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Quantum Mechanical Study of Photoinduced Charge Transfer in FMN Binding Protein[||]

Nadtanet Nunthaboot,[†] Fumio Tanaka,*,[†] Sirirat Kokpol,[‡] Haik Chosrowjan,[§] Seiji Taniguchi,[§] and Noboru Mataga[§]

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CT interactions between Iso* and nearby aromatic amino acids in FBP were investigated by a semiempirical MO method. Atomic coordinates of lumiflavin as Iso, 3-methylindole as Trp, and 4-methylphenol as Tyr, used for MO calculations, were obtained from crystal, 20 NMR structures and 40 MD structures (20 ps time intervals). Geometries of Iso-Trp32, Iso-Trp106 and Iso-Tyr35 systems were optimized by the PM3 method. The interaction energies (kcal/mol) of crystal structure were −16.9 in the Iso-Trp32 system, −7.4 in the Iso-Trp106 system and 1.4 in the Iso-Tyr35 system. The interaction energies (kcal/mol) of NMR structures were −16.5 ± 0.28 in the Iso-Trp32 system, −10.6 ± 0.14 in the Iso-Trp106 system, and 0.97 ± 0.09 in the Iso-Tyr35 system. The interaction energies (kcal/mol) of MD structures were −24.3 ± 0.19 in the Iso-Trp32 system, −10.2 ± 0.49 in the Iso-Trp106 system, and 0.285 ± 0.037 in the Iso-Tyr35 system. CT interaction from the aromatic amino acids to Iso* was judged from negative charge at Iso*. The charge in the Iso-Trp32 system was −0.490 in crystals, −0.439 ± −0.099 in NMR structures, −0.454 ± 0.048 in MD structures. The charge in the Iso and Trp106 system was −0.011 ± 0.004 in MD structures, but negligible in other structures. CT interactions in Iso-Tyr35 system were also negligible. The ET rate obtained with Kakitani and Mataga theory and MD decreased as the magnitude of the interaction energy decreased. Correlation between the ET rate and CT interaction in FBP was examined. The interaction energy (Y) was approximated with ln(ET rate) (X) by a function, $Y = 0.0036X^3 + 0.0306X^2 - 1.7822X - 21.177$.

Introduction

Fluorescence of riboflavin was first found by Weber.[1] The intense fluorescence of free flavins is quenched by various substances including aromatic amino acids as Trp and Tyr.[1,2] In most flavoproteins, the fluorescence is also markedly quenched. In these flavoproteins Trp and/or Tyr always exist near Iso.[3–6] It was demonstrated by means of a picosecond-resolved[7,8] and femtosecond-resolved[9,10] transient absorption spectroscopy that electron transfer (ET) from Trp and/or Tyr to Iso* was responsible for the remarkable quenching. Ultrafast fluorescence dynamics of various flavoproteins have been investigated by an up-conversion technique.[3–5] Donor−acceptor distance-dependent ET rates in these flavoproteins have been expressed with center to center distance rather than edge to edge distance.[11] The distance dependence of ET rates was analyzed with three kinds of ET theories.[12]

Flavoproteins mostly function in redox reactions and electron transport in various organs of mammals and plants, and in various bacteria.[13] Recently, a number of new flavoproteins have been found to function as photoreceptors.[14–20] Among six families of the photoreceptors, cryptochromes, phototropins and BLUF, contain flavins as the reaction centers.[14] In some of the flavin photoreceptors as AppA[15] and Slr1640[16] aromatic amino acids as Trp and/or Tyr exist near Iso. Because of without Tyr21 in AppA[15] it does not function as a photosensor, Tyr21 plays an essential role for its function of AppA.[15] It is also considered that three tryptophanes near Iso play an important role for photosensing function of *Arabidopsis* cryptochrome-1, because the replacement of two of the three tryptophanes by phenylalanines resulted in impairment of cryptochrome-1 dependent blue light responses in vivo.[17]

FMN binding protein (FBP) is considered to play an important role in the electron transport process in the bacterium, but the whole picture of the electron flow and coupling of the redox proteins is not known yet.[21] Three-dimensional structures of FBP were determined by X-ray crystallography[22] and NMR spectroscopy.[23] According to these structures, Trp32 was closest to Iso and then Tyr35 and Trp106.

Various ET theories have been modeled for ET processes in bulk solution.[24–32] It is not clear yet which factor is the most important for ET in proteins. There should be something different in ET processes in proteins from ones in bulk solution where a donor and an acceptor are surrounded by uniform solvent. The ET rate of FBP was much slower in the crystal than in solution,[33] which could not be explained only by the donor−acceptor distance. The fluorescence lifetimes of WT, W32Y and W32A have been measured and compared among

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|| Abbreviations used: FMN, flavin mononucleotide; FBP, FMN binding protein from *Desulfovibrio vulgaris* (Miyazaki F); Trp, tryptophan; Tyr, tyrosine; Iso, isoalloxazine ring; Iso*, the excited Iso; WT, wild type FBP; W32Y, FBP of which Trp32 is replaced by Tyr32; W32A, FBP of which Trp32 is replaced by alanine32; FAD, flavin adenine dinucleotide; MO, molecular orbital method; MD, molecular dynamic simulation; CT, photoinduced charge transfer; ET, photoinduced electron transfer; BLUF, photoreceptor of blue light using flavin; QM/MM, MO with nonempirical Gaussian method combined with classical molecular mechanic method; CASSCF, complete active space multiconfiguration self-consistent field method.
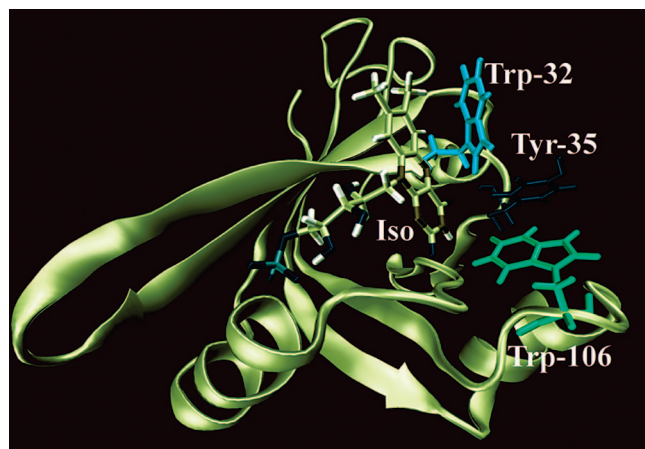
**Figure 1.** MD structure of FBP near FMN binding site. The structure was obtained by MD of FBP in water.
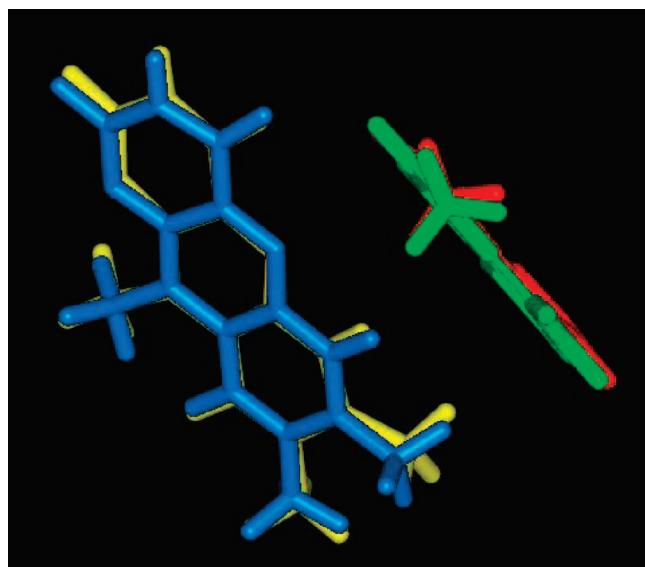
Figure 2. Comparison of Iso and Trp32 structures obtained by MD and by QM. Configurations of front (blue) and behind (yellow) Iso were obtained by QM and MD, respectively. Configurations of front Trp32 (green) and behind (red) Trp32 were obtained by QM and MD, respectively. QM structure was obtained by optimizing geometry with PM3 method. ε_0 used was 9.86. Dipole moment obtained by QM was 16.84 D, and amount of charge transfer from Trp-32 to Iso was 0.534. Center to center distance between Iso* and Trp-32 was 0.678 nm by QM and 0.682 nm by MD. Interplaner angle between Iso and Trp-32 was 61.7 deg by QM and 64.4 deg by MD.

them.[34] Ultrafast fluorescence decay of WT was analyzed with three kinds of ET theories and MD.[35] Those of WT, W32Y and W32A were also simultaneously analyzed with Kakitani and Mataga theory and MD.[36] In these works all of ET parameters contained in the ET theories were determined by means of a nonlinear least-squares method. Agreements between the observed and calculated fluorescence decays of WT, W32Y and W32A were all satisfactory. It was suggested that the ET rate was fastest from Trp32, then from Trp106 and from Tyr35.

In these analyses both methodologies of ET theory and MD of the proteins were based upon classical mechanics. It is required to ensure quantum mechanically that ET or charge transfer (CT) can really occur in the flavoproteins with very short lifetimes. In this regard, ET from Tyr in flavin reductase and flavodoxin reductase was investigated by QM/MM method.[37,38] Recently, the initial step of photosensing reaction in the BLUF domain of AppA from *Rhodobacter sphaeroides* was investi-
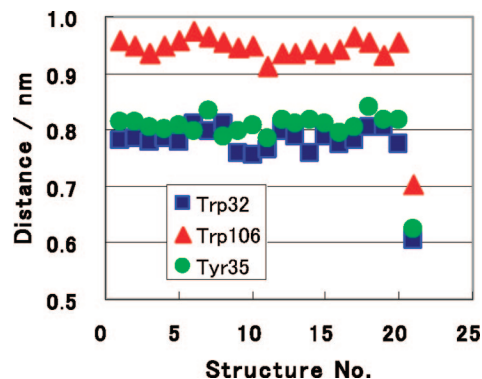
Figure 3. Center to center QM distances between Iso and nearby aromatic amino acids in crystal and NMR structures. Structure Nos. 1−20 represent NMR structure; No. 21 represents crystal structure. QM distance implies quantum mechanically optimized distance by PM3.
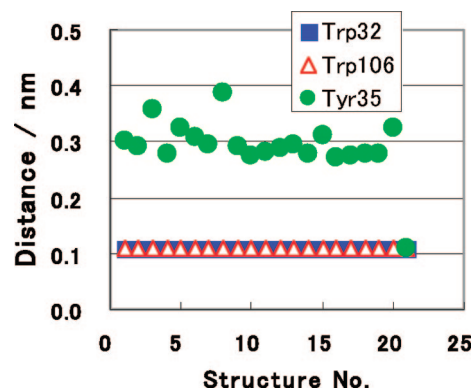
Figure 4. Edge to edge QM distances between Iso and aromatic amino acids in crystal and NMR structures. Structure Nos. 1−20 represent NMR structure; No. 21 represents crystal structure. QM distance implies quantum mechanically optimized distance by PM3.

gated by the QM/MM method with CASSCF at a higher level.[39] Here we understand that ET means 100% irreversible electron transfer defined by ET theory, and CT means full or partial electron transfer obatined by MO. In the present work, we have studied CT interaction between Iso* and nearby aromatic amino acids in FBP with a semiempirical MO of PM3, using atomic coordinates obtained from crystal, NMR and MD structures. Correlation between CT and the ET rate were also examined.

Methods

Details of MD calculation were described in the previous works.[35,36] Iso was substituted with lumiflavin, Trp with 3-methylindole, and Tyr with 4-methylphenol. Atomic coordinates of these chromophores were obtained from the crystal, 20 NMR and 40 MD structures with 20 ps time intervals up to 800 ps. Molecular orbital calculations were performed with a software of WinMOPAC (Professional, Version 3.9; Fujitsu, Tokyo). Geometries of Iso-Trp32, Iso-Trp106 and Iso-Tyr35 systems were optimized with a semiempirical MO of PM3. Keywords used for PM3 were EF, PM3, PRECISE, EXCITED, XYZ, GEO-OK and EPS (visit for meaning of these keywords: http://openmopac.net/). The value of ε_0 (static dielectric constant) was 9.86 which was obtained in the previous work.[36] Interaction energy (E) between the excited Iso and Trp or Tyr was evaluated by eq 1.

$$E = \Delta H_f(\text{Iso*AA}) - \{\Delta H_f(\text{Iso*}) + \Delta H_f(\text{AA})\} \quad (1)$$

TABLE 1: Various Geometries between Iso and Nearby Aromatic Amino Acids in FBP[a]

	center to center distance (nm)			edge to edge distance (nm)			interplaner angle (deg)		
structure	2 (SE)	Iso106(SE)	Iso35(SE)	Iso32(SE)	Iso106(SE)	Iso35(SE)	Iso32 (SE)	Iso106 (SE)	Iso35 (SE)
crystal[b]	0.705	0.855	0.774	0.350	0.382	0.341	−48.0	57.3	−69.8
NMR[b]	0.842	0.814	0.736	0.331	0.361	0.358	−18.9	75.1	−66.2
	(0.004)	(0.003)	(0.004)	(0.001)	(0.002)	(0.002)	(1.17)	(8.4)	(1.6)
MD[c]	0.639	0.983	1.050	0.260	0.249	0.433	14.7	83.9	79.5
QM crystal[d]	(0.004)	(0.005)	(0.006)	(0.002)	(0.005)	(0.007)	(1.1)	(0.6)	(1.2)
	0.604	0.703	0.624	0.109	0.109	0.110	−71.3	71.5	84.7
	0.775	0.947	0.809	0.109	0.110	0.299	42.2	−71.0	−27.6
QM NMR[d]	(0.010)	(0.003)	(0.003)	(0.000)	(0.000)	(0.006)	(1.7)	(0.7)	(17.3)
QM MD[d]	0.642	0.958	1.04	0.224	0.234	0.411	−51.4	47.5	59.5
	(0.003)	(0.008)	(0.007)	(0.004)	(0.004)	(0.007)	(1.9)	(9.8)	(1.2)

[a] Iso32, Iso106 and Iso35 represent the Iso-Trp32, Iso-Trp106, Iso-Tyr35 systems, respectively. SE means standard error. [b] Atomic coordinates were taken from PDB (code: 1FLM for crystal structure and 1AXJ for NMR structures). The averages were taken over 20 NMR structures. [c] The averages were taken over 40 MD structures at 20 ps time intervals up to 800 ps. [d] The structures were energetically optimized by PM3 method.
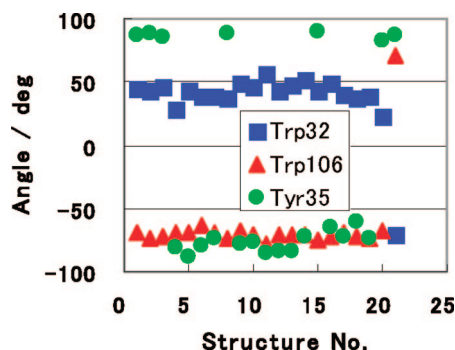

Figure 5. Interplaner QM angles between Iso and nearby aromatic amino acids. Structure Nos. 1−20 represent NMR structure; No. 21 represents crystal structure. QM angles were obtained from quantum mechanically optimized geometries.

In eq 1 $\Delta H_f(Iso*AA)$ denotes heat of formation in a system of Iso* and aromatic amino acid as Trp or Tyr (AA) in the ground state. $\Delta H_f(Iso*)$ and $\Delta H_f(AA)$ are heat of formation in the systems of only Iso* and of only AA in the ground state, respectively. The heat of formation contains total electronic energy, core−core repulsion energy and heat of formation of all constituent atoms.

Results

Comparison of Structures at Iso Binding Site among Crystal, NMR and QM Geometries Optimized from them. Local structure of Iso binding site in FBP is illustrated by Figure 1, which was obtained by one of MD snapshots. QM geometry means quantum mechanically optimized geometry with PM3 method. Figure 2 shows comparison of mutual configurations of Iso and Trp32 obtained by MD and one obtained by QM. In Figure 2 the QM structure was energetically optimized starting from MD structure. In these QM and MD geometries of Figure 2, the center to center distances between Iso and Trp32 were both 0.68 nm, and the interplaner angle between both aromatic rings was 62° in QM structure and 64° in MD structure. QM structure in Figure 2 did not differ very much with its original MD structure.

Figures 3 and 4 show center to center QM distances and edge to edge QM distances between Iso and nearby aromatic amino acids, respectively, obtained from crystal and 20 NMR structures. Mean distances and angles between Iso and nearby aromatic amino acids in various structures of FBP are listed in Table 1. The mean center to center QM distances of Iso32, Iso106 and Iso35 systems were shorter by more than 0.1 nm

than those of original crystal structure. Edge to edge QM distances of Iso32, Iso106 and Iso35 systems were all about 0.11 nm, and those in the original crystal structure were about 0.36 nm. The center to center QM NMR distances were shorter in Iso32 by ca. 0.07 nm, longer in Iso106 by ca. 0.13 nm, and longer in Iso35 by ca. 0.17 nm, compared to those of respective systems in original NMR structures.

Comparison between MD and QM Structures. QM geometries were also obtained from 40 MD structures at 20 ps time intervals up to 800 ps. Structural parameters of MD and QM geometries are also listed in Table 1. The mean center to center distances over MD time ranges were 0.639 nm between Iso and Trp32, 0.983 nm between Iso and Trp106, and 1.05 nm between Iso and Tyr35. Figure S1 (Supporting Information) shows a time-dependent change in the center to center distance obtained by QM starting from MD structures. The mean distance between Iso and Try32 was 0.642 nm by QM. The mean QM center to center distances were 0.958 nm in Iso-Trp106 system and 1.040 nm, respectively. These distances did not differ much with the mean distance obtained by original MD. Figure S2 (Supporting Information) illustrates time-dependent change in edge to edge QM distance between Iso and Trp32, between Iso and Trp106 and between Iso and Tyr35. The mean QM edge to edge distances were 0.224 nm between Iso and Trp32, 0.234 nm between Iso and Trp106 and 0.411 between Iso and Tyr35.

Figure S3 (Supporting Information) displays interplaner angles between Iso and Trp or Tyr obtained by QM. The angles between Iso and Trp32 obtained by QM were around −50°, which was very different from those obtained by MD, around 15°. In QM calculation Iso (substituted by lumiflavin), Trp (substituted by 3-methylindole) and Tyr (substituted by 4-methylphenol) can freely rotate, whereas in the real protein system they cannot freely rotate because of covalent bonds between the chromophores and the peptide chain and also steric hindrance by the other amino acid residues. This may be reason why the angles obtained by QM differed very much from those by MD, of which situation was similar to crystal and NMR structures.
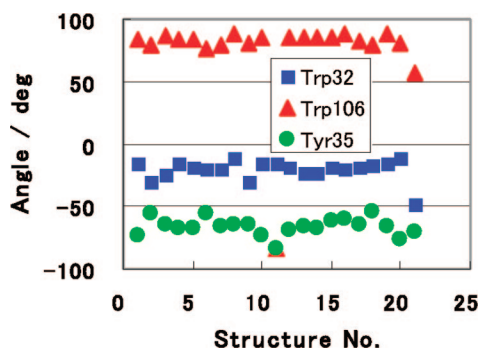
Interaction Energy between Iso* and Trp or Tyr. Interaction energies between Iso* and nearby aromatic amino acids were obtained starting from NMR and crystal structures and listed in Table 2. The interaction energies (kcal/mol) in crystal structure were −16.91 in the Iso-Trp32 system, −7.35 in the Iso-Trp106 system and 1.41 in the Iso-Tyr35 system. Mean interaction energies (kcal/mol) of NMR structures were −16.49 in the Iso-Trp32 system, −10.63 in the Iso-Trp106 system and 0.97 in the Iso-Tyr35 system. The interaction energy was highest
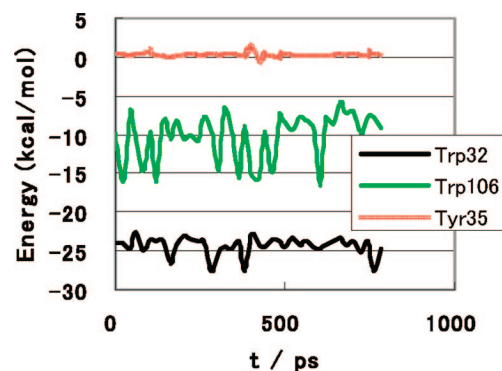
TABLE 2: Interaction Energy between the Iso* and Nearby Aromatic Amino Acids and Charge at Iso of NMR and Crystal Structures[a]

structure	Iso-Trp32		Iso-Trp106		Iso-Tyr35	
	raction Energy (kcal/mol)	charge at Iso	interaction energy (kcal/mol)	charge at Iso	interaction energy (kcal/mol)	charge at Iso
crystal	−16.91	−0.490	−7.35	0.0232	1.41	0.0006
NMR						
1	−17.00	−0.910	−10.25	0.0001	0.55	−0.0951
2	−16.16	−0.161	−10.36	0.0002	0.69	0.0010
3	−17.28	−0.990	−11.13	0.0003	0.40	0.0002
4	−15.57	−0.063	−10.49	0.0003	1.46	0.0007
5	−16.82	−0.953	−10.13	0.0002	0.54	0.0003
6	−14.53	−0.066	−9.55	0.0003	0.65	0.0004
7	−15.45	−0.085	−9.82	0.0000	1.45	0.0004
8	−14.38	−0.030	−10.38	−0.0002	0.40	0.0001
9	−17.78	−0.990	−10.64	0.0003	1.22	0.0006
10	−18.93	−0.995	−10.50	0.0001	1.43	0.0008
11	−17.46	−0.203	−12.23	−0.0004	1.41	0.0008
12	−15.51	−0.125	−11.09	0.0005	0.78	0.0006
13	−16.62	−0.919	−11.19	0.0004	0.68	0.0006
14	−18.52	−0.916	−10.89	0.0003	1.38	0.0008
15	−16.68	−1.015	−10.95	0.0002	0.51	0.0008
16	−17.46	−0.174	−10.89	0.0002	1.28	0.0010
17	−17.18	−0.018	−10.00	0.0001	1.31	0.0009
18	−15.28	−0.033	−10.10	0.0001	1.34	0.0007
19	−14.90	−0.078	−11.28	0.0002	1.34	0.0007
20	−16.30	−0.052	−10.33	0.0003	0.57	0.0003
mean	−16.49	−0.439	−10.63	0.0002	0.97	−0.0042
SE[b]	0.28	0.099	0.14	0.0000	0.09	0.0048

[a] Atomic coordinates were taken from X-ray structure (PDB code: 1FLM) and 20 NMR structures (PDB code: 1AXJ). Interaction energies were calculated from eq 1 in text. [b] Standard error among 20 NMR structures.

Figure 6. Interplaner angle between Iso and nearby aromatic amino acids obtained from PDB. Structure Nos. 1−20 represent NMR structures; No. 21 represents crystal structure. Atomic coordinates were obtained from PDB (Code: 1AXJ for NMR structures, 1FLM for crystal structure).
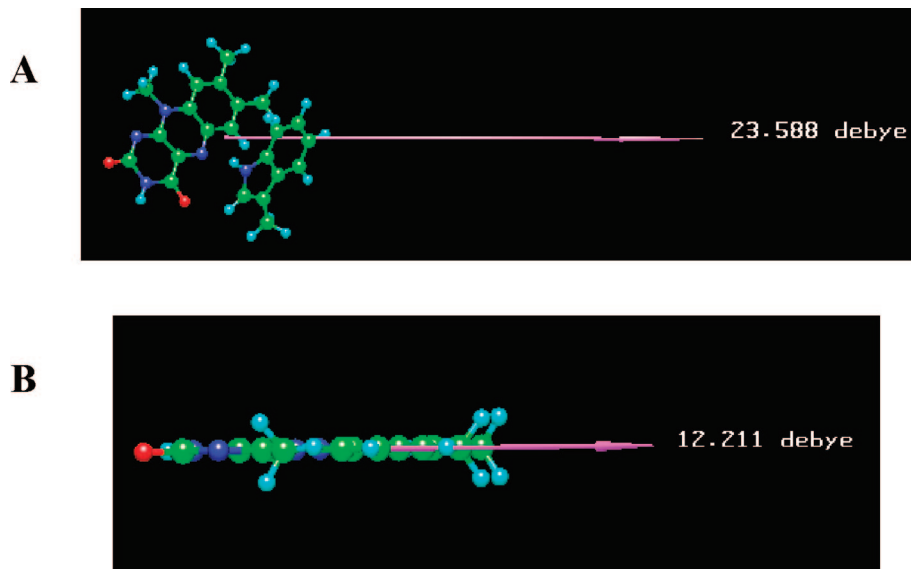

Figure 7. Interaction energy between Iso* and Trp or Tyr. The interaction energies were obtained by eq 1, according to MD structures.

in the Iso-Trp32 system and was lowest in the Iso-Tyr35 system both in crystal and in NMR structures.

Figure 7 shows time-dependent changes in the interaction energies. The mean interaction energies (kcal/mol) over MD time range were −23.5 in the Iso-Trp32 system, −10.2 in the Iso-Trp106 system and 0.28 in the Iso-Tyr35 system. The interaction energy was greatest in the Iso-Trp32 system, and then in the Iso-Trp106 system, and least in the Iso-Tyr35 system. As described above, the center to center QM distance was shortest in Iso-Trp32 system among three systems, and the mean edge to edge distance did not differ much between Trp32 and Trp106 cases. It is noted that the interaction energy between Iso* and Tyr35 was very little.

CT Interaction from the Aromatic Amino Acids to Iso*. Figure 8 illustrates direction and magnitude of dipole moments of Iso* in one of QM structures. Panel A shows the direction and magnitude of dipole moment in the Iso-Trp32 system, and
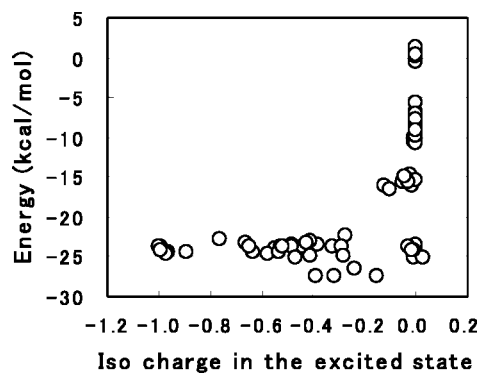
panel B those in the Iso system. The magnitude of the dipole was 23.6 D in A and 12.2 D in B. The direction of the dipole moment in A was from Iso to Trp32, but it was within the plane of Iso. Recent MO study (symmetry adapted-cluster-configuration interaction method) revealed that the dipole moment of lumiflavin was 9.6 D in the ground state and 11.2 D in the lowest excited state.[40] The results of the dipole moments reveal the presence of the CT interaction from Trp32 to Iso*. Figure 9 illustrates the time-dependent change of charge at Iso* in unit of *e*. The charge was obtained as sum of Mulliken charges at all atoms in Iso*. The charge of Iso* changes markedly from 0 to −1 with time in the Iso-Trp32 system, but it changed little in the Iso-Trp106 system. The charge in the Iso-Tyr35 system was almost zero at any time. The mean charge over MD time range was −0.45 in the Iso-Trp32 system, −0.011 in the Iso-Trp106 system, and 0.000 in Iso-Tyr35. Figure 10 shows a correlation between the interaction energy and charge of Iso* in all the systems Iso-Trp32, Iso-Trp106 and Iso-Tyr35.

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Figure 8. Dipole moments of Iso*. Panel A shows dipole moment of Iso* and Trp32 system. Panel B shows dipole moment of Iso* alone. In (A) direction of the dipole moment is from Iso ring to the Trp32 ring, and in B it is within the plane of the Iso ring. This shows the presence of charge transfer interaction from Trp32 to Iso* in the Iso-Trp32 system.
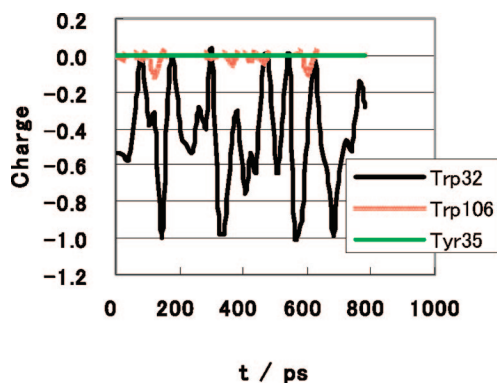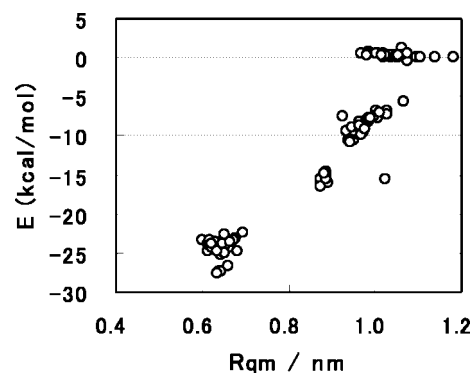

Figure 9. Time-dependent change in charge at Iso* in the Iso-Trp or -Tyr system obtained by MD structures. The charge at Iso* was generated as a consequence of charge transfer from Trp32, Trp106 and Tyr35 to Iso*. The charge was expressed as a sum of Mulliken charge densities at all atoms of lumiflavin.

Figure 11. Correlation between the interaction energy and the center to center distance obtained by QM. Rqm denotes center to center distance obtained by QM. The interaction energies and the distances were obtained from all the systems Iso-Trp32, Iso-Trp106 and Iso-Tyr35.
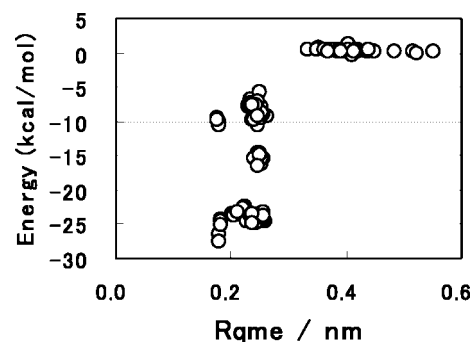

Figure 10. Correlation between the interaction energy and Iso* charge. The energies and charges are displayed for all systems of Iso-Trp32, Iso-Trp106 and Iso-Tyr35.

Figure 12. Correlation between the interaction energy and the edge to edge distance obtained by QM. Rqme denotes edge to edge distance obtained by QM. The interaction energies and the edge to edge distances were obtained from all the systems of Iso-Trp32, Iso-Trp106 and Iso-Tyr35.

Absolute magnitude of the interaction energy tends to increase as amount of the charge increased. However, in many cases the interaction energy took various values at zero charge. According to Figure 9 charge in Iso -Trp32 system frequently took zero or nearly zero, even though the interaction energy was much.

Figure 11 demonstrates a correlation between the interaction energy and the center to center distance obtained by QM in the all systems of Iso-Trp32, Iso-Trp106 and Iso-Tyr35. The magnitude of interaction energy tends to increase as the center to center QM distance becomes shorter. Correlation of the interaction energy with the edge to edge distance obtained by
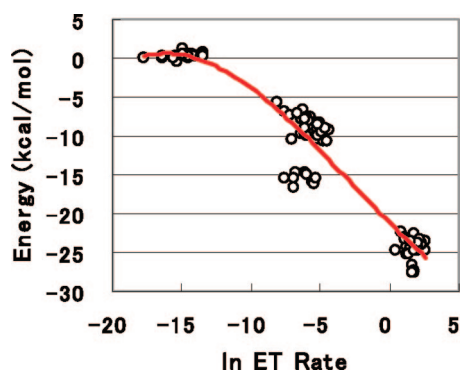
Figure 13. Correlation between the interaction energy and the ET rate. The ET rate is expressed in unit of ps^{-1}, which was obtained with ET parameters by KM theory. This contains all data of Trp32, Trp106 and Tyr35. Approximate equation with a 3rd order polynomial, Y (energy) vs X [ln(ET rate)], was expressed as $Y = 0.0036X^3 + 0.0306X^2 - 1.7822X - 21.177$.
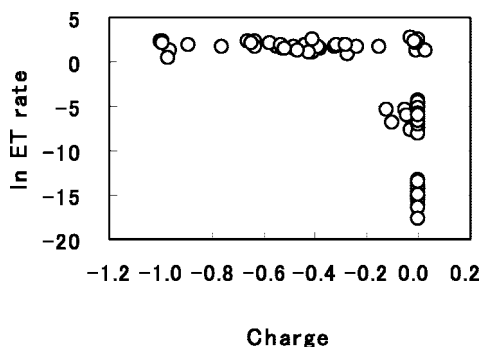
Figure 14. Correlation between the ET rate and charge at Iso*. The ET rate was expressed in unit of ps^{-1}. The ET rate was not always proportional to the charge.

QM is shown in Figure 12. The interaction energy was almost zero when the edge to edge distance longer than 0.35 nm. It suddenly increased as the edge to edge distance became shorter than 0.35 nm, but it decreased gradually with the center to center distance.

Correlation of the Interaction Energy with the ET Rate from Trp or Tyr to Iso*. ET rates were obtained with Kakitani and Mataga theory in the previous work.[36] In this work fluorescence decays of WT, W32Y and W32A were simultaneously analyzed using the center to center distances obtained by MD. ET parameters in this theory, frequency ν_0, a coefficient for ET processes β, critical distance between adiabatic and nonadiabatic ET processes R_0, and $-\Delta G_0$ were determined separately between Trp and Tyr by means of a nonlinear least-squares method.[36] The ET rate was fastest from Trp32 to Iso* and then from Trp106 to Iso*.[36] The ET rate was slowest from Tyr35 to Iso*.[36] This result is in good accord with the interaction energy obtained in the present work. Figure 13 shows a correlation between the interaction energy and the ET rate. When the ET rate was faster, the interaction energy was greater (with negative sign), and vice versa. Figure 14 shows a correlation between the ET rate and charge at Iso*. In Figures 13 and 14 the ET rate was expressed in ps^{-1} unit. The relationship between ln(ET rate) (X) and the interaction energy (Y) was approximated by a function, $Y = 0.0036X^3 + 0.0306X^2 - 1.7822X - 21.177$. The ET rate was not always proportional to the charge at Iso*. From Figure 9 sometimes the charge was zero even in Iso-Trp32 system, where the ET rate was very fast.

Discussion

The relationship between the ET rate and CT has not been very clear yet. Callis et al. have been trying to obtain ET rates or relative quantum yields of Trp fluorescence from the excited Trp to peptide bonds in proteins by means of quantum mechanical transition probability and QM/MM methods.[38,41,42] The present work may also provide a quantum mechanical elucidation of ultrafast charge separation. Remarkable CT occurred in Iso-Trp32 system, but only a little CT in Iso-Trp106 system, as judged from the amount of charge at Iso*. In Iso-Tyr35 system, no CT was found. The interaction energies between the donor and acceptor correlated well with the amount of negative charges at Iso*, with the donor−acceptor distances and with ET rates.

In the Iso-Trp32 MD system, sometimes the Iso charge oscillated between 0 and −1, though the interaction energy was much higher than those of Iso-Trp106 and Iso-Tyr35 systems. The remarkable change in the charge from almost zero to −1 was also found among 20 NMR structures. Even though the charge was nearly zero, the interaction energy was not zero as in Iso-Tyr35 system. Although relationships between the charge and the interplaner angle and between the charge and edge to edge QM distance were examined, any correlations could not be found. In the lowest excited state of Iso, a local excited state may be mixed with a CT state. Sometimes the local excited state (charge equal to 0) may be the lowest or the CT state (charge equal to −1) may be lowest in the excited state energy depending on the mutual geometrical arrangement. The oscillatory behavior of CT may be reasonably elucidated by this hypothesis (we appreciate the reviewer's comment about it). It may be also related to an oscillatory fluorescence decay in CT complex.[43,44]

It was evident that the interaction energies in Iso-Tyr35 system were much lower than those in the Iso-Trp106 system, even though the center to center distances in Iso-Tyr35 system did not differ much with those in Iso-Trp106 system. The following results may be elucidated from the QM interaction energy. The lifetime of W32Y, where Trp32 was replaced by Tyr32, was quite long, ca. 15 ps, compared to lifetime of WT, ca. 220 fs,[34] despite that the center to center distance in Iso-Tyr32 system of W32Y did not differ much from one in Iso-Trp32 system of WT, 0.66 and 0.64 nm, respectively, according to MD data.[36] An electronic energy part in Kakitani and Mataga theory may be represented by ν_0, which is proportional to energy. ν_0^{Trp} for Trp was 5 time higher than ν_0^{Tyr} for Tyr.[36] Though the interaction energy between Iso* and Tyr was very little in FBP, Tyr itself has been still efficient fluorescence quencher in other flavoproteins. van der Berg et al. demonstrated that a shortest lifetime of fluorescence of wild-type glutathione reductase disappeared when Tyr177 was replaced by phenylalanine.[43] They also observed CT interaction between Iso* and Tyr177 in this flavoprotein, by means of time-resolved fluorescence anisotropy.[45] Iso in flavodoxin from *Desulfobivrio vulgaris* (Miyazaki) is sandwiched by Trp60 and Tyr98. The ET rate was 4.78 ps^{-1} from Trp60 to Iso* in Y98F (Tyr98 was replaced by Phe98) and 3.73 ps^{-1} from Tyr98 to Iso* in W60F (Trp60 is replaced by Phe60).[5] In the case the center to center distances were 0.64 nm between Iso and Trp60 and 0.50 nm between Iso and Tyr98. Despite that the distance between Iso and Tyr98 is shorter than one between Iso and Trp60, the ET rate was 1.3 times faster in the Iso-Trp60 system of flavodoxin than in the Iso-Tyr98 system. The CT interaction energy may become larger even in the Iso-Tyr system, when the donor−acceptor distance becomes shorter.

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The relationship between the interaction energy and the ultrafast ET rate is especially of interest, because it connects ET theory obtained by classical continuum model with quantum chemical MO theory. The relationship may be useful to obtain the ET rate from the interaction energy. It is quite difficult to measure the ET rate with very short lifetime of a flavoprotein. By the function, $Y = 0.0036X^3 + 0.0306X^2 - 1.7822X - 21.177$, where Y is the interaction energy and X is ln(ET rate), the ET rate in a flavoprotein with very short lifetime may be evaluated from the calculated interaction energy, though it may be improved with data of other flavoproteins with very short lifetimes. Normally, a few Trp and/or Tyr contain in these flavoproteins. By using the equation ET rate from each, Trp or Tyr may be separately evaluated.

In some flavin photoreceptors as AppA, Slr1640 and cryptochrome-1, aromatic amino acids as Trp and Tyr exist near Iso. According to three-dimensional structure of flavin-binding AppA[15] and Slr1640,[16] center to center distances both between Iso and nearby Tyr and between Iso and nearby Trp are about 0.8−0.9 nm, at which distance ET could occur from the Tyr or Trp to Iso* as other flavoproteins investigated.[11,12] The ET in AppA BLUF domain was found by means of an ultrafast transient absorption spectroscopy by Dragnea et al.[46] and Toh et al.[47]

Supporting Information Available: Figure S1 (time-dependent change in the center to center distance), Figure S2 (time-dependent change in edge to edge QM distance), Figure S3 (interplaner angles between Iso and Trp or Tyr). This information is available free of charge via the Internet at http://pubs.acs.org.

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