Structural Variety of Copper(II)-Peroxide Adducts and Its Relevance to DNA Cleavage

Satoshi Nishino, Teruyuki Kobayashi, Mami Kunita, Sayo Ito and Yuzo Nishida

Institute for Molecular Science, Myodaijimachi Okazaki 444–8585, Japan

Z. Naturforsch. **54c,** 94–99 (1999); received August 4/October 16, 1998

Copper(II)-peroxide Adduct, DNA Cleavage, Effect of Peripheral Group

The reactivity of copper(II) compounds with several tetradentate ligands towards some spin-trapping reagents was studied in the presence of hydrogen peroxide. The compounds used in this study are roughly divided into two groups based on the reactivity towards 2,2,6,6-tetramethyl-4-piperidinol(and also 2,2,6,6-tetramethyl-4-piperidone), which are trapping agents for singlet oxygen, $^1O_2(^1\Delta_g)$; The A-group compounds exhibited a high activity to form the corresponding nitrone radical, which was detected by ESR spectroscopy, but corresponding activity of the B-group compounds was very low. The A-group compounds defined as above exhibited high activity for cleavage of DNA(supercoiled Form I) in the presence of hydrogen peroxide, yielding DNA Form II (relaxed circular) or Form III (linear duplex) under our experimental conditions ([Cu(II)]=0.1~0.5 mM). On the other hand, the B-group compounds effected complete degradation of the DNA (double-strand scission) under the same experimental conditions, formation of Form II or Form III DNA was negligible. Two different DNA cleavage patterns observed for A- and B-group compounds were elucidated by the different structural property of the copper(II)-peroxide adducts, which is controlled by the interaction through both DNA and the peripheral group of the ligand system.

Among the strategies available for chemotherapeutic treatment of cancer is the use of naturally occurring antitumor antibiotics (Neidle and Waring, 1993). These complex molecules create a variety of different types of DNA lesions, ranging from DNA cross-linking to strand scission. The antitumor agents whose mode of action entails DNA strand scission fall into separate categories. Those which generate a bifunctional carbon-based diradical cleaving moiety (Goldberg, 1993) and those which form activated oxygen species (Stubbe and Kozarich, 1987). Both types of antibiotics can create both single-strand breaks(ss-breaks) and double-strand breaks(ds-breaks) in duplex DNA. Double-strand breaks are thought to be biologically more significant than are ss-breaks's as sources of cell lethality, because they apparently are less readily restored by DNA repair mechanism (Steighner and Povirk, 1990).

Because of the biological and clinical importance of double-strand cleavage as a mode of cell lethality, we have investigated the design of a copper(II)-based transition metal compound which performs double-strand cleavage of DNA in a nonrandom fashion. A monofunctional transition metal-based double-stranded cleavage agent must produce four events; nick formation, binding at the nicked site, activation, and complementary strand scission to degrade DNA (Pamatong *et al.,*

Figure 1

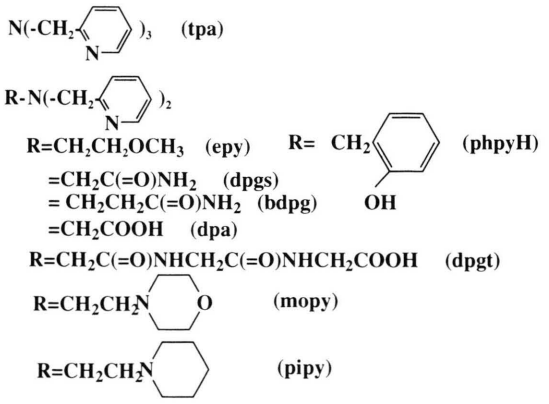


Fig. 1. Chemical structures of the ligands cited in this paper.

Reprint requests to Prof. Y. Nishida.
Fax: +81–564–55–5245.
E-mail: yuzo@ims.ac.jp

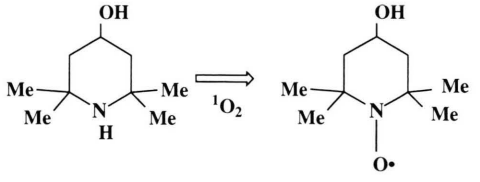
0939–5075/99/0100–0094 $ 06.00

1996). We have begun our study using square-planar copper(II)-containing metal compounds that include various peripheral groups, as shown in Fig. 1, and investigated the cleavage pattern of supercoiled DNA by copper(II) complexes in the presence of hydrogen peroxide; part of the present work has been already reported (Kobayashi *et al.,* 1996). In the course of this work, we have observed that some complexes show a high activity to nick the supercoiled DNA, yielding Form II (relaxed circular) or Form III (linear duplex) DNA (Micklos and Freyer, 1990), but some result in the complete degradation of the DNA(ds-breaks) under the same experimental conditions (Kobayashi *et al.,* 1996, 1998).

Experimental

Materials

The ligands used in this study are illustrated in Figure 1, and copper(II) compounds used in this study and structural properties of several compounds were already reported; Cu(tpa)$ClPF_6$ (Karlin *et al.,* 1982), Cu(dpgs)$ClClO_4$ (Okuno *et al.,* 1997), Cu(bdpg)Cl_2 (Okuno *et al.,* 1997), Cu(mopy)$ClClO_4$ (Kobayashi *et al.,* 1996), Cu(pipy)$ClClO_4$ (Kobayashi *et al.,* 1996), Cu(phpyH)$ClClO_4$ (Ito *et al.,* 1998) Cu(dpgt)$ClClO_4$ (Kobayashi *et al.,* 1998). Spin-trapping reagents, PBN (a-phenyl-N-*t*-butylnitrone, one of the spin-trapping reagents for $OH^{\bullet}$ radical) (Greenstock and Wiebe, 1982), 2,2,6,6-tetramethyl-4-piperidinol (abbreviated hereafter as TMPN), and 2,2,6,6-tetramethyl-4-piperidone, one of the spin-trapping agents for singlet oxygen, $^1O_2(^1D_g)$ (Lion *et al.,* 1976) (see the following figure), were purchased from Tokyo Kasei Co. DNA(pBR322 and ϕ174) were purchased from Wako Chemicals.



ESR spectral measurements

ESR spectra of the equivolume mixtures of a copper(II) complex(1/10~1/1000 M in 1/1 water/acetonitrile solution), hydrogen peroxide(1/10 M in 1/4 water/acetonitrile solution), and TMPN (1/10 M in 1/4=water/ acetonitrile solution) (or PBN(a-phenyl-N-*t*-butylnitrone; 1/10 M in 1/4=water/ acetonitrile solution) were measured with a JEOL ESR apparatus Model RE-2X using an X-band at 298 K. In our experiments, $[H_2O_2]/[Cu^{2+}]$= 10~100.

DNA cleavage by iron(III) complex and hydrogen peroxide systems

In a typical run, aqueous solution of the copper(II) complex (4 μl of 0.1–1.0 mmol dm^{-3}), DNA (4 μl of 0.1 mg per ml), tris buffer (3 μl of 0.1 mol dm^{-3}, pH=7.8), and H_2O_2 (4 μl of 0.1 mol dm^{-3}) were mixed and allowed to stand for 1 hour at 25 °C. The extent of DNA cleavage was assessed by analysis on 0.9% agarose gel containing ethidium(3,8-diamino-5-ethyl-6-phenylphenanthridinium) bromide (Micklos and Freyer, 1990). The bands were photographed with Polaroid 667 film, and the band intensities were quantified on an ATTO Densitograph Model AE-6920-M/W/V (for Mac). In this experiment, Cu(phpyH)$ClClO_4$ was not used because of its low solubility in water.

Results and Discussion

ESR Signal due to nitrone radical of TMPN(2,2,6,6-tetramethyl-4-piperidinol)

In this study we cannot detect the formation of PBN-OH in any solutions containing a copper(II)/H_2O_2 system investigated. No formation of the nitrone radical of TMPN was detected in the solution of Cu(bdpg)Cl_2 complex, H_2O_2, and TMPN (data not shown), and the similar facts are found for the compounds with (dpgs), (pipy), and (epy), etc. On the other hand, formation of the nitrone radical was detected for the compounds with (tpa) (see Fig. 2) and also (mopy), (phpyH) complexes. The signal intensities have increased with the time.

In our previous paper (Okuno *et al.,* 1997), we have investigated the formation of the oxygenated products of cyclohexane in the presence of a copper(II) compound and hydrogen peroxide, and reported that Cu(bdpg)Cl^+ exhibits the highest activity for oxygenation of cyclohexane, and concluded that its oxygenation activity should be due to facile formation of a peroxide adduct, adduct-I in the figure below, where the peroxide ion is activated through the electronic interaction with the amide group of the ligand system (bdpg) (Okuno *et al.,* 1997).

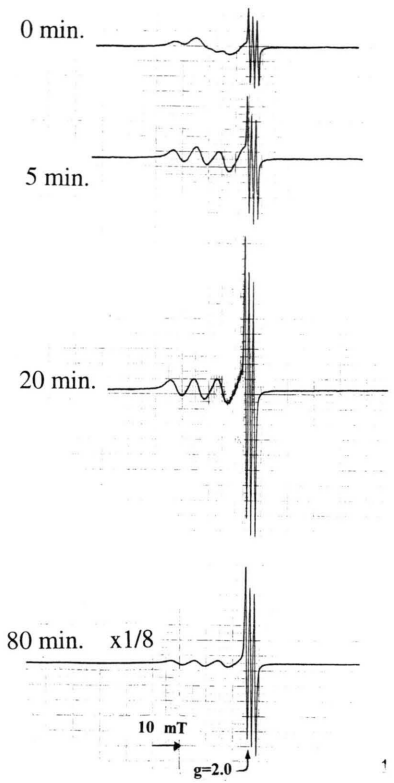
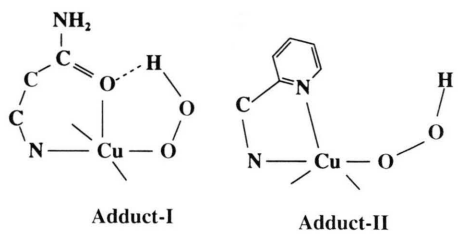


Fig. 2. Time course of ESR spectra of the solution containing Cu(tpa)$ClClO_4$(1/500 M in 1/1=water/acetonitrile), H_2O_2, and TMPN.



The negligible activity for the hydroxylation of cyclohexane by the complexes with (tpa), (mopy), (pipy), and other has been attributed to unfavorable formation and activation of the peroxide adduct (Okuno *et al.*, 1997) (see adduct-II; in these cases the complex has no peripheral group which promotes formation of a peroxide adduct through hydrogen bonding). The fact that Cu(bdpg)Cl^+, which exhibits high hydroxylation activity for the alkanes, shows no activity for formation of the nitrone radical of TMPN suggests that the nitrone radical formation is not due to the oxygenation reaction of TMPN.

In the formation of the nitrone radical, the difference in the activity between the (mopy)-complex (active) and (pipy)-complex (not active) is noteworthy, because both compounds have a very similar structure, except for the absence of ethereal oxygen in the (pipy)-ligand (Kobayashi *et al.*, 1996). Thus, it seems quite likely that the presence of the morpholine ring of Cu(mopy)Cl^+, i.e., presence of the ethereal oxygen in (mopy) ligand, is closely related with the formation of an activated peroxide adduct to generate the nitrone radical. As the TMPN is one of the spin-trapping reagents for singlet oxygen, it seems likely that reactivity of a peroxide adduct in the solution containing Cu(mopy)Cl^+ is similar to that of the singlet oxygen, and that structure of the peroxide adduct should be different from that of adduct-I.

The phenol group of Cu(phpyH)Cl^+ may also play a similar role in the formation of the peroxide adduct. In addition to this, we have observed that ESR spectrum of the solution containing Cu(phpyH)Cl^+ and H_2O_2 is different from that in the mixture containing Cu(phpyH)Cl^+, H_2O_2, and TMPN, i.e., the average value of hyperfine splitting due to nuclear spin of ^{63,65}Cu is 7.66 mT in the solution containing Cu(phpyH)Cl^+ and H_2O_2, but it increases to 8.86 mT when TMPN is added to the above solution (see Fig. 3, **A**). This indicates that formation of a reactive copper(II)-peroxide adduct occurs only when TMPN is added to the system.

A similar result was obtained with the Cu(tpa)Cl^+ complex. As shown in Fig. 3, **B**), the ESR signal due to the copper(II) ion changes with the addition of TMPN to the solution; appearance of clear hyperfine lines due to nuclear spin of ^{63,65}Cu(see trace **d** in Fig. 3, **B**) occurred on the addition of TMPN. This also suggests that TMPN is playing an important role in formation of a reactive peroxide adduct in this case, because in the case of Cu(tpa)Cl^+, formation of a peroxide adduct is unfavorable due to no interaction with the ligand system, as illustrated in adduct-II in the figure above (Okuno *et al.*, 1997). No remarkable change was observed in the ESR spectra of the solution containing Cu(bdpg)Cl^+ on the addition of TMPN (data not shown). This demonstrates that in the cases of (tpa)-, (mopy)-, and H(phpy)-

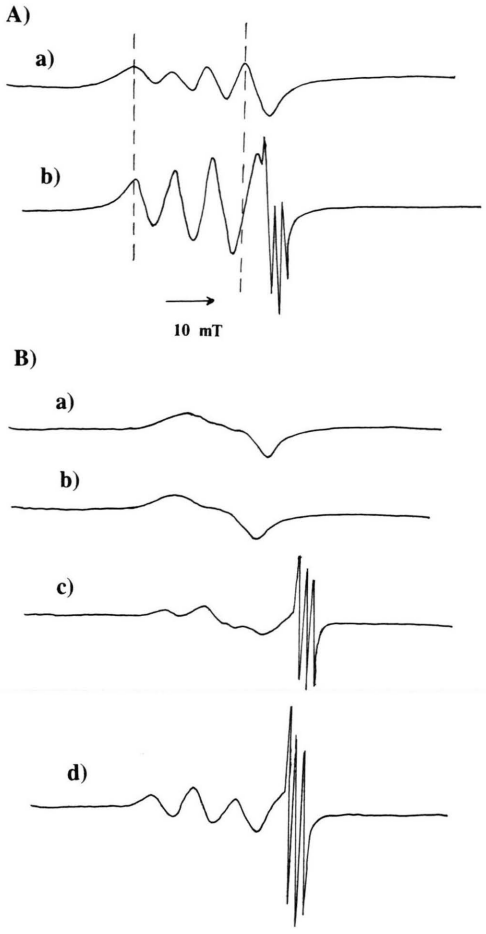
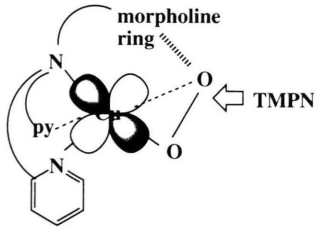


Fig. 3. ESR spectra of solution containing **A**) Cu(phpyH)$ClClO_4$(1/500 M in 1/1=water/acetonitrile) at 298 K.
a: Cu(phpyH)$ClClO_4$ and H_2O_2
b: Cu(phpyH)$ClClO_4$, H_2O_2, and TMPN (5 minutes after addition of TMPN) and **B**) Cu(tpa)$ClClO_4$(1/250 M in 1/1=water/acetonitrile)
a: Cu(tpa)$ClClO_4$
b: Cu(tpa)$ClClO_4$ and H_2O_2,
c: Cu(tpa)$ClClO_4$, H_2O_2, and TMPN (0 minute after addition of TMPN)
d: Cu(tpa)$ClClO_4$, H_2O_2, and TMPN (10 minutes after addition of TMPN)

compounds, the formation of a reactive peroxide adduct is promoted through the interaction with both TMPN and the peripheral group, such as morpholine, phenol, or pyridine ring (see the figure below, in this case the coordination of the peroxide ion is assumed to be of pseudo side-on type).



DNA cleavage by Copper(II)/H_2O_2 systems

As shown in Fig. 4, the DNA cleavage pattern by the copper(II)/H_2O_2 systems depends on the copper(II) complex used (Kobayashi *et al.*, 1996). It should be noted here that the complexes which show high activity for formation of nitrone radical of TMPN, (tpa)- and (mopy)-complexes, give Form II or III DNA in the reaction with Form I DNA (supercoiled form) (see lanes 7 and 9 in Fig. 4, **A**), but other compounds which evidence low activity for the TMPN-radical formation, for example (bdpg)- or (dpgs)-compounds, effect complete degradation of DNA under the same conditions (see lanes 5 and 11 in Fig. 4, **B**). This suggests that the difference in the DNA cleavage reaction observed for $Cu(tpa)Cl^+$ and $Cu(bdpg)Cl^+$ should be due to the difference in the reactivity of the peroxide adduct formed in the reaction course. The different DNA cleavage patterns observed for (mopy)- and (pipy)-complex (Kobayashi *et al.*, 1996) can be elucidated by the same discussion as described above. These clearly indicate that there are two pathways in the DNA cleavage by a copper(II) compound in the presence of hydrogen peroxide.

The DNA cleavage by the Fe-bleomycins has been investigated by Stubbe *et al.* (Stubbe and Kozarich, 1987; McGall *et al.*, 1992). They have reported that there are two pathways in the DNA cleavage reaction, and thus our present result is consistent with their results, but the origin for the presence of two pathways has never been discussed. It seems quite likely that a cationic copper(II)-peroxide adduct may bind to the DNA through electrostatic and hydrophobic interactions (Wu *et al.*, 1996; C.-Cortes *et al.*, 1997). According to the results obtained in this study, it seems quite likely that the structure of the peroxide adducts in the (tpa)- and (mopy)-compounds are different from that of the (bdpg)-complex, and the reactive

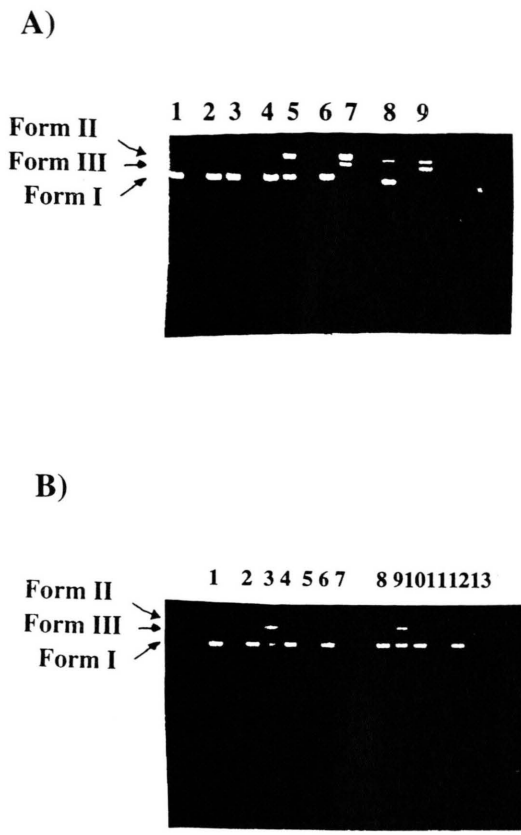
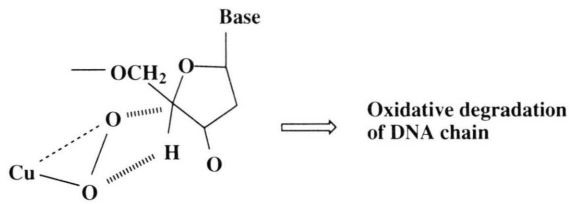
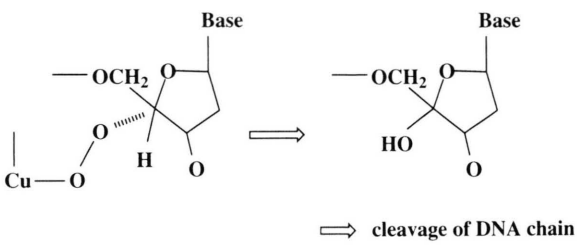


Fig. 4. electrophoresis containing DNA(pBR322), copper(II) complex and hydrogen peroxide.
A: lane 1, DNA alone; lane 2, $CuCl_2 \cdot 2H_2O$ (0.1 mM solution); lane 3, $CuCl_2 \cdot 2H_2O$ (0.1 mM solution) and H_2O_2; lane 4, Cu(dpa) Cl(0.1 mM solution); lane 5, Cu(dpa) Cl (0.1 mM solution) and H_2O_2; lane 6; Cu(tpa) $ClClO_4$ (0.1 mM solution) and H_2O_2; lane 7, Cu(tpa) $ClClO_4$ (0.1 mM solution) and H_2O_2; lane 8, Cu(tpa) $ClClO_4$ (0.5 mM solution); lane 9, Cu(tpa)$ClClO_4$ (0.5 mM solution) and H_2O_2; and **B**: lane 1, DNA alone; lane 2, Cu(bdpg)$ClClO_4$ (0.1 mM solution); lane 3, Cu(bdpg)$ClClO_4$ (0.1 mM solution) and H_2O_2; lane 4, Cu(bdpg)$ClClO_4$ (0.5 mM solution); lane 5, Cu(bdpg) $ClClO_4$ (0.5 mM solution) and H_2O_2; lane 6; Cu(bdpg) $ClClO_4$ (1.0 mM solution); lane 7, Cu(bdpg)$ClClO_4$ (1.0 mM solution) and H_2O_2; lane 8, Cu(dpgs)$ClClO_4$ (0.1 mM solution); lane 9, Cu(dpgs)$ClClO_4$(0.1 mM solution) and H_2O_2; lane 10, Cu(dpgs)$ClClO_4$ (0.5 mM solution); lane 11, Cu(dpgs)$ClClO_4$ (0.5 mM solution) and H_2O_2; lane 12, Cu(dpgs)$ClClO_4$ (1.0 mM solution); lane 13, Cu(dpgs)$ClClO_4$ (1.0 mM solution) and H_2O_2.

peroxide adduct of the former two compounds can form only when they are interacting with DNA. If the peroxide adduct in the mixture with DNA is similar to that in the mixture with added TMPN, the peroxide adduct of the former two compounds may behave as a singlet oxygen, inducing oxidative cleavage (McGall *et al.*, 1992) of the DNA chain through formation of an intermediate shown below; this also gives ss-break. If the oxidatively degraded DNA fragment has no site for copper(II) binding, especially a phosphate back-bone, the cleavage of DNA by the copper(II) complex does not proceed further, yielding only an ss-break.



Present results strongly suggest that in this case the Cu(bdpg)Cl^+/H_2O_2 system, DNA cleavage occurs through hydroxylation of the carbon atom at 4'-position(ss-break) through the intermediate formation shown below, associated with heterolytic cleavage of the peroxide O-O bond into atomic oxygen and oxo oxygen, and insertion of the atomic oxygen into C-H bond at C4'-position (Nishida *et al.*, 1997); formation of the intermediate and heterolysis of the O-O bond is promoted by the interaction with the sugar moiety of DNA (Nishida *et al.*, 1997).



After this reaction, if the copper(II) complex can reside at the nicked site, and there occurs another DNA cleavage through formation of a new peroxide adduct, leading to ds-break, as observed. The DNA cleavage catalyzed by other compounds, such as Cu(dpgs)Cl^+, may be explained by the similar way.

In conclusion, it seems quite apparent that there are two pathways in DNA cleavage reaction cata-

lyzed by the copper(II)/H_2O_2 system and the cleavage reaction is highly controlled by the reactivity of a peroxide adduct formed in the reaction course. These may give an important information on the elucidation of DNA cleavage mechanism by the metal compounds, and also give many hints to design new chemotherapeutic agents.

C.-Cortes J., Sugiyama H., Ikudome K., Saito I. and Wang H.-J. (1997), Interactions of deglycosylated cobalt(III)-peplemycin (green form) with DNA based on NMR structural studies. Biochemistry **36**, 9995–10005.

Goldberg I. H. (1993), Molecular Aspects of Anticancer Drug-DNA Interactions., Vol. **1** (S. Neidle and M. Waring Eds.). CRC Press, Boca Raton, FL.

Greenstock G. L. and Wiebe R. H. (1982), Substituent effects in kinetic analysis of free radical reaction with nitrone spin traps, Can. J. Chem., **60,** 1560–1564.

Ito S., Nishino S., Ohba S. and Nishida Y. (1998), Copper(II) compound with long Cu(II)-phenolic oxygen atom bonding as galactose oxidase model, Polyhedron, **17**, 1637–1642.

Karlin K. D., Hayes J. C., Hutchington J. P. and Zubieta J. (1982), Tetragonal vs. trigonal coordination in copper(II) compounds with tripodal ligands. Inorg. Chem. **21,** 4106–4108.

Kobayashi T., Ito S., Ohba S. and Nishida Y. (1996), Relaxation of pBR322 Form I DNA by copper(II) complex and hydrogen peroxide. Chem. Lett. **1996**, 347–348.

Kobayashi T., Okuno T., Suzuki T., Kunita M., Ohba S. and Nishida Y. (1998), DNA degradation by the copper(II) complex with tripodal-ligands containing peptide group. Polyhedron **17**, 1553–1559.

Lion Y., Delmeles M. and van de Vorst A. (1976), New method of detecting singlet oxygen production. Nature **263,** 442–443.

McGall G. H., Rabow L. E., Ashley G. W., Wu S. H., Kozarich J. W. and Stubbe, J. (1992), New insight into the mechanism of base propenal formation during Bleomycin-mediated DNA degradation. J. Am. Chem. Soc. **114,** 4958–4967.

Micklos, D. A. and Freyer, G. A. (1990), DNA Science. Cold Spring Harbour Laboratory Press, New York.

Neidle S. and Waring M. (1993), Molecular Aspects of Anticancer Drug-DNA Interactions., Vol. **1** (S. Neidle and M. Waring Eds.). CRC Press, Boca Raton, FL.

Nishida Y., Ito S., Okuno T. and Ohba S. (1997), New insight into reaction of iron(III)peroxide adduct with alkanes; an alternative model for cytochrome P-450 and methane monooxygenase. Z. Naturforsch. **52C**, 615–622.

Okuno T., Ohba S. and Nishida Y. (1997), Oxidation of cyclohexane with hydrogen peroxide catalyzed by copper(II) complexes conatining N,N-bis(2-pyridylmethyl)-b-alanineamide ligands. Polyhederon **16,** 3765–3774.

Pamatong F. V., Detmer III C. A. and Bocarsly J. R. (1996), Double-strand cleavage of DNA by a monofunctional transition metal cleavage agent. J. Am. Chem. Soc. **118,** 5339–5345.

Steighner R. J. and Povirk L. F. (1990), Bleomycin-induced DNA lesin at mutational hot spots; Implications for the mechanism of double-strand cleavage. Proc. Natl. Acad. Sci. U. S. A. **87**, 8350–8354.

Stubbe J. and Kozarich J. W. (1987), Mechanisms of Bleomycin-induced DNA degradation. Chem. Rev. **87,** 1107–1136.

Wu W., Vanderwall D. E., Turner C. J., Kozarich J. W. and Stubbe J. (1996), Solution structure of Co-Bleomycin A2 green complexed with d(CCAGGCCTGG). J. Am. Chem. Soc. **118**, 1281–1294.