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Classification of the mechanisms of photoinduced electron transfer from aromatic amino acids to the excited flavins in flavoproteins

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Abstract

In many flavoproteins a photoinduced electron transfer (ET) efficiently takes place from aromatic amino acids as tryptophan or tyrosine to the excited isooalloxazine, so that the fluorescence lifetimes of isooalloxazine in some flavoproteins become ultrashort. The mechanism of ET in the flavoproteins were classified into four classes from the relationship between logarithmic ET rates (ln Rate) and the donor-acceptor distances ($R_c$), using reported data. A physical quantity, $GT$, was defined as a sum of solvent reorganization energy, electrostatic energy between a donor cation and Iso anion, standard free energy gap between the photoproducts and reactants, and net electrostatic energy between the photoproducts and other ionic groups in the flavoproteins ($NetES$). When $GT$ fluctuates around zero with $R_c$, ET rate becomes fastest (faster than 1 ps$^{-1}$) in Kakitani and Mataga rate. In the ultrafast ET processes the ln Rate becomes parabolic function (Category 1) of $R_c$ as in FMN binding proteins and pyranose 2-oxidase at the shorter emission wavelengths, when $NetES$ is negligible compared to the other quantities in $GT$ function. In the ultrafast ET processes the ln Rate does not display any clear function of $R_c$ (Category 2) when $NetES$ is dominant in $GT$ function, because of no direct relation between $NetES$ and $R_c$. ET in flavodoxin from *Helobacor pylori* may be classified into Category 2. When $GT$ linearly varies with $R_c$ around a certain positive value, the ET rates become much slower ($< 1$ ps$^{-1}$). In this case the ln Rate linearly decreases with $R_c$ (Category 3), as Tyr224 in D-amino acid oxidase dimer. It is also conceivable that the ln Rate decreases with much scattered function of $R_c$ (Category 4), when $NetES$ is dominant in $GT$ function, as in Tyr314 in D-amino acid oxidase dimer. In ET processes of Category 1, ET rates decrease as $R_c$ becomes shorter than the distance at the maximum values of ln Rate, where $GT$ displays negative. Condition and physical meaning were discussed for the $GT$-negative region.
I. Introduction

Flavoproteins play an important role for electron transport, oxidation-reduction reactions in oxidases, oxygenases, dehydrogenases, etc.\(^1\) Photochemistry and photobiology of flavins and flavoproteins are also important fields in science,\(^2\) since a number of new flavin photoreceptors have been found in the last decade.\(^3\) It is considered that initial steps of photoregulation functions by AppA in photosynthetic systems\(^4,5\) and TePixD in pili-dependent cell motility\(^6\) are photoinduced electron transfer (ET) from tyrosine (Tyr) to the excited isoalloxazine (Iso*) through hydrogen bonding chain by glutamine. ET phenomena have been central field in photochemistry and photobiology.\(^7,8\)

Fluorescence of flavins was first observed by Weber.\(^9\) Fluorescence quenching of flavin fluorescence by indole ring was reported with isoalloxazine (Iso) -(CH\(_2\)_n-indole compounds by McCormick.\(^10\) Time-resolved fluorescence spectroscopy of flavins and flavoproteins has been reviewed by Berg and Visser.\(^11\) A number of flavoproteins displays practically no fluorescence. However, they emit with very short lifetimes (sub-picoseconds) upon the excitation with an ultra-short pulse laser.\(^12-16\) In these flavoproteins, tryptophan (Trp) and/or Tyr always exist near isoalloxazine ring. The remarkable fluorescence quenching in these flavoproteins was demonstrated to be due to ET from Trp and/or Tyr to the excited Iso (Iso*), by means of picosecond\(^17,18\) and femtosecond\(^19\) transient absorption spectroscopy.

ET phenomena including dark electron transfer in proteins have been amply reported by many workers during years 1970-2000.\(^20-29\) Moser et al.\(^25\) have experimentally demonstrated linear relationship of the logarithmic ET rates (ln Rate) with the donor-acceptor distances in photosynthetic proteins, which is called the Dutton rule. We have been working on the ET mechanisms in flavoproteins from the aromatic amino acids to Iso* with atomic coordinates
of the proteins determined by molecular dynamics simulation (MDS) and ultrafast fluorescence decays or lifetimes as experimental data.\textsuperscript{30-38}

The protein systems dealt in the present work are pyranose 2-oxidase from Tetrametes multicolor (P2O), flavodoxin from Helcobactor pylori (HPFD), flavin mononucleotide (FMN) binding proteins from Desulfovibrio vulgaris, Miyazaki F (FBP), and D-amino acid oxidase from porcine kidney (DAAO). The P2O is a homotetramer (Mw; 67 kDa per subunit), covalently bound flavin adenine dinucleotide (FAD) as a cofactor. It catalyses oxidative degradation of lignin to produce hydrogen peroxide.\textsuperscript{39} Crystal structure of wild type P2O has been determined by Kujawa et al.\textsuperscript{40} HPFD is a small flavoproteins (Mw; 18 kDa) which contains flavin mononucleotide (FMN) as cofactor, and functions as electron transport among proteins.\textsuperscript{41} HPFD is a pathogen for type-B gastritis and peptic-ulcer diseases,\textsuperscript{41} and gastric carcinoma.\textsuperscript{42} FBP (Mw; 18 kDa) also binds FMN and functions as electron transport like flavodoxins.\textsuperscript{43} DAAO (Mw; 39 kDa per monomer) is in an equilibrium state between monomer and dimer at relatively low concentrations,\textsuperscript{44} and binds one mole of FAD per monomer. DAAO catalyses oxidative degradation of D-amino acids into corresponding imino compounds, ammonium and hydrogen peroxides. Mammalian DAAO associates with the brain D-serine metabolism and with the regulation of the glutamatergic neurotransmission.\textsuperscript{45,46}

In the present work we have described on a classification of the ET mechanisms from the aromatic amino acids to Iso* in P2O, HPFD, FBPs and DAAO dimer based upon the relationship between the ln Rate and $R_c$, using reported ET rates and related physical quantities.

\textbf{METHODS OF ANALYSES}
**Procedure for determination of the ET rate from fluorescence decay of a flavoprotein.**

Principle of the method to determine ET rates from aromatic amino acids to Iso* in flavoproteins are described in a review article.\textsuperscript{47} The procedure is illustrated as a Diagram in Supporting Information. Here the method is outlined below.

1) Time-dependent atomic coordinates are determined by MDS method.
2) ET rates are calculated with appropriate initial values of ET parameters contained in an ET theory. Sometimes the initial ET parameters are taken from the reported works.
3) Fluorescence decays and lifetimes of Iso* in flavoproteins are calculated with the ET rates.
4) Value of chi-square (\(\chi^2\)) is obtained between the observed and calculated decays or lifetimes.
5) New set of ET parameters are evaluated to get smaller values of the chi-square by the non-linear least square method according to Marquardt algorithm.
6) ET rates are calculated with the new set of ET parameters.
7) The procedures 2) to 6) are repeated to obtain the minimum value of the chi-square.
8) Convergence criteria is that the chi-square does not appreciably decrease from one iteration to the next. Numerically the criteria is determined by an equation,

\[
\frac{\chi^2(k) - \chi^2(k+1)}{\chi^2(k)} < \varepsilon
\]

where \(\chi^2(k)\) and \(\chi^2(k+1)\) are chi-square at \(k\)-th and \((k+1)\)-th iterations. Normally \(\varepsilon\) is taken to be 10\(^{-6}\).
9) When the criteria is satisfied, ET rates and related physical quantities are calculated with the best-fit ET parameters.

**ET rates from Trp168 to Iso* in P2O.**

Method of MDS for P2O is described in the previous work.\textsuperscript{38} Original Marcus theory\textsuperscript{49-51} has been modified in various ways for ET processes.\textsuperscript{52-57} In the present analysis, KM model\textsuperscript{55-57}
was used, because it is applicable both for adiabatic and non-adiabatic ET processes, and has been found to satisfactorily reproduce experimental fluorescence decays and lifetimes of flavoproteins. In P2O the observed fluorescence decays were non-exponential, and expressed with two-exponential decay functions (see Table S2 in Supporting Information). The lifetimes of fast component in the wild type P2O is emission-wavelength (λ) dependent, while that of the slow component is emission-wavelength independent, 358 ps. The mean lifetime of the fast component is 88 fs over all emission wavelengths. The method of ET analysis for P2O are described in the previous work (see Supporting Information).

**Decomposition of logarithmic ET rate into three terms.**

The ln Rate may be decomposed as eq. 1.

\[
\ln k_{ET} = \ln EC + \ln SQRT + GTLAM
\] (1)

Here

\[
\ln EC = \ln \left( \frac{v_0}{1 + \exp\{\beta (R_e - R_o)\}} \right)
\] (2)

\[
\ln SQRT = \ln \left( \frac{k_B T}{4\pi\lambda_s} \right)
\] (3)

\[
GT = \Delta G^0 - e^2 / \varepsilon D_{aR} + \lambda_s + E_{Net}
\] (4)

\[
GTLAM = -\frac{(GT)^2}{4\lambda_s k_B T}
\] (5)

The \(k_{ET}\) is ET rate expressed with KM model. The ET rates for P2O are given by eq. S1 for fast component and eq S4 for the slow component (Supporting Information). \(EC\) is an electronic coupling term. \(SQRT\) denotes a square root term. \(GT\) is total free energy gap, and \(GTLAM\) exponential term, and are dependent on emission wavelength \(j\) in P2O in the fast component of P2O. Among these quantities \(EC\) (\(\ln EC\)) and \(SQRT\) (\(\ln SQRT\)) are
emission-wavelength independent even in P2O. In eq 4 the following abbreviations are used; $SFEG$ for $\Delta G^0$, $ESDA$ for $-e^2/\varepsilon_{DA} R$, $SROE$ for $\lambda_S$, and $NetES$ for $E_{Net}$.

RESULTS

Protein structure of P2O.

The quaternary structure (tetramer) and local structure near FAD are shown in Fig. S1 (Supporting Information). The Trp168 locates near Iso, and closest to Iso among aromatic amino acids. Ionic amino acids, Glu358, Asp452, Lys91 and Arg472, are located near Iso within 1 nm. Mean $Rc$ values between Trp168 and Iso are listed in Table S1 (Supporting Information), which are 0.75 nm in subunit A (Sub A), 0.73 nm in Sub B, 0.76 nm in Sub C and 0.75 nm in Sub D. The distances in aqueous solution obtained by MDS are longer by 0.12 – 0.15 nm than those in crystal structure.

ET rates in P2O.

It was identified that the slow fluorescent component is from Sub A and the fast component with the emission-wavelength dependent lifetimes are from Sub B, Sub C and Sub D (see Table S3 in Supporting Information). Free energies related to electron affinity of Iso* determined in the previous work are listed in Table S4 (Supporting Information), ET parameters in Table S5. Mean ET rates from Trp168 to Iso* in Sub A, Sub B, Sub C and Sub D obtained in the previous work are also listed in Table S6. The ET rates of the fast component were 10 - 12 ps$^{-1}$ at 580 nm, 8 - 10 ps$^{-1}$ at 555 nm and 530 nm, 14 – 15 ps$^{-1}$ at 500 nm, and 17.5 ps$^{-1}$ at 480 nm. In the slow component of Sub A, it was 0.003 ps$^{-1}$.

Relationship between ln Rate and Rc in P2O.

Fig. 1 shows the ln Rate vs Rc relations in Sub B and Sub D of P2O. The relationships for Sub A and Sub C obtained in the previous work are illustrated in Fig. S2 (Supporting Information). At 530 nm of the emission wavelength the ln Rate linearly decreased with Rc.
both in Sub B and Sub D. The slope was -5.4 in Sub B and -5.3 in Sub D. At 480 nm the ln Rate vs Rc function was parabolic, not linear. Approximate function was $Y = -12.98 X^2 + 12.21 X - 4.24$, where Y is ln Rate ($\ln k'^f$) and X Rc. The ln Rate vs Rc relation for Sub A and Sub C are reported in the previous work.\textsuperscript{38} In Sub A the values of ln Rate are expressed with a linear function. In Sub C the values of ln Rate are also approximated with linear functions at 580, 555 and 530 nm. However, they were parabolic at 500 and 480 nm. The coefficients of these approximated functions obtained in the previous work\textsuperscript{38} are listed in Table S7 (Supporting Information).

**Relationship between $GT$ and $Rc$ in P2O.**

Fig. 2 shows dependence of $GT$ on Rc in Sub A (emission-wavelength independent), Sub B and Sub D at 530 nm and 480 nm (the fast components). In all systems $GT$ approximately linearly increased with Rc. Table 1 lists the slopes of the approximated linear functions and the range of $GT$. The slopes were 1.52 in Sub A, 1.59 in Sub B, 1.53 in Sub C and 1.39 in Sub D. The slopes did not vary appreciably with the emission wavelength. The $GT$ varied in the ranges of 1.14 – 1.34 in Sub A. In Sub B the variation ranges in $GT$ were 0.15 - 0.37 at 580 nm, 0.20 – 0.43 at 555 and 530 nm, 0.04 – 0.27 at 500 nm, and -0.15 – 0.08 at 480 nm. In Sub C the variation ranges were 0.23 – 0.43 at 580 nm, 0.20 – 0.29 at 555 nm, 0.28 – 0.48 at 530 nm, 0.12 – 0.32 at 500 nm, and -0.07 – 0.13 at 480 nm. In Sub D the ranges were 0.21 – 0.43 at 580 nm, 0.27 – 0.48 at 555 nm, 0.26 – 0.47 at 530 nm, 0.11 – 0.32 at 500 nm and -0.08 – 0.12 at 480 nm. Mean values of $GT$ over 25000 snapshots are listed in Table S6 (Supporting Information). In the fast component the range of $GT$ (Table 1) and the mean value of $GT$ shifted toward lower values at 500 nm and 480 nm, compared to those at other emission wavelengths (Table S6; Supporting Information). At 480 nm the ranges extended from negative to positive values in all fast subunits, which implies that $GT$ values fluctuate around zero.
ET processes in any subunits in P2O are adiabatic, because the values of $R_0^1$ and $R_0^2$ are 1.30 and 1.21 nm (see Table S5, Supporting Information). Accordingly, the values of $\ln EC$ given by eq. 2 are almost constants (see Fig. S5, Supporting Information). Variations of $\ln SQRT$ given by eq. 3 are shown in Fig. S3 (Supporting Information). Amplitudes of the variation are little and almost constant with time.

Fig. 3 shows relationships between $GTLAM$ and $Rc$ in Sub B and Sub D of P2O. At 530 nm the $GTLAM$s can be expressed by linear functions of $Rc$ both in Sub B and Sub D, while at 480 nm they were approximated with parabolic functions. Behaviour of $Rc$-dependent $GTLAM$ in Sub B and Sub D was similar with Sub C (see Fig. S4 in Supporting Information). Coefficients of the approximate functions of $GTLAM$ against $Rc$ in P2O are given in Table S7. In P2O ET processes are all adiabatic, so that $\ln EC$ terms did not change appreciably with $Rc$ (see Fig. S5 in Supporting Information). The slopes of $\ln Rate$ vs $Rc$ and $GTLAM$ vs $Rc$ functions are similar. In another word behaviour of $\ln Rate$ vs $Rc$ relation in P2O are determined by the $GTLAM$ vs $Rc$ relationships.

**Relationships of GT and GTLAM with Rc in HPFD.**

Protein structure of HPFD near FMN is shown in Fig. S6 (A) (Supporting Information). ET rates and related physical quantities in HPFD are listed in Table S8 (Supporting Information). In HPFD Tyr91 is fastest ET donor to Iso* among aromatic amino acids.\(^{35}\) All physical quantities including the ET rate are taken from Ref 35. Fig. 4A shows a relationship of $\ln EC$ with $Rc$ in HPFD. Variation of $\ln EC$ was little (from 6.365 to 6.395), because ET process of Tyr91 in HPFD is almost adiabatic ($R_0 = 1.14$ nm, while $Rc$ varies from 0.5 to 0.65 nm).\(^{35}\) Variation of $\ln SQRT$ was also little with 0.1 width as shown in Fig. 4B. On the other hand the variation of $GT$ was quite wide from -0.4 to 0.3 eV as shown in Fig. 4C. Fig. 4D shows dependence of $GTLAM$ on $Rc$. The variation range was remarkable from zero to -7. The $\ln Rate$ is obtained as a sum of $\ln EC$, $\ln SQRT$, and $GTLAM$ as in eq. 1. The $\ln Rate$ vs $Rc$
relationship is shown in Fig. S7 (Supporting Information). Marked fluctuation of \(GTLAM\) as in Fig. 4D was due to the great variation of \(GT\) with \(Rc\) as in Fig. 4C.

Here a quantity, \(GP\) is defined by eq. 6.

\[
GP = SROE + ESDA + SFEG
\]  

(6)

Here \(SROE\) is solvent reorganization energy as noted below eq 5 (see also eqs. S2 and S5 for P2O in Supporting Information), \(ESDA\), ES energy between Iso anion and donor cation, \(SFEG\) (eqs. S3 and S6 for P2O in Supporting Information), standard free energy gap.

\[
SROE + ESDA = e^2 \left( \frac{1}{2a_{iso}} + \frac{1}{2a_q} \right) \left( \frac{1}{\varepsilon_{\infty}} - \frac{1}{\varepsilon_{DA}} \right) - \frac{e^2}{\varepsilon_{\infty} R_c}
\]  

(7)

Hence \(GP\) in eq. 6 and \(GT\) in eq. 4 increases linearly with \(Rc\) (more exactly, hyperbolic function of \(Rc\)), because \(SFEG\) term (eqs. S3 or S6 for P2O in Supporting Information) and \(Rc\) in eq. S8 for P2O (Supporting Information) are independent of \(Rc\) in the present model.

Then why \(GT\) displayed remarkable fluctuation with \(Rc\) in HPFD? Fig. 5 shows dependence of each component in \(GT\) on \(Rc\). The \(SROE\) and \(ESDA\) increased with \(Rc\) (Figs. 5A and 5B). This is natural because the both terms vary with \(1/Rc\). In fact \(SROE + ESDA\) increases with \(Rc\) as eq. 7. The \(Rc\)-dependence of \(GP\), which is a sum of \(SROE\), \(ESDA\) and \(SFEG\) \((\Delta G_{Tyr91}^0 = E_{Tyr}^{iso} - G_{iso}^0 = 0.841 \text{ eV}\) as in eq. 7, is shown in Fig. 5C. It is noted that the values of \(GP\) in eq. 6 varies around zero with \(Rc\). The \(NetES\) also vary around zero with \(Rc\) as in Fig. 5D, but the variation amplitude was much greater than that of \(GP\). The \(NetES\) does not have any explicit relation with \(Rc\), so that it markedly fluctuates with \(Rc\). This is the reason why the \(GT\) markedly fluctuates with \(Rc\), since sum of \(GP\) and \(NetES\) is equal to \(GT\) (see eq. 4).

**Relationship between \(GT\) and \(Rc\) in FBPs.**
Protein structures of wild type (WT) FBP, E13K (Glu13 is replaced by Lys) FBP, E13R (Glu13 is replaced by Arg) FBP, E13T (Glu13 is replaced by Thr) FBP and E13Q (Glu13 is replaced by Gln) FBP are shown in Fig. S8 (Supporting Information). In FBPs Trp32, Tyr35 and Trp106 are plausible ET donors. $R_c$s to Iso are ca. 0.66 - 0.75 nm in Trp32, 0.85 - 1.0 nm in Tyr35 and 0.91 – 1.05 nm Trp106. Distances between the amino acid residue-13 and Iso were around 1.5 nm, while between the amino acid residue-13 and Trp32, Tyr35 and Trp106 they were around 1.0 nm, 1.2 nm and 1.8 nm, respectively. Best-fit ET parameters are listed in Table S9 (Supporting Information). ET rates calculated from the best-fit ET parameters are listed in Table S10 (Supporting Information). The ET rates are fastest from Trp32. The rate from Trp32 were 6.11 in WT, 8.65 in E13K, 8.25 in E13R, 6.70 in E13T and 5.02 ps$^{-1}$ in E13Q. Physical quantities of Trp32 as $\text{NetES}$, $\text{SROE}$, $\text{ESDA}$, and $\text{SFEG}$ ($\Delta G_{\text{Trp}}^{\text{ES}}$) are listed in Table S11 (Supporting Information).

Relationship between $GT$ and $R_c$ are shown in Fig. 6. $GT$s increased with $R_c$, which were approximated with linear functions. The coefficients of the linear functions ($Y = A \times X + B$) are listed in Table 2, where $Y$ is $GT$ and $X$ is $R_c$. The values of $A$ were 1.49 in WT, 1.96 in E13K, 2.35 in E13R, 1.75 in E13T and 1.56 in E13Q.

**Relationships of $GTLAM$ with $R_c$ and $\ln$ Rate with $R_c$ in FBPs.**

Dependences of $GTLAM$ on $R_c$ in five FBP isoforms are shown in Fig. 7. In the all isoforms the $GTLAM$ could be approximated with parabolic functions, $Y = A \times X^2 + B \times X + C$, where $Y$ is $GTLAM$ and $X$ is $R_c$. The coefficients, $A$, $B$, and $C$, are listed in Table 2. In Table 2 the coefficients in the parabolic functions of $\ln$ Rate with $R_c$ are also listed for comparison. The coefficient of $A$ in $GTLAM$ vs $R_c$ function was considerably larger than that of $A$ in $\ln$ Rate vs $R_c$ function in the every isoform, whereas the $B$ coefficient in $GTLAM$ vs $R_c$ function was much smaller than the $B$ coefficient in $\ln$ Rate vs $R_c$ function. The result reveals that ET mechanisms in the FBP isoforms are quite different from those in the fast component at 500
nm and 480 nm of the emission wavelength in P2O (see Table S7; Supporting Information), where every coefficient in the parabolic function are similar between those in ln Rate vs Rc function and GTLAM vs Rc function. The difference in the ET mechanisms is ascribed to different dependencies of ln EC term on Rc between FBPs and P2O. Fig. 8 shows relationship between ln EC vs Rc in five FBPs. In the FBP isoforms ln EC term appreciably decreased with Rc, whereas in the fast component at 500 and 480 nm in P2O did not depend on Rc. If eq. 1 is taken into account, ln EC considerably contributes to ln Rate in FBP isoforms.

**Rc-dependencies of ln Rate, GTLAM and GT in DAAO dimer.**

Best-fit ET parameters to calculate ET rates are listed in Table S12 (Supporting Information). Fastest ET rates in DAAO dimer are Tyr224 at 10 °C in Sub A and at 30 °C in Sub B, and Tyr314 at 10 °C in Sub B and at 30 °C in Sub A. Rc-dependencies of ln Rate, GTLAM and GT were examined for Tyr224 and Tyr314 using the MDS snapshots. Fig. 9A, 9B and 9C show the relationships between ln Rate and Rc, between GTLAM and Rc, and between GT and Rc in Tyr224 at 10 °C in Sub A. Red lines indicate approximate linear functions, of which are represented as Y = A X + B, and X is Rc in nm unit, Y is ln Rate in A, GTLAM in B and GT in eV unit in C, as shown in the inserts. In the inserts coefficients of determination for the linear functions (R²) are also indicated. The values of R² should be 1 when the data of Y and X are completely linear, and zero when the data show no linear relation at all. In the all relationships the values of R² were quite high (> 0.6) in Tyr224, which suggests that the linear functions are good approximations. Similar relations for Tyr314 are shown in Fig. 10A, 10B and 10C. Apparently, the linear relations in Tyr314 are poor for the three quantities, ln Rate, GTLAM and GT. In fact the values of R² were all very small (0.18 in ln Rate, 0.0001 in GTLAM and 0.0008 in GT), compared to those in Tyr224. In GTLAM and GT the slopes were almost zero. In the other temperature and subunit the slope
(A value) and the R² values are listed in Table 3. The R² values of GTLAM and GT were all little in Tyr314 at 10 °C in Sub B, at 30 °C in Sub A and Sub B, and also in Tyr224 at 30 °C in Sub A. In these cases the slopes of ln Rate vs Rc functions were all less than -9, while in the other cases the slopes were greater than -14. Especially the slopes in Tyr314 at 10 °C in Sub A and at 30 °C in Sub A were -6.2 and -6.4, respectively, which were very close to -β_Tyr (6.25 nm⁻¹). These results suggest that the contribution of GTLAM to the ln Rate vs Rc relation is negligible when GT (and GTLAM) do not display any linear relation with Rc, so that the slope of the ln Rate vs Rc function should be close to -β_Tyr. Then why GT does not display any linear relation with Rc in some cases? As described at HPFD section (see Fig. 5D), NetES does not show any direct relation with Rc. Accordingly, GT in eq. 4 may not display any linear relation with Rc, when 1) GP in eq. 6 is close to zero, 2) NetES is dominant among four quantities in GT, 3) if NetES displays slight negative slope with Rc, then it may be cancelled with the linear function of GP vs Rc (it always shows a positive slope). In the case of Tyr314 in Sub A (at both 10 °C and 30 °C), GT did not display appreciable Rc-dependence due to the reason 3) above, because the slopes of GP vs Rc functions were 0.56 at 10 °C and 0.60 at 30 °C, while the slopes of NetES vs Rc plots were -0.51 at 10 °C and -0.53 at 30 °C. Accordingly, the slopes of the both approximate linear functions were cancelled out against Rc.

**Classification of the mechanism of ET in flavoproteins from the relationship between the ln Rate and Rc.**

ET mechanism in flavoproteins may be classified from two points of view, A) GT function, and B) NetES. When GT varies with Rc around zero and further NetES is negligible compared to GP, the ln Rate display a parabolic function of Rc. If NetES is not negligible compared to GP and GT varies with Rc around zero, then the ln Rate do not display linear nor parabolic function of Rc, and scattered with Rc. When GT varies with Rc around a positive value, ET
rates becomes much slower than those in the above cases, because $GTLAM$ values are always negative (see eqs. 1 and 5). Accordingly, there should be four classes as follows: 1) $GT$ (eq. 4) varies around zero, and $NetES$ is negligible compared to absolute values of $GP$, 2) $GT$ varies around zero, and $NetES$ is dominant compared to absolute values of $GP$, 3) $GT$ varies around a positive value, and $NetES$ is negligible compared to absolute values of $GP$, and 4) $GT$ varies around a positive value, and $NetES$ is dominant compared to absolute values of $GP$. Table 4 shows criteria of the four categories, and examples in the flavoproteins. In Table 4 adiabatic and non-adiabatic ET processes are separately considered for $\ln \text{Rate vs } Rc$ function, and the slopes are shown when the approximate functions are linear.

DISCUSSION

The donor-acceptor distance has been considered to be most influential parameter upon ET rates. In the present work the individual parameter has been determined by means of the non-linear least squares methods, using MDS snapshots and fluorescence decays or fluorescence lifetimes as experimental data. Now it is possible to ponder on the ET mechanisms in flavoproteins from the ET parameters thus obtained. Maximum ET rates are obtained when the exponential term is zero, because this term always reduces the ET rates due to its negative sign ($GTLAM$, see eq. 5). When $GT$ varies around zero, $GTLAM$ becomes a parabolic function of $Rc$ ($GP \gg NetES$) or no relation with $Rc$ ($GP \ll NetES$). These conditions correspond to Categories 1) and 2), respectively (see Table 4). ET processes classified as Category 1) are found in ET from Trp168 to Iso* in P2O in adiabatic process, and ET from Trp32 to Iso* in FBP isoforms in non-adiabatic processes. The ET from Tyr91 to Iso* in HPFD may be classified as Category 2), because no clear relation was found between $\ln \text{Rate}$ and $Rc$. A peak $Rc$ may be defined as $Rc$ with the peak value of $\ln \text{Rate}$. When $GT$ varies around a certain positive value, ET rates become much slower than those in
Categories 1) and 2). In P2O ET from Trp168 to Iso* in the slow subunit, and in the fast subunits at 530 nm, 555 nm and 580 nm of the emission wavelength in P2O. Here ET processes are adiabatic and classified as Category 3). ET processes from Tyr35 to Iso* in FBP isoforms were mostly non-adiabatic and also classified as Category 3). ET processes from Tyr224 and Tyr314 to Iso* in DAAO dimer were also non-adiabatic, but the ln Rate vs Rc function was much scattered though ln Rate decreased with Rc, because the values of NetES were not negligible compared to GP. ET in this case may be also classified as Category 3), while in ET process from Tyr314 in DAAO dimer the linear relationship between ln Rate and Rc were not clear due to dominant NetES compared to GP which may be classified as Category 4). The slopes of the linear functions in ln Rate vs Rc are determined by the slopes (-Sg in Table 4) of GTLAM vs Rc functions in adiabatic ET processes, while the slopes in ln Rate vs Rc functions may be expressed as sums of the slopes in GTLAM vs Rc function (-Sg) plus -βw (w is Trp or Tyr) in EC term. The linear relations may hold in Categories 3) and 4) in the present model.

In the previous work it was pointed out that KM rate is not valid anymore in the region of Rc where GT is negative, because ET rate decreases with decreasing Rc, despite that the interaction energy between the donor and acceptor should increase with decreasing Rc. It is of interest to discuss more in detail on the negative region of GT in Category 1), where the NetES is negligible compared to GP. Then GT is nearly equal to GP. The condition for the negative GT may be expressed as eq. 8 in KM rate.

\[ \frac{e^2}{\varepsilon_c R_c} + G_{bo}^{0} > e^2 \left( \frac{1}{2a_{bo}} + \frac{1}{2a_q} \right) \left( \frac{1}{\varepsilon_c} - \frac{1}{\varepsilon_{Da}} \right) + E_{ip}^{q} \]  \( \text{(8)} \)

The negative values of GT may be attained when 1) \( R_c \) is quite short, 2) \( G_{bo}^{0} \) (electron affinity of Iso*) is greater than \( E_{ip}^{q} \) (ionization potential of a donor q), 3) \( \varepsilon_{Da} \) is close to \( \varepsilon_c \).
The charge transfer complexes between Trp32 and Iso* in FBP, and between Trp59 or Tyr97 and Iso* were studied by a semi-empirical molecular orbital method. In these systems the charge transfer interactions are considered to take place in GT-negative region. It is also worthy to discuss on a charge transfer complex and ET phenomena between N,N’-dimethylaniline (DMA) and the excited pyrene (Py*) in DMA-(CH2)n-pyrene diads. The fluorescence from the charge transfer complex between DMA and Py* in the diads was observed only in non-polar solvents, and instead a transient absorption band of pyrene anion in polar solvents was observed as a consequence of ET from DMA to the Py*. In the charge transfer complex, the conditions of 1) and 3) may be fulfilled, and accordingly the interaction between DMA and Py* in the charge transfer complex should be quantum chemically dealt. From a classical view the transferred electron from DMA to Py* could take place recombination to the donor cation from pyrene anion, and from the donor to Py* again, which forms a kind of equilibrium state as the charge transfer complex. As Rc increases a potential surface of Iso* could intersect with that of a charge separation state, and so the charge separation could occur at Rc longer than the intersection distance.

CONCLUSION

Not only electronic coupling term but also nuclear term play an important role on the ln Rate vs Rc relations. The ln Rate vs Rc function is linear or parabolic or noisy without any clear relations in ET processes from aromatic amino acids to Iso* in flavoproteins. The behaviour was classified into four Categories with GP and NetES quantities. When GT quantity fluctuates around zero, ET rates are ultrafast (< 1 ps⁻¹). In this case ln Rate vs Rc function becomes a parabolic when GP > NetES (Category 1), no clear function (Category 2) when GP < NetES. When GT fluctuates around a positive values, the ET rate becomes much slower than 1 ps⁻¹. In this case case the ln Rate vs Rc function becomes a linear function when GP <
NetES (Category 3). It is also no clear function when \( GP < NetES \) (Category 4), even though the ET rate is slow.

Reference


530 nm: Sub B

\[ Y = -5.3973X + 6.2794 \]

530 nm: Sub D

\[ Y = -5.2802X + 6.129 \]

480 nm: Sub B

\[ Y = -12.979X^2 + 19.211X - 4.2387 \]
Figure 1  Relationship between ln Rate and Rc in Sub B and Sub D of P2O.

P2O consists of four subunits, Sub A, Sub B, Sub C and Sub D. Among these subunits, Sub B, Sub C and Sub D are the fast subunits with the fluorescence lifetimes of 0.11 ps at 530 nm and 0.057 ps at 480 nm, due to ET from Trp168 to Iso*. ET rates are expressed in unit of ps\(^{-1}\). Inserts indicate approximate functions of ln Rate (Y) and Rc (X). Physical quantities used were taken from Ref 38.
Slow: Sub A

Y = 1.52X + 0.112

Fast: 530 nm Sub B

Y = 1.59X - 0.842

Fast: 480 nm Sub B

Y = 1.59X - 1.19
Figure 2  Dependence of $GT$ on $Rc$ in P2O.

The $GT$ is defined by eq. 4 in text. Fluorescence lifetimes of Sub B are Sub D were 110 fs at 530 nm and 57 fs at 480 nm. Inserts indicate approximate functions of $GT$ ($Y$) and $Rc$ ($X$). Physical quantities used were taken from Ref 38.
530 nm: Sub B

\[ Y = -5.1247X + 3.2055 \]

480 nm: Sub B

\[ Y = -13.42X^2 + 20.13X - 7.5498 \]

530 nm: Sub D

\[ Y = -5.024X + 3.0672 \]
Figure 3  Relationship between GTLAM and Rc in P2O.

GTLAM is defined by eq. 5 in text. Inserts indicate approximate functions of GTLAM (Y) with Rc (X). Physical quantities used were taken from Ref 38.

\[
Y = -9.7738X^2 + 14.441X - 5.3353
\]
Figure 4  Dependence of the physical quantities related to ET rate of Tyr91 on Rc in HPFD.

Rc is distance between Tyr91 and Iso. Panel A shows ln EC vs Rc plot, panel B ln SQRT vs Rc plot, panel C GT vs Rc plot and panel D GTLAM vs Rc plot. Physical quantities used were taken from Ref 35.
Figure 5  Dependence of individual component in $GT$ term on $Rc$ in HPFD.

Panel A indicates the relationship between $SROE$ and $Rc$. Panel B between $ESDA$ and $Rc$, Panel C between $GP$ and $Rc$, and Panel D between $NetES$ and $Rc$. $GP$ is defined by eq. 6 in text. Physical quantities used were taken from Ref 35.
\[ Y = 1.49X - 1.14 \]

\[ Y = 1.96X - 1.35 \]

\[ Y = 2.35X - 1.48 \]
Figure 6  Relationship between $GT$ and $R_c$ of Trp32 in five FBP isoforms.

$GT$ is defined by eq. 4 in text. Inserts indicate approximate function of $GT$ ($Y$) with $R_c$ ($X$).

Physical quantities used were taken from Refs 36 and 37.
Figure 7  Relationship between $GTLAM$ and $Rc$ of Trp32 in five FBP isoforms.

$GTLAM$ is defined by eq. 5 in text. Inserts indicate approximate functions of $GTLAM$ ($Y$) with $Rc$ ($X$). Physical quantities used were taken from Refs 36 and 37.
Figure 8  Relationship between logarithmic $EC$ and $R_c$ in five FBP isoforms.

The logarithmic $EC$ is defined by eq. 2 in text. The ln $EC$ linearly decreases with $R_c$ longer than $R_0$ in all FBP isoforms, which shows ET process non-adiabatic in the region of $R_c$.

Physical quantities used were taken from Refs 36 and 37.
Figure 9  Dependencies of ln Rate, \( GTLAM \) and \( GT \) on \( Rc \) of Tyr224 at 10 °C in Sub A of DAAO dimer.

The ET rates are obtained with KM rate. \( GTLAM \) and \( GT \) are defined by eqs. 5 and 4 in text. Inserts indicate approximate linear functions and determination coefficients (\( R^2 \)). Physical quantities used were taken from Ref. 60.
**A**

\[ Y = -6.19X + 1.94 \]

\[ R^2 = 0.18 \]

**B**

\[ Y = 0.133X - 2.08 \]

\[ R^2 = 0.0001 \]

**C**

\[ Y = 0.047X + 0.53 \]

\[ R^2 = 0.0008 \]
Figure 10  Dependencies of ln Rate, GTLAM and GT on Rc of Tyr314 at 10 °C in Sub A of DAAO dimer.

Inserts indicate approximate linear functions of Y (In Rate in Panel A, GTLAM in Panel B, and GT in Panel C) with X (Rc). R² in the inserts represents determination coefficient.

Physical quantities used are taken from Ref 60.
<table>
<thead>
<tr>
<th>Wave-length (nm)</th>
<th>Sub A</th>
<th>Range</th>
<th>Sub B</th>
<th>Range</th>
<th>Sub C</th>
<th>Range</th>
<th>Sub D</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>580</td>
<td>-</td>
<td>-</td>
<td>1.59</td>
<td>0.15 – 0.37</td>
<td>1.53</td>
<td>0.23 – 0.43</td>
<td>1.39</td>
<td>0.21 – 0.42</td>
</tr>
<tr>
<td>555</td>
<td>-</td>
<td>-</td>
<td>1.59</td>
<td>0.20 – 0.43</td>
<td>1.53</td>
<td>0.20 – 0.29</td>
<td>1.39</td>
<td>0.27 – 0.48</td>
</tr>
<tr>
<td>530</td>
<td>-</td>
<td>-</td>
<td>1.59</td>
<td>0.20 – 0.43</td>
<td>1.53</td>
<td>0.28 – 0.48</td>
<td>1.39</td>
<td>0.26 – 0.47</td>
</tr>
<tr>
<td>500</td>
<td>-</td>
<td>-</td>
<td>1.59</td>
<td>0.04 – 0.27</td>
<td>1.53</td>
<td>0.12 – 0.32</td>
<td>1.39</td>
<td>0.11 – 0.32</td>
</tr>
<tr>
<td>480</td>
<td>-</td>
<td>-</td>
<td>1.59</td>
<td>-0.15 – 0.08</td>
<td>1.53</td>
<td>-0.07 – 0.13</td>
<td>1.39</td>
<td>-0.08 – 0.12</td>
</tr>
<tr>
<td></td>
<td>1.52</td>
<td>1.14 – 1.34</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

a  The linear functions are expressed by $Y = A X + B$, where $Y$ is $GT$ and $X$ is $Rc$. 

Table 1 Coefficients of approximate linear function of $GT$ with $Rc$ and range of $GT$ in P2O.
Table 2  Coefficients of approximate functions of $GT$ and $GTLAM$ with $Rc$ of Trp32 as ET donor in five FBP isoforms$^a$

<table>
<thead>
<tr>
<th>FBP</th>
<th>$GT$</th>
<th>$GTLAM$</th>
<th>ln Rate$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>A</td>
</tr>
<tr>
<td>WT</td>
<td>1.49</td>
<td>-1.14</td>
<td>-108</td>
</tr>
<tr>
<td>E13K</td>
<td>1.96</td>
<td>-1.35</td>
<td>-153</td>
</tr>
<tr>
<td>E13R</td>
<td>2.35</td>
<td>-1.48</td>
<td>-187</td>
</tr>
<tr>
<td>E13T</td>
<td>1.75</td>
<td>-1.18</td>
<td>-118</td>
</tr>
<tr>
<td>E13Q</td>
<td>1.56</td>
<td>-1.06</td>
<td>-82.6</td>
</tr>
</tbody>
</table>

$^a$ Approximate functions for $GT$ was expressed by $Y = AX + B$, where $Y$ is $GT$ and $X$ is $Rc$. Approximated functions for $GTLAM$ and ln Rate are expressed by parabolic functions, $Y = AX^2 + BX + C$, where $Y$ is $GTLAM$ and $X$ is $Rc$.

$^b$ Data were taken from Ref 36.
Table 3  The slope of linear function of physical quantities with $Rc$ in Tyr224 and Tyr314 of DAAO dimer\(^a\).

| Subunit (T / °C) | Quantity | Tyr224 | | Tyr314 | |
|-----------------|----------|--------|---|---|
|                 |          | $A^b$  | $R^{2c}$ | $A^b$  | $R^{2c}$ |
| A (10) In Rate  |          | -17.7  | 0.829 | -6.19  | 0.174 |
| $GTLAM$         |          | -3.79  | 0.631 | 0.133  | 0.0001 |
| $GT$            |          | 1.075  | 0.853 | 0.0465 | 0.0008 |
| B (10) In Rate  |          | -14.2  | 0.806 | -7.61  | 0.478 |
| $GTLAM$         |          | -2.94  | 0.5   | -1.1   | 0.0265 |
| $GT$            |          | 0.704  | 0.541 | 0.234  | 0.0463 |
| A (30) In Rate  |          | -8.85  | 0.297 | -6.38  | 0.278 |
| $GTLAM$         |          | -0.747 | 0.0157| -0.07  | 0.00007|
| $GT$            |          | 0.214  | 0.025 | 0.0686 | 0.0021 |
| B (30) In Rate  |          | -14.36 | 0.797 | -8.64  | 0.496 |
| $GTLAM$         |          | -2.88  | 0.495 | -2.02  | 0.066 |
| $GT$            |          | 0.802  | 0.546 | 0.357  | 0.0998 |

\(^a\) Data of In Rate and $Rc$ were taken from Ref 60.
b Slope in the linear functions of the quantities vs Rc.

c Determination coefficient ($R^2$) obtained as square of Pearson’s correlation coefficient.
Table 4  Classification of the behavior on the relationship between logarithmic ET rate and $Rc^a$

<table>
<thead>
<tr>
<th>In Rate (Lifetime)</th>
<th>Category$^b$</th>
<th>$GP^c$</th>
<th>$GT^d$ vs $Rc$</th>
<th>$GTLAM^e$ vs $Rc$</th>
<th>Adiabatic (Slope)</th>
<th>Example</th>
<th>Non-adiabatic (Slope)</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faster than 1 ps$^{-1}$ (Shorter than 1 ps)</td>
<td>1) » NetES</td>
<td>Linear around zero</td>
<td>parabolic</td>
<td>parabolic</td>
<td>Trp168 of fast subunits at 480 and 500 nm in P2O</td>
<td>Skewed parabolic</td>
<td>Trp32 in five FBP isoforms</td>
<td></td>
</tr>
<tr>
<td>Slowly than 1 ps$^{-1}$ (longer than 1 ps)</td>
<td>2) « NetES</td>
<td>Scattered around zero</td>
<td>Scattered</td>
<td>No relation</td>
<td>Tyr91 in HPFD</td>
<td>Scattered linear ($S_g$)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Slower than 1 ps$^{-1}$ (longer than 1 ps)</td>
<td>3) » NetES</td>
<td>Linear with positive values</td>
<td>Linear ($-S_g$)</td>
<td>Linear ($-S_g$)</td>
<td>Trp168 in Sub A, and fast subunits at 530, 555, and 580 nm of P2O</td>
<td>Linear ($-S_g$-$eta$)</td>
<td>Tyr35 in five FBP isoforms.</td>
<td></td>
</tr>
<tr>
<td>4) « NetES</td>
<td>Scattered linear with positive values</td>
<td>Scattered linear ($-S_g$)</td>
<td>Scattered linear ($-S_g$)</td>
<td>-</td>
<td>Scattered linear ($-S_g$-$eta$)</td>
<td>-</td>
<td>Tyr314 in DAAO dimer</td>
<td></td>
</tr>
</tbody>
</table>

a The behavior of $\ln$ Rate vs $Rc$ relationship was observed with $Rc$ fluctuations of MDS snapshots.

b see text.

c $GP = SROE + ESDA + SFEG$ (eq. 6 in text), where SROE, ESDA, and SFEG are solvent reorganization energy, electrostatic energy between Iso anion and a donor cation, and standard free energy gap.

d $GT$ is given by eq. 4.

e $GTLAM$ is given by eq. 5.