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Dynamics of Intramolecular Electron Transfer Reaction of FAD Studied by Magnetic Field Effects on Transient Absorption Spectra

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The kinetics of intermediates generated from intramolecular electron-transfer reaction by photo irradiation of the flavin adenine dinucleotide (FAD) molecule was studied by a magnetic field effect (MFE) on transient absorption (TA) spectra. Existence time of MFE and MFE action spectra have a strong dependence on the pH of solutions. The MFE action spectra have indicated the existence of interconversion between the radical pair and the cation form of the triplet excited state of flavin part. All rate constants of the triplet and the radical pair were determined by analysis of the MFE action spectra and decay kinetics of TA. The obtained values for the interconversion indicate that the formation of cation radical promotes the back electron-transfer reaction to the triplet excited state. Further, rate constants of spin relaxation and recombination have been studied by the time profiles of MFE at various pH. The drastic change of those two factors has been obtained and can be explained by SOC (spin−orbit coupling) induced back electron-transfer promoted by the formation of a stacking conformation at pH > 2.5.

Introduction

Radical pair is the state in which two radicals situate relatively close and is important in the biological or artificial photochemical reaction centers[1,2] and in the process of the redox reaction of an enzyme.[3] In such a system after the production of radical pairs, competition between a charge recombination process to an original state and a following charge separation process is very essential for the efficiency of photoactive devices in optoelectronics[4,5] and artificial biological systems.[6−10]

The external magnetic field effect (MFE) based on a radical pair mechanism (RPM) has been studied in various ways.[11,12] The MFE is caused by the modulation of electron spin dynamics by an applied magnetic field and a spin-selective charge recombination process in a radical pair. The biradicals generated by the intramolecular photochemical reaction of a polymethylene-linked system generally have a large MFE[13,14] because the possibility of geminate recombination is much larger than that in homogeneous solutions. In such systems, the reaction dynamics based on the RPM was well studied not only by the MFE but also by various magnetic resonance spectroscopic methods.[15−19]

Previously, experimental and theoretical studies on the MFE in singlet-born ionic biradicals have been given by Weller, Staerk et al.,[20,21] and Tanimoto et al.[22−24] In those systems, the existence of interconversion between the exciplex state and the singlet radical pair enhances the MFE because of the reproduction of a radical ion pair. However, this fact prevents us from direct analysis of the spin lifetime of the radical pair by means of a time-resolved spectroscopic method. Recently, Enjo et al.[25] have demonstrated that the precise analysis of RYDMR (reaction yield detected magnetic resonance) spectra give us information of the spin lifetime in the polymethylene-linked phenantherene/*N*,*N*-dimethylaniline system. This result stimulates us to study unknown complicated dynamic process in photochemical reactions by means of techniques of spin chemistry.

As discussed above, the effect of molecular dynamics on the MFE has been studied in various model systems. Therefore, the basic knowledge of the RPM as the mechanism of the MFE is applicable to explore the dynamics of the radical pair generated in complicated reaction systems. In other words, the analysis of MFE gives important information about the dynamics of the radical pair which is not obtained by the conventional spectroscopic methods. Previously, demonstrations of the application of MFE and its action spectrum to the study of photoinduced electron-transfer reactions have been performed.[26,27] Using MFE-action spectrum, one can selectively observe the spectrum and the kinetics of the radical pair and species generated from the radical pair avoiding the overlap of the spectra of excited states.

Flavin adenine dinucleotide (FAD) is one of the most important co-enzymes for electron transportation and the important photoreceptor in biological systems. Especially, in the photoactive flavoproteins, the radical pair formation by photoinduced charge transfer of the flavin cofactor is an essential process and has been discussed as the basic mechanism of the biological interesting effects, such as DNA repair by DNA photolyase[28−35] and orientation recognition of animals.[36−38]

Besides the biological applications, the FAD itself produces a biradical state by an intramolecular electron transfer reaction because FAD is a linked system of an electron donor (adenine) and an acceptor (flavin). Previously, photo-CIDNP (photo chemically induced dynamic nuclear polarization) spectra have been reported in the photoinduced intramolecular electron-transfer reaction of FAD by Kaptein et al.[39,40] The photo-CIDNP signal was observed in the pH region of 1.5−4.0 and indicates that the radical pair is produced in the photo irradiation. The disappearance of the photo-CIDNP signal at higer pH than 4.0 was interpreted by the strong exchange interaction due to the

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formation of stacking conformation in the ground state.[41,42] However, the relationship of the ground state conformation and the dynamics in the excited states is still unclear because real-time observation of the radical pair by time-resolved spectroscopy has not been performed. Therefore the reaction kinetics of the intramolecular electron-transfer reaction has not been revealed.

Accordingly, we have tried to observe the radical pair in the FAD system directly with the MFE on time-resolved transient absorption (TA) spectroscopy. In the previous paper, we reported that the MFE-action spectra were observed in various pH conditions and clarified the existence of the interconversion between the radical pair and the excited triplet state.[43] In the present paper, we reveal the kinetics and the molecular dynamics of the intramolecular electron transfer by the further analysis of the MFE time profile and MFE-action spectra.

Experimental Section

The TA spectra were observed by a homemade setup. A flow system was used for transferring a sample solution into a quartz optical cell where photochemical reactions were initiated by a laser pulse. The third harmonics ($\lambda = 355$ nm) of a Nd:YAG Laser (Spectra Physics GCR-3) was used as an exciting light source. The energy of the laser was adjusted to 15 mJ per pulse. A 500 W Xe lamp (Ushio UXL-500SX) was used as a probe light source. The TA signal was detected by a photomultiplier tube (Hamamatsu R928) fixed with a monochromator (Jasco CT-25) and was recorded by a digital oscilloscope (LeCroy LT-344) and was analyzed by a personal computer.

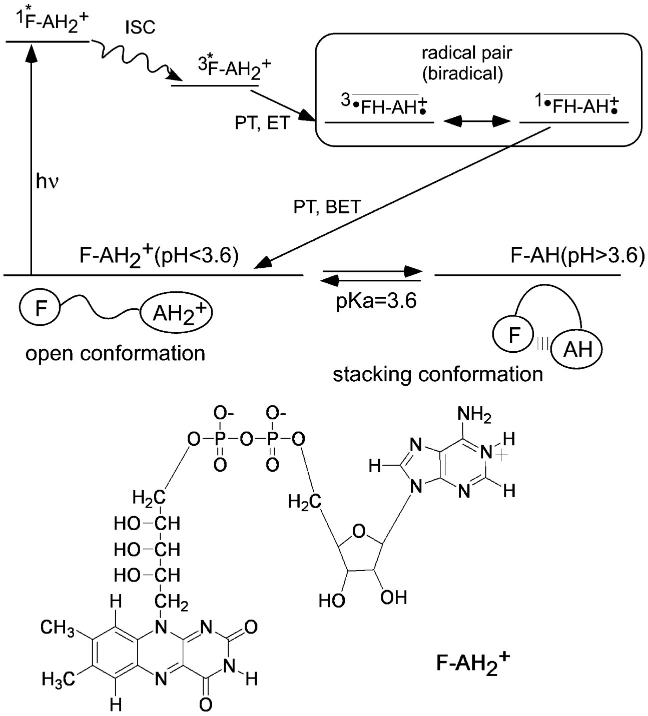
FMN and adenosine (Sigma) and FAD (Sigma and Tokyo Kasei) were used without purification. The concentrations of FAD and FMN were adjusted to 0.2 and 0.18 mM, respectively. Buffer solutions ($Na_2HPO_4 \cdot 12H_2O$: 4−83 mM/citric acid monohydrate: 98−59 mM, pH = 2.2−4.2[44]) of distilled water (Kishida) were used for the solvent. The sample solutions were deoxidized by Ar gas bubbling.

Cyclic voltammetry (CV) experiments were performed by using 100B/W (CV-50W) by Bioanalytical Systems. $LiClO_4$ was used by electrolyte, and the concentration of the object molecule was 15 mM in buffer solutions mentioned in the previous paragraph.

Results

TA Spectrum of FAD. The reaction scheme of the photoinduced intramolecular electron-transfer reaction of FAD proposed by Kaptein and co-workers is shown in Scheme 1.[39,40] The radical pair composed of a flavin radical and an adenine radical is generated by the electron transfer following the proton transfer from the adenine moiety to the excited triplet state of the flavin part. The TA spectra of FAD observed in the laser flash photolysis at pH = 2.3 are shown in Figure 1a. Two components, the short-lived component ($\lambda_{max} = 680$ nm, $\tau = 0.7$ μs) and the long-lived component ($\lambda_{max} = 510$ nm, $\tau = 50$ μs), can be recognized. The spectrum of the former component is assigned to the T−T absorption of the cation form of the flavin part ($^3FH^+$) from the similarity to that of FMN.[45] Since the proportion of the latter component increases by increasing the laser power, it can be assigned to an intermediate generated from a bimolecular reaction.[46] The lifetime of the short-lived component became short at higher pH as shown in Figure 1b, whereas the spectral shape does not have a characteristic or clear difference in the region of 1.9−4.1. The TA signal of neutral radical of flavin ($FH^\bullet$, $\lambda_{max} = 502$ and 580 nm[47]) could not be recognized clearly because of the interference of the triplet absorption, which is strong and broad in the range of 500−730 nm.

SCHEME 1: Reaction Scheme of the Intramolecular Electron Transfer Induced by Light Irradiation of FAD Molecule in the Region of pH < 3.6[a]



[a] The reaction scheme is proposed by Kaptein et al. The structure formula is included in the scheme.

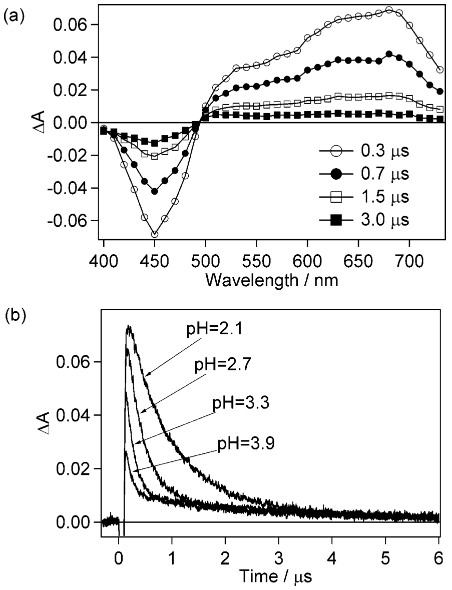


Figure 1. (a) Transient absorption spectra observed in the laser flash photolysis of FAD molecule at pH = 2.3. Open circle, filled circle, open square, and filled square are the spectra observed at $t = 0.3$, 0.7, 1.5, and 3.0 μs after laser pulse, respectively. (b) pH dependence of the time profiles of the transient absorption observed at $\lambda = 680$ nm without a magnetic field.

Magnetic Field Effect (MFE) on Photoinduced Intramolecular Electron-Transfer Reaction of FAD. *(a) MFE Time Profile.* An example of the MFE on the decay kinetics of TA spectra of FAD is shown in Figure 2a. The TA signal observed at 600 nm increases in the presence of an external magnetic field. The MFE time profile is calculated by the subtraction of

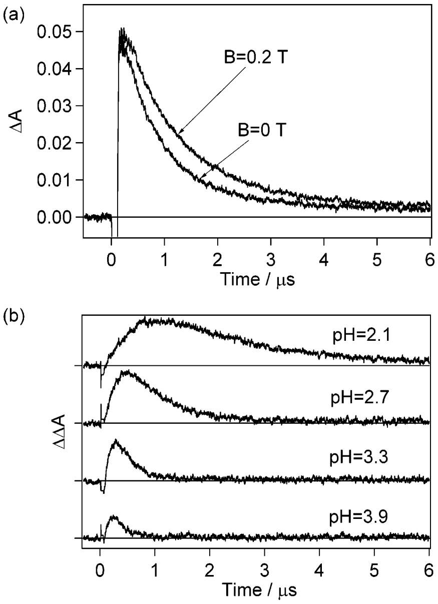


Figure 2. (a) Time profiles of the transient absorption at $\lambda = 580$ nm with (upper, $B = 0.2$ T) and without (lower, $B = 0$ T) magnetic field. (b) pH dependence of the MFE time profiles given by the subtractions of the data without magnetic field from that with magnetic field ($B = 0.2$ T). These MFE time profiles were obtained by averaging the data in the region of $\lambda = 550\sim650$ nm.

the TA signal observed in zero magnetic field, $\Delta A(B = 0T,t)$ from that in the presence of the magnetic field, $\Delta A(B = 0.2T,t)$ that is given by

$$\Delta\Delta A(B = 0.2T,t) = \Delta A(B = 0.2T,t) - \Delta A(B = 0T,t) \quad (1)$$

Calculated MFE time profiles in various pH are shown in Figure 2b. The MFE time profiles strongly depend on pH. The MFE at pH = 1.9 exists until about 5 μs after the laser pulse, whereas that disappears within 1 μs at pH = 3.9. The continuous change of the MFE time profiles by pH in Figure 2b is noteworthy because the continuous change cannot be explained only by the conformational change in the ground state of the FAD molecule. The dynamics of the excited state and the radical pair in the FAD system is modulated continuously by pH.

The MARY (magnetic field effect on reaction yield) curve obtained by plotting the increase of TA versus applied external magnetic field (shown in the Supporting Information) was characteristic of the hyperfine mechanism[11,12] and the relaxation mechanism,[12,48] which are in the category of the RPM. On the basis of the RPM, the positive MFE indicates that the radical pair is produced from a triplet precursor molecule if the back electron-transfer reaction takes place in the singlet radical pair. The MFE time profile should reflect the existence time of the radical pair state and its dynamics should depend on acidity of the solution.

(b) MFE Action Spectrum. An action spectrum of MFE is obtained by plotting the MFE obtained by the subtraction versus wavelength of the reference light. Generally, MFE action spectra should gives us spectra of the radical pair itself and species generated from the radical pair[26] avoiding the overlap of spectra of precursor molecules.

The action spectra of FAD observed at various pH are shown in Figure 3a. The action spectra were calculated by averaging of the MFE in the whole range of time. The spectral shape depends on pH as shown in Figure 3a. The spectrum at pH = 2.3 in Figure 3a has a peak around $\lambda = 650$ nm and is similar to the T−T absorption spectrum of the cation form of flavin (^{3}FH$^+$). In contrast, at pH = 3.9, the spectrum has turned into a broad spectrum around 550−600 nm, which is similar to the absorption spectrum of the neutral flavin radical (FH$^\bullet$). We successfully reproduced the observed spectra in various pH by linear combinations of the two template spectra of the triplet excited state of flavin $\epsilon_T(\lambda)$ and the neutral radical $\epsilon_R(\lambda)$, which are shown in Figure 3b

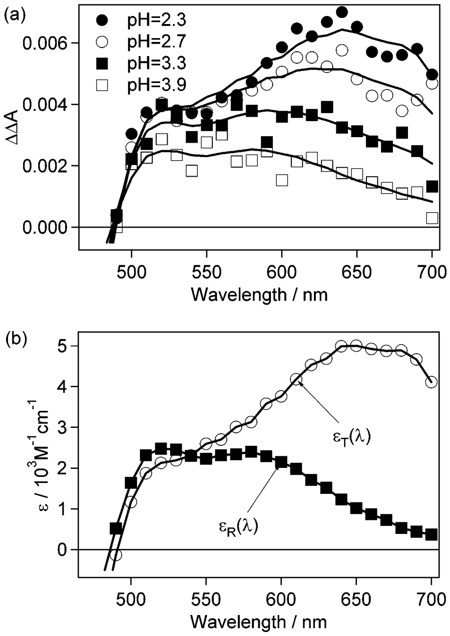


Figure 3. (a) pH dependence of the MFE action spectra given by averaging the data in the region of the time to be observed the MFE: filled circle, pH = 2.3; open circle, pH = 2.7; filled square, pH = 3.3; open square, pH = 3.9. Solid lines are simulation spectra by the linear combination of the template spectra. (b) Template spectra for the fitting of the MFE action spectra by eq 2. $\epsilon_T(\lambda)$ was obtained at 0.5 μs after laser flash photolysis of an aqueous solution (pH = 3.7) of FMN. $\epsilon_R(\lambda)$ was obtained at 5 μs after laser flash photolysis of an aqueous solution (pH = 3.7) of FMN and adenosine (15 mM).

$$\Delta\Delta A = \Delta C_R\epsilon_R(\lambda) + \Delta C_T\epsilon_T(\lambda) \quad (2)$$

where ΔC_R and ΔC_T represent the contribution of FH$^\bullet$ and ^{3}FH$^+$ on the MFE action spectra, respectively. The template spectra, $\epsilon_R(\lambda)$ and $\epsilon_T(\lambda)$, in Figure 3b have been obtained by the TA measurements in the water solution of FMN (pH = 3.7) and the mixture of FMN and adenosine under same conditions. The absolute value reported in the system of riboflavin (pH = 2.2) has been used as the absolute extinction coefficients.[46] The simulated spectra reproduced the experimental data at each pH as shown in Figure 3a. The pH dependence of the value, $\Delta C_T/\Delta C_R$, is used for the analysis in the next section. In the FAD system, the MFE was observed to the triplet and the radical pair.

Time-resolved MFE action spectra observed at pH = 2.3 are shown in Figure 4a. The time evolution of the spectra can be recognized within 0.5 μs after laser pulse. This fact is due to the transformation of the MFE created in RP to the triplet state. Such time evolution of the spectra have been clearly recognized only in the low pH region (<2.3). This result suggests that the chemical process from the radical pair to the triplet excited state is promoted by the protonation reaction of radicals. Such a process could not be observed by the conventional TA spectra because the strong signal of the triplet excited state produced just after a laser flash had been overlapped in the spectra as shown in the solid line in the figure. The MFE action spectra

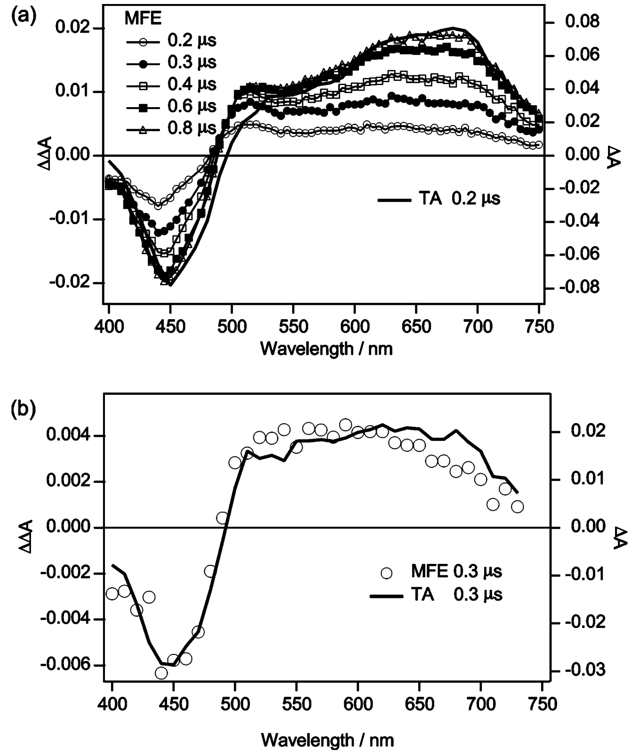


Figure 4. (a) Time-resolved MFE action spectra at pH = 2.3: open circle, $t = 0.25$ μs; filled circle, 0.35 μs; open triangle, 0.45 μs. Solid line is the TA spectrum at 0.25 μs after laser pulse. (b) Time-resolved MFE action spectra (open circle, $t = 0.3$ μs) at pH = 3.3. Solid line is the TA spectrum at 0.3 μs after the laser pulse.

provide us the information of the photochemical process following after production of the radical pair. At pH = 3.3, we could not recognize clear time evolution of the action spectra because of the short duration of the MFE. However, the MFE action spectrum (open circle) and the TA spectrum (solid line) observed at the same time (0.3 μs) are not identical as shown in Figure 4b. This result indicates that the relative concentration of the RP and the triplet state had not attained to a quasi steady state at 0.3 μs yet.

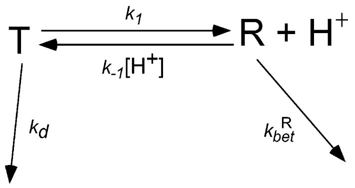
Discussion

Analysis by a Kinetic Model. The fact that the two components of the triplet excited state and the radical pair were overlapped in the MFE action spectra indicates the reproduction of the triplet excited state from the radical pair, which strongly depends on pH. The time-resolved spectra in Figure 4a shows the time evolution of the system toward the quasi-steady-state, in which the ratio of the triplet and the radical pair and their MFEs should not depend on time. For instance, at pH = 2.3, the action spectra do not depend on time later than 0.5 μs even the MFE prolongs about 5 μs. We have assumed that ΔC_T and ΔC_R are in proportion to the concentration of the excited triplet state (C_T) and radical pair (C_R) on the reaction, that is

$$\frac{\Delta C_T}{\Delta C_R} \cong \frac{C_T}{C_R} \tag{3}$$

To analyze the results, we have used a kinetic model shown in Scheme 2. The kinetic parameters k_1 and k_{-1} are the rate constants for the interconversion and the k_d and k_{bet}^R are the rate constants for the decay of the triplet excited state and the charge recombination of the radical pair to the ground state, respectively.

SCHEME 2: Schematic Representation of the Kinetic Model for the Interconversion between the Excited Triplet State (T) and the Radical Pair (R)



Rate equations in this model are written by

$$\frac{dC_T}{dt} = -(k_d + k_1)C_T + k_{-1}[H^+]C_R \tag{4}$$

$$\frac{dC_R}{dt} = -(k_{bet}^R + k_{-1}[H^+])C_R + k_1 C_T \tag{5}$$

Under the steady-state condition ($dC_R/dt = 0$) and the assumption of eq 3, two following relationships can be derived

$$\frac{\Delta C_T}{\Delta C_R} \cong \frac{C_T}{C_R} = \frac{k_{bet}^R}{k_1} + \frac{k_{-1}}{k_1}[H^+] \tag{6}$$

$$k_0 = k_d + k_{bet}^R \frac{C_R}{C_T} \cong k_d + k_{bet}^R \frac{\Delta C_R}{\Delta C_T} \tag{7}$$

where k_0 is a first-order decay rate constant of the triplet excited state in the steady-state condition.

Plots of $\Delta C_T/\Delta C_R$ versus $[H^+]$ and k_0 versus $\Delta C_T/\Delta C_R$ are shown in Figure 5, panels a and b, respectively. The plots have a linear relationship in the region of pH = 1.9−3.1. The plots were analyzed by eq 6 and 7 under the kinetic model in the region of pH = 1.9−3.1. From the slopes and the segments of the plots, all kinetic parameters k_1, k_{-1}, k_d, and k_{bet}^R in the model have been determined to be 7×10^6 s^{-1}, 2×10^9 M^{-1} s^{-1}, 0.3×10^6 s^{-1}, and 3×10^6 s^{-1}, respectively.

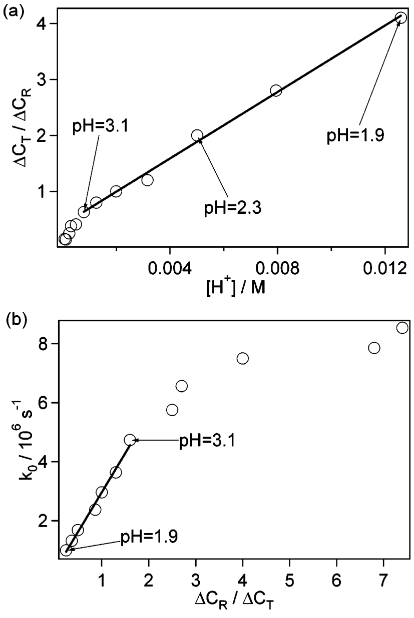
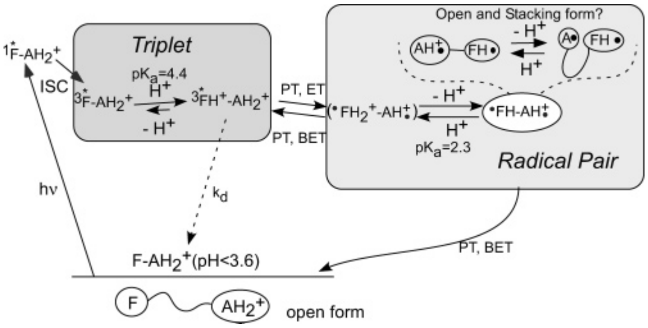


Figure 5. (a) Plot of the value of $\Delta C_T/\Delta C_R$ versus the proton concentration of the sample solution. (b) Plot of the overall decay rate (k_0) of the excited triplet state of the flavin part of FAD at each pH versus the value of $\Delta C_T/\Delta C_R$.

SCHEME 3: Proposed Reaction Scheme by the Present Investigation



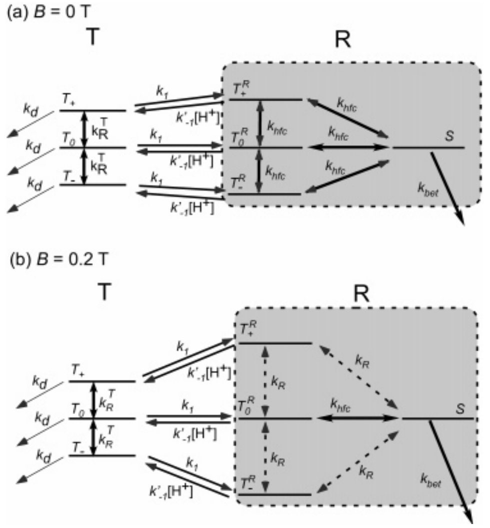
The equilibrium constant of the excited triplet and the radical pair can be calculated by the equation, $K = k_{-1}/k_1$. The calculated value of $-\log K$ (pK) is 2.5, which is similar to the pK_a (=2.3) value of the cation form of the flavin radical.[49] This result indicates that the formation of the cation form of the flavin radical plays an important role in the formation of the triplet excited state from the biradical. The obtained k_d value is reasonable because it is similar to the separately measured decay rate constant, which is 0.26×10^6 s^{-1}, of the triplet excited state of the cation form of flavin mononucleotide (FMN).

The plots in the region of pH > 3.1 have been out of the linear relationship. This is due to the failure of the steady-state approximation in eq 6. This fact can be confirmed by the comparison of the TA spectra and the MFE action spectra shown in Firure 4b. Figure 4b indicates that $\Delta C_T/\Delta C_R$ observed in the action spectrum is smaller than C_T/C_R in the TA spectrum. The similar difference of the action spectra and the TA spectra also appears in the very early time event within 500 ns in low pH region as shown in Figure 4a. Totally, we conclude that the system attains to the steady state in the time scale of the several hundreds ns and the triplet excited state is produced initially before production of the radical pair. This conclusion is consistent with the direction of the MFE.

Interconversion between the Radical Pair and the Excited Triplet State of the Flavin Part. The proposing reaction scheme obtained by the analysis of the MFE action spectra is shown in Scheme 3. The excited triplet state of the cation form of flavin part (^{3}FH $^+$$-AH_2^+$) and biradical •FH $-$ AH•$^+$ interconvert with each other. The pK_a value of the protonation of neutral form (^{3}F) was estimated to be 4.4 by Sakai et al.[45] Therefore, the cation form of triplet flavin is generated in the region of pH < 4.0. The pH dependence on the action spectra indicates that the formation of •FH$_2^+$$-$AH•$^+$ plays an important role to the reproduction of the triplet flavin. However, the absorption of •FH$_2^+$ ($\lambda_{max} = 488$ nm[39]) was not clearly observed in the MFE action spectra. It is because the bleaching of ground-state overlaps to the absorption of •FH$_2^+$ and/or the biradical •FH$_2^+$$-$AH•$^+$ is unstable to release a proton to the neutral radical or produce the triplet excited state by back electron-transfer reaction rapidly.

The fact that MFE was observed in both the excited triplet state and the radical pair indicates that the excited triplet state is reproduced from the radical pair. This dynamics prolongs the existence time of the radical pair especially in the low pH region. Ito and co-workers[51] previously reported that the long lifetime of the radical ion pair in the system of the retinal$-$C$_{60}$ dyad is due to the equilibrium between radical ion pair and the excited triplet state. Such mechanism to prolong the existence time of radical ion pair has been studied by Enjo et al. in the polymethylene-linked system of phenanthrene$-$$N$,$N'$-dimethylaniline.[25] In that case, the interconversion between singlet exciplex and radical ion pair takes place. In both cases, the interconversion between relatively long-lived electronic states and the radical pair cause the radical pair to prolong the existence time. However, the pure lifetime of the electron spin state in the radical pair is different from the existence time and is hardly determined by the conventional spectroscopic measurements. In our system of FAD, we can calculate it from $(k_{-1}[\mathrm{H}^+] + k_{\mathrm{bet}}^{\mathrm{R}})^{-1}$.

SCHEME 4: Spin Chemical Model for the Interconversion in the Case of (a) Presence (B = 0.2 T) and (b) Absence (B = 0 T) of External Magnetic Field



The interconversion reaction between the excited triplet state and the radical pair is energetically probable. Cyclic voltammetry (CV) experiments have been performed in aqueous solution of Adenosine and FMN at pH = 2.6. Since the oxidation potential of adenosine ($\Delta E_{1/2}^{\mathrm{ox}}$(Adn)) and the reduction potential of FMN ($\Delta E_{1/2}^{\mathrm{red}}$(F)) are +1.51 and -0.61 eV (vs AgCl), respectively, the potential energy of the radical pair composed of the neutral radical of flavin and the cation radical of adenine is estimated to be 2.1 eV. This value is similar to the triplet energy of cationic form of FMN (2.2 eV) measured by phosphorescence spectrum.[52]

Spin Dynamics. The kinetic model discussed in the previous section is applicable only in the steady state condition. Therefore, we could not analyze the time profiles of the MFE especially in the region of pH > 3 because the system has not attained to the steady-state condition. Here we have performed model calculations and fitting on the time profiles of the MFE obtained by eq 1. The MFE on the dynamics of the radical pair are interpreted by the hyperfine mechanism[11,12] and the relaxation mechanism.[12,48] The model schemes that take into account the spin sublevels of the radical pair and the triplet excited state under the condition of $B = 0$ and 0.2 T are shown in Scheme 4, parts a and b, respectively. At zero magnetic field (Scheme 4a), all spin states of the radical pair (S, T_+^{R}, T_0^{R}, T_-^{R}) can interconvert each other by the rate constant k_{hfc} that can be estimated from the hyperfine coupling constants of the couple of radicals. In contrast at the presence of external magnetic field ($B = 0.2$ T), the T_+^{R} and T_-^{R} states are energetically separated from the S,T_0^{R} mixed states because of Zeeman interaction. In this condition, the electron spin relaxation (k_{R}) between T_+^{R} and T_-^{R} states and S, T_0^{R} mixed states are sufficient for the reaction dynamics of the radical pair.

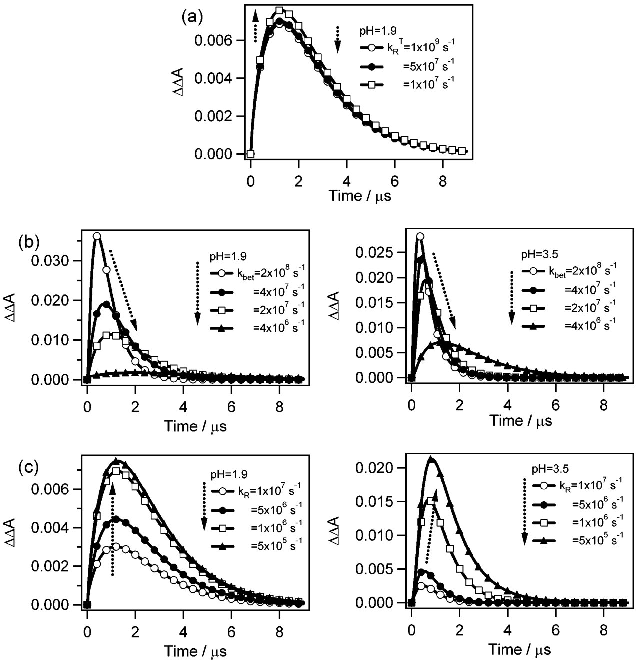


Figure 6. (a) Calculated k_R^T dependence of MFE time profile at pH = 1.9. Kinetic parameters: $k_1 = 7 \times 10^6$ s^{-1}, $k'_{-1} = 2.7 \times 10^9$ M^{-1} s^{-1}, $k_d = 0.3 \times 10^6$ s^{-1}, $k_{hfc} = 8 \times 10^7$ s^{-1}, $k_R = 1 \times 10^6$ s^{-1}, $k_{bet} = 1.2 \times 10^7$ s^{-1}, fac = 0.07 were used for the calculation. Open circle, $k_R^T = 1 \times 10^9$ s^{-1}; filled circle, $k_R^T = 5 \times 10^7$ s^{-1}; open triangle, $k_R^T = 1 \times 10^7$ s^{-1}. (b) Calculated k_{bet} dependence of MFE time profile at pH = 1.9 and pH = 3.5. Kinetic parameters: $k_1 = 7 \times 10^6$ s^{-1}, $k'_{-1} = 2.7 \times 10^9$ M^{-1} s^{-1}, $k_d = 0.3 \times 10^6$ s^{-1}, $k_{hfc} = 8 \times 10^7$ s^{-1}, $k_R^T = 1 \times 10^8$ s^{-1}, $k_R = 1 \times 10^6$ s^{-1}, and the proportional factor: fac = 0.07 were used for the calculation. Open circle, $k_{bet} = 2 \times 10^8$ s^{-1}; filled circle, $k_{bet} = 4 \times 10^7$ s^{-1}; open square, $k_{bet} = 2 \times 10^7$ s^{-1}; filled triangle, $k_{bet} = 4 \times 10^6$ s^{-1}. (c) Calculated k_R dependence of MFE time profile at pH = 1.9 and pH = 3.5. Kinetic parameters: $k_1 = 7 \times 10^6$ s^{-1}, $k'_{-1} = 2.7 \times 10^9$ M^{-1} s^{-1}, $k_d = 0.3 \times 10^6$ s^{-1}, $k_{hfc} = 8 \times 10^7$ s^{-1}, $k_R^T = 1 \times 10^8$ s^{-1}, $k_{bet} = 1.2 \times 10^7$ s^{-1} and the proportional factor: fac = 0.07 were used for the calculation. Open circle, $k_R = 1 \times 10^7$ s^{-1}; filled circle, $k_R = 5 \times 10^6$ s^{-1}; open square, $k_R = 1 \times 10^6$ s^{-1}; filled triangle, $k_R = 5 \times 10^5$ s^{-1}.

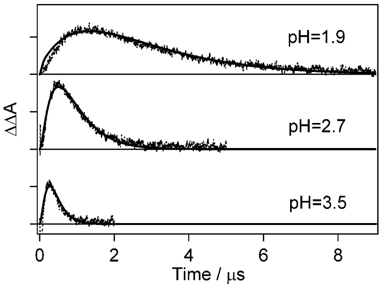


Figure 7. Fitting of the MFE time profiles by spin chemical model. Dash lines are the experimental data. Solid lines are the simulated data.

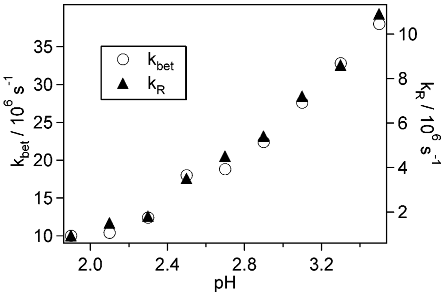


Figure 8. pH dependence of the values of k_R and k_{bet} given by the fitting of the MFE time profiles.

We have simulated the observed time profiles by the calculation shown in appendix A. The rate constant of the hyperfine induced spin mixing k_{hfc} is calculated to be 8×10^7 s^{-1} from the hyperfine coupling constants of flavin radical and adenine radical reported previously.[53−55] The rate constant of the relaxation of the triplet excited state of flavin part, k_R^T, is estimated by eq a-10. The rotational correlation time τ_c has been calculated to be 58 ps by the Einstein−Stokes relation under the assumption that the radius a of FAD molecule is 0.4 nm. The calculation by eq a-11 indicates that k_R^T decreases about 30% in the magnetic field of 0.2 T. We have estimated the absolute value of k_R^T at $B = 0.2$ T to be 1×10^8 s^{-1} by eq a-10, using D and E values ($D = 1.98$ GHz, $E = 0.56$ GHz) reported by Lhoste et al.[52]

We have investigated the dependence of the MFE time profiles on the parameters (k_R^T, k_R, k_{bet}). In the case of $k_R^T > 5 \times 10^7$ s^{-1}, the MFE time profiles were independent of k_R^T value as shown Figure 6a. This result indicates that the triplet mechanism is not sufficient on the MFE as compared with the radical pair mechanism. This conclusion is consistent with the conclusion of the MARY spectra. On the other hand, k_{bet} and k_R have large contributions to the MFE time profile as shown in Figure 6, parts b and c, respectively. Those two parameters govern the existence time and the scale of the time profiles of the MFE. At low pH, k_R affects to the intensity of the MFE only.

The MFE time profile of each pH could be reproduced by our model calculations as shown in Figure 7. The same proportional factor fac = 0.07 in eq a-17 has been used for all time profiles in the region of pH = 1.9∼3.5. At first, we had tried to reproduce the experimental results in various pH by single set of the parameters of k_{bet} and k_R. However, in fact, we could not succeed the simulation unless we use the pH dependent k_{bet} and k_R values. Especially in the high pH region, the time profiles directly reflect the radial pair dynamics because the equilibrium between the triplet and the radical pair shifts to the radical pair states. In such condition, rise and decay of the MFE subtraction curve is explained by the double exponential function of k_{bet} (rise) and k_R (decay), respectively, and they are strongly dependent on pH. This fact indicates that the spin and molecular dynamics of the radical pair is modulated by pH in addition to the back electron-transfer reaction to the triplet excited state. The pH dependence of k_{bet} and k_R are shown in Figure 8. Those two parameters are increased by increasing of pH (pH > 2.3). The synchronized increase of the parameters suggests the formation of stacked radical pair as shown in Scheme 3. In the stacking form of the radical pair, spin−orbit coupling (SOC) promotes $S - T$ mixing of the radical pair[56−59] and simultaneously back electron transfer reaction to the ground state takes place. Those processes are reflected effectively in both parameters, k_R and k_{bet}. At higher pH, the MFE is small and disappeared in the short time scale. It is because the contribution of SOC in the stacked radical pair overcomes that of the hyperfine coupling mechanism. The distance between the flavin moiety and adenine part in the stacking conformation ($\pi-\pi$ stacking) in the ground state of FAD is reported to be shorter than 0.6 nm by van den Berg et. al.[60] If the distance of the stacking conformation of the radical pair is similar to that of ground state, SOC would attribute the $S - T$ mixing of the radical pair. In the case of higher pH (pH > 2.5), the contribution of strong exchange interaction would be too large to cause $S - T_0$ mixing by hyperfine interaction. In the condition of large exchange interaction, the possible spin mixing process by hyperfine interaction is $S - T_{-}$ mixing. However, the MARY spectra are very similar at pH = 2.3 and 3.0. Therefore, we conclude that the SOC mechanism overcomes the HFM for spin mixing in the stacking form of the radical pair. This conclusion is not contradictory to diminishment of the photo-CIDNP signal at higher pH reported by Kaptein et al.[40]

Concluding Remarks

The MFE on the radical pair has been applied for the analysis of the radical pair dynamics and has informed us of the various kinetic parameters. Especially in this study, pH dependence was helpful for the analysis. The protonation process of flavin and adenine radicals individually induces the various changes of the dynamics of the radical pair and the excited states. That is not only due to the conformational change in the ground state but ones in triplet excited state and radical pair state. The present results show us the possibility and the applicability of the MFE on TA and its action spectra for precise analysis of photochemical reaction dynamics.

At the moment, it is unclear how the interconversion mechanism of the triplet and the radical pair contribute in the photochemical system of FAD binded. However, the MFE in the triplet excited state of flavin has the potential for various applications because one can transfer this MFE into various molecular systems by using a quencher molecule of the triplet flavin. We have observed (not shown) quenching of the MFE due to transferring it to a radical species of an amino acid molecule (tryptophan) and nucleic-acid base molecules: GMP (guanosine 5′-monophosphate) and adenosine (not shown). The similar quenching of the magnetic field effect also has been observed in nondeoxidized solution. On the basis of them, for instance, using oxygen as a quencher of the triplet flavin part, we have a possibility to transfer the MFE of the triplet to singlet oxygen. Using this mechanism, we may control the singlet oxygen concentration by the external magnetic field. This project is now underway.

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Supporting Information Available: MARY spectra in the laser flash photolysis of FAD at pH = 2.3 and 3.0. This material is available free of charge via the Internet at http://pubs.acs.org.

Appendix

The model scheme in the absence of magnetic field ($B = 0$ T) is shown in Scheme 3a. At zero magnetic field (Scheme 3a), all spin states of the radical pair (S, T_{+}^{R}, T_{0}^{R}, T_{-}^{R}) can interconvert each other by hyperfine interaction with the rate constant k_{hfc}. The rate equations on the basis of this scheme are represented by eqs a-1∼a-5.

$$\frac{d[S]}{dt} = -(k_{bet} + 3k_{hfc})[S] + k_{hfc}[T_{p}^{R}] + k_{hfc}[T_{0}^{R}] \quad \text{(a-1)}$$

$$\frac{d[T_{p}^{R}]}{dt} = 2k_{hfc}[S] - (k'_{-1}[H^{+}] + 2k_{hfc})[T_{p}^{R}] + 2k_{hfc}[T_{0}^{R}] + k_{1}[T_{p}] \quad \text{(a-2)}$$

$$\frac{d[T_{0}^{R}]}{dt} = k_{hfc}[S] + k_{hfc}[T_{p}^{R}] - (3k_{hfc} + k'_{-1}[H^{+}])[T_{0}^{R}] + k_{1}[T_{0}] \quad \text{(a-3)}$$

$$\frac{d[T_{p}]}{dt} = k'_{-1}[H^{+}][T_{p}^{R}] - (k_{d} + k_{1} + k_{R}^{T})[T_{p}] + 2k_{R}^{T}[T_{0}] \quad \text{(a-4)}$$

$$\frac{d[T_{0}]}{dt} = k'_{-1}[H^{+}][T_{0}^{R}] + k_{R}^{T}[T_{p}] - (k_{d} + k_{1} + 2k_{R}^{T})[T_{0}] \quad \text{(a-5)}$$

where

$$[T_{p}] = [T_{+1}] + [T_{-1}], \quad [T_{p}^{R}] = [T_{+1}^{R}] + [T_{-1}^{R}] \quad \text{(a-6)}$$

$[T^{+}]$, $[T_0]$, $[T_{-1}]$, and $[S]$ represent the population of the each spin state in the radical pair and $[T_{+1}^{R}]$, $[T_{0}^{R}]$, and $[T_{-1}^{R}]$ represent that in the triplet excited state.

The rate constant k_{hfc} is estimated from the $B_{1/2}$ by the nucleus in the couple of radicals in the radical pair[12,61,62]

$$k_{hfc} \approx \frac{g\mu_{B}B_{1/2}}{h} \quad \text{(a-7)}$$

and the $B_{1/2}$ value is calculated by the effective local magnetic field of each radical $B_i(i = 1,2)$

$$B_{1/2} = \frac{2({B_1}^2 + {B_2}^2)}{B_1 + B_2} \quad \text{(a-8)}$$

$$B_i(i = 1,2) = (\sum_k I_{ik}(I_{ik} + 1){a_{ik}}^2)^{1/2} \quad \text{(a-9)}$$

where I_{ik}, and a_{ik} represents a nuclear spin quantum number and a hyperfine coupling constant of the kth nucleus in the ith radical, respectively.

The kinetic parameter k'_{-1} is the rate constant of the reproduction from the triplet state of the radical pair to the triplet excited state. As the assumption, we put $k'_{-1} = (4/3)k_{-1}$, where k_{-1} is the rate constant from the radical pair to the triplet excited state and is determined in the analysis by the kinetic model under the steady-state condition.

k_{R}^{T} is the rate constant of electron spin relaxation in the triplet excited state. This value is estimated by the theory of the rotating triplet by Atkins[63]

$$k_{R}^{T} = (D^2 + 3E^2)j(\nu_0\tau_c) \quad \text{(a-10)}$$

where $j(\nu_0\tau_c)$ is

$$j(\nu_0\tau_c) = \frac{2}{15}\left\{\frac{4\tau_c}{1 + 4{\nu_0}^2{\tau_c}^2} + \frac{\tau_c}{1 + {\nu_0}^2{\tau_c}^2}\right\} \quad \text{(a-11)}$$

D and E values are zero field splitting (ZFS) parameters and τ_c and ν_0 are rotational correlation time and zeeman frequency, respectively.

The model scheme in the presence of magnetic field ($B = 200$ mT) is shown in Scheme 3b. The triplet sublevels splits because of Zeeman interaction. In this condition only the T_{0}^{R} sublevel is able to interconvert to S state by k_{hfc}. The spin relaxation process between T_{+}^{R}, T_{-}^{R} states and S state is taken into account by the rate constant k_R. Rate equations in the presence of magnetic field are represented by eqs a-12∼a-16

$$\frac{\mathrm{d}[S]}{\mathrm{d}t} = -(k_{\mathrm{bet}} + k_{\mathrm{hfc}} + 2k_{\mathrm{R}})[S] + k_{\mathrm{R}}[T_{\mathrm{p}}^{\mathrm{R}}] + k_{\mathrm{hfc}}[T_0^{\mathrm{R}}] \quad \text{(a-12)}$$

$$\frac{\mathrm{d}[T_{\mathrm{p}}^{\mathrm{R}}]}{\mathrm{d}t} = 2k_{\mathrm{R}}[S] - (k'_{-1}[\mathrm{H}^+] + 2k_{\mathrm{R}})[T_{\mathrm{p}}^{\mathrm{R}}] + 2k_{\mathrm{R}}[T_0^{\mathrm{R}}] + k_1[T_{\mathrm{p}}] \quad \text{(a-13)}$$

$$\frac{\mathrm{d}[T_0^{\mathrm{R}}]}{\mathrm{d}t} = k_{\mathrm{hfc}}[S] + k_{\mathrm{R}}[T_{\mathrm{p}}^{\mathrm{R}}] - (k_{\mathrm{hfc}} + 2k_{\mathrm{R}} + k'_{-1}[\mathrm{H}^+])[T_0^{\mathrm{R}}] + k_1[T_0] \quad \text{(a-14)}$$

$$\frac{\mathrm{d}[T_{\mathrm{p}}]}{\mathrm{d}t} = k'_{-1}[\mathrm{H}^+][T_{\mathrm{p}}^{\mathrm{R}}] - (k_{\mathrm{d}} + k_1 + k_{\mathrm{R}}^{\mathrm{T}})[T_{\mathrm{p}}] + 2k_{\mathrm{R}}^{\mathrm{T}}[T_0] \quad \text{(a-15)}$$

$$\frac{\mathrm{d}[T_0]}{\mathrm{d}t} = k'_{-1}[\mathrm{H}^+][T_0^{\mathrm{R}}] + k_{\mathrm{R}}^{\mathrm{T}}[T_{\mathrm{p}}] - (k_{\mathrm{d}} + k_1 + 2k_{\mathrm{R}}^{\mathrm{T}})[T_{\mathrm{p}}] \quad \text{(a-16)}$$

Solving the couple of simultaneous differential eqs a-1∼a-5 and eqs a-12∼a-16 numerically, we could calculate the time evolution of the triplet excited state and the radical pair. The time profiles of MFE of the radical pair and the triplet excited state (ΔP_{R} and ΔP_{T}) can be calculated by subtraction of the solution of the couple of equations. The total signal intensity of the MFE is calculated by a linear combination of the contributions of the neutral radical ($FH^{\bullet}$) and the excited triplet state ($^3\mathrm{FH}^+$). Therefore, the fitting function for the experimentally observed time profiles of the MFE is expressed by

$$\Delta\Delta A = \mathrm{fac}\left(\Delta P_{\mathrm{R}} + \frac{\epsilon_{\mathrm{T}}}{\epsilon_{\mathrm{R}}}\Delta P_{\mathrm{T}}\right) \quad \text{(a-17)}$$

The value of $\epsilon_{\mathrm{T}}/\epsilon_{\mathrm{R}} = 2.0$ has been calculated from Figure 3b in the wavelength ($\lambda = 550$∼650 nm) in which we have measured the time profiles of the MFE. In the calculation, we have used a software MATLAB (MathWorks Software) and have used an algorism for automatic optimization of the parameter k_{R} and k_{rec}.

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