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DNA Cleavage by Pentadentate Iron(II) Complexes Containing Fluoro-Substituted Phenyl Groups

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Abstract—Four pentadentate iron(II) complexes containing non- or fluoro-substituted phenyl group (**2b**–**2e**) were synthesized and cleaving activity of them to pUC19 DNA was evaluated in the presence of hydrogen peroxide. DNA cleavage activity increased with the number of substituted fluorine atoms on the phenyl group of **2b**.

Introduction

The activation of dioxygen or hydrogen peroxide and action of DNA cleavage by iron complexes including non-heme, heme and simple models are a great topic of bioinorganic, medical and pharmaceutical fields.[1–5] For example, bleomycins (BLMs) are a family of antitumor antibiotics used clinically for the treatment of testicular carcinomas[6] and non-Hodgkin's lymphomas.[7] BLM binds to iron(II) to form an iron(II) complex, Fe^{II}–BLM. The resulting Fe^{II}–BLM uptakes dioxygen at the sixth site and activates it to generate so-called 'activated BLM', formally, ferric hydroperoxide,[8] which can also be formed from Fe^{III}–BLM with hydrogen peroxide[9] and is characterized by electrospray mass spectrometry (EMS)[10] and X-ray absorption spectroscopy (XAS).[11] This activated BLM is thought to be responsible for the oxidative damage of DNA.[12,13]

In the past few years, several iron(III)–hydroperoxide complexes also have been synthesized and characterized by various techniques such as UV–vis and EPR spectroscopies.[14–22]

Recently, we reported the syntheses, X-ray crystal structures, and spectroscopic properties of the tetra- and pentadentate iron(II) complexes, **1** and **2** (Scheme 1).[23–25] We found that the tetradentate complex **1** is converted into a pentadentate complex **2** with new C–C bond formation between the central carbon of 1,3-diimine part and various nitrile solvents such as acetonitrile and benzonitrile by addition of triethylamine. Moreover, complex **2a** (R=CH_3) can cleave plasmid pUC19 DNA with non-specificity at the concentration of 10 μM in the presence of hydrogen peroxide.[26]

In this study, we synthesized four iron(II) complexes, **2b**–**2e**, where fluoro-substituent is introduced into the phenyl ring of **2b** for **2c**–**2e**, and investigated spectral properties of the iron(II) complexes in the presence of hydrogen peroxide and DNA cleavage activity.

Results and Discussion

Synthesis and physicochemical properties

The iron(II) complexes, **2b**–**2e**, were prepared in yield of 50–80% from the reaction of **1** with the corresponding nitrile (benzonitrile for **2b**, 4-fluorobenzonitrile for **2c**, 3,4-difluorobenzonitrile for **2d**, and pentafluorobenzonitrile for **2e**) in the presence of triethylamine according to the earlier published method.[23,24,27]

Infrared spectra of the complexes showed the stretching bands between 3256 and 3270 cm^{-1} due to N–H of the newly formed imino group –C(R)=NH, where R is C_6H_5, 4-F-C_6H_4, 3,4-$F_2C_6H_3$, and C_6F_5. In the ^1H NMR spectra of complexes in acetonitrile-d_3, the chemical shift of –C(R)=N*H* moiety moves toward down-field with increasing the number of fluorine atom on the phenyl ring: 12.15 ppm for **2b**, 12.10 ppm for **2c**,

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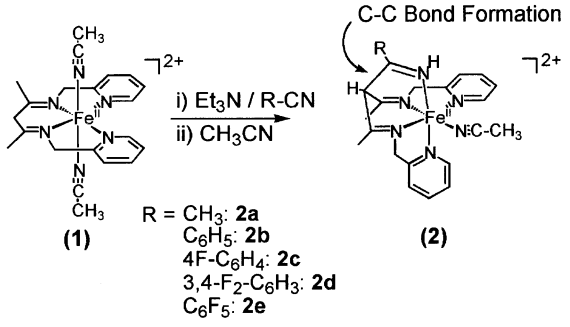
12.30 ppm for **2d**, and 13.18 ppm for **2e**, indicating that the electron-withdrawing effect of fluorine atom reduces the electron density on the phenyl and the imino groups.

Cyclic voltammetry of **2b** to **2e** revealed the redox potentials for Fe^{II}/Fe^{III} change from $E^0 = +0.55$ for **2b** to +0.58 for **2c**, +0.61 for **2d** and +0.71 V versus Ag/Ag^+ for **2e** with increasing the number of fluorine atoms.[24] This anodic shift is the consequence of the electron-withdrawing effect of fluorine atom and Fe^{II} state is most stabilized for **2e** compared with those for **2a**–**2d**.

In **2b**, X-ray structure analysis was carried out; the X-ray crystal structure of **2b** revealed that the iron center was octahedrally coordinated with *cis*-β configuration. The five nitrogen atoms from the ligand coordinate to the metal ion, and the remaining terminal coordination site is filled by a molecule of acetonitrile.[24]

Reaction of 2e with hydrogen peroxide

Treatment of **2e** with 50 equimolar of hydrogen peroxide in acetonitrile at 25 °C resulted in the appearance of bluish violet color ($\lambda_{max} = 587$ nm). This absorption reached maximum after 300 s and then gradually diminished (Fig. 1). Multiple introduction of fluorine atoms gave rise to red-shift of the transient absorption maxima; λ_{max} 532 nm for **2b**; 571 nm for **2c**; 573 nm for **2d**; 587 nm for **2e**.



Scheme 1.

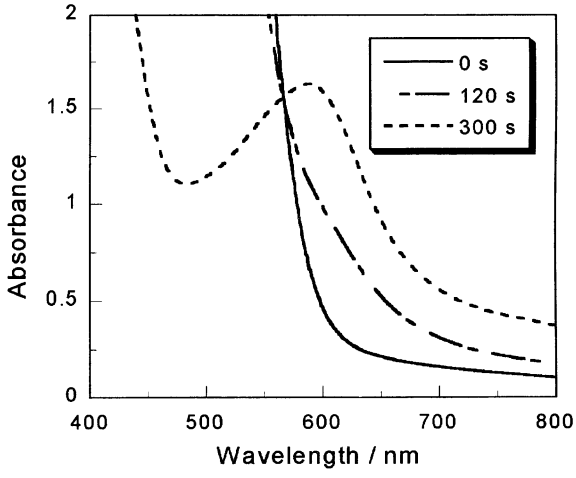


Figure 1. Visible spectral change of **2e** in acetonitrile in the presence of 50 equimolar amounts of H_2O_2 at 25 °C.

An aliquot of sample was taken after 300 s and EPR measurement was carried out at 77 K. EPR spectrum of an oxygenated species derived from **2e** with hydrogen peroxide showed the characteristic of a low-spin iron(III) complex ($S = 1/2$) with g values at 2.11, 2.08, and 1.99 (Fig. 2).

Similar behavior has been reported for related iron(III)–hydroperoxide complexes containing pyridyl-pendant in the molecules and the appearance of transient absorption observed in the range of 531–592 nm moves to lower energy with the increase in number of pyridine arms: 531–541 nm with three pyridines, 548 nm with four pyridines and 592 nm with five pyridines.[15–21]

These results suggest that oxygenated species are an iron(III)–hydroperoxide complex and their spectroscopic data resemble those of the characterized iron (III)–hydroperoxide complexes[14–22] and that hydrogen bonding fortified with electron withdrawing substituents stabilizes the iron(III)–hydroperoxide intermediate in **2e** complex (Fig. 3) in comparison of those with **2b**–**2d**. Such internal hydrogen bonding model was proposed by Valentine and co-workers in a Fe–BLM model, Fe–PMAH [PMAH = 2-((*N*-(aminoethyl)amino)methyl)-4-(*N*-(2-(4-imidazoyl)ethyl)carbamoyl)-5-bromopyrimidine] and Fe–cyclam complex [cyclam = 1,4,8,11-tetraazacyclotetradecane].[28,29]

DNA cleavage activity

In order to determine the fluoro-substituent effect on the DNA cleavage efficiency, the DNA-cleaving reaction by pUC19 DNA was carried out in the presence of hydrogen peroxide in Tris–borate buffer (50 mM, pH 8.1) for 1 h at 15 °C. The DNA cleavage efficiency was evaluated with the formation of relaxed (Form II) and linear (Form III) forms from supercoiled pUC19 DNA (Form I) (judged by densitometric analysis after UV imaging; Fig. 4). Non fluoro-substituted **2b** (lane 2) produced DNA cleavage to afford Form II (62%) at 10 μM whereas mono and difluoro-substituted **2c** and **2d** completely cleaved supercoiled pUC19 DNA to produce Form II and Form III (72 and 28% for **2c** and 63 and 34% for **2d**) under the same conditions (lanes 3 and 4).

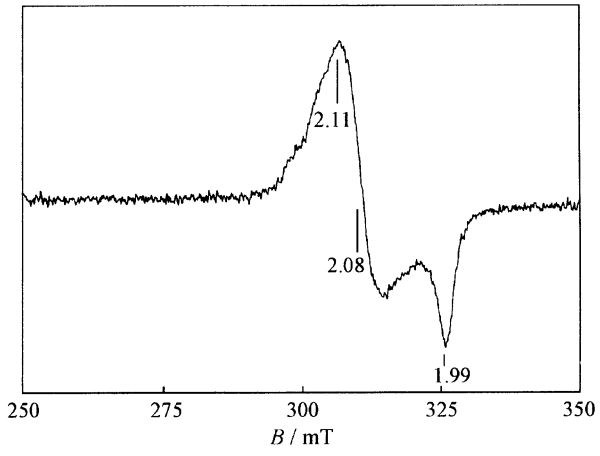


Figure 2. EPR spectrum of **2e** in acetonitrile in the presence of H_2O_2 at 77 K.

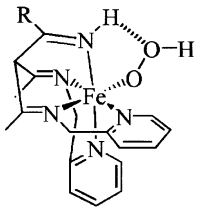


Figure 3. Proposed structure of an iron(III)–hydroperoxide complex of **2e**.

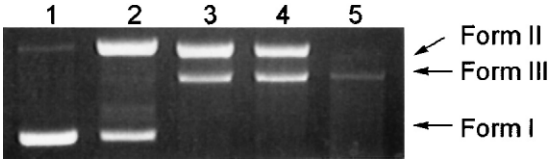


Figure 4. (a) Agarose gel electrophoresis of cleavage reaction of pUC19 DNA by **2b**, **2c**, **2d**, and **2e** in the presence of H_2O_2. Reaction conditions: solutions (total volume 24 μL) contained 0.34 μg pUC19 plasmid DNA. The DNA cleaving reactions were run for 1 h at 15 °C and electrophoresis was conducted at 70 V (10 min) and 120 V (2 h) on a 1.0% agarose gel. Lane 1, DNA alone 97% Form I (supercoiled), 3% Form II (relaxed); lane 2, 10 μM **2b** + 3 mM H_2O_2, 38% Form I, 62% Form II; lane 3, 10 μM **2c** + 3 mM H_2O_2, 72% Form II, 28% Form III; lane 4, 10 μM **2d** + 3 mM H_2O_2, 63% Form II, 34% Form III; lane 5, 10 μM **2e** + 3 mM H_2O_2, 100% Form III (linear).

Pentafluoro-substituted **2e** gave the best DNA cleavage efficiency to produce Form III (100%) (lane 5). Thus, introduction of fluorine atoms on the phenyl ring in **2b** increased the DNA cleavage efficiency.

In summary, we have prepared four the pentadentate iron(II) complexes, **2b**–**2e**, and examined the substituent effect on DNA cleavage activity by the iron complexes in the presence of hydrogen peroxide. The relative efficiency in pUC19 DNA cleavage was found to be: **2e** > **2d**≈**2c** > **2b**.

Acknowledgements

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25. Abbreviations: **1**, Di(acetonitrile)[1,3-dimethyl-1,3-propanediylidenebis(2-pyridylmethanamine)]iron(II) perchlorate; **2a**, (acetonitrile)[3-(1-iminoethyl)-2,4-bis(2-pyridylmethylimino)-pentane]iron(II) perchlorate; **2b**, (acetonitrile)[3-(1-imino-1-phenylmethyl)-2,4,-bis(2-pyridylmethylimino)pentane]iron(II) perchlorate; **2c**, (acetonitrile){3-[1-(4-fluorophenyl)-1-iminomethyl]-2,4-bis(2-pyridylmethylimino)pentane}iron(II) perchlorate; **2d**, (acetonitrile){3-[1-(3,4-difluorophenyl)-1-iminomethyl]-2,4-bis(2-pyridylmethylimino)pentane}iron(II) perchlorate; **2e**, (acetonitrile)[3-(1-imino-1-pentafluorophenylmethyl)-2,4-bis (2-pyridylmethylimino)pentane]iron(II) perchlorate.
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27. All the complexes were analyzed by ^{1}H NMR, IR spectroscopy and elemental analysis. Analytical data: **2b**: Anal. calcd for $FeC_{26}N_6H_{28}Cl_2O_8{\cdot}0.5C_4H_8O$: C, 47.01; H, 4.51; N, 11.75. Found: C, 46.74; H, 4.55; N, 12.03. Selected IR ν (N–H), 3262 cm^{-1}. **2c**: Anal. calcd for $FeC_{26}N_6H_{27}Cl_2O_8F_1{\cdot}0.5C_4H_8O$: C, 45.86; H, 4.26; N, 11.46. Found: C, 45.71; H, 4.56; N, 11.33. Selected IR ν (N–H), 3270 cm^{-1}. **2d**: Anal. calcd for $FeC_{26}N_6H_{26}Cl_2O_8F_2{\cdot}0.5C_4H_8O_1{\cdot}0.5H_2O$: C, 44.23; H, 4.11; N, 11.05. Found: C, 43.85; H, 4.48; N, 11.41. Selected IR ν (N–H), 3256 cm^{-1}. **2e**: Anal. calcd for $FeC_{26}N_6H_{23}Cl_2O_8F_5$: C, 40.60; H, 3.01; N, 10.93. Found: C, 40.88; H, 3.01; N, 10.77. Selected IR ν (N–H), 3263 cm^{-1}.
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