Unexpected enhancement of the photonuclease activity of $Ru(bpz)_3^{2+}$ by Cu/Zn superoxide dismutase

Etienne Gicquel, Nicole Paillous*† and Patricia Vicendo

Laboratoire IMRCP, UMR 5623, Université Paul Sabatier, 118 route de Narbonne, 31062 Toulouse Cedex 4, France

Cu/Zn SOD led to an unexpected enhancement of the photonuclease activity of $Ru(bpz)_3^{2+}$ *via* an increase in the production of singlet oxygen.

The ruthenium complexes possess versatile photoredox properties, which are exploited in a wide variety of applications including the photolysis of water,[1–3] development of novel photonucleases[4–8] and more recently for modelling chemical photolyases.[9] The photonuclease[4] activity of these complexes stems in part from their redox potential in the excited state. If this is above that of guanine,[10] the nucleobase with the lowest oxidation potential, under irradiation the polypyridyl ruthenium complexes may induce an electron transfer from DNA to the dye in the excited state, giving rise to single strand breaks in the double helix of DNA.[11,12] The efficiency of this type I mechanism for producing single strand breaks is higher than that of the type II mechanism.[13] Furthermore, it has been shown that electron transfer leads to formation of covalent photoadducts between ruthenium complexes such as tris(1,4,5,8-tetraazaphenanthrene)ruthenium(II) [$Ru(tap)_3^{2+}$][14] and tris(2,2′-bipyrazyl)ruthenium(II) [$Ru(bpz)_3^{2+}$][15] and DNA.

In the case of $Ru(bpz)_3^{2+}$, the mechanism of single strand breaking is complex, involving electron transfer, formation of singlet oxygen and radical species.[15] In order to identify the various oxygenated species involved in the photonuclease activity of $Ru(bpz)_3^{2+}$, and the superoxide anion in particular, we used the Cu/Zn superoxide dismutase (Cu/Zn SOD). While this enzyme should give rise to a reduction in efficiency of the DNA cleavage by $Ru(bpz)_3^{2+}$, *via* a dismutation of $O_2^{\cdot -}$, an unexpected increase in photonuclease activity of this complex was observed. Here, we report the main characteristics of this unexpected effect.

As shown in a previous study,[15] $Ru(bpz)_3^{2+}$ (1.8 μM) in solution [5 mM phosphate buffer (pH 7.4), 10 mM NaCl] and irradiated at 436 nm in the presence of supercoiled phage φX174 DNA (18 μM) induced single strand breaks with a quantum yield of 10^{-5} (Fig. 1). In the presence of Cu/Zn SOD (Cu/Zn SOD from human erythrocytes, 22 U ml^{-1}, 0.299 μM), the number of single strand breaks was 2.4-fold higher at 60 s. This enzyme is not able to induce alone DNA breakage upon irradiation at 436 nm. The same finding was obtained, under similar experimental conditions, with Cu/Zn from bovine erythrocytes. Furthermore, the plot of the number of photoinduced DNA single strand breaks against time in the presence of SOD was not linear. There was an increase in rate with duration of irradiation. After 90 s irradiation, nearly all the supercoiled form of the plasmid had been transformed into the relaxed circular form. This was not observed in the absence of SOD even after 30 min of irradiation.

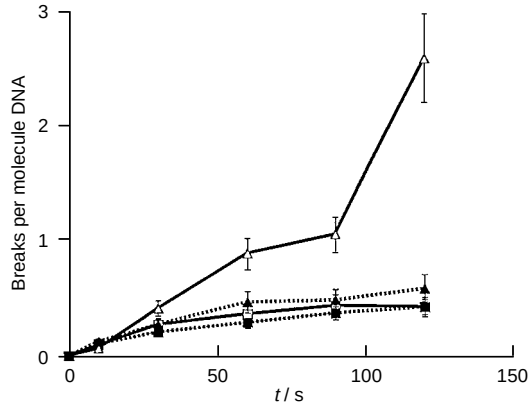

Fig. 1 Plot of number of DNA single strand breaks per molecule of φX174 plasmid DNA photoinduced by $Ru(bpz)_3^{2+}$ at 436 nm against irradiation time: (□) $Ru(bpz)_3^{2+}$, (■) $Ru(bpz)_3^{2+}$ in the absence of oxygen, (△) $Ru(bpz)_3^{2+}$ with Cu/Zn SOD and (▲) $Ru(bpz)_3^{2+}$ with Cu/Zn SOD in the absence of oxygen

In the absence of oxygen, SOD had no significant effect on the DNA single strand breaks induced by $Ru(bpz)_3^{2+}$ (Fig. 1). This indicated that the action of SOD depended on the presence of oxygen, and was mediated by the production of oxygenated species. Catalase (22 U ml^{-1}) and desferioxamine (17 mM) were not found to modify the number of DNA single strand breaks induced by $Ru(bpz)_3^{2+}$ in the presence of Cu/Zn SOD. This observation indicates that the effect of Cu/Zn SOD did not result from the formation of $OH^{\cdot}$ *via* a Fenton reaction. Furthermore, the photonuclease activity of $Ru(bpz)_3^{2+}$ was not augmented in the presence of iron SOD (from *Escherichia coli*). It thus appeared that the action of Cu/Zn SOD was specific to this particular enzyme.

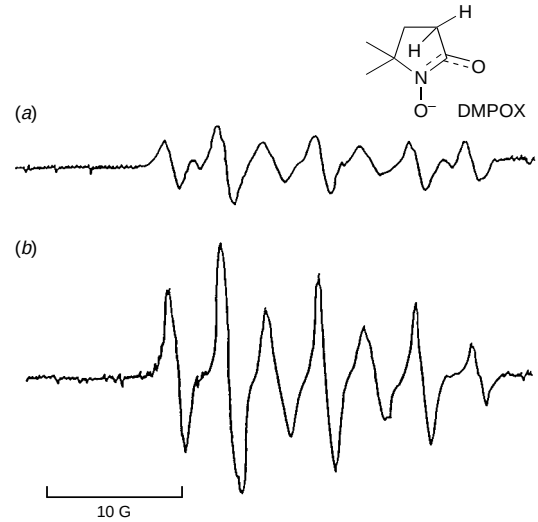

Fig. 2 EPR spectra of UV irradiated (λ > 400 nm) solutions containing DMPO and ruthenium complexes: (*a*) $Ru(bpz)_3^{2+}$ and (*b*) $Ru(bpz)_3^{2+}$ with Cu/Zn SOD

In order to better understand the function of oxygenated radical species in the photoactivation of $Ru(bpz)_3^{2+}$ by Cu/Zn SOD, EPR studies have been performed. EPR studies on $Ru(bpz)_3^{2+}$ (10^{-3} M) irradiated (λ > 400 nm) in the presence of 5,5-dimethyl-2-pyrolidine 1-oxide (DMPO) (150 mM) failed to detect $OH^{\cdot}$ radicals, and indicated the presence of 5,5-dimethyl-2-pyrolidone 1-oxyl radicals (DMPOX) (a_N = 4.1, a_H = 7.2) derived from the oxidation of DMPO by singlet oxygen[16]

(Fig. 2). The formation of this radical was increased almost three-fold by addition of Cu/Zn SOD (22 U ml^{-1}) (Fig. 2). EPR studies of irradiated Cu/Zn SOD in the presence of DMPO did not show the formation of radicals. It thus appeared that the action of Cu/Zn SOD on $Ru(bpz)_3^{2+}$ was mediated by an increase in the production of singlet oxygen, which is responsible for the enhanced photonuclease activity of this complex. This hypothesis was supported by the results obtained on addition of *N*-acetylhistidine (50 mM) and DABCO (20 mM), singlet oxygen scavengers. They led to a 100% inhibition in production of DMPOX radicals in the presence or absence of Cu/Zn SOD. Moreover, *N*-acetylhistidine abolished totally the increase in DNA single strand breaks obtained after photosensitization of DNA by $Ru(bpz)_3^{2+}$ in the presence of this enzyme (Fig. 3).

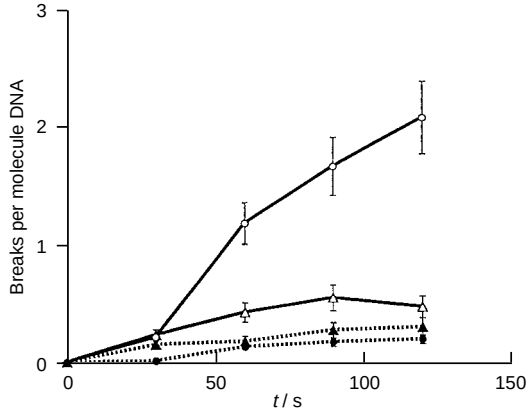

Fig. 3 Plot of number of DNA single strand breaks per molecule of ϕX174 plasmid DNA photoinduced by $Ru(bpz)_3^{2+}$ at 436 nm against irradiation time: (△) $Ru(bpz)_3^{2+}$, (▲) $Ru(bpz)_3^{2+}$ with *N*-acetylhistidine, (○) $Ru(bpz)_3^{2+}$ with Cu/Zn SOD and (●) $Ru(bpz)_3^{2+}$ with Cu/Zn SOD and *N*-acetylhistidine

The action of Cu/Zn SOD (22 U ml^{-1}) on the electron transfer process was investigated by determining the efficiency of $Ru(bpz)_3^{2+}$ (1.5×10^{-4} M) covalent addition to calf thymus DNA (1.5×10^{-3} M in nucleotides) upon irradiation at 436 nm in the presence or absence of this enzyme. Quantification of ruthenium bound to DNA by ICP[15] analysis failed to identify any difference in the kinetics or equilibrium of the addition of $Ru(bpz)_3^{2+}$ to DNA. After 2 h of irradiation at 436 nm in the presence or absence of SOD, the photoadditions reached a plateau corresponding to 1.4×10^{-7} mol of $Ru(bpz)_3^{2+}$ per mg of calf thymus DNA or one molecule of complex for 11 ± 1 nucleotides. SOD thus did not appear to alter the photoreactivity of $Ru(bpz)_3^{2+}$ mediated by electron transfer.

Analysis by UV–VIS spectroscopy of the photochemical behavior of $Ru(bpz)_3^{2+}$ (10^{-5} M) in 5 mM phosphate buffer (pH 7.4) and 10 mM NaCl irradiated at 436 nm showed that SOD did not affect the rate of photodecomposition of $Ru(bpz)_3^{2+}$ (Fig. 4). This observation suggested that the increase in production of singlet oxygen by $Ru(bpz)_3^{2+}$ in the presence of SOD was not due to enhanced photostability of the complex. EPR studies on solutions of complexes of ruthenium of various redox potentials such as tris(2,2′-bipyridyl)ruthenium(II) [$Ru(bipy)_3^{2+}$] [$E(Ru^{2+*}/Ru^{+})$ = 0.77 V *vs*. SCE in MeCN][17] (10^{-3} M) and tris(2,2′-bipyrazyl)(dipyrido[3,2-*a*:2′3′-*c*]phenazine-N^4,N^5)ruthenium(II) [$Ru(bpz)_2dppz^{2+}$] [$E(Ru^{2+*}/Ru^{+})$ = 1.21 V *vs*. SCE in MeCN[13] (10^{-3} M) revealed that, in the absence of SOD, these two compounds generated under irradiation the same radical DMPOX as $Ru(bpz)_3^{2+}$ [$E(Ru^{2+*}/Ru^{+})$ = 1.45 V *vs*. SCE in MeCN].[17] In the presence of Cu/ZnSOD, the increase in production of this radical was, with $Ru(bipy)_3^{2+}$ and $Ru(bpz)_2dppz^{2+}$, 1.4- and 2.6-fold higher, respectively, than in the absence of Cu/Zn SOD. These results show that the increase in production of the DMPOX radical generated by these

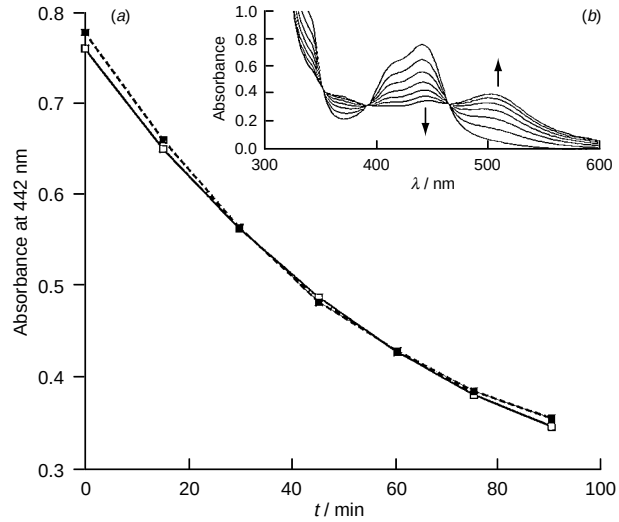

Fig. 4 (*a*) Absorbance at 442 nm of UV irradiated (λ = 436 nm) solutions of $Ru(bpz)_3^{2+}$ (□) without additive and (■) with SOD. (*b*) Variation in absorption spectra of $Ru(bpz)_3^{2+}$ in 5 mM phosphate buffer (pH 7.4) and 10 mM NaCl upon irradiation at 436 nm.

ruthenium complexes in the presence of Cu/Zn SOD partly depends on the redox potential of their excited state.

In conclusion, Cu/Zn SOD led to an unexpected enhancement of the photonuclease activity of $Ru(bpz)_3^{2+}$, which may result from an increase in singlet oxygen production. This unusual phenomenon may be correlated to the strongly oxidizing nature of this dye.

We thank Mr Alain Mari for his technical assistance with the EPR studies.

Notes and References

† E-mail: paillous@ramses.ups-tlse.fr

1 K. Kalyanasundaram, *Coord. Chem. Rev.*, 1982, **46**, 159.
2 J. M. Lehn, J. P. Sauvage and R. Ziessel, *Nouv. J. Chim.*, 1980, **4**, 355.
3 R. J. Crutchley and A. B. P. Lever, *J. Am. Chem. Soc.*, 1980, **102**, 7128.
4 J. M. Kelly, A. B. Tossi, D. J. McConnell and C. OhUigin, *Nucleic Acids Res.*, 1985, **13**, 6017.
5 H. Y. Mei and J. K. Barton, *Proc. Natl. Acad. Sci. USA*, 1988, **85**, 13 395.
6 M. B. Fleisher, K. C. Waterman, N. J. Turro and J. K. Barton, *Inorg. Chem.*, 1986, **25**, 3549.
7 J. M. Kelly, D. J. McConnell, C. OhUigin, A. B. Tossi, A. Kirsch-De Mesmaeker, A. Masschelein and J. Nasielski, *J. Chem. Soc., Chem. Commun.*, 1987, **24**, 1821.
8 A. B. Tossi and J. M. Kelly, *Photochem. Photobiol.*, 1989, **49**, 54.
9 P. J. Dandliker, R. E. Holmlin and J. K. Barton, *Science*, 1997, **275**, 1465.
10 P. Subramanian and G. J. Dryhurst, *J. Electroanal. Chem. Interfac. Electrochem.*, 1987, **224**, 137.
11 A. Kirsch De Mesmaeker, A. G. Orellana, J. K. Barton and N. J. Turro, *Photochem. Photobiol.*, 1990, **52**, 461.
12 J. P. Lecomte, A. Kirsch De Mesmaeker, J. M. Kelly, A. B. Tossi and H. Görner, *Photochem. Photobiol.*, 1992, **55**, 681.
13 C. Sentagne, J. C. Chambron, J. P. Sauvage and N. Paillous, *J. Photochem. Photobiol. B: Biol.*, 1994, **26**, 165.
14 L. Jacquet, J. M. Kelly and A. Kirsch-De Mesmaeker, *J. Chem. Soc., Chem. Commun.*, 1995, 913.
15 P. Vicendo, S. Mouysset and N. Paillous, *Photochem. Photobiol.*, 1997, **65**, 647.
16 M. M. Mossoba, Ionel Rosenthal, A. J. Carmichel and P. Riesz, *Photochem. Photobiol.*, 1984, **39**, 731.
17 M. A. Haga, E. S. Dodsworth, G. Eryavec, P. Seymour and A. B. P. Lever, *Inorg. Chem.*, 1985, **24**, 1091.

Received in Cambridge, UK, 20th January 1998; 8/00545A