Effect of Coulomb Interaction on the Dynamics of the Radical Pair in the System of Flavin Mononucleotide and Hen Egg-White Lysozyme (HEWL) Studied by a Magnetic Field Effect

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A large magnetic field effect (MFE) up to 13% of the yield of the free radical formation is found in the photoinduced electron-transfer reaction from the tryptophan residue of the hen egg-white lysozyme (HEWL) to the flavin mononucleotide (FMN). The magnetic field effect is special in the water solution of FMN, and that is reduced to 5−7% by the addition of sodium chloride to the solution and in the system of riboflavin. The MFE up to 13% coincides with the appearance of the fast quenching process of the excited singlet state. The large MFE is attributed to association by Coulomb interaction between the ionic phosphate group of FMN and the cationic surface of the protein molecule.

1. Introduction

The magnetic field effect (MFE) in chemical kinetics has been studied from various points of view.[1,2] One of the most essential mechanisms of MFE is the 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 a recombination reaction. In the proposed mechanisms for MFE, the mechanism observed in the weakest magnetic field is the hyperfine mechanism, which belongs to the category of the RPM. This mechanism is observable in the range of the magnetic field that is comparable to hyperfine coupling constants, which are mainly less than 10 mT. Recently, Hore et al.[3] proposed the low-field mechanism due to the interference of the spin mixing processes. They showed that the MFE appears for magnetic fields that are smaller than hyperfine coupling constants.

The characteristic time scale of the spin mixing by hyperfine interaction is several nanoseconds in the system of organic radical pairs. The cage lifetime of the radical pair in homogeneous solution is normally shorter than that. Therefore, it is hard to observe the magnetic field effect by the hyperfine mechanism in homogeneous solution, and the main mechanism observed under such a condition is the Δg mechanism, which can be observed in the large external magnetic field ($\sim$1 T).[4,5] To observe the MFE in small magnetic fields, many scientists produced the associated systems that restrict the diffusion process of the radical pair, for instance, micellar systems, biradicals, membranes, and viscous solutions.[1]

The reaction dynamics of the radical pair formed in various biological environments is one of the essential problems in the analysis of the magnetic effect in biological environments.[6,7] Here we focus on the MFE caused by a low magnetic field in the biological model systems because the low-field condition is more general in our living environment. In biological systems, association between a protein molecule and a small molecule in water solution is one of the essential models of the enzyme reactions. If there are some associations between the protein and the small molecule, then one can produce a confined radical pair, which lets us observe the MFE due to the hyperfine mechanism.

In the present paper, we studied the photoinduced electron-transfer reactions of hen egg-white lysozyme (HEWL) and flavin dyes, which is one of the interesting model systems of the radical reactions in water solutions. In addition to the basic interest, radical pair formation by the photochemical reaction of flavin and an amino acid residue has been applied to the analysis of the protein structure by the CIDNP technique.[8,9] The CIDNP technique is sensitive for studying protein structures and was recently applied to the real-time observation of the protein folding process.[10,11] The kinetics of the formation and decay of the radical pair was studied by the time-resolved CIDNP method.[12,13] The information of the confined radical pair in the present paper is important to the analysis of these experimental results.

2. Experimental Section

The transient absorption (TA) spectra were observed by a homemade setup. A flow system was used to transfer the sample solutions into a quartz optical cell where the reactions were initiated by a laser pulse. The third harmonic ($\lambda = 355$ nm) of a 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 monochromator (JASCO CT-25). The signal from the photomultiplier was recorded by a digital oscilloscope (LeCroy LT-344) and analyzed by a personal computer. The sample was deoxygenated by bubbling Ar gas.

Flavin mononucleotide (FMN), riboflavin (RF), and lysozyme (hen egg-white lysozyme:14 300 Da HEWL) were purchased from Sigma (St. Louis, MO). NaCl was purchased from Wako. All samples were used without further purification. Distilled water (Kishida) was used for the solvent. The concentration of sensitizer (FMN or RF) was 0.2 mM.

3. Results and Discussion

TA spectra observed in the system of FMN and HEWL in water solution (pH 6.1) are shown in Figure 1. Initially, the

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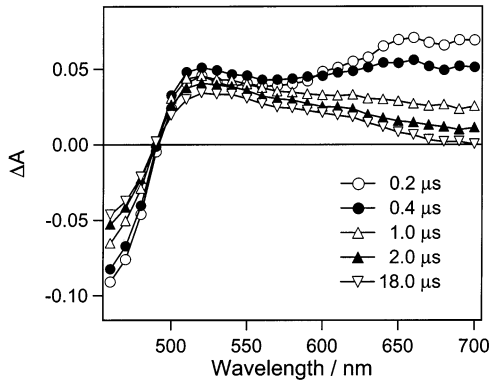

Figure 1. Time-resolved transient absorption spectra observed in the photochemical reaction of FMN (0.2 mM) and HEWL (0.5 mM) in water solution.

absorption band of the triplet excited state of FMN is observed around 650 nm, and according to the decay of that, the absorption of the intermediate radical is recognized at 500−600 nm.[14] The observed absorption band at 500−600 nm can be assigned to the neutral radical of flavin that is generated from the electron-transfer reaction from the aromatic residue of HEWL to FMN followed by the proton-transfer reaction from a water molecule. The CIDNP experiment by Hore and Kaptein[15] has indicated that the flavin derivatives mainly react with tryptophan residues (tryptophan-62 and tryptophan-123) in the native state of HEWL.

The effect of applying an external magnetic field ($B = 250$ mT) on the decay kinetics observed at 520 nm in the system of FMN and HEWL in water solution (FMN−HEWL system) is shown in Figure 2a. The yield of the free radical is increased by applying an external magnetic field. The MFE is calculated by

$$\mathrm{MFE}(\%) = \frac{\Delta A(B = 250\ \mathrm{mT}) - \Delta A(B = 0\ \mathrm{mT})}{\Delta A(B = 0\ \mathrm{mT})} \times 100 \quad (1)$$

where $\Delta A(B = 250$ mT) and $\Delta A(B = 0$ mT) represent the TA signals of free radicals observed in the external magnetic field and at zero magnetic field, respectively. We averaged the absorption of the time range from 5 to 18 μs and obtained the MFE by eq 1. The MFE is about 13%. This value is larger than the expected value for the general systems in homogeneous solutions.

Figure 2b shows the MARY spectra, which are obtained by plotting MFEs versus the applied magnetic field. The MFE rises rapidly with the increase in the external magnetic field and saturates at about 50 mT. This MFE is assigned to a mixture of the hypefine mechanism and the relaxation mechanism because the observed $B_{1/2}$ value (the midpoint of the MFE) is about 10 mT, which is a little higher than that calculated from the effective hyperfine coupling constants (3 mT).[1,2] The time evolution of the MARY spectra has not been noticed. The experimental results indicate that the cage lifetime of the radical pair is rather long, even in the homogeneous water solution. If the radical pair lifetime were longer than the spin relaxation time scale, then the MARY spectra would be changed by the delay time after laser irradiation, as observed in the system of flavin derivatives and indole derivatives in SDS micelles.[16,17] Therefore, the radical pair lifetime in our system should be comparable to or shorter than the time scale of the spin relaxation in the applied magnetic field.

We have also performed the same experiments in the riboflavin system (riboflavin−HEWL system). All features of

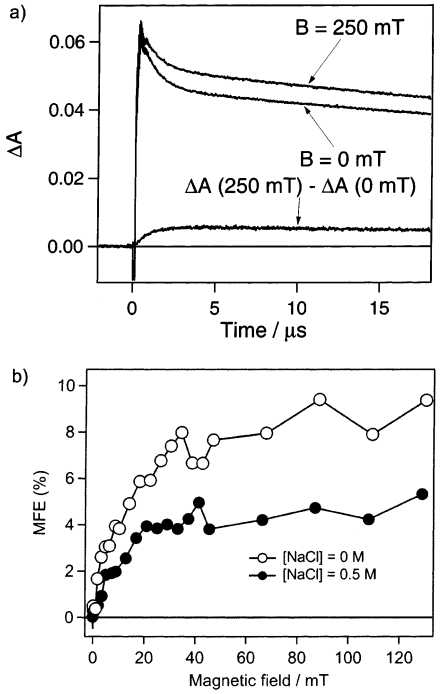

Figure 2. (a) Time profiles of transient absorption observed at $\lambda =$ 520 nm in the FMN−HEWL system with (top, $B = 250$ mT) and without (middle, $B = 0$ mT) a magnetic field. The MFE time profile, $\Delta\Delta A(t)$, calculated by the subtraction of the data without a magnetic field, $\Delta A(B = 0$ mT), from that with a magnetic field, $\Delta A(B = 250$ mT), is added to the bottom. (b) Magnetic field dependence on the MFE in water solution (FMN−HEWL system, ○) and in sodium chloride solution (FMN−HEWL−NaCl system, [NaCl] = 0.5 M, ●). The MFE in the time region of 5−18 μs was averaged for all data.

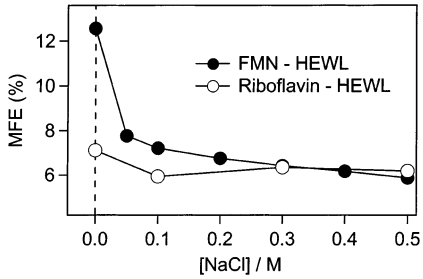

Figure 3. Concentration dependence of NaCl on the MFE in the FMN (●) and riboflavin (○) systems.

the TAs are similar to that in the FMN system. However, the MFE has been reduced to 7%. Additionally, when we added NaCl to the solution of the FMN−HEWL system (FMN−HEWL-NaCl system), the MFE was reduced to 6%. Figure 3 shows the concentration dependence of NaCl on the MFE. The MFE in the FMN−HEWL system rapidly decreased by the addition of salt and reached a value similar to that observed in the riboflavin system. The reduction of the MFE with the increasing ionic strength of the solution by the addition of salt indicates that the Coulomb force is the origin of the additional MFE from 6 to 13%.

The quenching features were studied by a detailed analysis of the TAs of the triplet excited state of flavin derivatives and a Stern−Volmer plot. The decay-time profiles observed at 690 nm with various concentration of HEWL are shown in Figure 4. In FMN−HEWL, not only the dynamic quenching process to the decay profiles but also the quenching to the initial concentration of the triplet excited state is noticeable (Figure 4a and the absorption intensity at $t = 0$). In contrast to this, such a feature has not been observed in riboflavin−HEWL

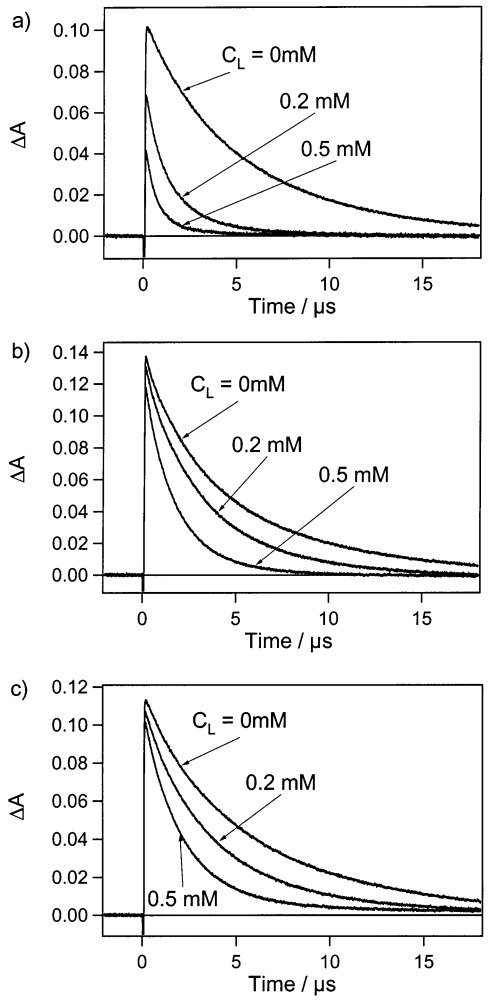

Figure 4. Concentration dependence of HEWL on the time profiles of transient absorption observed at 690 nm for the (a) FMN−HEWL, (b) riboflavin−HEWL, and (c) FMN−HEWL−NaCl (0.5 M) systems.

(Figure 4b) and FMN−HEWL−NaCl (Figure 4c). The quenching to the initial concentration of the triplet state coincides with the appearance of the large MFE up to 13%.

The observed decay-time profiles were analyzed by a kinetic model

$$\frac{d[T]}{dt} = -(k_0 + k_q C_L)[T] - k_{TT}[T]^2 \qquad (2)$$

where the contribution of the triplet−triplet annihilation by a rate constant, k_{TT}, was taken into account. k_0, k_q, and C_L represent a first-order relaxation rate constant of the triplet state of the flavin molecule by all processes except the reaction with protein and the triplet−triplet annihilation, a quenching rate constant, and the concentration of HEWL molecules, respectively. Substituting $k_0 + k_q C_L$ by a single value k_1, one can solve eq 2 and give the time profile of the transient absorption,

$$\Delta A(t) = \epsilon[T] = \frac{\epsilon \exp(-k_1 t)}{\frac{k_{TT}}{k_1}\{1 - \exp(-k_1 t)\} + \frac{1}{[T]_0}} \qquad (3)$$

ϵ and $[T]_0$ represent the extinction coefficient and the initial concentration of the triplet molecules, respectively. Using eq 3, we have analyzed the decay curve and determined k_1 and $[T]_0$ by curve fitting, where ϵ and k_{TT} were constant for the

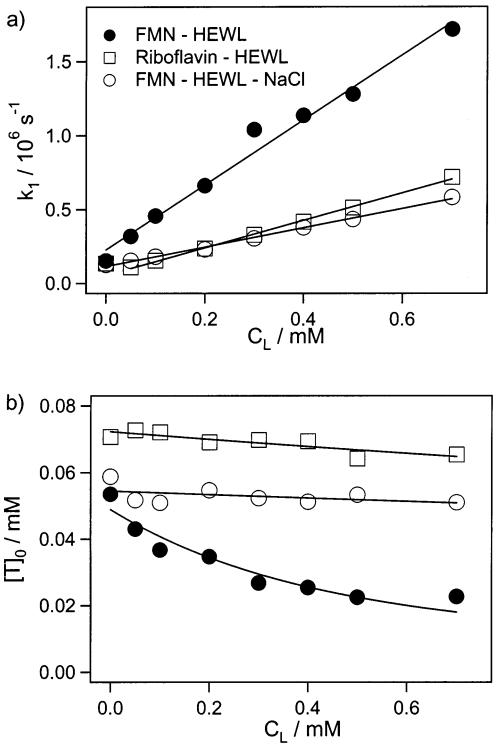

Figure 5. (a) Stern−Volmer plots of the first-order kinetic rate constant k_1 versus the total concentration of HEWL (C_L) in the FMN−HEWL (●), FMN−HEWL−NaCl (○), and riboflavin−HEWL (□) systems. (b) Plots of the initial concentration of the triplet excited state ($[T]_0$) versus the total concentration of HEWL (C_L) in the FMN−HEWL (●), FMN−HEWL with NaCl (○), and riboflavin−HEWL (□) systems. Solid lines are fitting lines given by eq 5.

results with various concentrations of HEWL. The ϵ value (5×10^3 M^{-1} cm^{-1}) was measured from the quantitatively observed TA spectrum that was previously reported.[18] The k_{TT} values are determined from the curve fitting of the data observed at $C_L = 0$ mM by eq 3 and are 1.3×10^9 M^{-1} s^{-1}, 1.5×10^9 M^{-1} s^{-1}, and 2.2×10^9 M^{-1} s^{-1} for FMN−HEWL, FMN−HEWL−NaCl, and riboflavin−HEWL, respectively. These values are reasonable because they are a little smaller than the estimated value (6×10^9 M^{-1} s^{-1}) of the diffusion-controlled reactions in water solution. k_1 and $[T]_0$ versus C_L are plotted in Figure 5a and b, respectively.

The quenching features observed in the decay-time profiles of the triplet excited state in the system of FMN lead us to believe that there are two types of triplet-state quenching: (1) quenching by the diffusion process of HEWL and FMN (dynamic quenching process) and (2) quenching in the associated HEWL and FMN. Types 1 and 2 are shown in Figure 5a and b, respectively. The dynamic quenching feature of the triplet excited state of flavin is analyzed by the Stern−Volmer plot shown in Figure 5a. The plots have a linear relationship, and the quenching rate constant, k_q, values are determined to be 2.2×10^9, 0.65×10^9, and 0.93×10^9 M^{-1} s^{-1} for FMN−HEWL, FMN−HEWL−NaCl, and riboflavin−HEWL, respectively. k_q for the FMN−HEWL system is much larger than for other systems. This indicates that the attractive Coulomb force works on the quenching process of the triplet state of the flavin molecule.

Figure 5b indicates that the initial concentration of the triplet excited state is quenched only in the FMN−HEWL system in water solution. As a simple model, we considered an association of the flavin molecule with HEWL by an equilibrium constant K as shown in eq 4.

$$\text{flavin} + \text{HEWL} \overset{K}{\rightleftharpoons} \text{F}-\text{HEWL} \quad (4)$$

In this model, we assume that the excited state of flavin in F−HEWL is quenched quickly after laser irradiation. Under this assumption, the initial concentration $[T]_0$ observed in the decay-time profile should proportional to the concentration of the free flavin molecule [flavin]. In this model, the initial concentration of the free excited triplet state of flavin is given by

$$[T]_0 = f[\text{flavin}] = \frac{f}{2}\left\{C_f - C_L - \frac{1}{K} - \sqrt{(C_f - C_L)^2 + \frac{1}{K}\left(\frac{1}{K} + 2(C_f - C_L)\right)}\right\} \quad (5)$$

where C_f is the total concentrations of flavin molecules, which is 0.2 mM under our experimental conditions. The relationship of $[T]_0$ versus C_L shown in Figure 5b has been fitted by eq 5. By this fitting, the association constant K in the F−HEWL system in water solution has been determined to be K(FMN−HEWL) = 3×10^3 M^{-1}. The K values in the FMN−HEWL−NaCl and ribaflavin−HEWL systems are negligibly small as compared with that in the FMN−HEWL system.

From the analysis of the quenching feature of the triplet state and the MFE, the association in the ground state is correlated with the appearance of the additional MFE up to 13 %. However, which quenching process is the main source of the MFE is still unclear. The MFE subtraction time profiles $\Delta A(B = 250\text{ mT}, t) - \Delta A(B = 0\text{ mT}, t)$ in various systems are compared to the decay of the triplet excited state in Figure 6. The rise time of the MFE depends on the quenching process of the triplet excited state and follows the decay profiles of the triplet state. The lifetime of the triplet state analyzed by fitting with a single-exponential function corresponds to the rise time of the subtraction in Figure 6. Such correspondence does not depend on the protein concentrations (not shown). These results indicate that the MFE is produced mainly in the slow dynamic quenching process. Details of the fast quenching process have been studied by fluorescence and its decay measurements.[19] The results indicate that a fast static quenching process of the excited singlet state is one cause of the fast quenching. It is considerable that the possibility of the formation of a singlet radical ion pair occurs via fast quenching. However, that might not be detected by the magnetic field effect on the transient absorption because of the radical's short lifetime. It is apparent at least that the attractive Coulomb interaction works on the molecular dynamics of the ground state and the radical pair in the FMN−HEWL system.

The attractive Coulomb force between the radicals of HEWL and FMN is essential to the large MFE observations. The isoelectric point of HEWL is 11.4. Therefore, at neutral pH, the surface of HEWL is positively charged and interacts with the negatively charged phosphate group of the FMN molecule. In the FMN−bovine serum albumin (BSA) system, only 5−6% of the MFE has been observed, and the large magnetic field effect such as the one that is present in the HEWL system has not been observed.[19] That is reasonable because the isoelectric point of BSA is 4.2 and protein is negatively charged at neutral pH.

Finally, we summarize the quantitative comparison of the MFE in various systems. The MFE in the mixture of the FMN and tryptophan as a free amino acid was about 2%. In the riboflavin−HEWL system, the MFE increases to 5−7%. This increment of the MFE can be rationalized by slower diffusion and an increase in the re-encounter probability of large protein molecules. In the FMN−HEWL system, the MFE is up to 13% and decreases rapidly upon the addition of NaCl. The only difference between FMN and riboflavin is a phosphoric acid group. In conclusion, Coulombic interaction between the protein surface and the ionic phosphoric acid group at the side chain of the FMN molecule plays an important role in the dynamics of the radical pair. The present results suggest that the calculation of the radical pair dynamics containing molecular dynamics and intermolecular interactions is an interesting subject in the investigation of the chemical kinetics and magnetic spin effects in biological environments.

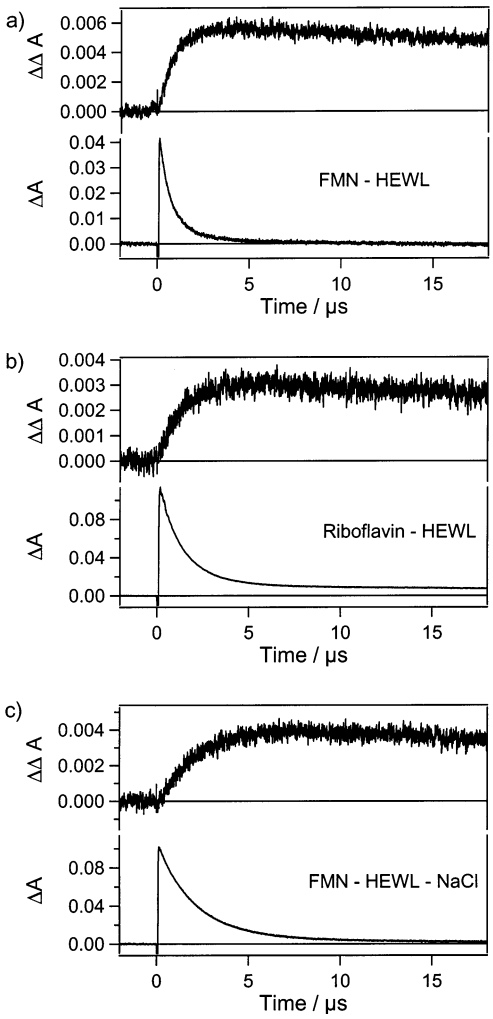

Figure 6. Comparison of the MFE subtraction time profiles (upper), $\Delta A(B = 250\text{ mT}, t) - \Delta A(B = 0\text{ mT}, t)$, observed at $\lambda = 520$ nm and the time profiles of TA observed at $\lambda = 690$ nm (lower). (a) FMN−HEWL. (b) Riboflavin−HEWL. (c) FMN−HEWL−NaCl (0.5 M).

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