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Role of Iron(III)—Nucleoside Interaction in the Photochemically—Induced Oxidative Degradation of Adenosine
アデノシンの光酸化分解反応におけるFe(III)—ヌクレオシド相互作用の役割

Koji ARAKI* and Shinsaku SHIRAISHI*
荒 木 孝 二・白 石 振 作

1. Introduction

Nucleic acids and their constituents, nucleotides and nucleosides, are one of the most important class of biological molecules in living systems, and effect of photoirradiation on these molecules, especially on the base moieties of these molecules, have been actively studied[1]. However, relatively few has been known as to the photoreactivity of the ribose moieteis of these molecules, and, moreover, their photoproducts are often not well characterized[2]. We previously reported that one of the common nucleosides, adenosine, was oxidatively degraded mostly into adenine and adenosine-5'-aldehyde by irradiation of near-UV light in the presence of iron(III) under either aerobic or anaerobic atmosphere[3]. Iron ion attached to variuos biological and synthetic molecules like bleomycin[4] or methidiumpropyl-EDTA[5] has been recognized as a potential oxidant of the sugar moiety of DNA. Since out results showed that simple iron(III) ion can cause destruction of nucleoside, we further extended the study in order to clarify the role of an iron-adenosine interaction in the photodegradation of adenosine.

2. Experimental

Materials and experimental procedures were essentially the same as those reported previously[3]. ^{1}H-and ^{13}C-N.m.r. spectra were measured at 27°C in dimethyl sulfoxide (DMSO)-d_6 by a JEOL GX-270 spectrometer operated at 270 and 67.8 MHz, respectively. Mass spectrum was recorded on a JEOL JMS-DX303 with a FAB-ionization method using glycerin as a liquid matrix.

*Dept. of Industrial Chemistry and Metallurgy, Institute of Industrial Science, University of Tokyo

Photoirradiation to an acidic adeosine-$FeCl_3$ solution, analyses of the irradiated solution by a high-performance liquid chromatography (HPLC), and isolation of the photoproduct were carried out according to the procedures reported previously[3]. The product was obtained as a white solid (4mg) from 20 ml of the irradiated solution, and were subjected to FAB-mass and n.m.r. measurements.

For the reaction in a weakly basic solution, an adenosine (0.7 mmol)-$FeCl_3$ (0.28 mmol) solution (70 ml), whose pH was initially adjusted at 9.6 by addition of a small amount of conc NaOH, was irradiated for 6 h at 20°C with airbubbling through the solution[6]. Though freshly prepared solution was clear, brown precipitate of iron hydroxide was formed during irradiation with concomitant pH decrease. Therefore, a small amount of conc. NaOH solution was added every an hour to keep the pH of the solution within the range between 7 and 9.6. To the irradiated solution (20ml), 50mg of benzoic hydrazine in 3ml of water was added, and the solution was kept at 4°C to yield a white microcrystalline precipitate. This was recrystallized from water and dried at 80°C *in vacuo* for 2 days (yield 2.2 mg, mp. 177-178°C), whose i.r. and ^{1}H-n.m.r. spectra were found to be identical with those of 2-adenyl-3,5-dihydroxy - 4 - benzoylamino - 6 - hydoxylmethylmorpholin (1)[7]. **1** was prepared separately by the periodate-oxidation of adenosine and subsequent treatment of the resultant adenosine-2',3'-dialdehyde (2) with benzoyl hydrazine according to the method of Hansske *et al*[7].; yield 75%; mp. 177-178°C (lit. 180°C[5]); Found: C, 51.15; H, 4.70; N, 24.45%; Calcd for $C_{17}H_{19}O_5N_7$: C, 50.87; H, 4.77; N, 24.43.

3. Results

3.1. Identification of the Photoproducts in Acidic Media

As reported in the previous report[3], irradiation of Pyrex-filtered light (> 300nm) to an aqueous adenosine-$FeCl_3$ solution (pH 1.8-2.5) under either aerobic or anaerobic conditions caused destruction of adenosine mostly into adenine and adenosine-5'-aldehyde(3), which in total comprised more than 75% of the adenosine degraded. However, HPLC analysis of the irradiated solution showed the presence of another minor product, which was eluted shortly after the unreacted adenosine (Fig. 1). Column-chromatographic separation successfully gave this product as a white solid. Unlike adenosine-5'-aldehyde, this product was stable during chromatographic separation and subsequent isolation processes, and chromatographic analysis confirmed the solid contained nearly 98% of the photoproduct.

Molecular-ion peak (MH^+) observed in the FAB-MS spectrum of this compound was 284 showing that the molecular weight of this compound was higher by 16 than that of adenosine (Mw. 267). ^1H-and ^{13}C-N.m.r. spectra of the compound in DMSO-d_6 (Table 1) revealed that the compound has the intact ribose moiety. In addition, the proton signal at 8-position (8.2 ppm) of the adenine ring of adenosine was replaced by a broad peak at around 10 ppm, which disappeard upon addition of D_2O. Therfore, the photoproduct was concluded to be 8-hydroxyadenosine (4, Mw. 283). Amounts of 8-hydroxyadenosine in the irradiated solutions were estimated by HPLC along with those of adenine and adenosine-5'-aldehyde. Unlike other two major products, 8-hydroxyadenosine has the intact ribose moiety, and it comprised only 10% of the adenosine degraded. All three products increased as irradiation time became longer, and, therefore, it is likely that three products were formed simultaneously rather than successively.

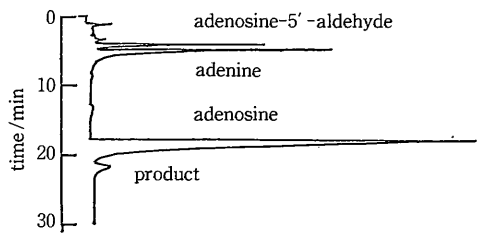


Fig. 1 Typical HPLC Profile of the Irradiated Solution Analyzed by a JASCO Twicle-type HPLC (monitored by absorbance at 260 nm) with Develosil ODS-5 column (Nomura Chemical Co., 250x4 mm; CH_3CN (2 vol%)+ammonium acetate (0.1mol dm^{-3})/H_2O).

Table 1 ^1H-and ^{13}C-Nmr Spectral Data of Isolated Photoproduct in DMSO-d_6 at 27°C

^1H		^{13}C	
3.52(*d-d*, 2H)	$H_{5'}$	63.2	$C_{5'}$
3.85(*s*, 1H)	$H_{4'}$	71.6	$C_{2'}$, $C_{3'}$
4.12(*s*, 1H)	$H_{3'}$	71.8	
4.85(*d*, 1H)	$H_{2'}$	86.5	$C_{1'}$, $C_{4'}$
5.0-5.2 (*m*, 3H)	$OH_{2'}$,$OH_{3'}$, $OH_{5'}$	86.8	
5.66(*d*, 1H)	$H_{1'}$	105.1	adenine ring
6.55(*s*, 2H)	NH_2	147.3 148.2	
8.00(*s*, 1H)	OH_8	151.9	
9.97(*b*, 1H)	H_2	153.0	

Chemical shifts are reported in ppm from thet of TMS, and *s*, *d*, *m*, and *b* represent singlet, doublet, multiplet, and broad signals, respectively.

3.2. Photoirradiation in a Weakly Alkaline Solution

In the presence of a small amount of iron ion at neutral to weakly alkaline pH, monosaccharides are susceptible to the photochemically-induced oxidation with oxygen by coupling with the oxidation-reduction cycle of iron[6]. Terefore, we carried out photoirradiation to an adenosine (0.75 mmol)-$FeCl_3$ (0.30 mmol) aqueous solution (75 ml) whose pH was maintained within 7 and 9.6 for 6 h. The irradiated solution after removal of the precipitate was analyzed by HPLC, which showed that 0.082 mmol of adenosine was degraded during photoirradiation and that another photoproduct was present in addition to adenine (0.041 mmol, 50%) and trace amounts of adenosine-5'-aldehyde. Though attempts to isolate this unidentified photoproduct were unsuccessful because of instability of the compound during chromatographic separation, addition of benzoyl hydrazine to the irradiated solution gave white microcrystalline solid, which was identified to be 2-adenyl-3,5-dihydroxy-4-benzoyl-amino-6-hydoxylmethylmorpholine(1). Therefore,

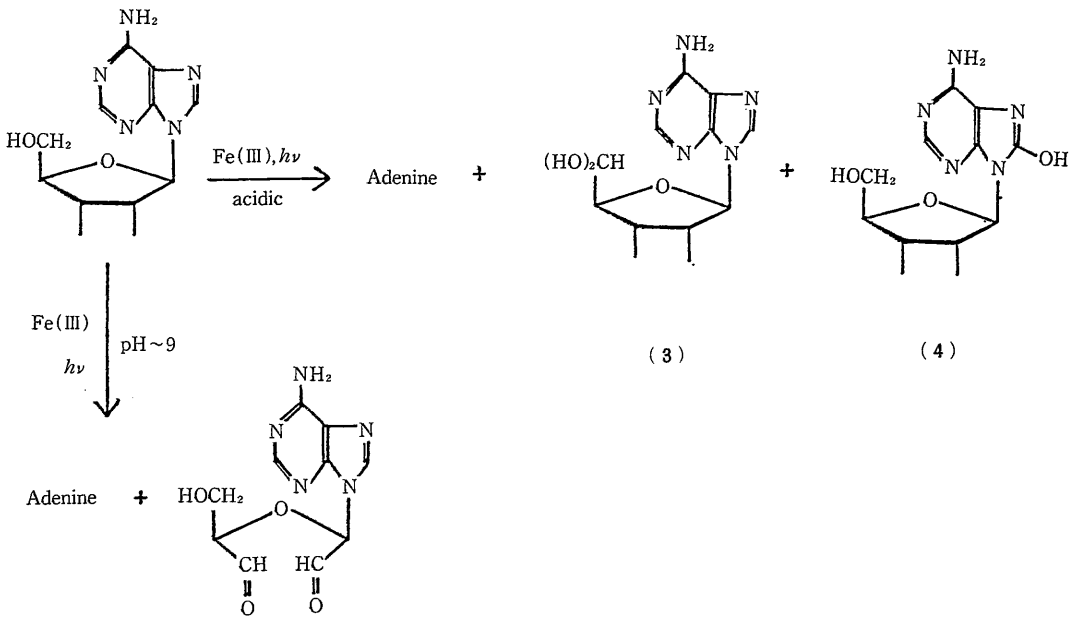
the product was adenosine-2',3'-dialdehyde(2)[7], which was reported to be unstable. Since adenosine-2',3'-dialdehyde prepared separately also gave similar peak as that of the photoproduct in chromatogram, the product was concluded to be adenosine-2',3'-dialdehyde. As it was difficult to isolate adenosine-2',3'-dialdehyde[7], the amounts of this compound in the irradiated solution were eatimated from the amount of 1 recovered from the irradiated solution (0.021 mmol, 26%). No detectable decrease in adenosine concentration was observed without photoirradiaiton or in the absence of $FeCl_3$. Thus, adenosine in a weakly alkaline solution was shown to be susceptible to the photodestruction of its ribose moiety in the presence of iron (III) to give adenine (50%) and adenosine-2',3'-aldehyde (26%) as the photoproducts.

4. Discussion

Photodestruction of adenosine in the presence of iron(III) was summarized in the Scheme.

No reaction took place in the absence of $FeCl_3$ or without photoirradiation. Furthermore, in the case of the reaction in acidic solution, the action spectrum of the reaction agreed well with the absorption band of $FeCl_3$, and the presence of oxygen has little effect on the reaction. Therefore, the reaction is concluded to be proceed by the light-absorption of $FeCl_3$. Formation of 8-hydroxyadenosine in an acidic solution indicates that the adenine ring is also susceptible to the photoirradiation. However, amounts of 8-hydroxyadenosine is only 10% of the adenosine degraded, and most of the reaction takes place at the ribose moiety of adenosine.

In acidic solutions, a site-specific iron(III)-monosaccharide interaction has been shown to cause the sitespecific C-C bond cleavage(s) of monosaccharides in the photochemically-induced oxidation of monosaccharides by iron(III)[8]. In the case of D-ribose, it was converted to D-erythrose and D-glycelaldehyde via the C1-C2 and C2-C3 bond cleaveges, respectively[8], but the C5 position of D-ribose was not susceptible to this type of photooxidation. However, iron(III) is thought to interact preferentially to the adenine ring of adenosine rather than the ribose moiety in an acidic soilution[9], Thus, intro-



(2)

Scheme

duction of the adenine ring to D-ribose affected much to the reaction, yielding adenosine-5'-aldehyde and 8-hydroxyadenosine in addition to adenine. Since 8-hydroxyadenosine is known to be formed by γ-ray irradiation of aqueous adenosine via attack of hydroxyl radical[10], formation of 8-hydroxyadenosine is indicative of the participation of hydroxyl radical. Therefore, formation of adenosine-5'-aldehyde and 8-hdyroxyadenosine may best be explained by a site-specific formation of hdroxyl radical sensitized by the iron(III) coordinated to the adenine ring and subsequent attack of hydroxyl radical to the near-by 5'-or 8-position of adenosine. Recently, similar site-specific formation of hydroxyl radical due to an iron-sugar interaction has been reported in the presence of H_2O_2[11].

While, the hydroxyl groups at 2' and 3' positions are thought to be dissociated in some extent at weakly alkaline pH, and the dissociated *cis*-diol serves as a good chelation site to metal cations[12]. As periodate-oxidation of adenosine leads to the cleavage of this *cis*-diol moiety, formation of adenosine-2',3'-dialdehyde in neutral to weakly basic solutions may be the result of the direct interaction of iron(III) with the 2'-and 3'-hydroxyl groups and subsequent oxidative cleavage of this C-C bond. Thus, different photoproducts were obtained at different pH of the solution, and these results suggest that the mode of interactions between iron and adenosine plays important role in the photodestruction of adenosine.

5. Condluding Remarks

Action of bleomycin on DNA is interpreted to be the attack of hydroxyl radical formed at the iron-center of bleomycin toward the 4'-position of deoxyribose, which causes destruction of deoxyribose moiety leading to the strand scission of DNA. Currently, many efforts have been focused on developing synthetic antitumor agents that have bleomycin-like DNA-cleaving ability[13], but little attention has been paid for the location of metal-center in DNA-drug complex. Present results indicated that the mode of interactions between adenosine and Fe(III) plays important role in the photodestruction of adenosine. Terefore, location of the iron-center in DNA-drug complex can be the important factor for its antitumor activity, and may help designing synthetic bleomycin-like antitumor agent.

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