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Structure and kinetics of the intermediate biradicals generated from intramolecular electron transfer reaction of FAD studied by an action spectrum of the magnetic field effect

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Abstract

The intramolecular electron transfer reaction induced by light irradiation of flavin–adenine dinucleotide (FAD) was studied by an action spectra of the magnetic field effect on the transient absorption. The action spectra depended on pH in the region of 2.0–4.0. They are reproduced by the sum of two components: T–T absorption and radical form of flavin. The contribution of T–T absorption is dominant at $pH \leqslant 2.3$ whereas that of flavin radical is dominant at $pH > 3.3$. The existence of the equilibrium between the radical pair and triplet excited state is concluded. © 2002 Elsevier Science B.V. All rights reserved.

1. Introduction

The magnetic field effect (MFE) on chemical kinetics has been studied from various points [1,2]. One of the most general mechanisms of MFE is radical pair mechanism (RPM). This mechanism is based on the modulation of electron spin dynamics by the external magnetic field and the spin selectivity of recombination reaction. In the biradical generated in the polymethylene-linked system, the MFE on the kinetics [3–6] and the spin polarizations [7–11] increases because the lifetime of the radical pair becomes longer than that in homogeneous solutions.

In contrast to the case of neutral radical pairs, the effect of the polymethylene chain dynamics on the lifetime of radical ion pair is more or less complicated because a back electron transfer process takes place in both its singlet and triplet spin multiplicities. The lifetime of radical ion pair becomes shorter when the re-encounter probability increases by connecting the two radicals with polymethylene chain. Even in such cases, the MFE by RPM was well observed and was reported by Weller, Steark et al. [12–14], and Tanimoto et al. [15–17]. The reproduction of radical ion pair by interconversion reactions of radical ion pair and exciplex, or the equilibrium between them, makes

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the total existence time of radical ion pair longer as discussed by Enjo et al. [18].

As discussed above, the effect of molecular dynamics on MFE has been studied in various model systems. Therefore the basic knowledge about the mechanism of MFE is applicable in exploring the reaction mechanism of radical pairs in complicated reaction systems. In other words, the analysis of MFE should give us important information which is not obtained by conventional spectroscopy. The present Letter is one of the demonstrations of an application of MFE and MFE-action spectrum [19] to the study of photo-induced electron transfer reaction. With MFE-action spectrum, one can distinguish the radical pair and species generated from radical pair in the complicated and overlapped spectrum.

The flavin–adenine dinucleotide (FAD) is one of the most important co-enzymes for the electron transportation in the biological system, where an electron donor (adenine) and acceptor (flavin) are linked by covalent bond. Previously, the radical pair (biradical) by intramolecular photo-induced electron transfer reaction was studied by means of CIDNP techniques by Kaptein and coworkers [20,21]. The pH dependence of CIDNP was interpreted by the large exchange interaction due to the formation of stacking conformations at higher pH than 3.6 [22,23]. The CIDNP signal gives an evidence of the generation of radical pair. However, the direct and real-time detection of the radical pair by time-resolved spectroscopy has not been performed.

In the present Letter, we observed and analyzed the MFE-action spectra of the transient absorption (TA) spectrum to reveal the mechanism and the intermediates for intramolecular electron transfer reaction of FAD. In this process, it is clarified that the interconversion reactions of the radical pair and the triplet excited state, or the equilibrium between them, play an important role on the reaction kinetics of the radical pair.

2. Experimental

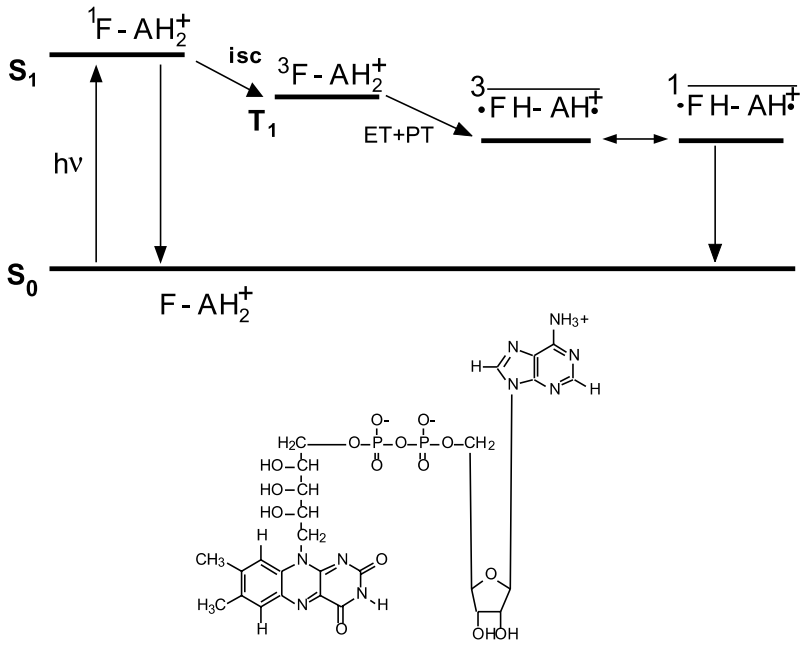
The TA spectra were observed by a home made setup. A flow system was used for transfer the sample solutions into a quartz optical cell where the reactions were initiated by laser pulse: Third harmonics ($\lambda = 355$ nm) of Nd:YAG Laser (Spectra Physics GCR-3) was used as an exciting light source. A 500 W Xe lamp (Ushio UXL-500SX) was used as a probe light source. The TA signal was detected by a photomultiplier (Hamamatsu R-928) fixed with a monochrometor (JASCO CT-25). The signal from the photomultiplier is recorded by a digital oscilloscope (LeCroy LT-344) and analyzed by a personal computer.

FAD, FMN, riboflavin, and adenosine were obtained from Sigma and were used without further purification. The concentrations of FAD, FMN, and riboflavin were adjusted to 0.2, 0.18, and 0.18 mM, respectively. Buffer solutions ($Na_2HPO_410H_2O$: 4–83 mM/citric acid monohydrate: 98–59 mM, pH = 2.2–4.2) of distilled water (Kishida) were used for the solvent. The sample was deoxigenated by bubbling of Ar gas.

Cyclic voltammetry (CV) experiment was performed by an equipment, 100B/W(CV-50W) by Bioanalytical Systems, $LiClO_4$ (0.1 M) was used as electrolyte, and the concentration of the object molecule was 15 mM in buffer solutions mentioned in the previous paragraph.

3. Results and discussion

The structure of FAD molecule and the reaction scheme proposed by Kaptein and coworkers [20,21] are shown in Scheme 1. The electron transfer reaction followed by a proton transfer generates the radical pair that is composed of the neutral radical of flavin and the cation radical of adenine. The TA spectra observed on laser flash photolysis of FAD at pH = 2.3 are shown in Fig. 1a. Two components, a short-lived component ($\tau = 0.7$ μs, $\lambda_{max} = 650$ nm) and a long-lived component ($\tau = 0.1$ ms, $\lambda_{max} = 510$ nm: see spectrum at $t = 3.0$ μs), are recognized in the spectra. The latter component strongly depended on the laser power and can be assigned to the long-lived intermediate or the product generated from the bimolecular reactions of intermediates. The short-lived component can be assigned to T–T absorption band of flavin molecule because it is similar to



Scheme 1. Structure of FAD molecule and the general reaction scheme of the photo-induced intramolecular electron transfer reaction of FAD in the range of pH < 3.5. This scheme is proposed by Kaptein and coworkers [21].

the T–T absorption of FMN [24,25]. Although the spectral shape does not depend on pH in the region of pH = 2.0–3.6, the lifetime of short-lived component becomes short at higher pH as shown in Fig. 1b. The decay profiles of the short-lived component are able to be analyzed by single exponential decay functions, and have no clear dependence on the monitoring wavelength. If the TA spectra contains absorption band of radical pair, it should be observed around 550 nm [26,27]. The TA signal of radical pair, however, has not been recognized apparently. It is considered that the TA signal of radicals is overlapped with strong and broad T–T absorption, and has similar decay kinetics with that.

An example of the MFE on the decay kinetics of TA at pH = 2.3 is shown in Fig. 2. In the external magnetic field ($B = 0.2$ T), TA signal, $\Delta A(B = 0.2, t)$, observed at 600 nm increased from that in zero magnetic field $\Delta A(B = 0, t)$. The MFE curve obtained by plotting the increment of TA versus the external magnetic fields is characteristic of the hyperfine mechanism [1,2] and the relaxation mechanism [2,28]. Therefore the presence of such MFE indicates the generation of radical pair by intramolecular electron transfer reaction.

The subtractions of TA kinetics

$$\Delta\Delta A(B = 0.2, t) = \Delta A(B = 0.2, t) - \Delta A(B = 0, t) \tag{1}$$

reflect the kinetic feature of radical pair and are shown in Fig. 2b. The time profiles of subtraction have strong pH dependence. At pH = 1.9 the radical pair was observed until about 5 μs after laser pulse. In contrast, at higher pH, the MFE becomes small and rapidly disappeared within several hundreds of ns. This change of MFE time profiles indicates the drastic change of the dynamics of the radical pair by pH.

The contribution of the magnetic field on the TA spectrum was obtained by the subtractions of the TA spectrum without magnetic field ($B = 0$) from that with magnetic field ($B = 0.2$ T). This spectrum is called MFE-action spectrum. The MFE-action spectra observed at various pH are shown in Fig. 3a. These spectra were analyzed by the sum of two absorption bands. One component

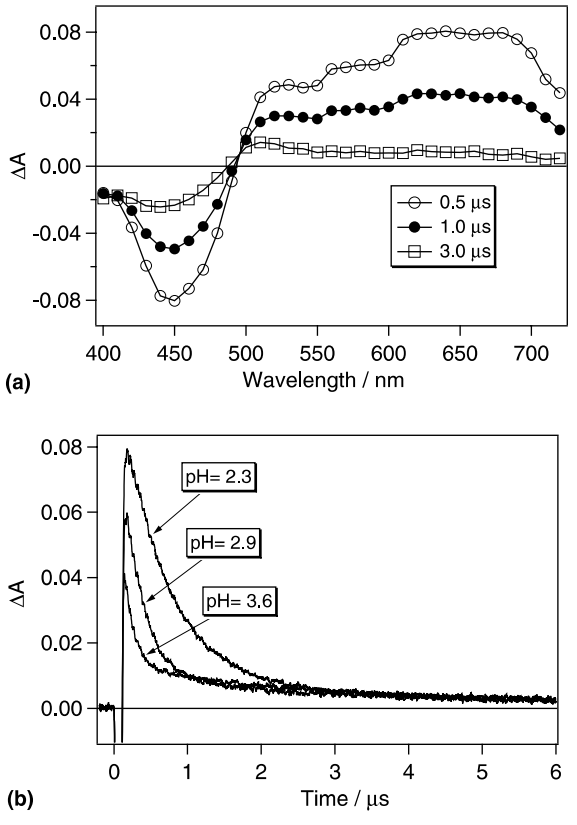


Fig. 1. (a) Transient absorption spectra observed in the laser flash photolysis of FAD at pH = 2.3. Open circle, filled circle, and open square are the spectra observed at $t = 0.5$, 1.0, and 3.0 μs, respectively. (b) pH dependence of the time profile of transient absorption observed at zero magnetic field. Observation wavelength $\lambda = 650$ nm.

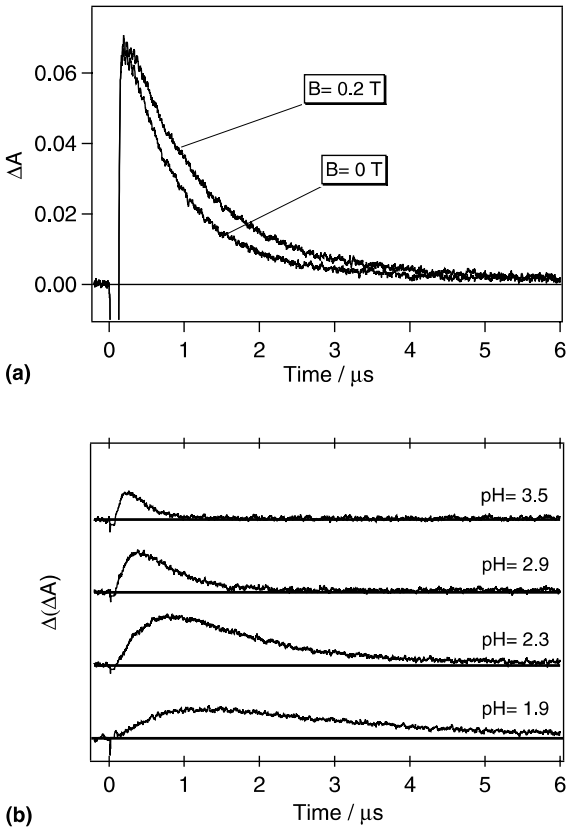


Fig. 2. (a) Time profiles of transient absorption observed at $\lambda = 600$ nm with (upper, $B = 0.2$ T) and without (lower, $B = 0$ T) magnetic field. (b) pH dependence of the MFE time profiles given by the subtractions of the data without magnetic field ($B = 0$ T) from that with magnetic field ($B = 0.2$ T).

has peak around 550–600 nm (see at pH = 4.1) and was dominant at higher pH. This component can be assigned to the neutral radical of flavin in FAD [26]. Laser flash photolysis of FMN and adenosine gave very similar transient spectrum as shown in Fig. 3b. The other component observed for FAD has peak around 650 nm, and appeared at lower pH. This band cannot be rationalized by any expected radical species: neutral radical ($\lambda_{max} = 502$, 580 nm) [26] or cation radical ($\lambda_{max} = 488$ nm) [27]. The spectral shapes observed at lower pH are similar to the T–T absorption spectrum observed in the laser flash photolysis of riboflavin as shown in Fig. 3b. In order to explain the pH dependence of the action spectra, we tried to reproduce the observed action spectra by linear combinations of two tempate spectra: spectrum observed for FMN and adenosine $R(\lambda)$, and that observed for riboflavin $T(\lambda)$. The simulation spectra are calculated by

$$\Delta\Delta A(\lambda) = C_R R(\lambda) + C_T T(\lambda), \quad (2)$$

where C_R and C_T are fitting coefficients indicating the contribution of the radical pair and triplet state, respectively. The experimental data is well reproduced by the simulation spectra as shown in Fig. 3a. These results indicate that both the radical pair and triplet state dynamics suffer MFE.

In Scheme 1, the triplet excited state is not generated from radical pair. Therefore the obser-

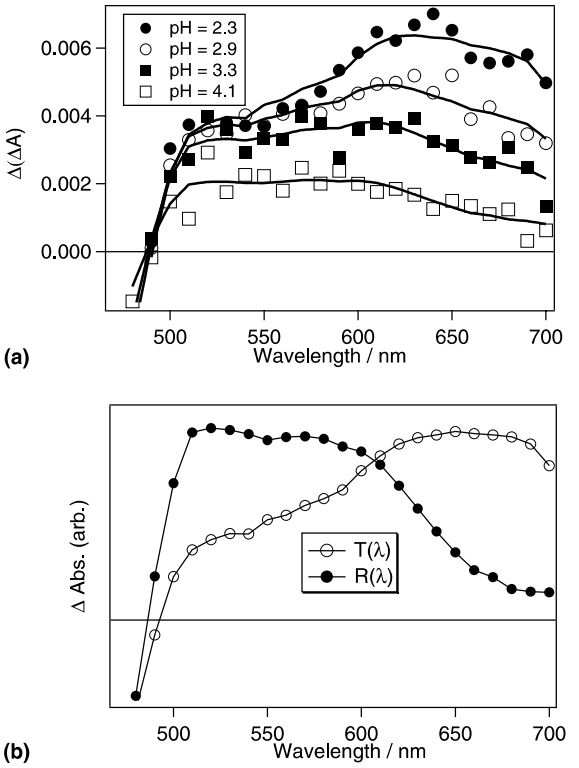
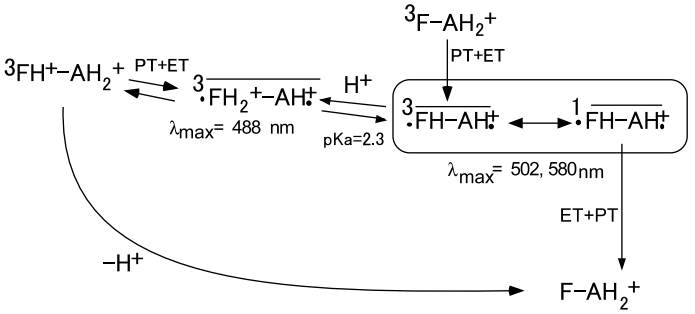


Fig. 3. (a) MFE-action spectra on the transient absorption at pH = 2.3: filled circle, pH = 2.9: open circle, pH = 3.3: filled square, and pH = 4.1: open square. Solid lines are simulation spectra by linear combination of the template spectra shown in (b). (b) Template spectra for fitting of the MFE-action spectra. $R(\lambda)$ was obtained at 8 μs after the pulsed laser flash photolysis of an aqueous solution (pH = 4.2) of FMN and adenosine. $T(\lambda)$ was obtained at 2 μs after laser flash photolysis of an aqueous solution (pH = 2.3) of riboflavin.

vation of the MFE on the T–T absorption cannot be expected from Scheme 1. On the contrary, strong MFE has been observed in the triplet state of flavin part at low pH value. The observation of MFE on T–T absorption of FAD is solved by the assumption that the triplet excited state of flavin is generated from the radical pair. CV measurements have been performed in aqueous solution of FMN and adenosine at pH = 2.6. Since reduction potential of FMN, ΔE_{Red}(FMN), and oxidation potential of adenosine, ΔE_{Ox}(Adn), are observed to be −0.61 and 1.52 eV vs. AgCl, respectively, the energy of the radical ion pair state over the neutral pair is calculated to be 2.1 eV. This value is similar to the triplet energy of riboflavin (2.05 eV) measured by a phosphorescence spectrum [29]. Therefore the interconversion reactions between the radical pair and the triplet state are probable in our system.

The time profiles of MFE observed at 550, 600 (shown in Fig. 2b) and 650 nm have similar shape at the pH region of 2.0–4.0. This means that the radical pair and the triplet excited state exhibited the same profile of time evolution of the concentration. By this, we conclude that the interconversion reactions are so fast that the system has been attained to the quasi-equilibrium condition in our observation time scale (∼0.05 μs). The pH dependence of MFE-action spectra shows that the critical pH value for appearance of the contribution of the T–T absorption is about 2.3. This value is identical with the pK_a value of flavin neutral radical to cation radical. This means that the formation of the cation form of flavin radical plays an important role for shifting the equilibrium to the



Scheme 2. Proposed modification of the reaction scheme by the present investigation.

triplet state of flavin. We propose that the back electron transfer reaction to the triplet excited state is achieved through the cation form of flavin radical as shown in Scheme 2.

The equilibrium between radical pair and triplet state is strongly suggested by the experimental observation that the existence time (apparent lifetime) of radical pair becomes longer at lower pH as shown in Fig. 2b. Ito and coworkers [30] reported that the long lifetime of the radical ion pair in the system of Retinal-C_{60} dyad was due to the equilibrium between the radical ion pair and the triplet state. Such mechanism to prolong the ion pair existence time has been observed also in the singlet-born radical ion pairs by Enjo et al. [18]. In this case, interconversion between radical ion pair and singlet exciplex should take place. In both cases, the fast interconversion between relatively long-lived electronic states and the radical pair are significant to prolong the existence time of the radical pair.

In the present Letter, we clearly distinguish between the existence time and the pure lifetime of radical pair. When the interconversion between the excited state and the radical pair state takes place in the photo-induced electron transfer reactions, the pure lifetime of the radical pair spin state seems to be much shorter than the existence time estimated by the time profiles of MFE. Although the estimation of the pure lifetime of the radical pair is difficult by optical spectroscopy, it is possible by the magnetic resonance spectroscopy. We have tried to observe the time-resolved ESR spectra in our system. However, it was hard to detect by conventional ESR because the pure spin lifetime is very short in the biradical system. The time-resolved absorption-detected magnetic resonance (ADMR) in a family of the reaction yield detected magnetic resonance (RYDMR) spectroscopy is capable of observing the short-lived radical pair. The analysis by this technique is now underway.

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References

[1] Yu.N. Molin (Ed.), Spin Polarization and Magnetic Effect in Radical Reactions, Elsevier, Amsterdam, 1984.
[2] U.E. Steiner, T. Ulrich, Chem. Rev. 89 (1989) 51.
[3] M.B. Zimmt, C. Doubleday Jr., N.J. Turro, J. Am. Chem. Soc. 107 (1985) 6726.
[4] M.B. Zimmt, C. Doubleday Jr., N.J. Turro, J. Am. Chem. Soc. 108 (1986) 3618.
[5] Y. Tanimoto, M. Takashima, K. Hasegawa, M. Itoh, Chem. Phys. Lett. 135 (1987) 307.
[6] Y. Tanimoto, M. Takashima, M. Itoh, Bull. Chem. Soc. Jpn. 62 (1989) 3923.
[7] G.L. Closs, M.D.E. Forbes, J.R. Norris, J. Phys. Chem. 91 (1987) 3592.
[8] G.L. Closs, C.E. Doubleday, J. Am. Chem. Soc. 95 (1973) 2735.
[9] K. Maeda, Q.-X. Meng, T. Aizawa, M. Terazima, T. Azumi, Y. Tanimoto, J. Phys. Chem. 96 (1992) 4884.
[10] K. Maeda, M. Terazima, T. Azumi, Y. Tanimoto, J. Phys. Chem. 95 (1991) 197.
[11] K. Maeda, N. Suzuki, T. Azumi, J. Phys. Chem. 97 (1993) 9562.
[12] H. Staerk, W. Kühnle, R. Treichel, A. Weller, Chem. Phys. Lett. 118 (1985) 19.
[13] H.-G. Busmann, H. Staerk, A. Weller, J. Chem. Phys. 91 (1989) 4098.
[14] H. Staerk, H.-G. Busmann, W. Kühnle, R. Treichel, J. Phys. Chem. 95 (1991) 1906.
[15] Y. Tanimoto, N. Okada, M. Itoh, K. Iwai, K. Sugiura, F. Takamura, R. Nakagaki, S. Nagakura, Chem. Phys. Lett. 136 (1987) 42.
[16] Y. Tanimoto, K. Hasegawa, N. Okada, M. Itoh, K. Iwai, K. Sugiura, F. Takemura, R. Nakagaki, S. Nagakura, J. Phys. Chem. 93 (1989) 3586.
[17] H. Cao, T. Fujiwara, T. Haino, Y. Fukazawa, C.-H. Tung, Y. Tanimoto, Bull. Chem. Soc. Jpn. 69 (1996) 1.
[18] K. Enjo, K. Maeda, H. Murai, T. Azumi, J. Phys. Chem. 101 (1997) 10661.

[19] S.S. Ali, K. Maeda, H. Murai, T. Azumi, Chem. Phys. Lett. 267 (1997) 520.
[20] C.G. van Schagen, F. Müller, R. Kaptein, Biochemistry 21 (1982) 402.
[21] S. Stob, J. Kemmink, R. Kaptein, J. Am. Chem. Soc. 111 (1989) 7036.
[22] J.C.M. Tsibris, D.B. McCormick, L.D. Wright, Biochemistry 4 (1965) 504.
[23] D.W. Miles, D.W. Urry, Biochemistry 7 (1968) 2791.
[24] M. Sakai, H. Takahashi, J. Mol. Struct. 379 (1996) 9.
[25] P.F. Heelis, B.J. Parsons, G.O. Phillips, J.F. McKellar, Photochem. Photobiol. 28 (1978) 169.
[26] F. Müller, M. Brüstlein, P. Hemmerich, V. Massey, W.H. Walker, Eur. J. Biochem. 25 (1972) 573.
[27] K.H. Dudley, A. Ehrenberg, P. Hemmerich, F. Müller, Helv. Chim. Acta 47 (1964) 1354.
[28] H. Hayashi, S. Nagakura, Bull. Chem. Soc. Jpn. 57 (1984) 322.
[29] J.M. Lhoste, A. Haug, P. Hemmerich, Biochemistry 5 (1966) 3290.
[30] M. Yamazaki, Y. Araki, M. Fujitsuka, O. Ito, J. Phys. Chem. A 105 (2001) 8615.