DALTON COMMUNICATION

8-Hydroxy-2′-deoxyguanosine formation and DNA damage induced by a dinuclear manganese(IV) complex and hydrogen peroxide

Teruyuki Kobayashi, Kazuyoshi Tsuchiya and Yuzo Nishida *

Department of Chemistry, Faculty of Science, Yamagata University, Yamagata, 990, Japan

The dinuclear manganese(IV) complex $[Mn_2O_3L_2][PF_6]_2$ exhibits high activity for 8-hydroxy-2′-deoxyguanosine formation and relaxation of pBR322 form I DNA in the presence of H_2O_2, where L = 1,4,7-trimethyl-1,4,7-triazacyclononane; the active species is postulated to be a unidentate peroxide adduct of the manganese(IV) complex.

Chemical methods for nicking DNA have several uses including probing structural variations in nucleic acids,[1,2] identifying binding sites of DNA ligands,[1,3] designing artificial nuclease[4-6] and serving as chemotherapeutic agents, such as bleomycin.[7] Co-ordination compounds have also been used to promote such DNA cleavage, *e.g.* $[Cu(phen)_2]^+$ (phen = 1,10-phenanthroline),[8] $[Fe(edta)]^{2-}$ (edta = ethylenedinitrilotetra-acetate) as a free complex[9,10] or linked to DNA binding agents[11,12] and agents based on oxoruthenium(IV).[13]

A manganese(II)–bleomycin compound can degrade DNA in the presence of hydrogen peroxide,[14] however the precise mechanism is not known. We have observed that a dinuclear manganese(IV) compound with three oxo bridges, $[Mn_2O_3L_2]^{2+}$,[15] exhibits high activity for nicking the plasmid DNA (pBR322) in the presence of hydrogen peroxide, where L = 1,4,7-trimethyl-1,4,7-triazacyclononane. We also found that this complex shows high ability for formation of 8-hydroxy-2′-deoxyguanosine (hguo) in the presence of H_2O_2 and 2′-deoxyguanosine (dG). Based on these facts, we have postulated an active species for hydroxylation of dG and double-strand breaks in DNA. This complex has been reported to be highly active for epoxidation of alkenes and oxidation of polyphenolic compounds in the presence of H_2O_2.[16]

2′-Deoxyguanosine (dG) was from Tokyo Kasei Co., DNA (pBR322) from Wako Chemicals. The complex $[Mn_2O_3L_2][PF_6]_2$ was prepared according to the published method.[15] The HPLC measurements were made using a Cosmosil $5C_{18}$-MS packed column (4.6 × 150 mm), and other experimental conditions were as reported by Kasai and Nishimura.[17] To a mixture (20 cm^3) of complex (60 mg) and dG (20 mg) was added H_2O_2 (5 cm^3 of 0.1 mol dm^{-3} solution), and the formation of hguo was examined by HPLC. The DNA double-strand breakage assay was carried out as described by Micklos and Freyer.[18] Aqueous solutions of the complex (4 μl of 0.5 or 1.0 mmol dm^{-3}), DNA (4 μl of 0.1 μg per μl), tris(hydroxymethyl)aminomethane (Tris) buffer (3μl of 0.1 mol dm^{-3}) and H_2O_2 (4 μl of 10 mmol dm^{-3}) were mixed and allowed to stand for 1 h 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. The bands were photographed with Polaroid 667 film. The catalase-like function of the complex was investigated in a similar manner to that reported at 25 °C.[19]

Fig. 1 (lane 1) shows DNA alone as a control. Incubation of DNA with the complex for 1 h induced little, if any, damage (lanes 2 and 4). Incubation of DNA with the complex and H_2O_2 caused DNA strand scission (lane 3); the conversion of form I (supercoiled) to II (relaxed circular) occurred. A higher concentration of the complex resulted in more damage, yielding forms II and III (linear duplex) (lane 5).[18] Evolution of dioxygen from the mixture (water) of the complex and H_2O_2 was monitored but it was found that this complex exhibits no catalase-like activity at room temperature.

Fig. 2 shows the time course of the formation of hguo in the presence of the complex, dG and H_2O_2. As the di-μ-oxo di-nuclear manganese(III, IV) complex $[Mn_2O_2(tpya)_2]^{3+}$ [tpya = tris(2-pyridylmethyl)amine][19] exhibits no formation of hguo under the same experimental conditions, the activity of the present complex is noteworthy.

Since the present dinuclear complex shows high activity for formation of hguo and DNA damage in the presence of H_2O_2, it seems reasonable to consider that the formation of a peroxide adduct of Mn^{IV} may occur. Two possible structures are shown in **A** and **B**, of the side-on and end-on types, respectively. Wieghardt and co-workers[20] have succeeded in obtaining a dinuclear managanese(IV)–peroxide adduct of type **A**, and

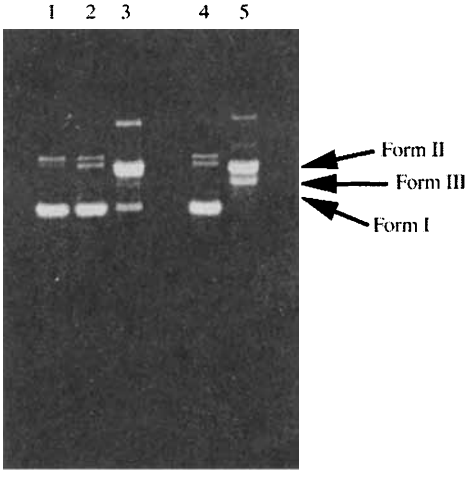
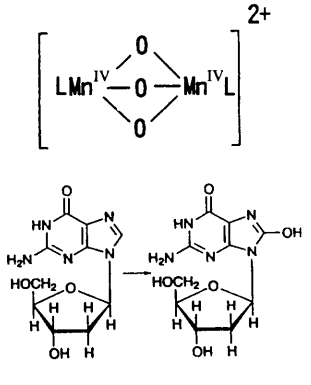


Fig. 1 Cleavage of DNA by the complex and H_2O_2. Lanes: 1, DNA (pBR322) alone; 2, 0.5 mmol dm^{-3} complex; 3, 0.5 mmol dm^{-3} complex and H_2O_2; 4, 1 mmol dm^{-3} complex; 5, 1 mmol dm^{-3} complex and H_2O_2



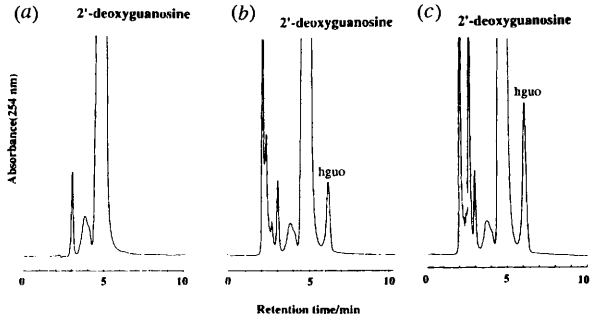


Fig. 2 High-performance liquid chromatography of the mixture of complex, dG and H_2O_2: (*a*) Mn^{IV} and dG; (*b*) Mn^{IV}, dG and H_2O_2 (measured immediately after addition of H_2O_2); (*c*) Mn^{IV}, dG and H_2O_2 (2 h after addition of H_2O_2)

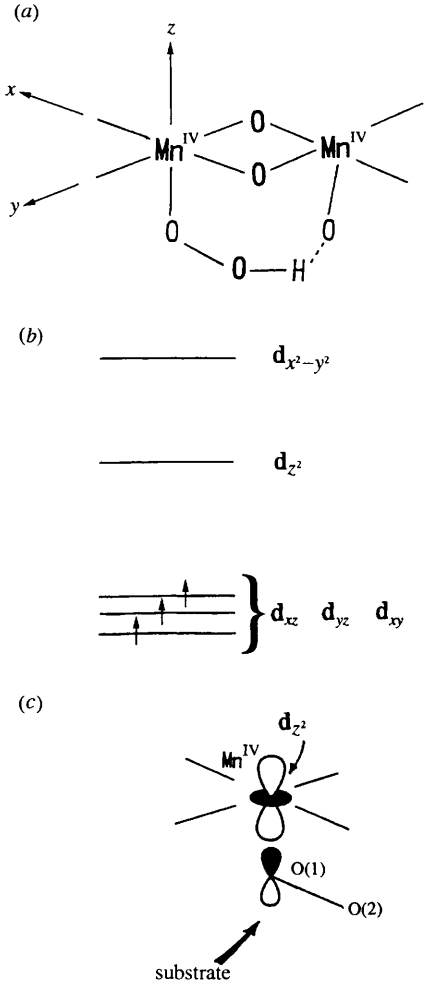
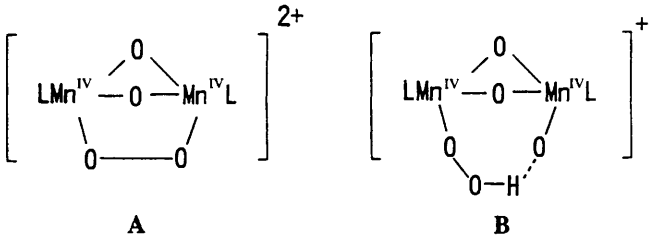
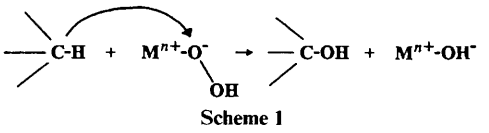


Fig. 3 (*a*) Possible geometry of the type **B** intermediate. (*b*) d-Orbital splitting scheme for this intermediate and electronic configuration of Mn^{IV}. (*c*) Attack of occupied orbital of substrate on d_{z^2} orbital of Mn^{IV}



reported that it is unstable at room temperature, decomposing in aqueous solution with evolution of dioxygen. Since we have observed that the present complex exhibits no catalase-like



Scheme 1

function in the presence of H_2O_2, it seems unlikely that the adduct **A** is present in solution. Previously we reported that a metal–peroxide adduct with type **B** configuration exhibits activity for the oxygenation of organic compounds, yielding hydroxylated products;[21–23] in the present case the d orbital which interacts with the oxygen of H_2O_2 is vacant [Fig. 3(*a*) and (*b*)], leading to facile interaction between it and the occupied orbital of the substrate, as shown in Fig. 3(*c*). Thus, the peroxide adduct **B** acts as an electrophile (two-electron acceptor), and interacts with the electrons in the bonding orbital of the organic substrate, to give the oxygenated product with concomitant, concerted, cleavage of the O–O bond,[21–24] as shown in Scheme 1.

Thus, the formation of hguo catalysed by the complex and H_2O_2 may be reasonably elucidated in terms of a peroxide adduct of type **B**. Since the structure of an 'activated bleomycin' has recently been confirmed to be an iron(III)–hydroperoxide adduct,[25] which corresponds to the peroxide adduct **B**, the present results may provide important information on the mechanism of DNA strand scission.

References

1 P. E. Neilsen, *J. Mol. Recognit.*, 1990, **3**, 1.
2 A. M. Pile and J. K. Barton, *Prog. Inorg. Chem.*, 1990, **38**, 413.
3 T. D. Tullius, in *Metal–DNA Chemistry*, ed. T. D. Tullius, American Chemical Society, Washington, DC, 1989, vol. 402, pp. 1–23.
4 B. C. F. Chu and L. E. Orgel, *Proc. Natl. Acad. Sci. USA*, 1985, **82**, 963.
5 P. B. Dervan, *Nature (London)*, 1992, **359**, 87.
6 L. Perrousult, U. Asseline, C. Rivalle, N. T. Thuong, E. Bisagni, C. Giovannangeli, C. T. Doan and C. Helene, *Nature (London)*, 1990, **344**, 358.
7 J. Stubbe and K. W. Kozarich, *Chem. Rev.*, 1987, **87**, 1107.
8 D. C. Sigman, *Acc. Chem. Res.*, 1986, **19**, 180.
9 T. D. Tullius and B. A. Dombroski, *Science*, 1986, **230**, 679.
10 T. D. Tullius and B. A. Dombroski, *Proc. Natl. Acad. Sci. USA*, 1986, **83**, 5469.
11 R. P. Hertberg and P. B. Dervan, *J. Am. Chem. Soc.*, 1982, **104**, 313.
12 R. P. Hertberg and P. B. Dervan, *Biochemistry*, 1984, **23**, 3934.
13 N. Gupta, N. Grover, G. A. Neyhart, P. Sigha and H. H. Thorp, *Inorg. Chem.*, 1993, **32**, 310.
14 R. M. Burger, J. H. Freedman, S. B. Horwitz and J. Peisach, *Inorg. Chem.*, 1984, **23**, 2215.
15 K. Wieghardt, U. Bossek, B. Nuber, J. Weiss, J. Bonvoisin, M. Corbella, S. E. Vitols and J. J. Girerd, *J. Am. Chem. Soc.*, 1988, **110**, 7398.
16 R. Hage, J. E. Ibung, J. Kerschner, J. H. Koek, E. L. M. Lempers, R. J. Martens, U. U. S. Racherla, S. W. Russell, T. Swarthoff, M. R. P. van Vllet, J. B. Warnaar, L. van der Wolf and B. Krijnen, *Nature (London)*, 1994, **369**, 637.
17 H. Kasai and S. Nishimura, *Nucleic Acids Res.*, 1984, **12**, 2137.
18 D. A. Micklos and G. A. Freyer, *DNA Science*, Cold Spring Harbor Laboratory Press, New York, 1990.
19 Y. Nishida, T. Akamastu, K Tsuchiya and M. Sakamoto, *Polyhedron*, 1994, **14**, 2251.
20 U. Bossek, T. Weyhermuller, K. Wieghardt, B. Nuber and J. Weiss, *J. Am. Chem. Soc.*, 1990, **112**, 6387.
21 Y. Nishida, H. Itoh and A. Yamazaki, *Polyhedron*, 1994, **14**, 2473.
22 Y. Nishida and S. Ito, *Z. Naturforsch., Teil C*, 1995, **50**, 571.
23 Y. Nishida and S. Ito, *Z. Naturforsch., Teil C*, 1995, **50**, 205; *J. Chem. Soc., Chem. Commun.*, 1995, 1211.
24 Y. Nishida, S. Takahashi, I. Watanabe, A. Yamazaki, H. Itoh and M. Sakamoto, *Polyhedron*, 1994, **14**, 1539.
25 J. W. Sam, X.-J. Tang and J. Peisach, *J. Am. Chem. Soc.*, 1994, **116**, 5250.

Received 5*th March* 1996; *Paper* 6/01563H