J|A|C|S
COMMUNICATIONS

Published on Web 09/21/2004

DNA Cleavage Induced by Thermal Electron Transfer from a Dimeric NADH Analogue to Acridinium Ions in the Presence of Oxygen

Shunichi Fukuzumi,* Kazusa Yukimoto, and Kei Ohkubo

Department of Material and Life Science, Graduate School of Engineering, Osaka University, and CREST, Japan Scicence and Technology Agency (JST), Suita, Osaka 565-0871, Japan

Received May 24, 2004; E-mail: fukuzumi@chem.eng.osaka-u.ac.jp

Photocleavage of DNA has attracted considerable interest because of the biological significance of DNA damage and repair.[1−6] A number of synthetic DNA cleavage agents that are activated photochemically have been developed. Hydroperoxides are typical sources of hydroxyl radicals under photoirradiation, leading to DNA cleavage.[2] Fullerenes are also used as DNA photocleavage agents with NADH and oxygen.[7] Photoinduced electron transfer from NADH to fullerenes in the presence of O_2 results in formation of superoxide anion ($O_2^{\bullet-}$).[7] Although $O_2^{\bullet-}$ itself does not cause DNA damage,[8] hydroxyl radicals are produced in photoinduced decomposition of hydrogen peroxide that is formed by dismutation of $O_2^{\bullet-}$.[7] Thus, any agent that is capable of reducing O_2 to $O_2^{\bullet-}$ may be a potential DNA photocleaver. However, there has been no report on thermal DNA cleavage using an agent which can produce $O_2^{\bullet-}$ without photoirradiation.

We report herein that acridinium ions, which can intercalate to DNA,[9] can act as thermal DNA cleavers in the presence of a dimeric NADH analogue and O_2 in an aqueous solution. The formation of $O_2^{\bullet-}$ in the present system is confirmed by ESR detection. The mechanism of acridinium ion-catalyzed thermal reduction of O_2 to $O_2^{\bullet-}$ by a dimeric NADH analogue is clarified on the basis of the detailed kinetic study.

A dimeric NADH analogue [a dimeric *N*-benzyldihydronicotinamide, $(BNA)_2$] is known to act as a two-electron donor in electron-transfer reactions with electron acceptors.[10] Initial electron transfer from $(BNA)_2$ to an electron acceptor (A) generates the dimer radical cation [$(BNA)_2^{\bullet+}$] and the electron acceptor radical anion ($A^{\bullet-}$). This step is followed by a fast cleavage of the C−C bond of the dimer to produce $BNA^{\bullet}$ and BNA^+. The second electron transfer from $BNA^{\bullet}$ to A should be by far faster than the first electron transfer, since $BNA^{\bullet}$ is a much strong reductant than $(BNA)_2$.[10,11]

The rates of electron transfer from $(BNA)_2$ to 10-methylacridinium ion ($AcrH^+$) and other electron acceptors were followed by monitoring the decay of the absorbance at 360 nm in 5 mM Tris-HCl buffer (pH 7.0) containing 20% DMSO.[12] The rate obeyed second-order kinetics: first-order with respect to each reactant concentration. The rate of photoinduced electron transfer from $(BNA)_2$ to the triplet excited state of 5,10,15,20-tetrakis(1-methylpyridinium-4-yl)porphyrin-*p*-toluenesulfonate (^{3}TMPyP*) was determined from the decays in the triplet−triplet absorption by laser flash photolysis. The observed second-order rate constants of electron transfer increase with increasing the one-electron reduction potentials (E^0_{red}) of electron acceptors[13] to approach a diffusion-limited value, as shown in Figure 1. This is a typical feature of outer-sphere electron-transfer processes.[14,15] Electron transfer from $(BNA)_2$ to $AcrH^+$ also takes place in the presence of DNA, although the rate constant in the presence of DNA becomes smaller than that in the absence of DNA (Figure 1) due to the intercalation of $AcrH^+$ with DNA.

The rate of electron transfer from $(BNA)_2$ to $AcrH^+$ was not affected by the presence of O_2. In the presence of O_2, formation of

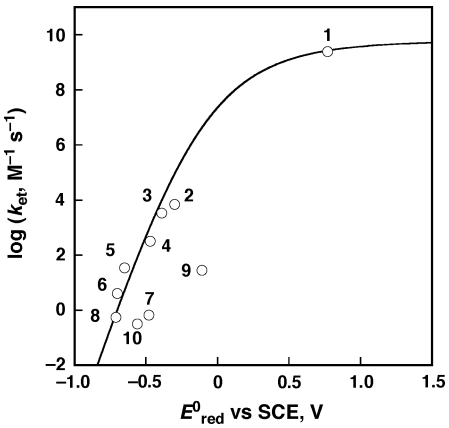
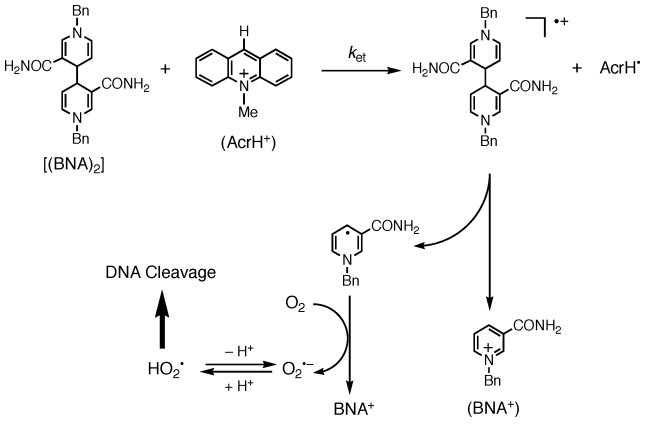


Figure 1. Plot of logarithm of the rate constants (log k_{et}) of electron-transfer reactions of $(BNA)_2$ with various electron acceptors at 298 K in 5 mM Tris-HCl buffer (pH 7.0) containing 20% DMSO vs the one-electron reduction potentials of the oxidants (E^0_{red}). 1, ^{3}TMPyP*; 2, $AcrH^+$; 3, *p*-benzoquinone; 4, *p*-toluquinone; 5, 2,5-dimethyl-*p*-benzoquinone; 6, 9-isopropyl-10-methylacridinium ion ($AcrPr^{i+}$); 7, 9-benzyl-10-methylacridnium ion; 8, tetramethyl-*p*-benzoquinone; 9, DNA-intercalated $AcrH^+$; 10, DNA-intercalated $AcrPr^{i+}$. The curve is drawn using the Rehm−Weller relationship for photoinduced electron transfer,[15] in which the one-electron oxidation potential of $(BNA)_2$ was determined as −0.18 V vs SCE.[13]

$O_2^{\bullet-}$ in the electron-transfer reaction from $(BNA)_2$ to $AcrH^+$ is confirmed directly by ESR. The rate-determining electron transfer from $(BNA)_2$ to $AcrH^+$ is followed by rapid C−C bond cleavage of $(BNA)_2^{\bullet+}$ to produce $BNA^{\bullet}$, which can readily reduce O_2 to $O_2^{\bullet-}$ (Scheme 1). The produced $O_2^{\bullet-}$ is detected by ESR, observed in frozen CH_3COOH/KOH buffer (pH 5.0) at 123 K after the reaction of $(BNA)_2$ with $AcrH^+$ at 298 K, as shown in Figure 2, where the observed ESR signal with $g_{\parallel} = 2.010$ and $g_{\perp} = 2.005$ is diagnostic of $O_2^{\bullet-}$.[16] No ESR signal due to $O_2^{\bullet-}$ was observed when $(BNA)_2$ was replaced by the monomer form, i.e., *N*-benzyl-1,4-dihydronicotinamide (BNAH), which undergoes hydride transfer with $AcrH^+$ rather than electron transfer.[17]

Scheme 1



10.1021/ja046955p CCC: $27.50

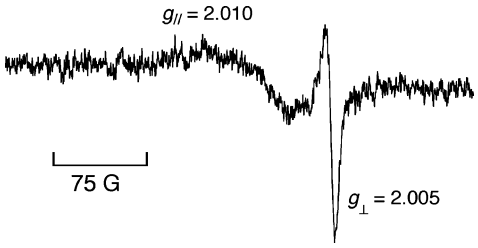


Figure 2. ESR spectrum of $O_2^{\bullet-}$ generated in the reaction of $(BNA)_2$ (1.0×10^{-2} M) with $AcrH^+$ (1.0×10^{-2} M) in 10 mM CH_3COOH/KOH buffer (pH 5.0) at 298 K and measured at 123 K.

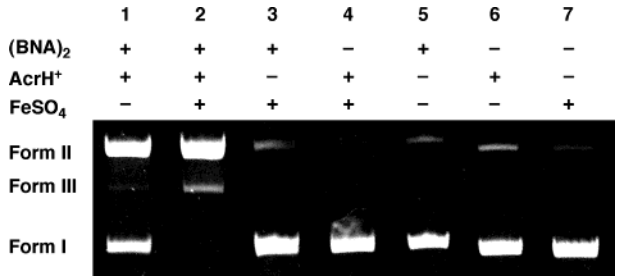


Figure 3. Agarose gel electrophoresis of cleavage of supercoiled pBR322 DNA (7.4×10^{-6} M) in the reactions of $(BNA)_2$ (2.0×10^{-3} M) with $AcrH^+$ (1.0×10^{-3} M) in the absence and in the presence of $FeSO_4$ (5.0×10^{-5} M) in oxygen-saturated 10 mM CH_3COOH/KOH buffer (pH 5.0) at 298 K. Each lane shows the result of the presence (+) and absence (−) of each component.

Since $O_2^{\bullet-}$ is produced in the electron-transfer reaction from $(BNA)_2$ to $AcrH^+$ in the presence of oxygen, we examined the DNA-cleavage activity of $AcrH^+$ in the presence of $(BNA)_2$ and O_2 using the widely used assay with pBR 322 supercoiled DNA.[7] Figure 3 shows that $AcrH^+$ can cleave DNA (supercoiled form I) to afford the nicked form II in the presence of $(BNA)_2$ and O_2 at pH 5.0 (lane 1). The agarose gel electrophoresis was performed after 1 h of reaction. At pH 8.0, DNA cleavage was observed only after prolonged reaction time. When $FeSO_4$ (5.0×10^{-5} M) is added to the $(BNA)_2-AcrH^+-O_2$ system after the reaction, the DNA cleavage is significantly accelerated to produce form III when no form I is left (lane 2). Without $AcrH^+$ or $(BNA)_2$, no or little cleavage is observed in the absence or presence of $FeSO_4$ (lane 3−7), showing sharp contrast with the case of the $(BNA)_2-AcrH^+-O_2$ system. When $(BNA)_2$ was replaced by BNAH, no DNA cleavage was observed under otherwise the same experimental conditions, when no $O_2^{\bullet-}$ was formed (vide supra). Thus, DNA is cleaved only in the presence of both $(BNA)_2$ and $AcrH^+$ with O_2, when $O_2^{\bullet-}$ is actually formed (Figure 2). The efficient DNA cleavage at pH 5.0 as compared with that at pH 8.0 indicates that $HO_2^{\bullet}$, which is in equilibrium with $O_2^{\bullet-}$, is the actual reactive species for the DNA cleavage, since the pK_a value of $HO_2^{\bullet}$ is 4.9.[18]

To examine the actual role of $O_2^{\bullet-}$ in the DNA cleavage, the DNA cleavage activity of the $(BNA)_2-AcrH^+-O_2$ system was examined in the presence of the superoxide dismutase (SOD) or catalase at pH 5.0. Addition of SOD shows a significant inhibitory effect on the DNA cleavage (see Supporting Information). Once $O_2^{\bullet-}$ is formed, H_2O_2 is formed by disproportionation of $HO_2^{\bullet}$, which is in equilibrium with $O_2^{\bullet-}$ at pH 5.0.[17] Addition of the H_2O_2-destroying enzyme catalase also significantly inhibits the DNA cleavage (Supporting Information). The OH radical is known as one of the most noxious reactive oxygen species, which induces the DNA cleavage.[19] The $OH^{\bullet}$ radical can be produced in a trace-metal-dependent Fenton reaction of H_2O_2, as reported for the DNA cleavage by the antitumor antibiotic leinamycin.[20] In the presence of $FeSO_4$, such a reaction is significantly accelerated, as shown in Figure 3 (lane 2).[21] Thus, the actual reactive species for the thermal DNA cleavage in the aqueous $(BNA)_2-AcrH^+-O_2$ system may be hydroxyl radical as well as $HO_2^{\bullet}$.

In conclusion, acridinium ions (DNA intercalators) can act as thermal DNA cleavers via *thermal* electron transfer from a dimeric NADH analogue to acridinium ions, followed by the electron-transfer reduction of O_2 to $O_2^{\bullet-}$ by the resulting $BNA^{\bullet}$ radical, leading to DNA cleavage by $HO_2^{\bullet}$ and $OH^{\bullet}$.

Acknowledgment. This work was partially supported by Grants-in-Aid (No. 16205020) from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

Supporting Information Available: DNA cleavage at pH 5.0 in the absence and in the presence of SOD and catalase. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Kochevar, I. E.; Dunn, D. A. *Bioorg. Photochem.* **1990**, *1*, 273.
(2) Armitage, B. *Chem. Rev.* **1998**, *98*, 1171.
(3) Nunez, M. E.; Rajski, S. R.; Barton, J. K. *Methods Enzymol.* **2000**, *319*, 165.
(4) (a) Kim, J.; Sistarc, M. F.; Carter, P. J.; Thorp, H. H. *Coord. Chem. Rev.* **1998**, *171*, 341. (b) Da Ros, T.; Spalluto, G.; Boutorine, A. S.; Bensasson, R. V.; Prato, M. *Curr. Pharm. Des.* **2001**, *7*, 1781.
(5) Bonnett, R. In *Metal Complexes for Photodynamic Therapy, Comprehensive Coordination Chemistry II*; McCleverty, J. A., Meyer, T. J., Eds.; Elsevier: Oxford, 2004; Vol. 9, pp 945−1003.
(6) (a) Dhar, S.; Senapati, D.; Das, P. K.; Chattopadhyay, P.; Nethaji, M.; Chakravarty, A. R. *J. Am. Chem. Soc.* **2003**, *125*, 12118. (b) Joseph, J.; Eldho, N. V.; Ramaiah, D. *Chem. Eur. J.* **2003**, *9*, 5926.
(7) (a) Yamakoshi, Y.; Umezawa, N.; Ryu, A.; Arakane, K.; Miyata, N.; Goda, Y.; Masumizu, T.; Nagano, T. *J. Am. Chem. Soc.* **2003**, *125*, 12803. (b) Yamakoshi, Y.; Sueyoshi, S.; Fukuhara, K.; Miyata, N.; Masumizu, T.; Kohno, M. *J. Am. Chem. Soc.* **1998**, *120*, 12363.
(8) Cadet, J.; Berger, M.; Douki, T.; Ravanat, J.-L. *Rev. Physiol. Biochem. Pharmacol.* **1997**, *131*, 1.
(9) Fukuzumi, S.; Nishimine, M.; Ohkubo, K.; Tkachenko, N. V.; Lemmetyinen, H. *J. Phys. Chem. B* **2003**, *107*, 12511.
(10) (a) Fukuzumi, S.; Suenobu, T.; Patz, M.; Hirasaka, T.; Itoh, S.; Fujitsuka, M.; Ito, O. *J. Am. Chem. Soc.* **1998**, *120*, 8060. (b) Patz, M.; Kuwahara, Y.; Suenobu, T.; Fukuzumi, S. *Chem. Lett.* **1997**, 567.
(11) Fukuzumi, S.; Koumitsu, S.; Hironaka, K.; Tanaka, T. *J. Am. Chem. Soc.* **1987**, *109*, 305.
(12) DMSO is used to increase the solubility of $(BNA)_2$ to an aqueous solution.
(13) The one-electron redox potentials were determined by second-harmonic ac voltammetry (SHACV) with a BAS 100B electrochemical analyzer.
(14) Kavarnos, G. J. *Fundamentals of Photoinduced Electron Transfer*; Wiley-VCH: New York, 1993.
(15) Rehm, D.; Weller, A. *Isr. J. Chem.* **1970**, *8*, 259.
(16) Bagchi, R. N.; Bond, A. M.; Scholz, F.; Stösser. R. *J. Am. Chem. Soc.* **1989**, *111*, 8270.
(17) Roberts, R. M. G.; Ostovic, D.; Kreevoy, M. M. *Faraday Discuss. Chem. Soc.* **1982**, *74*, 257.
(18) Sawyer, D. T.; Roberts, J. L., Jr. *Acc. Chem. Res.* **1988**, *21*, 469.
(19) Pogozelski, W. K.; Tullius, T. D. *Chem. Rev.* **1998**, *98*, 1089.
(20) (a) Mitra, K.; Kim, W.; Daniels, J. S.; Gates, K. S. *J. Am. Chem. Soc.* **1997**, *119*, 11691. (b) Behroozi, S. J.; Kim, W.; Dannaldson, J.; Gates, K. S. *Biochemistry* **1996**, *35*, 1768.
(21) $OH^{\bullet}$ radical may also be produced via bimolecular reaction of $HO_2^{\bullet}$.

JA046955P