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Reorganization Energy of Protein Electron Transfer Reaction: Study with Structural and Frequency Signature

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A new formalism is developed that enables us to analyze reorganization energy of the protein electron transfer reaction from a classic molecular dynamics trajectory with special emphasis on structural and frequency signature. In particular, energy contributions are divided into those from interactions of donor or acceptor sites with surrounding protein or water environments. This formalism is applied to cytochrome c. The protein environment is found to be soft as the water environment and, therefore, contributes similarly to the reorganization energy.

1. Introduction

Electron transfer mediated by protein molecules is one of the most fundamental processes in chemistry and biology. To understand how protein molecules controls electron transfer, many experimental[1] and theoretical studies have been done.

The reaction rate constant k for protein electron transfer reactions is generally given by[2]

$$k = \frac{2\pi}{\hbar}|T_{DA}|^2 FC \tag{1}$$

where T_{DA} is the electronic factor, or the tunneling matrix element, and FC is the nuclear factor, or the Franck−Condon factor. The latter, which is related to the fluctuations of classic nuclear motions in the thermal equilibrium, is given by[2]

$$FC = \sqrt{\frac{\beta}{4\pi\lambda}}\exp\left(-\beta\frac{(\lambda+\Delta G)^2}{4\lambda}\right) \tag{2}$$

where $\beta = 1/k_B T$ (k_B is the Boltzmann constant and T is absolute temperature), ΔG is the reaction free energy, and λ is the reorganization energy. In this paper, we focus on this key quantity λ. The reorganization energy λ is closely related to the coupling of protein and water nuclear fluctuation with the change of electronic state during electron transfer.

In recent years, a number of studies have attempted to reveal the character of electronuclear coupling by molecular dynamics simulation and subsequent analysis of the trajectory.[3−9] The key microscopic variable for understanding the electron transfer in these studies is the energy difference between the reactant and product electronic states with the same nuclear coordinate. The probability distribution of this energy difference and its autocorrelation function were used for a quantitative analysis of electronuclear coupling.

For electron transfer in proteins, the energy difference comes from the degrees of freedom of the protein as well as from the bulk solvent. However, in these recent papers,[3−9] attention has been paid only to the total energy difference, in which the protein molecule and the surrounding bulk water are treated as a single system. This treatment is inadequate for the ultimate purpose of understanding the mechanism of how the protein molecule performs its biological function. The three-dimensional structure of a protein molecule, which is determined by its amino acid sequence, controls its biological function. The mechanism of the biological function of a protein must therefore be elucidated in terms of its three-dimensional structure. In the case of electron-transfer proteins, this requirement means that one must account for the reorganization energy λ, with consideration of contributions from various aspects of the three-dimensional structure.

An initial attempt toward such a direction has been made by Basu et al.[10,11] in which the reorganization energy λ is broken down into the contribution from each normal mode. The result shows that low frequency modes generally have large contributions to the total reorganization energy. But, at the same time, there are some higher frequency modes with significant contributions. In this treatment, bulk water is treated by a continuum model that enables an analytical estimation of its contribution to the reorganization energy. The result shows that protein and water environments contribute about the same amount.

The model based on the normal-mode analysis has some limitations. Normal-mode analysis assumes that the protein conformational energy is harmonic within the range of thermal fluctuations. At the same time, bulk water is treated as a continuum in this model.[10,11] But, it has been shown that the real energy surface of protein surrounded by water solvent has considerable anharmonicity.[12,13] The energy surface has many local minima, and the thermal fluctuation induces transitions between these minima. Therefore, treatment based on normal-mode analysis must be extended to treat the anharmonic character of protein dynamics in water.

In this sense, molecular dynamics simulation, which was used in recent papers,[3−9] is the natural way to study protein electron transfer. However, for the purpose of understanding the mechanism of the protein electron transfer reaction in terms of the three-dimensional protein structure, total electronuclear coupling should be divided into various contributions, each with specific three-dimensional characteristics. First, such a division should be done between the protein and water molecules. Also, special attention should be paid to donor and acceptor sites because various experiments have been done by changing parameters

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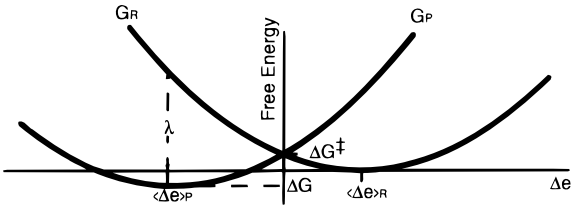

Figure 1. Free energy profile: λ and ΔG are the reorganization energy and the reaction free energy, respectively. We use Δe as the reaction coordinate. The values used for this figure are $\lambda = 1.2$ eV and $\Delta G = -0.2$ eV.

pertaining to the redox sites,[1] such as the metal atom type of the heme or the position of ligation of the ruthenium complex.

In this paper we propose a new analysis that has the benefits of both using molecular dynamics simulation and taking into account the character of the three-dimensional structure of protein. In the next section, we describe the formulation developed for this new analysis, and in subsequent sections, we analyze the result of molecular dynamics simulation with this new formalism.

2. Theory and Formulation

2.1. General Theory. The general theory of the Franck−Condon factor of nonadiabatic electron-transfer reaction has been reformulated.[14−16] The difference between energies of the reactant and product electronic states, U_R and U_P, with the same nuclear coordinate, q, is the key variable and we use this variable as the reaction coordinate:

$$\Delta e(q) = U_P(q) - U_R(q) \tag{3}$$

If the free energy of the system has a quadratic dependence on the reaction coordinate Δe, the Gibbs free energy of reactant and product states, $G_R(\Delta e)$ and $G_P(\Delta e)$, respectively, must have the following form with the same curvature[14]

$$G_R(\Delta e) = \frac{1}{4\lambda}(\Delta e - \Delta G - \lambda)^2 \tag{4}$$

$$G_P(\Delta e) = \frac{1}{4\lambda}(\Delta e - \Delta G + \lambda)^2 + \Delta G \tag{5}$$

The parameter λ in these parabolas has the classic meaning of reorganization energy as is clear from Figure 1. However, as is clear from eqs 4 and 5, λ is also related to the curvature of the parabolas. Therefore, we can define λ as also related to the equilibrium mean-square fluctuation of the reaction coordinate as:[15]

$$\lambda \equiv \frac{1}{2}\beta\langle(\Delta e - \langle\Delta e\rangle)^2\rangle \tag{6}$$

2.2. Calculation of the Energy Difference. The energy difference Δe between the reactant and the product states given by eq 3 is the important microscopic variable. As long as we are interested in the energy difference between the reactant and product states, we can reasonably assume that it comes only from electrostatic interactions. Therefore, we pay attention only to the electrostatic potential terms in the total potential. We can write the potential of the reactant state as

$$U_R = \sum_{i<j} q_i^R\, q_j^R f(\mathbf{r}_i,\mathbf{r}_j) = \sum_{i<j} \frac{1}{4\pi\epsilon}\frac{q_i^R\, q_j^R}{|\mathbf{r}_i - \mathbf{r}_j|} \tag{7}$$

where $\mathbf{r}_i$ is the coordinate vector of atom i and q_i^R is the partial charge of atom i in the reactant state. The function $f(\mathbf{r}_i,\mathbf{r}_j)$ is the coordinate dependence of electrostatic potential and its explicit form is given in the last expression of eq 7. We can also write an equation for the product state in the same way:

$$\Delta e = \sum_{i<j}(q_i^P\, q_j^P - q_i^R\, q_j^R) f(\mathbf{r}_i,\mathbf{r}_j) \tag{8}$$

where q_i^P is the partial charge of atom i in the product state.

The atoms in the system composed of protein and water molecules can be divided into two groups; that is, atoms whose partial charge changes during the reaction and the rest of atoms with no change. For the atoms in the former, we write

$$\Delta q_i = q_i^P - q_i^R \ (i \in \text{changed}) \tag{9}$$

and for the later,

$$q_i = q_i^P = q_i^R \ (i \in \text{unchanged}) \tag{10}$$

Then, we can write the energy difference as follows:

$$\begin{aligned}\Delta e &= \sum_{\substack{i\in\text{unchanged}\\ j\in\text{changed}}} q_i \Delta q_j f(\mathbf{r}_i,\mathbf{r}_j) + \sum_{\substack{i<j\\ i,j\in\text{changed}}} (q_i^R \Delta q_j + q_j^R \Delta q_i + \Delta q_i \Delta q_j) f(\mathbf{r}_i,\mathbf{r}_j) \\ &= \sum_{\substack{i\in\text{unchanged}\\ j\in\text{changed}}} q_i \Delta q_j f(\mathbf{r}_i,\mathbf{r}_j) + \sum_{\substack{i\neq j\\ i,j\in\text{changed}}} \frac{(q_i^P + q_i^R)}{2} \Delta q_j f(\mathbf{r}_i,\mathbf{r}_j)\end{aligned} \tag{11}$$

2.3. Component Analysis. For the purpose of understanding the reorganization energy in terms of clear structural characteristics, we divide the total energy difference into some components. The atoms in the system can be divided into four groups; they are, donor and acceptor sites in protein (D and A, respectively), nonredox site atoms in protein (P), and water atoms as the environment (W). During the electron transfer, the charges of atoms in groups D and A will change, and those of atoms in groups P and W will not change. Thus, each sum in eq 11 can be divided as

$$\sum_{\substack{i\in\text{unchanged}\\ j\in\text{changed}}} = \sum_{i\in P, j\in D} + \sum_{i\in P, j\in A} + \sum_{i\in W, j\in D} + \sum_{i\in W, j\in A} \tag{12}$$

and

$$\sum_{\substack{i\neq j\\ i,j\in\text{changed}}} = \sum_{i\in D, j\in A} + \sum_{i\in A, j\in D} \tag{13}$$

Note that self-energy terms, i.e., interactions between two atoms both in donor or both in acceptor sites, are not included, because they cancel when the second moment from the mean is considered in eq 6. They should be a part of the internal electronic energies of heme and ruthenium complex contributing to ΔG.[7]

In this way we can define five components of Δe as

$$\Delta e = \Delta e_{DP} + \Delta e_{AP} + \Delta e_{DW} + \Delta e_{AW} + \Delta e_{DA} \tag{14}$$

$$\equiv \Delta e' + \Delta e_{DA} \tag{15}$$

Each of the five components is related to certain structural character of protein and water. In particular, the last term, Δe_{DA},

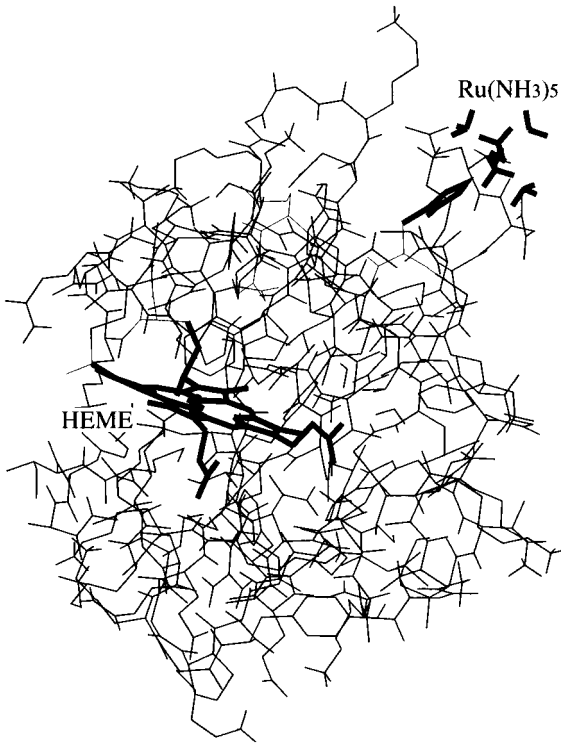

Figure 2. $Ru(NH_3)_5$ (His33)-cytochrome c structure.

stands for the direct interaction between the two redox sites, whereas $\Delta e'$, stands for the interaction of the redox sites with their environments. From the definition of the reorganization energy (eq 6) we need to calculate the fluctuation of the total energy difference. Because we have five components, the reorganization energy is given as a sum of variances and covariances of these component terms.

3. Method of Simulation

We have carried out a molecular dynamics simulation on a system of horse heart cytochrome c modified by the attachment of $Ru(NH_3)_5$ at His33. Related systems have been studied experimentally.[1] This particular system has also been recently studied theoretically.[10,11,16] The initial coordinates for the unmodified protein, along with four bound water molecules, were obtained from X-ray studies by Bushnell et al.[17] We[11] modified the residue His33 by attaching a $Ru(NH_3)_5$ by AMBER geometry and the molecular modeling package Insight II (Biosym Technologies, 1995). Figure 2 shows the prepared structure. Then, additional water molecules were placed around the protein molecule to fill a sphere of 25 Å radius. The total number of explicit water molecules included in the system is 1452. We used partial charges published previously for the reduced heme group.[18] For the oxidized heme group, we[16] prepared a charge set by using charge distribution of reduced and oxidized porphines.[19] The partial charges of the reduced and oxidized Ru complex [Im(His33)-$Ru(NH_3)_3$] were used as reported elsewhere.[20] All other parameters were taken from the AMBER−OPLS potential energy function[21,22] and the TIP3P water model.[23] The potential parameters employed here are exactly the same as those used by Miyashita and Go,[16] and have been deposited as electronic Supporting Information.

We used a newly developed program package that is based on the framework of the minimization/molecular dynamics program PRESTO.[25] Spherical Solvent Boundary Potential (SSBP) without reaction field,[26] and the Nosé Hoover algorithm[27,28] were employed. Long-range interactions were treated with no cutoff saving. We performed the energy minimization with a suitable combination of the steepest descent and the conjugate gradient techniques. Following the minimization, we performed a 40 ps-molecular dynamics simulation to equilibrate the system to 300 K and 1 atm. Then, we performed a 100-ps simulation and, during this simulation, the coordinate trajectory was stored at every 1 fs. The trajectory data were used for subsequent analyses. The minimization and the molecular dynamics simulation were performed with the reduced (Fe^{II}/Ru^{III}) protein charge set. Thus, in the following discussion, we assume the reaction of $Fe^{2+}Ru^{3+} \rightarrow Fe^{3+}Ru^{2+}$ (i.e., the donor is heme and the acceptor is the ruthenium moiety).

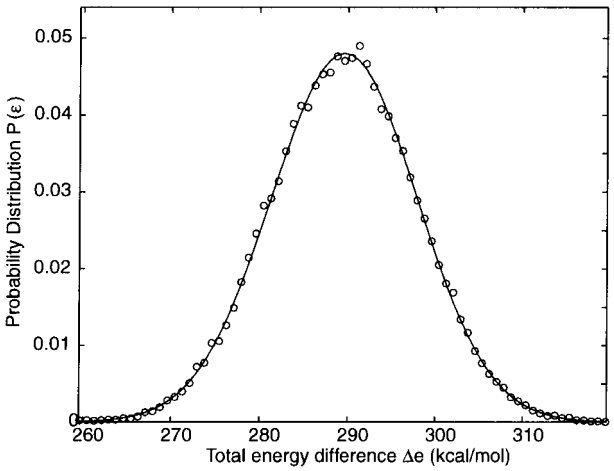

Figure 3. Probability distribution of the total energy difference Δe (the filled circles are results from the simulation; the continuous line is the Gaussian fit).

4. Results and Discussion

4.1. Total Energy Difference. From the distribution of the value of the total energy difference, we can check the validity of the harmonic assumption of the free energy profile embodied in eqs 4 and 5. For this purpose, we first define the probability distribution of energy difference as

$$P(\epsilon) = \langle \delta(\Delta e - \epsilon) \rangle \tag{16}$$

The corresponding diabatic free energy surface can be obtained from $P(\epsilon)$ according to

$$F(\epsilon) = -\beta \ln(P(\epsilon)) \tag{17}$$

If $P(\epsilon)$ is Gaussian, $F(\epsilon)$ is parabolic. In Figure 3 we show the result of probability distribution, which can be fitted very well by a Gaussian curve. This fact indicates the validity of the harmonic approximation embodied in eqs 4 and 5. When the harmonic assumption is valid, the quantity defined by eq 6 as the mean-square fluctuation of Δe has the classic meaning of the reorganization energy. From the distribution of the energy difference, its mean-square fluctuation of Δe is calculated as 68.98 $(kcal/mol)^2$. From the relation of eq 6, the reorganization energy is calculated as $\lambda = 57.87$ kcal/mol.

We note that the 100-ps simulation is long enough to obtain the statistical equilibrium value for the mean-square fluctuation of Δe because the average of the total fluctuation during each of three 33.3-ps trajectories is 68.13 $(kcal/mol)^2$, which is almost same as the one from the whole 100-ps trajectory.

This result is about twice the experimentally observed value, showing that the analysis is quantitatively inadequate. In this

TABLE 1: Variance and Covariance in (kcal/mol)^2 of Fluctuation of Δe_{DA} and $\Delta e'$ in Equation 15[a]

parameter	$\Delta e'$	Δe_{DA}
$\Delta e'$	68.53	−0.18
Δe_{DA}	*−0.024*	0.81

[a] Lower off-diagonal entry in italics is a correlation coefficient.

situation, we first examine possible sources of the inadequacy. From this examination we arrive at the conclusion that the theoretical analysis, regardless of its quantitative inadequacy, is rather robust as to relative importance of various component terms contributing to the reorganization energy. Because of this character of the theoretical analysis, it has reasonable power to discuss the molecular mechanism of the biological function.

The first obvious candidate for the source of the error is the charge parameters of the redox sites. The second candidate would be the rather small (1452) finite number of water molecules surrounding the protein molecule. However, the most serious source of error would be the use of the vacuum dielectric constant of $\epsilon = 1$. Theoretically speaking, the optical dielectric constant ϵ_{op} should be used in the classic molecular dynamics simulation in which all nuclear coordinates are treated as explicit independent variables and no electronic coordinates are treated explicitly. This choice of the independent variables in the classic molecular dynamics simulation assumes implicitly that the electronic wave function relaxes fast enough to follow any changes of nuclear coordinates. Therefore, the electrostatic interactions are partially screened by the relaxation of electronic wave function, which can be accounted for by the use of optical dielectric constant. However, the practically available force field for the molecular dynamics simulation presumes the use of a vacuum dielectric constant, and partial charges are determined under this presumption. Therefore, we are in a sense forced to use the vacuum dielectric constant of $\epsilon = 1$. The calculated value of the reorganization energy is very sensitive to the value of ϵ. If the same trajectory is used, but $\epsilon = \sqrt{2}$ is used just in eq 7, then the value of λ becomes half of the previously obtained value. After all, the uncertainty of the values of dielectric constant and partial charges makes the quantitatively precise evaluation of the reorganization energy very difficult. Fortunately, these main difficulties do not affect the relative importance of various component terms. This specific character endows theoretical reliability for understanding the molecular mechanism of the reaction.

4.2. Direct Term. To estimate the contribution from the direct term Δe_{DA} in eq 15, we calculated the variance and covariance of the direct term Δe_{DA} and the term $\Delta e'$ (interaction with environments). The result is given in Table 1. In this table, the diagonal elements are variances of corresponding terms. The upper off-diagonal is covariance of two corresponding terms and the lower off-diagonal in italics is a correlation coefficient of the two terms.

From the result, it can be seen that the contribution to total reorganization energy from the direct term Δe_{DA}, which is from the direct interaction between donor and acceptor, is small. The reason is simple; only a few atoms are included in this term and donor and acceptor are far apart. This result means that motions involving both donor and acceptor atoms do not have a particularly significant contribution to the total reorganization energy. The term $\Delta e'$, the interaction of redox sites with environments, mainly determines the total energy difference and its fluctuation.

In the study based on normal-mode analysis,[10,11] some modes that involve the direct interaction were found to have some conspicuous values in the reorganization energy spectrum. However, by the new analysis developed in this paper, we reached the conclusion that the direct interactions between the donor and acceptor sites were insignificant. These two conclusions are not contradictory to each other. In the normal-mode analysis, the reorganization energy was divided into contributions from several thousand modes and each term has a small contribution. The mode with the largest contribution turned out to have a significant direct contribution, but its magnitude is still very small compared with the total reorganization energy.

TABLE 2: Variance and Covariance in (kcal/mol)^2 of the Four Terms $\Delta e'$ in Equations 14 and 15[a]

parameter	Δe_{WA}	Δe_{PA}	Δe_{WD}	Δe_{PD}
Δe_{WA}	71.72	−25.28	1.33	−1.17
Δe_{PA}	*−0.498*	35.96	−2.54	1.64
Δe_{WD}	*0.043*	*−0.117*	13.12	−5.84
Δe_{PD}	*−0.041*	*0.081*	*−0.476*	11.48

[a] Lower off-diagonal entries in italics are correlation coefficients.

TABLE 3: Variance and Covariance in (kcal/mol)^2 of the Terms Related to Water, $\Delta e_{WA} + \Delta e_{WD}$, and to Protein, $\Delta e_{PD} + \Delta e_{PD}$[a]

parameter	$\Delta e_{WA} + \Delta e_{WD}$	$\Delta e_{PA} + \Delta e_{PD}$
$\Delta e_{WA} + \Delta e_{WD}$	86.50	−34.84
$\Delta e_{PA} + \Delta e_{PD}$	*−0.523*	50.71

[a] Lower off-diagonal entry in italics is correlation coefficient.

4.3. Variance−Covariance Matrix of the Four Main Terms. The term $\Delta e'$ for interaction of redox sites with environments can be divided into four terms, as in eq 14, and each term has a characteristic structural meaning. We also calculated the variance and covariance matrix, and the result is given in Table 2. From the result in Table 2 we can calculate the variance and covariance matrix for two terms related to protein and to water, $(\Delta e_{PA} + \Delta e_{PD})$ and $(\Delta e_{WA} + \Delta e_{WD})$, respectively. These results are given in Table 3. In these tables, the upper off-diagonals are covariances and the lower off-diagonals in italics are correlation coefficients, as in Table 1.

It can be seen that the variances of the two terms that are related to the acceptor site, Δe_{WA} and Δe_{PA}, are larger than those related to the donor site. In this simulation, the acceptor is the ruthenium complex. The large fluctuation of the terms that are related to the acceptor would be due to the flexibility of the ruthenium complex, which is exposed to water solvent, as shown in Figure 2.

By comparing Δe_{WA} and Δe_{PA}, we see that the fluctuation of the term for interaction of the acceptor with water is larger than that for acceptor interaction with protein. This result would be a natural consequence of the fact that the ruthenium moiety is more surrounded by water environment than protein. The variances of Δe_{WD} and Δe_{PD} are about the same. This result was contrary to our expectation (i.e., Δe_{PD} would be bigger because the donor site is surrounded almost by protein environment). The calculated result should therefore indicate larger structural flexibility of water. The results in Table 3 indicate that about two-thirds of the total fluctuation of Δe, or total reorganization energy, comes from water, and the rest from protein. Summarizing all these results, we see that the protein conformational flexibility as well as the structural flexibility of water is important for understanding the reaction.

Let us discuss the correlation coefficients. There is a strong correlation between Δe_{WA} and Δe_{PA}. On the other hand, the correlations between the two terms related to the donor, Δe_{PD} and Δe_{WD}, and the two terms related to the acceptor, Δe_{PA} and

Δe_{WA}, are small. These results indicate that division of reorganization energy into contributions from protein and water does not have clear meaning. On the other hand, the division into donor and acceptor sites has rather clear meaning.

In the study based on the normal-mode analysis,[10,11] it was assumed that the reorganization energy could be divided into contributions from bulk water and protein. In this new analysis, we can check the correlation between terms related to water and to protein, $(\Delta e_{WD} + \Delta e_{WA})$ and $(\Delta e_{PD} + \Delta e_{PA})$, respectively, from Table 3. The result, -0.52, indicates that the strong correlation between protein and water, and thus the division of the reorganization energy into protein and water contributions, was not appropriate.

4.4. Power Spectrum. Spectral characteristics of the dynamics of fluctuation in energy difference can be obtained from the Fourier transform of the energy difference $\Delta e(t)$

$$\Delta\hat{e}(\omega) = \int_0^T \mathrm{d}t \Delta e(t) e^{i\omega t} \tag{18}$$

and the power spectral density

$$P(\omega) = \frac{2}{T}|\Delta\hat{e}(\omega)|^2 \tag{19}$$

Figure 4a shows the power spectral density of the fluctuation of the total energy difference, Δe, and its cumulative sum. About 60% of the total reorganization energy is due to contributions from the low-frequency region (<150 cm^{-1}). There is a broad contribution from the region of 150 to 1000 cm^{-1} and it represents $\sim$37% of the total reorganization energy. We can see that the contribution from the low-frequency dynamics is dominant in the total reorganization energy.

As we have discussed, it is important to divide the total energy difference into various contributions. It is worth comparing the power spectrum obtained in this new analysis with the reorganization energy spectrum obtained from the normal-mode analysis.[10,11] Because the latter contains only contributions from protein, direct comparison can be done by dividing the total energy difference into a term related to water, $\Delta e_{WA} + \Delta e_{WD}$, and a term related to protein, $\Delta e_{PA} + \Delta e_{PD}$. Power spectra of these terms are shown in Figures 4b and c.

Power spectra related to the protein can be compared with the result of normal-mode analysis (compare Figures 4b and d with Figures 5 and 6 in ref 11). The comparison of these spectra shows that peaks in the two analyses are at the same frequencies. However, the contribution from the low-frequency region is more dominant in our new analysis. Note that comparison of two results of the normal-mode analysis with and without reaction field indicates that the contribution from the low-frequency region is more enhanced in the former treatment (compare Figures 5 and 6 of ref 11). These results indicate that solvent water induces slow and large conformational dynamics of protein.

In the spectrum of fluctuation of the term related to water (Figure 4c), the long tail contribution up to 1000 cm^{-1} is characteristic of water. These contributions are associated with librational motions of water molecules.[29] The difference observed between the power spectrum of the total energy difference (Figure 4a) and the reorganization energy spectrum (Figure 6a in ref 11) in the frequency range 150$-$1000 cm^{-1} is due to the dynamic characteristics of water.

Although our treatment and simulation have been done classically, we may need to discuss some quantum mechanical aspects.[8,9] One possible way to do so is to use the quantum correction of the second moments of the amplitude distributions,

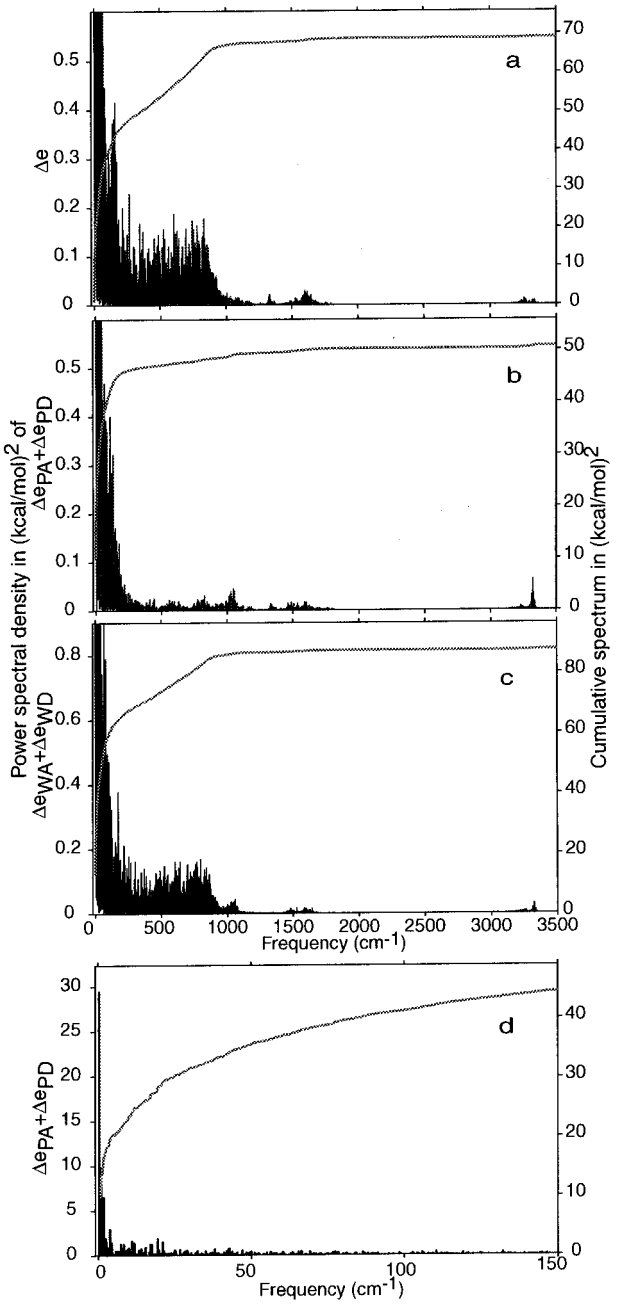

Figure 4. Power spectral density of the energy differences: (a) the power spectral density of the total energy difference Δe and its cumulative sum; (b, d) same pertaining to the energy difference related to protein, $\Delta e_{PD} + \Delta e_{PA}$; (c) same pertaining to the energy difference related to the water $\Delta e_{WD} + \Delta e_{WA}$.

that is;

$$\sigma^2_{\mathrm{quant}}(\omega)/\sigma^2_{\mathrm{class}}(\omega) = \frac{x}{2}\coth\left(\frac{x}{2}\right)$$

where $x = \hbar\omega/k_B T$. Therefore, we estimate the power spectrum with the quantum effect as,

$$P_{\mathrm{qm}}(\omega) = \frac{x}{2}\coth\left(\frac{x}{2}\right)P(\omega)$$

By integrating this power spectrum, the total fluctuation becomes 88.52 (kcal/mol)^2 at 300 K, which is $\sim$30% bigger than the calculation without the quantum effect. Figure 5 shows the difference of two power spectrum functions with and without

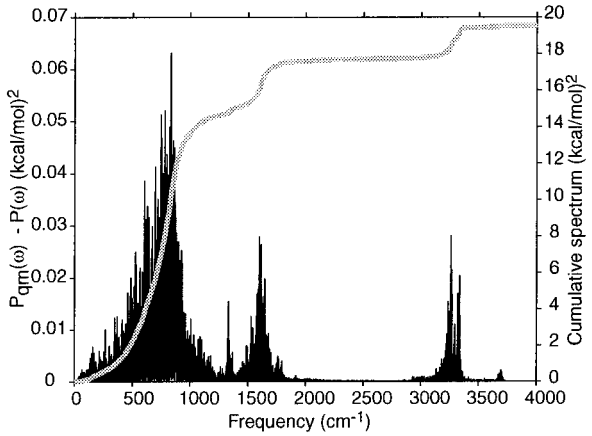

Figure 5. Difference of two power spectrum functions of total energy difference with and without quantum correction, $P_{qm}(\omega)-P(\omega)$, and its cumulative sum.

quantum correction $[P_{qm}(\omega) - P(\omega)]$. As is shown in Figure 5, the main correction of the quantum effect comes from frequency range between 500 and 1000 cm^{-1}. As already discussed, the contribution from this range is mostly due to the water solvent.

5. Conclusion

We developed a new formalism in which a key role is played by the difference (Δe) between energies of reactant and product electronic states with the same nuclear coordinate. The reorganization energy is related to the thermal mean-square fluctuation of this quantity. For the purpose of discussing the mechanism of the reaction in terms of the three-dimensional character of the protein, this energy difference, Δe, is divided into five terms, each of which is related to specific character of the structure. This new formalism allows direct calculation of the reorganization energy from the molecular dynamics trajectory, thus providing a solution of the limitation of the previous treatment based on the normal-mode analysis.

The reorganization energy is given as a sum of variances and covariances of the terms comprising the energy difference Δe. The result of the analysis shows that the component terms of Δe for interactions of redox sites, one with protein and the other with water, have a strong correlation, which indicates that the reorganization energy λ cannot be divided into contributions from protein and water in a simple way. On the other hand, the result indicates a rather clear division between one contribution from the donor site and the other from the acceptor site.

Further analysis indicates the following. The contribution from interactions involving the acceptor (ruthenium complex in this simulation) is larger than that involving the donor (heme moiety). About two-thirds of the total reorganization energy comes from water and the rest from protein. These results indicate that the structural flexibilities of water and the protein molecules are similarly important to understand the reaction. We also discussed spectral characteristics of various contributions to the reorganization energy. Some protein-specific peaks in the spectrum were obtained as in the previous normal-mode analysis.[10,11] However, the contribution from low-frequency dynamics becomes more pronounced in our new analysis in which the molecular dynamics simulation was used and the water molecules were treated explicitly.

The proposed new approach can be easily extended to further analyses. The division can be done, for example, into contributions from residues. Then, we can study the role of each residue in the electron-transfer reaction.

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