DEPURINATION OF ADENOSINE AND DEOXYADENOSINE MONOPHOSPHATES IN THE HEMIN-HYDROPEROXIDE SYSTEM

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The adenine base was liberated by the oxidative cleavage of the *N*-glycosidic bond of substrates (5'- and 3'-AMP, 5'- and 3'-dAMP) in the hemin-cumene hydroperoxide system, which was accelerated in the presence of 1-methylimidazole.

KEYWORDS hemin; adenine; peroxide; oxidation; cleavage; HPLC

The non-enzymatic cleavage of DNA has received considerable attention since it could be applied in many ways to gene manipulations such as DNA sequencing, chromosome analyses, gene isolation, and recombinant DNA techniques. The sequence-specific DNA-cleaving molecules are required to have two separate functions, recognition and cleavage of DNA. The cleaving functionalities are capable of oxidation of the deoxyribose,[1,2] modification of the bases,[3,4] or hydrolysis of the phosphodiester backbone.[5] It has been shown previously that attachment of the Fe-EDTA complex to a DNA-binding molecule creates an efficient DNA-cleaving molecule.[6] In the presence of dioxygen and reducing agents, the Fe-EDTA complex generates a hydroxyl radical to cleave DNA at a specific binding site through intercalators,[1,7] groove binders,[8] and oligonucleotides which are complementary to the target DNA sequence.[9-11] On the other hand, iron porphyrin could also be a sequence-specific DNA-cleaving reagent through the covalent linkage with intercalators.[12,13] A hemin complex was shown[14] to bind to DNA with sequence specificity through bis-acridine intercalators. Although porphyrins appear to be relatively inefficient DNA-cleaving agents,[12] it is fascinating that the electronic structure of the iron atom,[15,16] and hence the oxidative activity,[17] could be well controlled by the nature of the axial ligand. Here we report that the hemin complex can cleave the *N*-glycosidic bond of adenine nucleotides oxidatively to liberate the adenine moiety.

Figure 1A shows the time course[18] of the adenine production from 5'-AMP. CmOOH[19] was employed as an oxidant. The rate was slow enough to be determined by HPLC analysis. At least for 4 hours, the production was linear to time, and the initial velocity for the adenine release (V_0) was determined from the slope. The V_0 value was proportional to the concentrations of hemin and 5'-AMP with zero-intercepts. It was also linear to the CmOOH concentration below 100 mM (Fig. 1B). Further addition of CmOOH, however, decreased the V_0 value. This may indicate that a large amount of CmOOH decomposed the adenine and/or an active intermediate as discussed below, although the decomposed product could not be detected at 254 nm in our HPLC analysis. Since adenine was not produced in the absence of hemin and CmOOH, both of these components were indispensable to the reaction. Hydrogen peroxide oxidation was also examined, but the adenine release could not be detected. The water molecule contained in the commercial H_2O_2 seems to have inhibited the reaction.

Apparent rate constants (k_{app}) were determined (Table 1) at constant concentrations of hemin (596 μM) and CmOOH (70.5 mM). The k_{app} is defined as $k_{app} = V_0/[S]_0$, where $[S]_0$ is the initial concentration of a substrate. Since V_0 indicates the rate for adenine production and the V_0 value was proportional to the substrate concentrations, the k_{app} signifies the rate for the increase of the yield. The k_{app} values for dAMPs were two to three times as large as those for AMPs, and thereby the 2'-OH moiety on the furanose ring retarded the adenine release. The position of the phosphate group had less effect on the k_{app} values (compare 5'- vs 3'-AMP and 5'- vs 3'-dAMP).

Hemin-catalyzed oxidation is generally promoted in the presence of imidazole bases.[17] As seen in Table 1, the k_{app} values for the nucleotides were increased almost two-fold in the presence of 1-MeIm

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(3.4 mM). The V_0 dependency on the 1-MeIm concentration is shown in Fig. 2A. The V_0 decreased at a concentration of 1-MeIm greater than about 5 mM with a slope of -1. This indicates that one mol equivalent of 1-MeIm is responsible for the decrease in the V_0 value.[17] The binding property of 1-MeIm to the ferric hemin was then examined (Fig. 2B), and the absorbance change was analyzed. It was found that two equivalents of 1-MeIm bound to the hemin with a mid-point concentration of about 6 mM. The 1-MeIm concentration is very close to that obtained in the kinetic experiment (Fig. 2A). We have previously studied[17] the oxidation kinetics of ferrocyanide in the hemin-hydrogen peroxide system. It was found that the formation of an intermediate with a mono-imidazole ligand enhanced the oxidation rate, and that the coordination of the second imidazole inhibited the reaction. The kinetic and binding behaviors of imidazoles[17] were quite similar to those found in the present study.

The V_0 value was linear to the concentrations of hemin and CmOOH (less than 0.1 M) and thus hemin must react with CmOOH at 1:1 stoichiometry to produce an active intermediate. One mol equivalent of 1-MeIm was responsible for the rate enhancement, and hence the Fe^{4+}(PP)(O)(1-MeIm) complex is a plausible candidate for the intermediate. In this complex, the fifth ligand should be 1-MeIm and the sixth coordination site is occupied by the oxo ligand originating from CmOOH. The electron donation from the

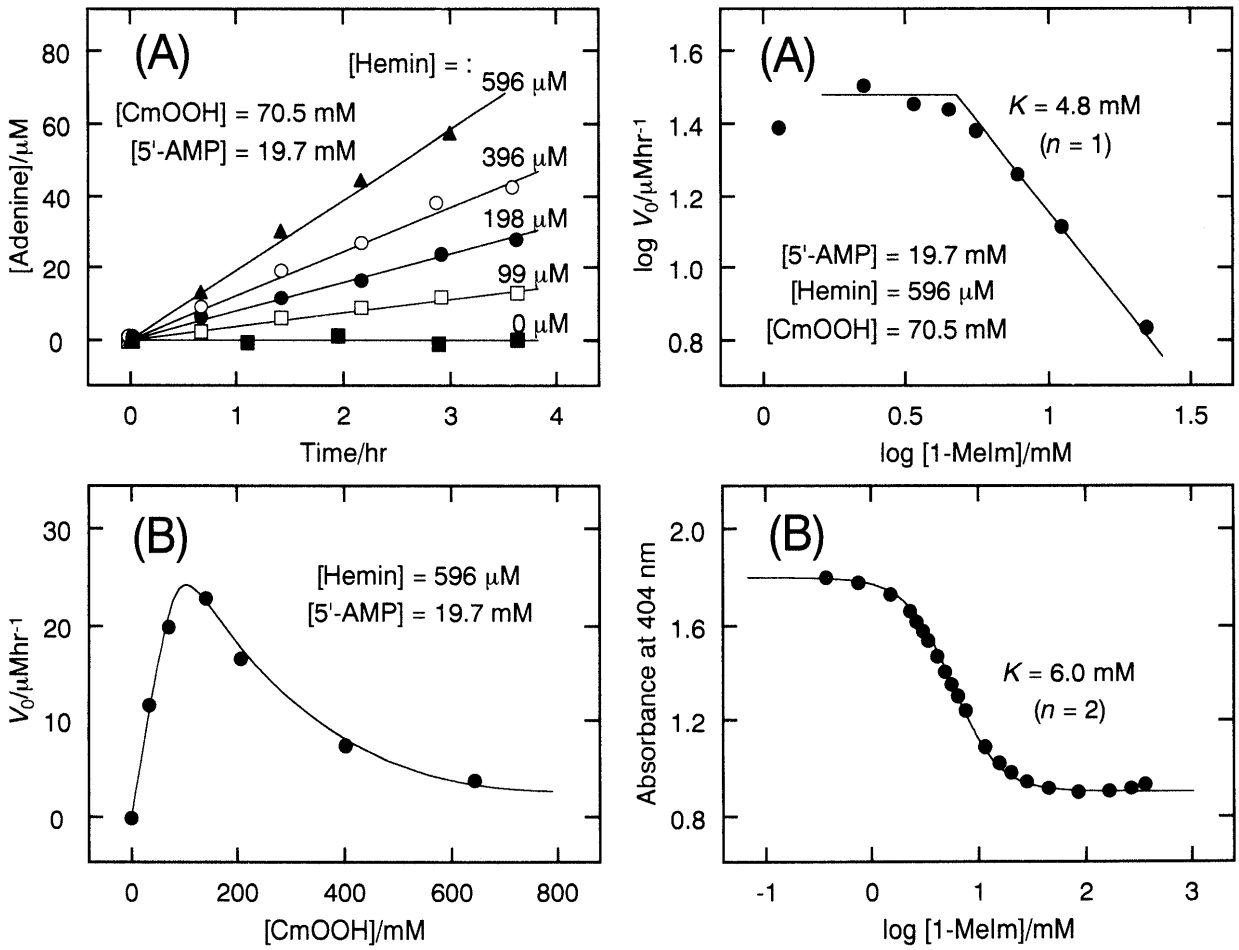

Fig. 1. (A) Effect of Hemin Concentration on the Kinetics of Adenine Production. The reaction was carried out in the presence of 19.7 mM 5'-AMP and 70.5 mM CmOOH at 25 °C. Hemin concentration; 0 (closed square), 99 (open square), 198 (closed circle), 396 (open circle), and 596 μM (closed triangle). (B) V_0 Dependency on the CmOOH Concentration. The reaction was carried out in the presence of 596 μM hemin and 19.7 mM 5'-AMP at 25°C.

Fig. 2. (A) Effect of 1-MeIm on the V_0 Value. The reaction was carried out in the presence of 596 μM hemin, 19.7 mM 5'-AMP, and 70.5 mM CmOOH at 25 °C. (B) Binding Property of 1-MeIm to the Ferric Hemin. Small aliquots of 1-MeIm solution was added into a Me_2SO solution containing ca. 10 μM hemin, and the absorbance change at 404 nm was monitored.

1-MeIm would activate the *trans* ligand, and a hydroxyl radical would be released to cleave the *N*-glycosidic bond. This mechanism accounts well for the low k_{app} values in AMPs, since the 2'-OH moiety could effectively scavenge a hydroxyl radical. The hydroxyl radical (·OH) is a potent oxidant but has short life span, especially in Me_2SO. The low yield of the adenine could partly be attributed to the nature of ·OH, and the yield would therefore be raised if the hemin were located close enough to the *N*-glycosidic bond.

In conclusion, the oxidative cleavage of the *N*-glycosidic bond of mononucleotides was examined kinetically and it was found that the 2'-OH moiety on the furanose ring reduced the rate. The modification of the porphyrin macrocycle, which can thereby direct a substrate properly, would result in a more effective agent for the oxidative cleavage of nucleotides.

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Table 1. The Apparent Rate Constant ($k_{app}/10^{-3}h^{-1}$)[a] in the Presence and Absence of 1-MeIm

	5'-AMP	3'-AMP	5'-dAMP	3'-dAMP
(-1-MeIm)	0.99	0.57	1.74	1.80
(+1-MeIm)[b]	1.46	1.13	3.46	3.05

[a] The reaction was carried out in the presence of 596 μM hemin and 70.5 mM CmOOH at 25°C. The concentration of the substrates (mM) was: 5'-AMP, 19.7; 3'-AMP, 19.7; 5'-dAMP, 9.9; 3'-dAMP, 9.9.
[b] The concentration of 1-MeIm was 3.4 mM.

REFERENCES AND NOTES

1) Hertzberg R. P., Dervan P. B., *Biochemistry*, **23**, 3934-3945 (1984).
2) Mack D. P., Dervan P. B., *J. Am. Chem. Soc.*, **112**, 4604-4606 (1990).
3) Baker B. F., Dervan P. B., *J. Am. Chem. Soc.*, **111**, 2700-2712 (1989).
4) Boger D. L., Munk S. A., Zarrinmayeh H., *J. Am. Chem. Soc.*, **113**, 3980-3983 (1991).
5) Basile L. A., Raphael A. L., Barton J. K., *J. Am. Chem. Soc.*, **109**, 7550-7551 (1987).
6) Dervan P. B., *Science*, **332**, 464-471 (1986).
7) Taylor J. S., Schultz P. G., Dervan P. B., *Tetrahedron*, **40**, 457-465 (1984).
8) Youngquist R. S., Dervan P. B., *Proc. Natl. Acad. Sci. U.S.A.*, **82**, 2565-2569 (1985).
9) Chu B. C. F., Orgel L. E., *Proc. Natl. Acad. Sci. U.S.A.*, **82**, 963-967 (1985).
10) Strobel S. A., Dervan P. B., *Nature (London)*, **350**, 172-174 (1991).
11) Singleton S. F., Dervan P. B., *J. Am. Chem. Soc.*, **114**, 6957-6965 (1992).
12) Lown J. W., Sondhi S. M., Ong C.-W., Skorobogaty, A., Kishikawa, H., Dabrowiak, J. C. *Biochemistry*, **25**, 5111-5117 (1986).
13) Hashimoto Y., Iijima H., Nozaki Y., Shudo K., *Nucleic Acids Res.*, **16**, 193-196 (1985).
14) Uno T., Sowa N., Shimabayashi S., *Chem. Pharm. Bull.*, **42**, 988-990 (1994).
15) Uno T., Hatano K., Nishimura Y., Arata Y., *Inorg. Chem.*, **29**, 2803-2807 (1990).
16) Uno T., Hatano K., Nishimura Y., *J. Am. Chem. Soc.*, **116**, 4107-4108 (1994).
17) Uno T., Takeda A., Shimabayashi S., *Inorg. Chem.*, **34**, 1599-1607 (1995) and papers cited therein.
18) Abbreviations used: CmOOH, cumene hydroperoxide; PP, hemin; 1-MeIm, 1-methylimidazole; Me_2SO, dimethyl sulfoxide.
19) CmOOH (Nacalai Tesque) was introduced at time = 0 into a Me_2SO (Merck) solution containing a substrate (Sigma, free acid), hemin chloride (Sigma), and 10 mM benzoic acid (Nacalai Tesque). At the respective times, the reaction mixture was injected onto an HPLC column (ODS-80Ts, 4.6 × 250 mm; Tosoh), and was eluted with 20 mM sodium phosphate buffer (pH 7.0) containing 7% acetonitrile (Merck, HPLC grade) at a flow rate of 1 ml/min (PU-980, Jasco). Absorbance at 254 nm was monitored (UV-970, Jasco) and the amount of adenine produced (retention time = 5.6 min) was determined. All the reactions were carried out at 25°C.

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