine Cu,Zn superoxide dismutase, *Biochem. Biophys. Res. Commun. 63*, 986−992.
34. Richardson, J. S., Thomas, K. A., Rubin, B. H., and Richardson, D. C. (1975) Crystal structure of bovine Cu,Zn superoxide dismutase at 3 Å resolution: Chain tracing and metal ligands, *Proc. Natl. Acad. Sci. U.S.A. 72*, 1349−1353.
35. Fridovich, I. (1974) Superoxide dismutases, *Adv. Enzymol. 41*, 35−97.
36. Fridovich, I. (1995) Superoxide radical and superoxide dismutases, *Annu. Rev. Biochem. 64*, 97−112.
37. Fridovich, I. (1997) Superoxide anion radical ($O_2^{-\cdot}$), superoxide dismutases, and related matters, *J. Biol. Chem. 272*, 18515−18517.
38. Rotilio, G., Bray, R. C., and Fielden, E. M., (1972) A pulse radiolysis study of superoxide dismutase, *Biochim. Biophys. Acta 268*, 605−609.
39. Klug-Roth, D., Fridovich, I., and Rabani, J. (1973) Pulse radiolytic investigations of superoxide catalyzed disproportionation. Mechanism for bovine superoxide dismutase, *J. Am. Chem. Soc. 95*, 2786−2790.
40. Fielden, E. M., Roberts, P. B., Bray, R. C., Lowe, D. J., Mautner, G. N., Rotilio, G., and Calabrese, L. (1974) Mechanism of action of superoxide dismutase from pulse radiolysis and electron paramagnetic resonance. Evidence that only half the active sites function in catalysis, *Biochem. J. 139*, 49−60.
41. Gicquel, E., Paillous, N., and Vicendo, P. (1998) Unexpected enhancement of the photonuclease activity of $Ru(bpz)_3^{2+}$ by Cu/Zn superoxide dismutase, *J. Chem. Soc. Chem. Commun. 9*, 998−999.
42. Ge, B., Scheller, F. W., and Lisdat, F. (2003) Electrochemistry of immobilized CuZnSOD and FeSOD and their interaction with superoxide radicals, *Biosens. Bioelectron. 18*, 295−302.
43. Getzoff, E. D., Tainer, J. A., Weineer, P. K., Kollman, P. A., Richardson, J. S., and Richardson, D. C. (1983) Electrostatic recognition between superoxide and copper, zinc superoxide dismutase, *Nature 306*, 287−290.
44. St. Clair, C. S., Gray, H. B., and Valentine, J. S. (1992) Spectroelectrochemistry of copper−zinc superoxide dismutase, *Inorg. Chem. 31*, 925−927.
45. Haga, M.-A., Dodsworth, E. S., Eryavec, G., Seymour, P., and Lever, A. B. (1985) Luminescence quenching of the tris(2,2′-bipyrazine)ruthenium(II) cation and its monoprotonated complex, *Inorg. Chem. 24*, 1901−1906.
46. Tainer, J. A., Getzoff, E. D., Richardson, J. S., and Richardson, D. C. (1983) Structure and mechanism of copper, zinc superoxide dismutase, *Nature 306*, 284−287.
47. Gicquel, E., Boisdenghien, A., Defrancq, E., Moucheron, C., and Kirsch-De Mesmaeker, A. (2004) Adduct formation by photo-induced electron transfer between photo-oxidising Ru(II) complexes and tryptophan, *J. Chem. Soc. Chem. Commun. 23*, 2764−2765.
48. Mailer, K., Addetia, R., and Livesey, D. L. (1989) UV spectroscopic studies of human erythrocyte superoxide dismutase, *J. Inorg. Biochem. 37*, 151−161.
49. Prasad, D. R., and Hoffman, M. Z. (1986) Photodynamics of the tris(2,2′-bipyrazine)ruthenium^{2+}/methylviologen/EDTA system in aqueous solution, *J. Am. Chem. Soc. 108*, 2568−2573.
50. Venturi, M., Mulazzani, Q. G., Ciano, M., and Hoffman, M. Z. (1986) Radiolytic and electrochemical reduction of tris(2,2′-bipyrazine)ruthenium^{2+} in aqueous solution. Stability, redox, and acid−base properties of tris(2,2′-bipyrazine)ruthenium^{1+}, *Inorg. Chem. 25*, 4493−4498.
51. Neumann-Spallart, M., Kalyanasundaram, K., Graetzel, C., and Graetzel, M. (1980) Ruthenium dioxide electrodes as suitable anodes for water photolysis, *Helv. Chim. Acta 63*, 1111−1118.
52. Li, H., Webb, S. P., Ivanic, J., and Jensen, J. H. (2004) Determinants of the relative reduction potentials of type-1 copper sites in proteins, *J. Am. Chem. Soc. 126*, 8010−8019.
53. Valentine, J. S., Pantoliano, M. W., McDonnell, P. J., Burger, A. R., and Lippard, S. J. (1979) pH-dependent migration of copper(II) to the vacant zinc-binding site of zinc-free bovine erythrocyte superoxide dismutase, *Proc. Natl. Acad. Sci. U.S.A. 76*, 4245−4249.
54. Morris, D. E., Hanck, K. W., and DeArmond, M. K. (1983) ESR studies of the redox orbitals in diimine complexes of iron(II) and ruthenium(II), *J. Am. Chem. Soc. 105*, 3032−3038.
55. Saito, I, Nakamura, T., Nakatani, K., Yoshioka, Y., Yamaguchi, K., and Sugiyama, H. (1998) Mapping of the hot spots for DNA damage by one-electron oxidation: Efficacy of GG doublets and GGG triplets as a trap in long-range hole migration, *J. Am. Chem. Soc. 120*, 12686−12687.
56. Schiemann, O., Turro, N. J., and Barton, J. K. (2000) EPR detection of guanine radicals in a DNA duplex under biological conditions: Selective base oxidation by $Ru(phen)_2dppz^{3+}$ using the flash-quench technique, *J. Phys Chem. B 104*, 7214−7220.
57. Cadet, J., Berger, M., Buchko, G. W., Joshi, P. C., Raoul, S., and Ravanat, J.-L. (1994) 2,2-Diamino-4-[(3,5-di-*O*-acetyl-2deoxy-β-D-*erythro*-pentofuranosyl)amino]-5-(2*H*)-oxazolone: A novel and predominant radical oxidation product of 3′,5′-Di-*O*-acetyl-2′-deoxyguanosine, *J. Am. Chem. Soc. 116*, 7403−7404.
58. Parman, T., Chen, G., and Wells, P. G. (1998) Free radical intermediates of phenytoin and related teratogens. Prostaglandin H synthase-catalyzed bioactivation, electron paramagnetic resonance spectrometry, and photochemical product analysis, *J. Biol. Chem. 273*, 25079−25088.

59. Steenken, S. (1989) Purines bases, nucleosides, and nucleotides: Aqueous solution redox chemistry and transformation reactions of their radical cations and e^- and OH adducts, *Chem. Rev. 89*, 503−520.
60. Chen, X. H., Syrstad, E. A., Nguyen, M. T., Gerbaux, P., and Turek, F. (2004) Distonic isomers and tautomers of the adenine cation radical in the gas phase and aqueous solution, *J. Phys. Chem. A 108*, 9283−9293.
61. DeFelippis, M. R., Murthy, C. P., Faraggi, M., and Klapper, M. H. (1989) Pulse radiolytic measurement of redox potentials: The tyrosine and tryptophan radicals, *Biochemistry 28*, 4847−4853.
62. Zhang, H., Andrekopoulos, C., Joseph, J., Chandran, K., Karoui, H., Crow, J. P., and Kalyanaraman, B. (2003) Bicarbonate-dependent peroxidase activity of human Cu,Zn-superoxide dismutase induces covalent aggregation of protein: Intermediacy of tryptophan-derived oxidation products, *J. Biol. Chem. 278*, 24078−24089.
63. Huang, P., Feng, L., Oldham, E. A., Keating, M. J., and Plunkett, W. (2000) Superoxide dismutase as a target for the selective killing of cancer cells, *Nature 407*, 390−395.

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