Chemical Physics Letters 501 (2011) 528–533

Contents lists available at ScienceDirect

Chemical Physics Letters

journal homepage: www.elsevier.com/locate/cplett

Temperature dependence of relaxation time of a stable radical pair in SyPixD investigated by pulsed EPR

Toru Kondo [a], Shinji Masuda [b,c], Kazuhiko Tsutsui [a], Hiroyuki Mino [a,*]

[a] Division of Material Science (Physics), Graduate School of Science, Nagoya University, Furo, Chikusa, Nagoya 464-8602, Japan
[b] Center for Biological Resources and Informatics, Tokyo Institute of Technology, Yokohama 226-8501, Japan
[c] PRESTO, Japan Science and Technology Agency (JST), Saitama 332-0012, Japan

ARTICLE INFO

Article history:
Received 11 August 2010
In final form 23 November 2010
Available online 26 November 2010

ABSTRACT

The temperature dependence of the spin–lattice relaxation of the radical pair of flavin and tyrosine in SyPixD was investigated by pulsed electron paramagnetic resonance (EPR) spectroscopy. Biphasic recovery with a fast component of the spin–lattice relaxation time, denoted as T_{1fast}, and slow component of spin–lattice relaxation time, denoted as T_{1slow}, was detected in the range of 4–80 K. Based on the temperature dependence of T_{1fast} and T_{1slow} below 16 K, the exchange coupling of the radical pair was estimated as $2J$ = 6–8 cm^{-1}, defined as $\mathscr{H} = -2J\boldsymbol{S}_1 * \boldsymbol{S}_2$.

© 2010 Elsevier B.V. All rights reserved.

1. Introduction

Many flavoproteins are known to function in biological redox processes. Recently, a new type of photoreceptor using flavin, referred to as the BLUF (blue-light using FAD) domain, was developed [1]. Four groups of the BLUF domain are known at present: PixD (SyPixD in *Synechocystis* sp. PCC6803 or TePixD in *Thermosynechococcus elongatus*), AppA and BlrB in *Rhodobacter sphaeroides* [1–3], PAC in *Euglena gracilis* [4], and YcgF in *Escherichia coli* [5]. PixD regulates pili-dependent cell motility [6], and AppA controls the expression of the photosynthesis gene [7]. The BLUF domain shows a reversible 10–20 nm red-shift of the flavin absorption bands upon light excitation at room temperature [2,3,6,8–10]. The red-shifted form (denoted as *F*) decays back to the dark-adapted state (denoted as *D*) within 10–30 min. The structures of TePixD, SyPixD, AppA, and BlrB have been determined by X-ray crystallography and nuclear magnetic resonance (NMR) spectroscopy [3,7,11–14]. Figure 1 shows (A) the protein structure of SyPixD around the flavin molecule and (B, C) the molecular structure of flavin and tyrosine.

Fourier transform infrared (FTIR) and Raman spectroscopic studies have shown that the vibration of the $C_4{=}O$ group in flavin is weakened in the red-shifted form, which suggest stronger hydrogen bonding [8,15–22]. It was proposed that Gln50 also forms a hydrogen bond with N_5 (or additionally O_4) of flavin in the dark and alters it during photoreaction because the mutant protein, which has Ala in place of Gln50 (Q50A), does not achieve the red-shifted form [14]. The dark-adapted state and the red-shifted state of PixD are denoted as *D* and *F*, respectively. The molecular structure of TePixD suggests that Tyr8 interacts with flavin through a FAD-Gln50-Tyr8 hydrogen-bond network [12], where Tyr8 is a hydrogen-bond donor to Gln50 in both the dark and light states [23]. Replacement of tyrosine with phenylalanine (Y8F in TePixD and SyPixD, Y21F in AppA, and Y472F in PACR-F2) abolished the photo-conversion activity at physiological temperatures [16,24–28]. Therefore, the electron transfer between flavin and tyrosine is proposed as being essential for the light reaction [29].

Recently, we have found an electron paramagnetic resonance (EPR) signal arising from TePixD [30]. The signal was light-induced at 5–150 K and stably trapped at low temperature. The signal occurred after the second photo-excitation of the photo-induced red-shifted states in the photocycle of TePixD. The lineshape of the signal shows typical magnetic dipole–dipole interaction (Pake doublet). Based on the pulsed ENDOR and site-directed mutation results, it was concluded that the signal was arising from the radical pair between the flavin neutral radical (FADH$^{\cdot}$) and tyrosine (Y8$^{\cdot}$). The distance between the radicals was estimated to be 6.9 Å, assuming point-dipole approximation. In this Letter, we have studied the EPR properties of the stable FADH$^{\cdot}$–Y8$^{\cdot}$ radical pair in order to clarify the reactions and the sensor mechanism in detail.

Abbreviations: BLUF, sensor of blue-light using FAD; SyPixD, *Synechocystis* BLUF domain; TePixD, *Thermosynechococcus elongatus* BLUF domain; FAD, flavin adenine dinucleotide; ESE, electron spin echo; CW, continuous wave; EPR, electron paramagnetic resonance; NMR, nuclear magnetic resonance; FTIR, Fourier transform infrared; ENDOR, electron nuclear double resonance; MES, 2-morpholinoethanesulfonic acid.

* Corresponding author. Fax: +81 52 789 2882.
E-mail address: mino@bio.phys.nagoya-u.ac.jp (H. Mino).

0009-2614/$ - see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.cplett.2010.11.073

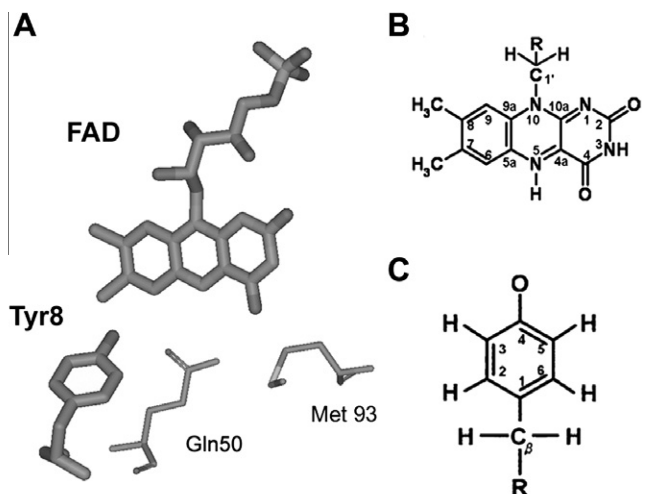

Figure 1. (A) Local structure of FAD and surrounding SyPixD (PDB entry 2HFO, chain A) [7]. Molecular structure of (B) neutral flavinosemiquinone (FADH) and (C) tyrosine.

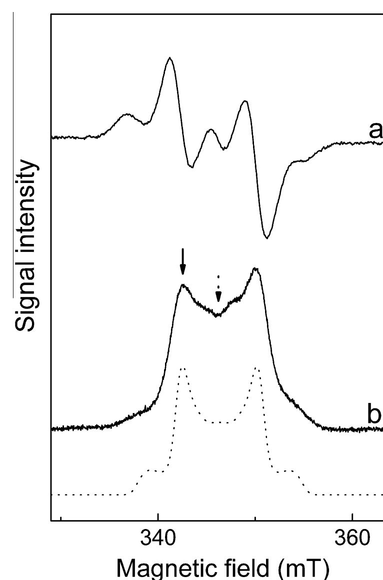

Figure 2. (a) CW and (b) primary ESE field-swept EPR spectra of SyPixD. Sample was illuminated at 150 K and subsequently cooled to 77 K under illumination. The horizontal axis for trace *a* was fitted to trace *b*. The dotted line is the simulated EPR spectrum based on the crystal structure [7]. Spin distributions of flavin and tyrosine molecules from Ref. [30] were used. The stick spectrum was convoluted using a Gaussian function with a width of 2.5 mT. Experimental conditions for CW EPR: microwave power, 1 mW; microwave frequency, 9.53 GHz; modulation amplitude, 0.3 mT; and temperature, 150 K. Experimental conditions for pulsed EPR: microwave frequency, 9.66 GHz; duration of the microwave pulses, 16 and 24 ns; the interval between the first and second pulse; 400 ns, repetition rate, 0.1 kHz; and temperature, 10 K.

2. Experimental

2.1. Sample preparation

SyPixD (Slr1694) was purified according to the previously reported method [31]. The protein was dissolved in a medium containing 50 mM Tris/HCl and 1 mM NaCl (pH 8.0).

Photosystem II membranes were prepared from spinach according to the previously described method [32] with a slight modification [33]. The membranes were dissolved in a medium containing 400 mM sucrose, 20 mM NaCl and 20 mM Mes/NaOH (pH 6.5).

2.2. EPR measurements

Continuous wave (CW) EPR measurements were performed using a Bruker ESP-300E EPR spectrometer with a gas flow temperature control system (CF935, Oxford Instruments, Oxford, GB). A standard resonator (ER4102, Bruker) was used. Electron spin echo (ESE) experiments were performed on a pulsed EPR spectrometer (ESP-380, Bruker) using a two-pulse (primary) ESE sequence. The spectrometer was equipped with a cylindrical dielectric cavity (ER4117DHQ-H, Bruker) and a gas flow temperature-control system (CF935, Oxford Instruments). Microwave pulses of 16 and 24 ns duration were used for the $\pi/2$ and π pulse sequence, respectively. Field-swept ESE spectra were measured by the $\pi/2$–τ–π sequence at a time interval τ of 200 ns between the microwave pulses. The spin–lattice relaxation time was measured by inversion recovery for the π–T–$\pi/2$–τ–π pulse sequence at a time interval τ of 200 ns.

Samples were illuminated using light from a 408 nm diode laser (ITC 510, Thorlabs) through a 1 mm diameter glass fiber.

3. Results and discussion

Figure 2 shows (a) CW and (b) ESE field-swept EPR signals of light-induced radicals in SyPixD. The sample was illuminated at 150 K and subsequently frozen into 50 K under illumination. The EPR lineshape consists of large inner peaks and small outer peaks, which typical for a magnetic dipole–dipole interaction (Pake doublet). The peak separations are 7.5 and 15 mT for the inner and outer peaks, respectively. An identical signal was found for TePixD [30], where the peak separations were 8.5 and 17 mT for the inner and outer peaks, respectively. The slight difference in the peak separations is ascribed to the slight difference in the local structure of SyPixD and TePixD. The dotted line in Figure 2 shows a simulated spectrum based on the crystal structure of SyPixD [7]. Previous reported [30] spin distributions for the flavin and tyrosine molecules were used. Based on the crystal structure, electron–electron dipolar interactions between spin density distributions on both radicals were calculated and summed. The *g*-factors for both molecules were assumed to be $g = 2$. The stick spectrum was convoluted using a Gaussian function with a width of 2.5 mT. The results show the protein structure with a Pake doublet, which is the same as the crystal structure. Nagai et al. reported a structural modification around flavin molecule of approximately 2.3 Å compared to crystal structure of TePixD [30]. However, there is no structural modification in TePixD after reexamination of EPR simulations based on the crystal structure of TePixD.

Figure 3 shows the H_1-dependence of the ESE-signal amplitude. Traces *a* and *b* were measured at the field position of the lower maximum peak (bold arrow in Figure 2) and the central field position of the peak-to-trough of the Pake doublet signal (dotted arrow in Figure 2), respectively. Trace *c* was measured in the Y_D signal of photosystem II as a standard of $\boldsymbol{S} = 1/2$ with $g = 2$. The horizontal axis is normalized by $H_{1\max}$, which is the maximum H_1 fed to the resonator. A density matrix formalism [34] for a two-pulse ESE sequence with pulses of the same amplitude and pulse-lengths of t_p and $2t_p$ shows that the ESE signal intensity for resonant spins is proportional to $\sin^3(2g\beta H_1\langle S_x\rangle t_p)$, where $\langle S_x\rangle$ is a matrix element of the transition operator for the case of $H_1 \| x$ and $H_0 \perp x$. Traces *d* and *e* represent calculations of the H_1-dependence of the ESE-signal amplitude. The H_1-dependence of spin $\boldsymbol{S} = 1/2$ with $g = 2$ (trace *d*) was fitted by trace *c*. Trace *e* shows calculated H_1-dependence of the spin $\boldsymbol{S} = 1$ with $g = 2$. The dot-dashed and dotted lines show the maximum amplitude of the $\boldsymbol{S} = 1/2$ and $\boldsymbol{S} = 1$, respectively. The concurrence of traces *a* and *e* shows that the Pake

Download English Version:

https://daneshyari.com/en/article/5384772

Download Persian Version:

https://daneshyari.com/article/5384772

Daneshyari.com