研究速報

UDC 541.143/.147 : 547.963.3+54

# Photodegradation of Nucleic Acid Constituents in the Presence of Iron(III)
## ——Adenosine Monophosphates——
## 鉄(III)存在下での核酸構成分子の光分解
## ——アデノシン一一リン酸——

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## 1. INTRODUCTION

Nucleic acids and their constituents, nucleotides and nucleoosides, are one of the most important class of molecules in living systems, which consist of sugar moieties (D-ribose or deoxy-D-ribose) and base moieties. Photochemistry of nucleotides and nucleosides has been extensively studied in order to get much better understanding of photodamage to living systems and also to explore new phototherapic method[1]. Most of the studies are focused on the photoreactivity of the base moieties, since the base moieties show intense light absorption in UV to near-UV region. However, relatively few has been known as to the photoreactivity of the ribose moiety. This negligence may be partly due to the fact that carbohydrates in general have no absorption bands in UV to near-UV region. In the presence of iron(III), however, highly site-specific photooxidation of monosaccharides takes place by irradiation of near-UV light via complex formation between monosaccharides and iron(III)[2]. We previously reported that the ribose moiety of adenosine, one of the most common nucleoside, was oxidatively degraded by irradiation of near-UV light in the presence of iron(III) under either aerobic or anaerobic atmosphere[3]. Because of the physiological importance of this finding, we further extended our studies to nucleotides, monomeric units of nucleic acids. Here, we wish to report the photodegradation of adenosine monophosphates in the presence of iron(III). The effect of the phosphate group on the reaction is also discussed.

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## 2. EXPERIMENTAL

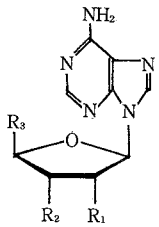
Adenosine and adenosine-5'-monophosphate were obtained from Tokyo Kasei Co. (Tokyo), and adenosine-2'-monophosphate and adenosine-3'-monophosphate from Sigma Chemical Co. (St. Louis, USA). They were used without further purification.

Experimental procedures were essentially the same as those reported previously[3]. A sample solution (75 ml, pH 2.5) was irradiated internally by a 100-W high-pressure mercury lamp through a Pyrex-made water-jacket at 20°C. For estimation of the quantum yield of the reaction, a sample solution in a quartz-made cell (3 ml) was subjected to the irradiation of light at 338 nm (band width 3 nm) in a JASCO CRM-FA spectroirradiator. Irradiated solutions were analyzed by a high-performance liquid chromatagraphy (HPLC) monitored by UV absorption at 260 nm with a $C_{18}$-silica gel column (Develosil ODS-5, Nomura Chemical Co., 4(i.r.) × 150 mm; 0.01 mol $dm^{-3}$ $KH_2PO_4$, 0.01 mol $dm^{-3}$ $K_2HPO_4$, 0.1 mol $dm^{-3}$ $NH_4Cl$ /$CH_3CN$(2%) + $H_2O$). Relative retention times of nucleotides and nucleisides are as follows; adenine =1, adenosine-5'-aldehyde 0.88, adenosine 3.9, adenosine - 2'- monophosphate ( 2'-AMP) 1.9, adenosine-3'-monophosphate(3'-AMP) 0.76, and adenosine-5'-monophosphate(5'-AMP) 0.46. Amounts of iron(II) in the irradiated solutions were determined by colorimetric method with 1, 10-phenanthroline.

## 3. RESULTS

### 3.1 Adenosine-5'-monophosphate (5'-AMP)

Photoirradiation to adenosine in the presence of iron(III) causes destruction of adenosine mostly into adenine, adenosine-5'-aldehyde, and another unidentified product[3]. Irradiation of a Pyrex-filtered



$R_1$=$OPO_3H_2$, $R_2$=OH, $R_3$=$CH_2OH$ : 2'-AMP
$R_1$=OH, $R_2$=$OPO_3H_2$, $R_3$=$CH_2OH$ : 3'-AMP
$R_1$=OH, $R_2$=OH, $R_3$=$CH_2OPO_3H_2$ : 5'-AMP
$R_1$=OH, $R_2$=OH, $R_3$=CHO : adenosine-5'-aldehyde

light(>300 nm) for 3 h at 20°C to an yellow-color solution (75 ml) containing 5'-AMP(0.3 mmol) and $FeCl_3$(0.75 mmol) gave a colorless solution. The absorption band due to iron(III) was almost disappeared in the electronic spectrum of the resultant solution (Fig. 1). However, the irradiated solution still showed the absorption at 260 nm presumably due to the adenine ring, indicating that the adenine ring of 5'-AMP remained intact even after irradiation. Chromatographic analysis showed that photoirradiation caused destruction of 5'-AMP mostly into adenine and adenosine-5'-aldehyde (Table 1). One hour of irradiation caused reduction of more than 70% of iron(III) into iron(II), and almost leveled off at that extent. No destruction of 5'-AMP occurred in the absence of $FeCl_3$ nor without photoirradiation, and only a little amount of iron(III) was reduced to iron(II) by irradiation in the absence of 5'-AMP. These results confirmed that the destruction of 5'-AMP took place by the photochemically-induced oxidation of the ribose moiety of adenosine with iron(III). The reaction under nitrogen atmosphere showed little difference

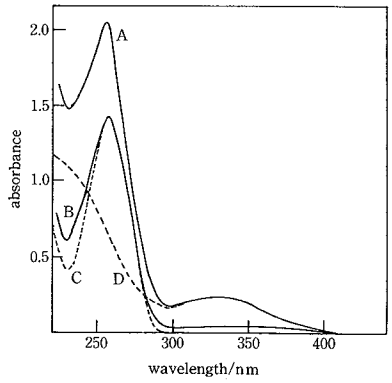



Fig. 1 Electronic Spectra of a 5'-AMP(0.3 mmol)-$FeCl_3$ (0.75 mmol) Solution (A) before and (B) after Photoirradiation for 3h at 20°C. Those of (C) 5'-AMP and (D) $FeCl_3$ at same concentrations are also shown for comparison.

from that under aerobic conditions, indicating that presence of oxygen has little effect on the reaction. These results are essentially the same as those of the photodestruction of adenosine[3], though the amount of adenosine-5'-aldehyde formed was much smaller in this case.

Quantum yield for destruction of 5'-AMP at 338 nm was roughly estimated to be $1\text{-}2\times10^{-3}$.

### 3. 2 Other Adenosine Monophosphates

Photoirradiation to 2'- and 3'-AMP solutions in the presence of $FeCl_3$ also brought about oxidative degradation of these nucleotides (Table 2). However, degradation products were mostly adenine, and formation of adenosine-5'-aldehyde was not detected chromatographically. For the purpose of comparison, adenosine was also irradiatied under the same conditions and the results are included in Table

Table 1 Photodestruction of 5'-AMP in Aqueous Solutions (pH 2.5) under Aeroboc Atmosphere at 20°C

| Initial/mmol | | Time | After Irradiation/mmol[a] | | | |
|---|---|---|---|---|---|---|
| 5'-AMP | Fe(III) | /h | Fe(II) | 5'-AMP consumed | Adenine | Adenosine-5'-aldehyde |
| 0.3 | 0 | 1 | — | 0.0075 | 0 | 0 |
| 0 | 0.75 | 1 | 0.03 | — | — | — |
| 0.3 | 0.75 | 1 | 0.56 | 0.11 | 0.065(59%) | 0.0098(9%) |
| 0.3 | 0.75 | 1[b] | 0.54 | 0.10 | 0.062(62%) | 0.0090(9%) |
| 0.3 | 0.75 | 2 | 0.71 | 0.12 | 0.075(63%) | 0.011 (9%) |
| 0.3 | 0.75 | 3 | 0.72 | 0.13 | 0.083(64%) | 0.011 (8%) |
| 0.3 | 0.3 | 1 | 0.16 | 0.060 | 0.038(63%) | 0.005 (8%) |

a) Conversions of the products given in parentheses are mol% to the 5'-AMP consumed.
b) Irradiated under nitrogen atmosphere.

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Table 2　Photodestruction of Adenosine and Adenosine Monophosphates in Aqueous Solutions under Aerobic Atmosphere at 20°C[a)]

| Substrate | After Irradiation/mmol[b)] | | | |
|---|---|---|---|---|
| | Fe(II) | Substrate consumed | Adenine | Adenosine-5'-aldehyde |
| 2'-AMP | 0.47 | 0.18 | 0.098(54%) | 0 |
| 3'-AMP | 0.53 | 0.16 | 0.098(61%) | 0 |
| 5'-AMP | 0.56 | 0.11 | 0.065(59%) | 0.010( 9%) |
| Adenosine | 0.60 | 0.17 | 0.071(42%) | 0.036(21%) |

a) Substrate(0.3 mmol)-$FeCl_3$(0.75 mmol) solutions(75 ml) were irradiated internally by a 100-W high-pressure mercury lamp for 1h. b) Conversions given in parentheses are mol% to the substrate consumed.

2. It is worth to note that the amount of 5'-AMP degraded after an hour of irradiation is smaller than that of 2'-AMP, 3'-AMP, or adenosine.

## 4. DISCUSSION

In the photochemically-induced oxidation of monosaccharides, interaction between iron(III) and hydroxyl groups of monosaccharides have shown to be the essential step, and light absorption due to the iron(III)-monosaccharide complex leads to the oxidative C-C bond cleavage[2)]. Therefore, introduction of a substituent like a nucleic acid base or phosphate, which has ability to interact with metal ion, into the sugar molecule is expected to alter the photochemical reaction. Transition metal ions are known to interact primarily with the nitrogen atoms of adenosine in neutral pH range, but the role of the ribose moiety in the complex formation is not so clear[4,5)]. In an acidic solution, the adenine-metal interaction might be weaker because adenine ring exists as a protonated form ($pK_a$=3.5—3.8). The ribose moiety of adenosine is shown to be susceptible to the photochemically-induced oxidation by iron (III) in our previous report. The results are much different from those of the photooxidation of ribose alone, and the adenine-iron(III) interaction is suggested to be important for the formation of adenosine-5'-aldehyde[3)]. Phosphate group, which exists as a mono-anion ($pK_{a1}$<1) in a pH 2.5 solution, is expected to interact strongly with transition metal ions. Indeed, formation of adenosine-5'-aldehyde was greatly suppressed in the reactions of adenosine monophosphates compared to that of adenosine (Table 2). Furthermore, 5'-AMP is less reactive compared to 2'-, 3'-AMP or event to adenosine. As is known for Ni(II) and some other transition metal ions[6,7)], interaction of metal ion with both the ring nitrogen and the phosphate group of 5'-AMP keeps the ribose moiety away from the coordinated metal ion, and this might be the reason for the low reactivity of 5'-AMP. Therefore, site of the interaction with iron(III) is indicated to be important for photodestruction of adenosine and adenosine monophosphates.

## 5. CONCLUSION

The effect of near-UV irradiation on the living organs, especially on DNA and RNA, have been actively studied[8)], and iron ion attached to various biological molecules like porphyrin and bleomycin has been recognized as a potential oxidant. We demonstrated here that simple iron(III) ion can also cause destruction of nucleoside or nucleotide by irradiation of near-UV light. Since iron(III) is ubiquitos in nature and in living systems, this finding might have physiological importance.

(Manuscript received, July 1, 1987)

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