VIP Coordination Modes

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Modulation of Characteristics of a Ruthenium-Coordinated Flavin Analogue That Shows an Unusual Coordination Mode**

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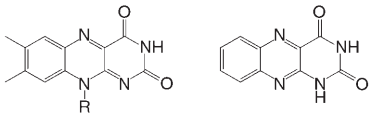
Flavins are redox-active fused heteroaromatic cofactors that are involved in a wide variety of biological redox processes. A flavin cofactor can undergo both stepwise one-electron processes through a radical intermediate and two-electron processes.[1] The ability to adjust their characteristics is indispensable to fulfill a specific function required for each flavoprotein, such as oxidation of substrates and electron-transfer reactions.[2] The redox potentials of flavin coenzymes are modulated by noncovalent interactions between apoproteins and flavin cofactors, including hydrogen bonds[3] and π–π interactions.[4] However, noncovalent interactions in biological systems are too intricate to evaluate directly.

To elucidate the effect of each interaction for controlling redox properties of flavin derivatives, Rotello and Niemz examined the effect of noncovalent interactions on the redox properties of flavin derivatives by using organic receptors.[5] They reported the adduct formation of 2,6-bis-(amido)pyridine derivatives as receptors that can form specific hydrogen bonds to flavins at 2-O, 3-NH, and 4-O positions and revealed that the hydrogen bonds stabilize the semiquinone radical state.[6] It has also been reported that hydrogen bonds exert some perturbation in the spin-density distribution of the naphthalimide radical anion by adduct formation with those receptors.[7] However, the effect of hydrogen bonds on the electronic structure of flavin radicals has yet to be disclosed.

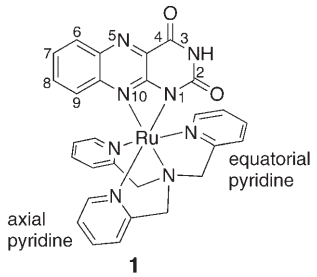
On the other hand, metal coordination can also stabilize the radical state of flavins. Flavins and their analogues have been known to bind to various metal ions by chelation, in many cases, through 4-O and 5-N atoms.[1] Among those metal complexes, the synthesis and characterization of ruthenium complexes,[8] including their structural determination by X-ray crystallography[9] and electrochemical measurements to reveal their redox behaviors,[8–10] has been reported. As for ruthenium complexes with flavins and their analogues, the detailed electronic structures of coordinated radical species have yet to be elucidated because of poor resolution of their electron spin resonance (ESR) spectra. In addition, the steric hindrance of the hydrogen-bond moiety of flavin owing to metal coordination has precluded examination of the effects of hydrogen bonds on the redox potentials of coordinated flavins.

We report herein the synthesis and characterization of a ruthenium(II) complex with alloxazine (Scheme 1), a flavin analogue, which forms an unusual four-membered chelate ring[14] with 1-N and 10-N atoms instead of a five-membered chelate ring with 4-O and 5-N atoms as reported for other flavin complexes.[1] Such novel 1,10-coordination in the ruthenium alloxazine complex allowed the formation of hydrogen-bonded complexes, through the available 4-O, 3-NH, and 5-N atoms, with hydrogen-bond acceptors. Thus, we could examine the effects of hydrogen bonds on the redox potentials of coordinated flavins and also on the electronic structure of the one-electron-reduced state of the hydrogen-bonded ruthenium alloxazine complex using ESR spectroscopy.



Scheme 1. Chemical structures of flavins (left) and alloxazine (right).

[Ru(Hallo)(tpa)]X (**1**)[11] (H_2allo = alloxazine; tpa = tris(2-pyridylmethyl)amine; X = ClO_4^-, PF_6^-; Scheme 2) was prepared by heating methanol solutions of [RuCl(tpa)]$_2X_2$[12] and H_2allo at reflux in the presence of excess NEt_3 under N_2. A single crystal of **1** (X = ClO_4) suitable for X-



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Scheme 2. Structure of complex **1** with alloxazine atom-numbering scheme.

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ray crystallography was obtained by recrystallization from methanol/2-propanol. The most remarkable feature of the crystal structure of **1** is the coordination mode of the alloxazine ligand (Figure 1a).[13a] It forms a four-membered

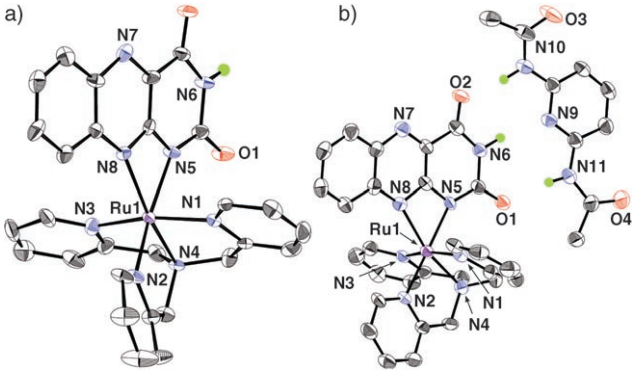

Figure 1. a) ORTEP drawing of **1** with 50% probability thermal ellipsoids. Selected bond lengths [Å] and angles [°]: Ru1–N1 2.054(5), Ru1–N2 2.081(4), Ru1–N3 2.054(4), Ru1–N4 2.064(4), Ru1–N5 2.116(4), Ru1–N8 2.201(4); N5-Ru1-N8 62.9(3), N1-Ru1-N4 83.8(3), N2-Ru1-N4 82.1(3), N3-Ru1-N4 80.7(3), N2-Ru1-N8 112.9(3), N4-Ru1-N5 102.6(3), N2-Ru1-N5 169.2(3), N4-Ru1-N8 164.9(3). b) ORTEP drawing of the adduct **1**·**2** with 50% probability thermal ellipsoids. Selected bond lengths [Å] and angles [°]: Ru1–N1 2.060(7), Ru1–N2 2.064(7), Ru1–N3 2.081(7), Ru1–N4 2.036(6), Ru1–N5 2.143(7), Ru1–N8 2.177(5); N5-Ru1-N8 62.6(2), N1-Ru1-N4 83.7(2), N2-Ru1-N4 81.4(2), N3-Ru1-N4 82.1(2), N2-Ru1-N8 113.8(2), N4-Ru1-N5 102.2(2), N2-Ru1-N5 174.1(2), N4-Ru1-N8 164.8(2).

chelate ring[14] with 1-N and 10-N atoms instead of a five-membered chelate ring with 4-O and 5-N atoms reported for other flavin complexes.[1] In the four-membered chelate ring of **1**, the Ru1–N8 bond (2.201(4) Å) is longer than the Ru1–N5 bond (2.116(4) Å) to reduce steric hindrance between the alloxazine ligand and the axial pyridine ring of the tpa ligand. In the case of the 4-O,5-N-chelating structure optimized by density functional theory (DFT) calculations at the B3LYP/LANL2DZ level, the distance between the 6-H atom of the axial pyridine ring of the tpa ligand and the neighboring 9-H atom of the alloxazine ligand (2.168 Å) is in close proximity compared to that of coordination through 1-N and 10-N (2.184 Å) for **1**. Moreover, the calculated 4,5-coordination structure has a larger dihedral angle between the axial pyridine moiety of tpa and the benzene moiety of the alloxazine ring (42.8° for 4,5-coordination and 32.2° for 1,10-coordination). These results indicate that the alloxazine ligand is subject to severe steric hindrance in the 4,5-coordination structure in the presence of the tetradentate tpa ligand. Thus, the unprecedented 1,10-coordination mode is characteristic of **1** by virtue of the Ru–tpa coordination environment.

The ^{1}H NMR spectrum of **1** was measured in CD_3CN and found to be consistent with that expected from the crystal structure. One singlet and two AX doublets are assigned to signals arising from the axial methylene and the equatorial methylene protons of the tpa moiety, indicating that the complex exhibits σ_h symmetry. A broad signal observed at $\delta =$ 8.88 ppm is ascribed to the imido proton of the alloxazine ligand as confirmed by its disappearance upon addition of D_2O.[15] Thus, the ^{1}H NMR spectrum of **1** indicates that the crystal structure of **1** is also maintained in solution.

The stability of **1** in solutions allowed us to investigate the recognition behavior for a hydrogen-bonding receptor. The alloxazine ligand of **1** bears hydrogen-bonding moieties that can bind a receptor as observed for free flavins through the 1,10-coordination mode. We used 2,6-bis(acetoamido)pyridine (**2**) as a hydrogen-bonding receptor to investigate the formation of the adduct and its influence on the redox potentials of the alloxazine ligand and also on the electronic structure of the alloxazine radical species. Diffusion of diethyl ether into a dichloromethane solution containing **1** and an excess amount (30 equiv) of **2** gave a single crystal of the 1:1 adduct (**1**·**2**; Figure 1b).[13b] The complex **1** and the receptor **2** form three-point intermolecular hydrogen bonds (O1–N11 2.845 Å, N6–N9 3.085 Å, O2–N10 2.959 Å).

The same adduct is expected to be formed in solution based on NMR spectroscopy measurements. In CD_2Cl_2, the signal of the imido proton of alloxazine in **1** at $\delta = 8.00$ ppm exhibits a downfield shift upon addition of the receptor **2** (Figure 2). The shift is attributed to adduct formation

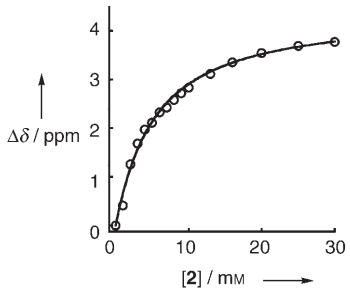

Figure 2. Change in the chemical shift of the imido proton of the alloxazine ligand in **1** (1 mM) upon addition of **2** in CD_2Cl_2 at room temperature.

between **1** and **2**, as the imido proton interacts with the basic pyridine nitrogen atom of **2** to be positively polarized, as revealed by the crystal structure of the adduct (Figure 1b). The binding constant, K_{ox}, was determined to be 205 M^{-1} by nonlinear least squares curve-fitting of the shift in the peak ($\Delta\delta$) as a function of the concentration of **2** (Figure 2).[16]

Cyclic voltammetry of free alloxazine exhibits irreversible redox waves at −1.32 and −1.89 V relative to the ferrocene/ferrocenium (Fc/Fc^+) redox couple in *N*,*N*-dimethylformamide (DMF). In contrast, the cyclic voltammogram of **1** in CH_3CN exhibits three reversible redox waves at 0.40 V ($\Delta E =$ 0.10 V), −1.34 V ($\Delta E = 0.10$ V), and −1.82 V ($\Delta E = 0.15$ V) relative to Fc/Fc^+ (see Supporting Information). ESR spectroscopy with potential-controlled bulk electrolysis allowed us to assign these waves to $Ru^{II/III}$,[17] $Hallo^{\bullet 2-}/Hallo^-$, and $Hallo^{3-}/Hallo^{\bullet 2-}$ redox couples, respectively. The reversibility of the redox processes is dramatically improved upon metal coordination. Addition of **2** to a solution of **1** in CH_2Cl_2 caused a positive shift (+43 mV) for the first reduction potential. The binding constant of **2** in the anion radical state, K_{rad}, was determined as 3300 M^{-1},[18] which was comparable to

those observed for uncoordinated flavins with 2,6-bis-(amido)pyridine derivatives by Rotello and co-workers.[6] The similarity of the binding constants for **1** with flavins lends credence to the significance of **1** as a model system to evaluate the electronic effects of hydrogen bonding on the electronic structure of flavins and their radical species.

For the one-electron-reduced species of **1** which was formed by electrolysis at −1.5 V (vs. $Ag/AgNO_3$) in CH_3CN at 243 K, its ESR spectrum showed a well-resolved nine-line signal at $g = 2.0012$. This signal is assigned to the coordinated alloxazine radical anion, $Hallo^{\bullet 2-}$ (Figure 3a). The g value is lowered relative to those of free flavin derivatives ($g = 2.0032$–2.0035)[19,20] as a result of spin–orbit coupling by coordination to the ruthenium center.[9,21] The hyperfine coupling constants (hfc) were estimated by DFT calculations (UB3-LYP/6-31G*) and show good agreement with the observed values (Figure 3c). The spin distribution on the alloxazine radical anion is centered at N5 and is similar to those observed for other flavin[19] or alloxazine radicals.[20] Metal coordination hardly affects the spin distribution of the coordinated alloxazine radical. Thus, flavin analogues bound to the Ru–tpa unit reveal almost the same electronic structures as their uncoordinated counterparts, which suggests that those derivatives can be recognized to be ruthenium-substituted flavins.

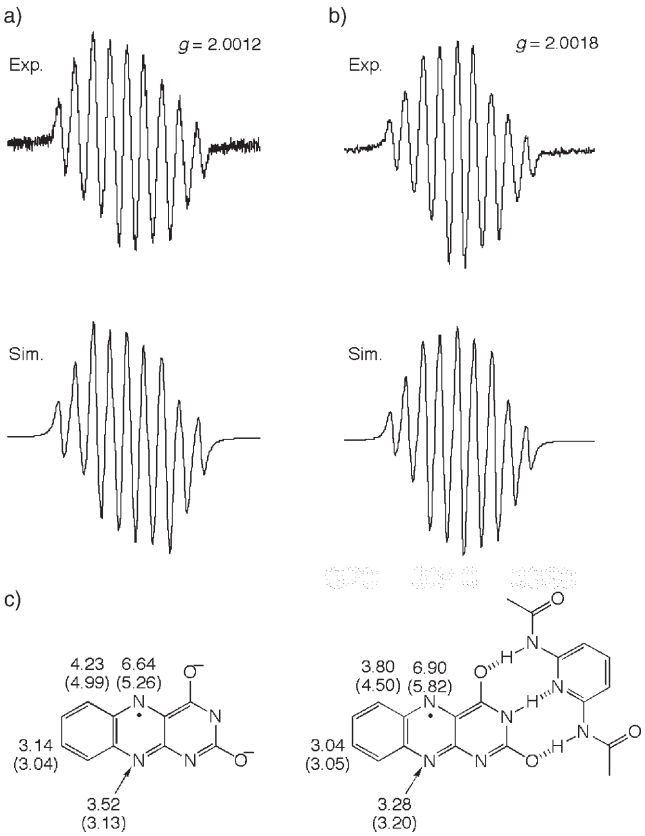

Figure 3. a, b) Experimental ESR spectra (upper) and computationally simulated spectra (lower; $\Delta H_{msl} = 1.0$ G) of a) the one-electron-reduced species of **1** (1.0 mM) and b) the one-electron-reduced species of **1** (1.0 mM) containing the hydrogen-bonding receptor **2** (30 mM). Both spectra were generated by electrolysis at −1.5 V (vs. $Ag/AgNO_3$) in CH_3CN containing 0.1 M Bu_4NPF_6 at 243 K. c) Observed hfc values [gauss] determined from the observed ESR spectra by the computer simulations; the calculated hfc values obtained by DFT calculations are indicated in parentheses.

We examined the effect of hydrogen bonds on the electronic structure of coordinated alloxazine (Figure 3b). The addition of receptor resulted in an increase in the g value from 2.0012 to 2.0018. This shift clearly shows the change of electronic structure of the coordinated alloxazine radical owing to perturbation of the spin–orbit coupling caused by the hydrogen bonds. The spin-density distribution in the hydrogen-bonded alloxazine radical anion was estimated by DFT calculations, which suggest its shift from the 8- and 10-positions to the 5- and 6-positions, which lie away from the ruthenium center. Thus, the interaction of the unpaired electron with the ruthenium center is weakened, leading to smaller spin–orbit coupling and thus a larger g value for the adduct.

In summary, we have synthesized a ruthenium(II) alloxazine complex that reveals an unprecedented coordination mode of alloxazine. The novel coordination mode makes possible multiple hydrogen-bonding interactions of the complex to form stable adducts with complementary receptors. These intermolecular hydrogen bonds allowed us to regulate the redox potentials and electronic structure of the alloxazine ligand. Thus, a variety of reactivities of flavin cofactors can be derived from those noncovalent interactions in various environments in flavoproteins.

Experimental Section

$[Ru(Hallo)(tpa)]ClO_4$ (**1**): $[\{RuCl(tpa)\}_2](ClO_4)_2$ (100 mg, 0.0950 mmol) was added to a degassed methanol solution containing H_2allo (45 mg, 0.21 mmol) and triethylamine (211 mg, 2.09 mmol). The mixture was heated at reflux for 6 h under N_2. After cooling, the solvent was removed, the residue was dissolved into CH_3CN, and the mixture was filtered. The solution was evaporated to dryness to obtain a purple solid. Recrystallization of the solid from methanol/2-propanol gave a violet precipitate (65% yield). Elemental analysis (%) calcd for $C_{28}H_{23}N_8O_6ClRu$ (**1**): C 47.77, H 3.29, N 15.92; found: C 47.47, H 3.31, N 16.12. A single crystal suitable for X-ray crystallography was obtained by slow recrystallization from methanol/2-propanol.

Preparation of the adduct **1**·**2**: Et_2O vapor was deposited at room temperature onto a solution of **1** and 2,6-bis(acetoamido)pyridine (30 equiv) in CH_2Cl_2 to give plate-shaped single crystals of the adduct. Elemental analysis (%) calcd for $C_{28}H_{23}N_8O_6ClRu$ (**1**·**2**·$2H_2O$): C 47.62, H 4.10, N 16.51; found: C 47.81, H 3.95, N 16.65.

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[11] ^{1}H NMR (300 MHz, CD_3CN): $\delta = 4.47$ (s, 2H; CH_2 (ax)), 4.87 and 5.91 (AX q, 4H, $J = 15$ Hz; CH_2 (eq)), 7.0–7.1 (m, 3H; py-H5 (eq) and py-H3 (ax)), 7.27 (t, 1H, $J = 6$ Hz; py-H5 (ax)), 7.43 (d, 2H, $J = 8$ Hz; py-H3 (eq)), 7.58 (t, 1H, $J = 7$ Hz; py-H4 (ax)), 7.67 (t, 2H, $J = 8$ Hz; py-H4 (eq)), 7.79 (t, 1H, $J = 7$ Hz; allo-H8), 8.10 (t, 1H, $J = 8$ Hz; allo-H7), 8.1–8.3 (m, 2H; allo-H6 and H9), 8.40 (d, 2H, $J = 5$ Hz, py-H6 (eq)), 8.88 (br, 1H, allo-H3), 9.65 ppm (d, 1H, $J = 5$ Hz, py-H6 (ax)).

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[13] a) Crystal data for **1**: orthorhombic, $Pna2_1$ (No. 33), $a = 17.2050(6)$, $b = 12.1332(4)$, $c = 13.3333(4)$ Å, $V = 2783.4(2)$ Å^3, $T = 20$ °C, $Z = 4$, $R(R_w) = 0.082(0.290)$ ($I > 2\sigma(I)$), GOF = 1.32; b) crystal data for the adduct **1**·**2**: triclinic, $P\bar{1}$ (No. 2), $a = 11.307(4)$, $b = 11.342(3)$, $c = 17.140(5)$ Å, $\alpha = 83.09(1)$, $\beta = 83.19(1)$, $\gamma = 67.70(1)$°, $V = 2013(1)$ Å^3, $T = -140$ °C, $Z = 2$, $R(R_w) = 0.070(0.190)$ ($I > 2\sigma(I)$), GOF = 1.06. CCDC 619273 (**1**) and 619274 (**1**·**2**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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[15] The pK_a value of the imido proton of **1** was determined to be 11.0 in Britton–Robinson buffer at room temperature based on the UV/Vis spectral change upon addition of 0.5 M NaOH. See Supporting Information.

[16] The data were fitted by using the following equation: $\delta_{obs} = \delta_H + \frac{(\delta_{HG} - \delta_H)\left[\left([H_t]+[G_t]+\frac{1}{K_a}\right) - \left(\left([H_t]+[G_t]+\frac{1}{K_a}\right)^2 - 4[H_t][G_t]\right)^{1/2}\right]}{2[H_t]}$ where $[H_t]$ and $[G_t]$ are total host and guest concentrations, respectively, δ_{obs} is the observed shift, δ_H is the chemical shift of the host, δ_{HG} is the chemical shift of the host–guest complex, and K_a is the association constant.

[17] The one-electron-oxidized species, $[Ru^{III}(Hallo)(tpa)]^{2+}$, which is produced by one-electron oxidation with $[Ru^{III}(bpy)_3]^{3+}$, exhibits an anisotropic ESR signal with $g = 1.72$, 2.33, and 2.47 at −150 °C (See Supporting Information).

[18] The K_{rad} value was determined by using the following equation: $\frac{K_{rad}+1}{K_{ox}+1} = \exp\left\{\frac{nF}{RT}(E_{1/2(bound)} - E_{1/2(unbound)})\right\}$.

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