Pressure Dependence of Protein Electron Transfer Reactions: Theory and Simulation

Osamu Miyashita and Nobuhiro Go*

Department of Chemistry, Graduate School of Science, Kyoto University, Kyoto 606-8502, Japan

Received: July 17, 1998; In Final Form: September 25, 1998

Pressure dependence of protein electron transfer rate constant is discussed. The rate constant is given as a product of two factors; electronic factor (electron tunneling matrix) and nuclear factor (Franck−Condon factor). Pressure affects the rate through both of these two factors. We analyzed effects of pressure on these two factors individually. The pressure dependence of the electronic factor is discussed by considering the dependence of through-space gaps in the tunneling pathway model. The effect of pressure on the nuclear factor is discussed in terms of the Marcus expression of the Franck−Condon factor and the pressure dependence of the two parameters, reorganization energy λ and reaction free energy ΔG, in it. We show that the dependence of λ is generally smaller than that of ΔG. To obtain a more quantitative picture, we carry out an analysis of pressure dependence of through-space gaps and ΔG by normal mode simulation on cytochrome *c*. In light of the results of these analyses, some experimental results in literature on pressure effects are discussed to identify their relative causes.

1. Introduction

Electron transfer is one of the most fundamental processes both in chemical and biological systems. In the latter the reaction rate constant is controlled by biopolymers, mostly proteins. Many studies have been done to elucidate the molecular mechanism of how protein molecules are involved in the control of the rate constant. Many physicochemical studies have been done especially on electron transfer reactions mediated by metalloproteins.[1]

For the purpose of revealing the molecular mechanism the rate constants have been measured experimentally by varying important parameters in the system, such as temperature, pressure,[2−5] and amino acid sequence of a protein. For the ultimate understanding of the mechanism, it is necessary to develop a theory that bridges the measured rate constants and the protein three-dimensional structures. In this paper we develop such a theoretical treatment on the basis of the three-dimensional structure of protein and apply it to the analysis of the pressure effect on the electron transfer rate constant.

In the cases of protein electron transfer reactions, interaction of the relevant electronic orbitals of the donor and acceptor sites is generally very weak. In this situation (the nonadiabatic limit) the reaction rate constant k is given by[6]

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

where T_{DA} is the electronic factor, or the tunneling matrix element, and FC is the nuclear factor, or the Franck−Condon factor.

We discuss the electronic factor T_{DA} in terms of the tunneling pathway model[7] in this paper. The Franck−Condon factor FC, which is related to classical nuclear motions in the thermal equilibrium state, is given by[6]

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

where λ is the reorganization energy and ΔG is the reaction free energy (see Figure 1). Theoretical analyses of the pressure dependence of these two parameters are developed in this paper.

This paper is structured as follows. Section 2 discusses the pressure dependence of electron transfer in a theoretical way. In section 2.1, we discuss the activation volume, which represents the pressure dependence of reaction rate constant. It is divided into two parts, the dependence of the coupling constant and that of the Franck−Condon factor. The former is discussed in section 2.2. Following three sections are about the pressure dependence of Franck−Condon factor. In section 2.6 we discuss an approximation which allows us to neglect the pressure dependence of λ. As a result of the discussions in these sections, it will be shown that the reaction volume is an important quantity to understand the activation volume and thus we discuss the reaction volume of the protein electron transfer reaction in section 2.7. Section 3 reports the result of simulations by normal mode analysis to understand the two important values in the theory. In section 4, following the above sections, we review some experimental results. Section 5 summarizes this paper.

2. Theory

2.1. Activation Volume. The pressure dependence is often discussed in terms of activation volume defined as

$$\Delta V^{\ddagger} = -k_{\mathrm{B}}T\frac{\partial}{\partial p}\log k \tag{3}$$

and then,

$$\Delta V^{\ddagger} = -\frac{1}{\beta}\frac{\partial}{\partial p}\log|T_{\mathrm{DA}}|^2 - \frac{1}{\beta}\frac{\partial}{\partial p}\log \mathrm{FC} \tag{4}$$

$$\overset{\mathrm{def}}{=} \Delta V^{\ddagger}_{\mathrm{cpl}} + \Delta V^{\ddagger}_{\mathrm{fc}} \tag{5}$$

The first term $\Delta V^{\ddagger}_{\mathrm{cpl}}$ is from the pressure dependence of the coupling constant $|T_{\mathrm{DA}}|^2$. If the high pressure causes a structural

10.1021/jp983064t CCC: $18.00
Published on Web 01/06/1999

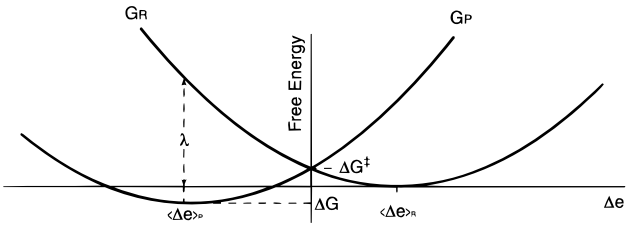

Figure 1. Free energy profile. λ and ΔG, respectively, are the reorganization energy and the reaction free energy. We use Δe as the reaction coordinate as defined in Appendix A. The used values for this figure are $\lambda = 1.2$ eV and $\Delta G = -0.2$ eV.

change, the electron tunneling matrix T_{DA} will be affected. The second term $\Delta V^{\ddagger}_{fc}$ stands for the pressure dependence of the Franck−Condon factor.

By using the relation of eq 2, the second term is given as

$$\Delta V^{\ddagger}_{fc} = \left(\frac{\lambda^2 - \Delta G^2}{4\lambda^2} + \frac{1}{2\beta\lambda}\right)\frac{\partial\lambda}{\partial p} + \frac{\lambda + \Delta G}{2\lambda}\frac{\partial}{\partial p}\Delta G \quad (6)$$

We see that the pressure dependence of the Franck−Condon factor is related to the pressure dependence of the reorganization energy λ and of the reaction free energy ΔG.

2.2. Pressure Dependence of the Coupling Constant. If the application of pressure causes change of protein structure, the electron tunneling matrix will also change. We discuss the pressure dependence of the electron tunneling matrix element based on the electron tunneling pathway model.[7−10] In this model the pressure effect is dominated by through-space gaps. For a case of the electron-tunneling pathway with only one through-space gap, the coupling constant is known empirically as being approximately given by[7]

$$|T_{DA}|^2 = |T^0_{DA}|^2 \exp[-3.4(R - 1.4)] \quad (7)$$

where R is the length of through-space gap in angstroms. When this relation is used, the activation volume $\Delta V^{\ddagger}_{cpl}$ due to the shortening of the gap is given by

$$-\frac{1}{\beta}\frac{\partial}{\partial p}\log|T_{DA}|^2 = \frac{3.4}{\beta}\frac{\partial R}{\partial p} \quad (8)$$

Later in section 3.2 for simulation, we will numerically estimate the magnitude of this term, thereby constructing a quantitative picture of relative importance of various terms for the pressure effect.

2.3. Pressure Dependence of the Franck−Condon Factor. As we saw in section 2.1, $\Delta V^{\ddagger}_{fe}$, the pressure dependence of the Franck−Condon factor, is given by a rather complex expression of eq 6. However, it is possible to show that this quantity $\Delta V^{\ddagger}_{fc}$ is indeed directly related to the volume characteristics of the system. For this purpose we employ the difference of the potential energy Δe between the reactant and product states as the reaction coordinate to describe the phenomenon of electron transfer reaction. This is done in detail in Appendix A.

From a second expression of the Franck−Condon factor given by eq A6, we can readily show that

$$\Delta V^{\ddagger}_{fc} = \frac{\partial}{\partial p}G_R(0) - k_B T\frac{\partial}{\partial p}\log\int d\Delta e\, e^{-\beta G_R(\Delta e)} \quad (9)$$

$$= \langle V\delta(\Delta e)\rangle_R - \langle V\rangle_R \quad (10)$$

where $\langle\ \rangle_R$ indicates an equilibrium average in the state of R. This equation shows that $\Delta V^{\ddagger}_{fc}$ is in fact the difference between the average volume of the system at the intersection region (states where potential energies of the reactant and product states are the same) and that in the equilibrium state.

In this way, the dependence of the Franck−Condon factor is closely related to the volume characteristics of the state in the intersection region. To describe the volume characteristics of the reaction system in a more general state, we consider the average volume of the system for any given value of Δe from eq A1

$$V(\Delta e) = \frac{\partial}{\partial p}G_R(\Delta e) = \frac{\partial}{\partial p}G_P(\Delta e) \quad (11)$$

If we assume the harmonic dependence of the free energy profile on Δe, we have from either eqs A3 or A4,

$$V(\Delta e) = -\frac{(\Delta e - \Delta G)^2 - \lambda^2}{4\lambda^2}\frac{\partial\lambda}{\partial p} - \frac{\Delta e - \Delta G - \lambda}{2\lambda}\frac{\partial}{\partial p}\Delta G + \frac{\partial G_0}{\partial p} \quad (12)$$

Thus, if the free energy profiles are harmonic, the volume profile should also have a quadratic dependence on the reaction coordinate Δe. When we calculate $V(0) - \langle V\rangle_R$ from this profile, we obtain exactly eq 6. Thus the relation of eq 10 is reconfirmed also for the case of harmonic energy profile.

The curvature of the volume profile depends only on $\partial\lambda/\partial p$ but not on $\partial\Delta G/\partial p$, and thus the functional form of the volume profile is determined by the ratio of these two derivatives. We, therefore, discuss relative magnitude of $\partial\lambda/\partial p$ and $\partial\Delta G/\partial p$. We do so by expressing these derivatives as some cumulant averages. From eq A5, the partial derivative of the reorganization energy λ with respect to pressure p is given by

$$\frac{\partial\lambda}{\partial p} = \frac{1}{2}\beta\frac{\partial}{\partial p}\{\langle\Delta e^2\rangle - \langle\Delta e\rangle^2\}$$

$$= -\frac{1}{2}\beta^2\{\langle V\Delta e^2\rangle - \langle V\rangle\langle\Delta e^2\rangle - 2\langle V\Delta e\rangle\langle\Delta e\rangle + 2\langle V\rangle\langle\Delta e\rangle^2\}$$

$$\equiv -\frac{1}{2}\beta^2\langle V\Delta e^2\rangle_c \quad (13)$$

Thus we see that $\partial\lambda/\partial p$ is given as a third-order cumulant quantity. To discuss the other derivative, we use the relation

$$\Delta G = \frac{1}{2}(\langle\Delta e\rangle_R + \langle\Delta e\rangle_P) \quad (14)$$

where $\langle\ \rangle_P$ indicates an equilibrium average in the state of P. This relation can be readily derived from eqs A3 and A4. From this relation, we have

$$\frac{\partial}{\partial p}\Delta G = -\frac{\beta}{2}(\langle V\Delta e\rangle_{R,c} + \langle V\Delta e\rangle_{P,c}) \quad (15)$$

From these equations, we can expect that the pressure dependence of the reaction free energy is larger than that of the reorganization energy, because the dependence of λ is given by a cumulant of higher order than that of ΔG. Therefore, pressure dependence of reorganization energy is expected to be small and the volume profile would have a simple nearly linear form (Figure 2).

At this point, we should also note that the insensitivity of the reorganization energy for various environmental changes has been implicitly assumed in analysis of various experiments.[1]

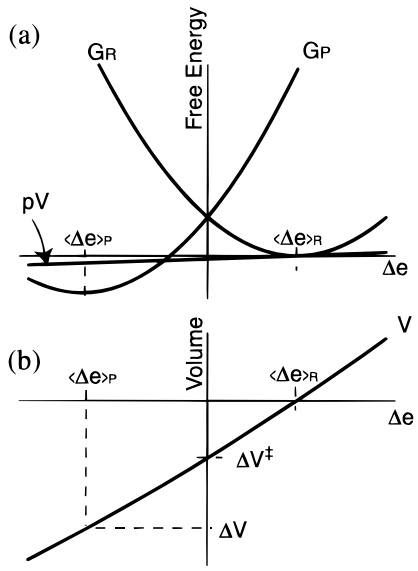

Figure 2. Free energy and volume profiles of a typical case. The curvature of the profile corresponds to the pressure dependence of the reorganization energy $\partial\lambda/\partial p$. If $\partial\lambda/\partial p$ is small enough, the volume profile has a simple nearly linear form. In such a case, the activation volume $\Delta V^{\ddagger}$ is simply related to the reaction volume ΔV. (a) $pV(\Delta e)$ is imposed onto the free energy profile. We used in this figure $\partial\Delta G/\partial p = \Delta V = -40$ cm^3/mol, $\partial\lambda/\partial p = -4$ cm^3/mol, $p = 1000$ atm, and the same values for λ and ΔG as in Figure 1. (b) Volume profile.

For example, it has been assumed that the change of types of ruthenium ligands have an effect on the reaction free energy but not on the reorganization energy.

2.4. Pressure Dependent Hamiltonian: One-Dimensional Case. So far the analysis has been done without referring to three-dimensional structure of a protein. To construct a theory to bridge between three-dimensional structure and reaction rate constant, we treat protein dynamics by the normal mode analysis in the next section. This means that the energy $U_R(q)$ and $U_P(q)$ will be assumed as a quadratic function of many conformational variables within the range of thermal fluctuation in the native state. On the basis of such a model, we will again discuss the relative magnitudes of the two quantities of eqs 13 and 15. In this section, as a preparation for the next section, we treat a one-dimensional case. In other words, we assume that the Hamiltonian in eq A1 to have a one-dimensional quadratic form, which leads exactly to eqs A3 and A4.

We consider one-dimensional harmonic potentials for the reactant and product states:

$$U_\sigma = \frac{1}{2}\omega^2\left(q + \frac{1}{2}\sigma\tilde{q}\right)^2 + \frac{1}{2}\epsilon\sigma \tag{16}$$

where σ is a *c*-number parameter

$$\sigma = \begin{cases} +1 \text{ for reactant state} \\ -1 \text{ for product state} \end{cases} \tag{17}$$

and q, $\tilde{q}$, ω, and ϵ are, respectively, the coordinate, the shift of the energy minimum coordinate, the frequency of the harmonic oscillator, and the reaction energy. It is assumed that the reactant state and the product state have the same frequency. These two potentials can be written in a compact form:

$$U_\sigma = \frac{1}{2}\omega^2 q^2 + \frac{1}{2}\sigma C q + \frac{1}{2}\epsilon\sigma \tag{18}$$

where $C = \omega^2\tilde{q}$.

To discuss the influence of pressure environment, we consider the model potential:

$$U_\sigma = \frac{1}{2}\omega^2 q + \frac{1}{2}\sigma C q + \frac{1}{2}\epsilon\sigma + pV(q) \tag{19}$$

where p is the pressure parameter and $V(q)$ is *the effective volume*. Justification of this expression is given in Appendix B. We assume that the effective volume is a function of coordinate q, in other words, an effective size of a large molecule as a function of the nuclear coordinates. In a practical application, one may assume that $V(q)$ is given by the excluded volume or by the partial molar volume.

We expand $V(q)$ in powers of q and approximate it to the second order,

$$V(q) \sim V_0 + \frac{\partial V}{\partial q}q + \frac{1}{2}\frac{\partial^2 V}{\partial q^2}q^2 \tag{20}$$

Note that this expansion is done not around minimum of U_σ, which is $-\sigma C/2\omega^2$, but around $q = 0$, i.e., the midpoint of the two minima. Then

$$U_\sigma = \frac{1}{2}\omega^2 q^2 + \frac{1}{2}\sigma C q + \frac{1}{2}\epsilon\sigma + pV_0 + pV'q + \frac{1}{2}pV''q^2 \tag{21}$$

$$= \frac{1}{2}(\omega^2 + pV'')q^2 + \left(\frac{1}{2}\sigma C + pV'\right)q + \frac{1}{2}\epsilon\sigma + pV_0 \tag{22}$$

where "′" stands for the partial derivative with respect to pressure p. Then the partition function is given by

$$Z = \int \mathrm{d}q\, \mathrm{e}^{-\beta U_\sigma} = \sqrt{\frac{2\pi}{\beta(\omega^2 + pV'')}}\exp\left[\frac{\beta}{8}\left(\frac{(2pV' + \sigma C)^2}{\omega^2 + pV''} - 4\epsilon\sigma - 8pV_0\right)\right] \tag{23}$$

The free energy is given by

$$G = -\frac{1}{\beta}\log Z = -\frac{(2pV' + \sigma C)^2}{8(\omega^2 + pV'')} + \frac{\epsilon\sigma}{2} + pV_0 - \frac{1}{\beta}\log\sqrt{\frac{2\pi}{\beta(\omega^2 + pV'')}} \tag{24}$$

The average coordinate of each state can be calculated as

$$\bar{q}_\sigma = -\frac{1}{Z}\frac{2}{\beta\sigma}\int \mathrm{d}q \frac{\partial}{\partial C}e^{-\beta U_\sigma} = \frac{2\partial G}{\sigma\partial C}$$

$$= -\frac{2pV' + \sigma C}{2(\omega^2 + pV'')} \tag{25}$$

$$= -\frac{1}{2}\frac{C}{\omega^2 + pV''}\sigma - \frac{pV'}{\omega^2 + pV''} \tag{26}$$

and then the difference of average coordinates of the reactant and the product state is

$$\Delta\bar{q} = \bar{q}_P - \bar{q}_R = \frac{C}{\omega^2 + pV''} \tag{27}$$

The curvature of the effective volume pV'' is added to the curvature of the potential ω^2 and it can affect the dynamics of

the system. On the other hand, V' does not affect to $\Delta\bar{q}$. It only changes each of average coordinates toward the same direction. These relations provide us prototypes of the way system dynamics can be related to structural information carried over into the harmonic model.

In order now to discuss electron transfer rate constant of the system, for which Hamiltonian is given by eq 21, we convert the independent variable from q to $\Delta e = U_P - U_R$ as is done in Appendix A. Thus,

$$\Delta E(q) = -Cq - \epsilon \tag{28}$$

By substituting eq 21 into eq A1, we have exactly eqs A3 and A4, where

$$\Delta G = \frac{CpV'}{\omega^2 + pV''} - \epsilon \tag{29}$$

$$\lambda = \frac{1}{2}\frac{C^2}{\omega^2 + pV''} \tag{30}$$

Then, their partial derivatives are given explicitly by

$$\frac{\partial}{\partial p}\Delta G = \frac{C\omega^2}{(\omega^2 + pV'')^2}V' \tag{31}$$

$$\frac{\partial}{\partial p}\lambda = -\frac{1}{2}\frac{C^2}{(\omega^2 + pV'')^2}V'' \tag{32}$$

When we expand eqs 27, 31 and 32 in terms of p and retain only the lowest order terms, we have the following low-pressure expressions.

$$\Delta\bar{q} = \bar{q}_P - \bar{q}_R = \tilde{q} \tag{33}$$

$$\frac{\partial}{\partial p}\Delta G = \tilde{q}V' \tag{34}$$

$$\frac{\partial}{\partial p}\lambda = -\frac{1}{2}\tilde{q}^2V'' \tag{35}$$

These low-pressure expressions are valid as long as pV'' is negligible compared with ω^2. Numerical normal mode calculation shows that, up to $p \sim 1000$ atm, pV'' is at most a few percent of ω^2.

The pressure dependence of ΔG, which was given by a second order cumulant of eq 15, is now seen to depend on V'. That of λ, reflecting the fact that it is given by a third order cumulant of eq 13, depends only on V'' but not on V'. Thus, by assuming that the second order term in the expansion of eq 20 is negligible in the range of $\tilde{q}$, we can conclude that the pressure dependence of λ is negligible against that of ΔG.

2.5. Pressure Dependent Hamiltonian: Multi Dimensional Case. In this section we treat the realistic case of normal mode Hamiltonian.[11,12] Following eq 21, we consider a quadratic potential which includes the pressure effect

$$U \equiv \frac{1}{2}\omega_i^2 q_i^2 + \frac{1}{2}\sigma C_i q_i + pV^0 + p\frac{\partial V}{\partial q_i}q_i + \frac{1}{2}p\frac{\partial^2 V}{\partial q_i \partial q_j}q_i q_j + \frac{1}{2}\epsilon\sigma \tag{36}$$

where q_i is the coordinate of the ith normal mode, and in this section repeated indices are to be summed in all cases. The set of normal modes is defined as the mode in the absence of pressure effect; if there is no pressure effect, the oscillators are independent of each other. But in the presence of pressure, these normal modes are no longer independent due to the term containing $\partial^2 V/\partial q_i \partial q_j$. The parameter C_i is determined by the relation[11]

$$C_i = -\frac{\partial}{\partial q_i}\Delta E(q) \tag{37}$$

where the difference of the potential energy $\Delta E(q)$ is

$$\Delta E(q) = U_P(q) - U_R(q) = -\frac{\partial U}{\partial(\sigma/2)} = -C_i q_i - \epsilon \tag{38}$$

We rewrite the potential of eq 36 as

$$U = \frac{1}{2}a_{ij}q_i q_j + b_i q_i + pV^0 + \frac{1}{2}\epsilon\sigma \tag{39}$$

where

$$a_{ij} = \omega_i^2\delta_{ij} + p\frac{\partial^2 V}{\partial q_i \partial q_j} \tag{40}$$

$$b_i = \frac{1}{2}\sigma C_i + p\frac{\partial V}{\partial q_i} \tag{41}$$

The partition function of this system is given by

$$Z = \int \mathrm{d}q \exp\left[-\beta\left\{\frac{1}{2}a_{ij}q_i q_j + b_i q_i + pV^0 + \frac{1}{2}\epsilon\sigma\right\}\right] \tag{42}$$

$$= (\det a)^{-1/2}\left(\frac{2\pi}{\beta}\right)^{N/2} \mathrm{e}^{-\beta(pV^0 + 1/2\epsilon\sigma)} \exp\left[\frac{\beta}{2}b_i a_{ij}^{-1} b_j\right] \tag{43}$$

where a_{ij}^{-1} is the ij element of the inverse matrix of a. From this partition function, we obtain the free energy

$$G = -\frac{1}{\beta}\log Z \tag{44}$$

$$= -\frac{1}{2}b_i a_{ij}^{-1} b_j + \frac{1}{2\beta}\log\det a + pV^0 + \frac{1}{2}\sigma\epsilon \tag{45}$$

The reaction free energy and the reorganization energy are given by

$$\Delta G = G|_{\sigma=-1} - G|_{\sigma=1} = pC_i a_{ij}^{-1}\frac{\partial V}{\partial q_j} - \epsilon \tag{46}$$

$$\lambda = \frac{1}{2}\beta\langle\Delta e^2\rangle_c = \frac{1}{2}C_i a_{ij}^{-1} C_j \tag{47}$$

To obtain the latter, we used the following relation

$$\langle\Delta e^2\rangle_c = -\frac{\partial^2 G}{\partial(\sigma/2)^2} \tag{48}$$

Finally, we obtain the pressure dependence of the reaction free energy, which equals the reaction volume:

$$\frac{\partial}{\partial p}\Delta G = C_i a_{ij}^{-1}\frac{\partial V}{\partial q_j} - pC_i a_{ij}^{-1}\frac{\partial^2 V}{\partial q_j \partial q_k}a_{kl}^{-1}\frac{\partial V}{\partial q_l}$$

$$= C_i a_{ij}^{-1}\omega_j^2 a_{jk}^{-1}\frac{\partial V}{\partial q_k} \tag{49}$$

and the dependence of the reorganization energy:

$$\frac{\partial}{\partial p}\lambda = -\frac{1}{2}C_i a_{ij}^{-1}\frac{\partial^2 V}{\partial q_j \partial q_k}a_{kl}^{-1}C_l \tag{50}$$

Here we obtain low-pressure expressions by expanding these expressions in terms of p and retaining the lowest order terms

$$\frac{\partial}{\partial p}\Delta G = \tilde{q}_i\frac{\partial V}{\partial q_i} \tag{51}$$

$$\frac{\partial}{\partial p}\lambda = -\frac{1}{2}\tilde{q}_i\tilde{q}_j\frac{\partial^2 V}{\partial q_i \partial q_j} \tag{52}$$

where $\tilde{q}_i$ is given by C_i/ω_i^2. These equations mean that pressure dependences of ΔG and λ are related to the gradient and curvature, respectively, of V along the direction of q_k. As in the one-dimensional case, we see that the pressure dependence of λ is negligible against that of ΔG, if the second order term in the expansion of V is negligible in the range of $\tilde{q} = (\tilde{q}_i)$.

2.6. Linear Volume Approximation. As will be shown later by numerical calculation, there are some indication suggesting that the second derivative of the system volume is negligible against the linear term. In this case, we arrive at the following relations:

$$\frac{\partial}{\partial p}\Delta G = \Delta V = \sum_i \frac{C_i}{\omega_i^2}\frac{\partial V}{\partial q_i} \tag{53}$$

$$\frac{\partial}{\partial p}\lambda = 0 \tag{54}$$

In this and subsequent sections, we describe simple and clear messages from the above relations. In this approximation the pressure dependence of reaction free energy, or the reaction volume, is represented as a sum of contributions from various normal modes. Later in section 3.1, we will numerically discuss the magnitude of the reaction volume, based on this relation.

This linear volume approximation has the very simple message that the reorganization energy does not depend on pressure. It also means that, in this model, the volume profile of eq 12 is exactly linear on Δe. From eq 6 it follows that the pressure dependence of the Franck−Condon factor $\Delta V_{\mathrm{fc}}^{\ddagger}$, one of the two contributions to the activation volume, is given by a simple relation:

$$\Delta V_{\mathrm{fc}}^{\ddagger} \sim \frac{\lambda + \Delta G}{2\lambda}\Delta V \tag{55}$$

Thus, in this approximation, we can represent $\Delta V_{\mathrm{fc}}^{\ddagger}$ in terms of λ, ΔG, and the new parameter ΔV. Therefore, the reaction volume ΔV is a key quantity to understand $\Delta V_{\mathrm{fc}}^{\ddagger}$.

2.7. Reaction Volume. In this section, we study the make up of the reaction volume ΔV. For an intraprotein electron transfer reaction, we assume as the first approximation that the reaction volume ΔV is given as a sum of the protein part and the solvation part:

$$\Delta \mathrm{V} = \Delta V_{\mathrm{prot}} + \Delta V_{\mathrm{wat}} \tag{56}$$

where ΔV_{prot} and ΔV_{wat} are the volume changes between the reactant and the product states, respectively, of the protein molecule and the bulk water. The protein part results from the changes of intrinsic size of a protein molecule caused by the configurational changes of protein nuclei. The solvation part represents all volume changes associated with electrostriction during the reaction. It can be divided further into two terms, the donor and the accepter parts, each for partial molar volume change caused by changes, respectively, of the donor site and of the accepter site.

$$\Delta V_{\mathrm{wat}} = \Delta V_{\mathrm{D,wat}} + \Delta V_{\mathrm{A,wat}} \tag{57}$$

Division of the protein part is not so simple. A protein molecule has many various oscillating modes. We classify them into three parts. (1) Low-frequency modes which invariably involve collective global motions; they couple to both donor and accepter sites. (2) High-frequency modes localized at the donor site; they can be affected by changes of the donor site, but not by changes of the accepter site. We call them the donor site modes. (3) High-frequency modes localized at the accepter site; we call them the accepter site modes. According to the above classification, we divide the protein part of the reaction volume into three parts,

$$\Delta V_{\mathrm{prot}} = \underbrace{\Delta V_{\mathrm{D,prot}} + \Delta V_{\mathrm{A,prot}}}_{\text{high-frequency modes}} + \underbrace{\Delta V_{\mathrm{DA,prot}}}_{\text{low-frequency modes}} \tag{58}$$

After all, the reaction volume ΔV can be considered as a sum of five types of contributions

$$\Delta V = \Delta V_{\mathrm{DA,prot}} + \Delta V_{\mathrm{D,prot}} + \Delta V_{\mathrm{A,prot}} + \Delta V_{\mathrm{D,wat}} + \Delta V_{\mathrm{A,wat}} \tag{59}$$

This equation is in a sense our proposal as to how we understand the reaction volume. We are proposing to understand the reaction volume by understanding each of the five types of partial molar volume change for charge change of either of the redox sites.

On the other hand, experimental approach to understand the reaction volume is done by changing some controllable parameters in a system of protein electron transfer reaction, such as metal atom type of the heme and position of an engineered redox site (such as liganded ruthenium complex), and by measuring ΔV as functions of such controllable parameters. The five types of partial molar volume change generally in a complex way for changes of such controllable parameters, the situation making understanding the reaction volume difficult. It is therefore important to design a prudent experiment in such a way that only a selected types of partial molar volume terms change for changes of chosen controllable parameters.

At this point, we should note that we have done the division of the reaction volume ΔV into five terms in eq 59 but not of the activation volume $\Delta V^{\ddagger}$. Each of the five terms of ΔV has a clear physical meaning. For example, $\Delta V_{\mathrm{D,wat}}$ is determined by the local characteristics of water and donor site and this value would not change even if we change other conditions such as acceptor site. However, $\Delta V^{\ddagger}$is given by eq 55, which means that also λ and ΔG affect the value of $\Delta V^{\ddagger}$. Thus, each term in $\Delta V^{\ddagger}$ is related not only to relevant local characteristics but also to the characteristics of the whole system through the dependence of λ and ΔG. Thus, the division of $\Delta V^{\ddagger}$ has no clear meaning as ΔV does.

Characteristics of ΔV that it is divisible into five types of terms further imply that value of each term may be transfered to that of a corresponding term in a different chemical system of electron transfer, as long as a relevant part for the term is invariant. This concept of transferability of components of ΔV can be a key in analysis of the experimental data.

3. Simulation by Normal Mode Analysis: Cytochrome *c*

So far we have developed a theoretical framework to understand the phenomenon of pressure effect on protein

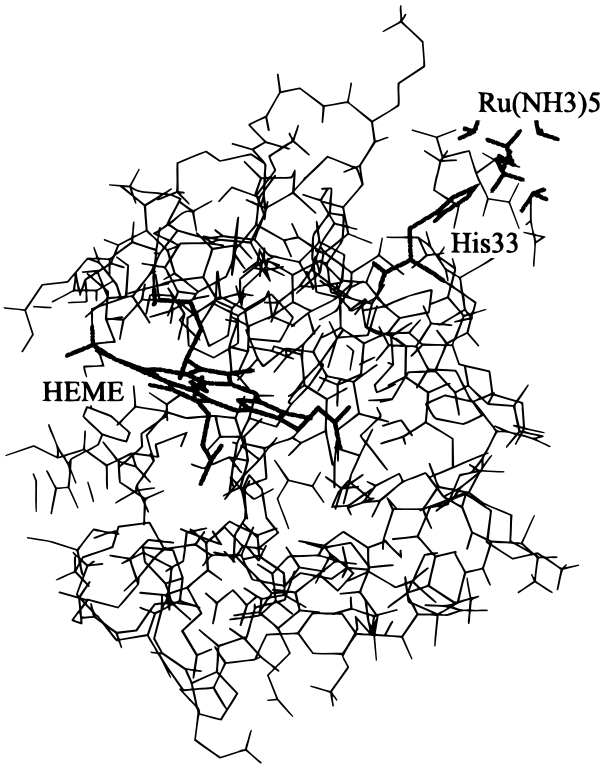

Figure 3. $Ru(NH_3)_5$(His33)−cytochrome *c*.

electron transfer. However, it is not easy to have a quantitative picture of the phenomenon only from the framework. The methods of computer simulation of conformational dynamics of proteins have now been developed powerful enough to provide us useful and reliable qualitative picture to some function-related properties.

In this section we carry out a conformational dynamics simulation, based on the normal mode picture on a system of horse heart cytochrome *c* modified by the attachment of $Ru(NH_3)_5$ at His33. The related systems have been studied experimentally.[2,3,13,14] This particular system has been recently studied theoretically.[11,12] The initial coordinates for the unmodified protein, along with four bound water molecules, were obtained from X-ray studies by Bushnell et al.[15] We modified[12] the residue His33 by attaching of a $Ru(NH_3)_5$ using AMBER geometry and the molecular modeling package Insight II (Biosym Technologies, 1995). Figure 3 shows the prepared structure. We used previously published partial charges for the reduced heme group.[16] For the oxidized heme group, we prepared a charge set with information of charge distribution of reduced and oxidized porphines.[17] The partial charges of the reduced and oxidized Ru complex [Im(His33)−$Ru(NH_3)_3$] were used as reported elsewhere.[18] All other parameters were taken from AMBER-OPLS potential energy function.[19,20] The potential parameters employed here have been deposited as electronic Supporting Information.

We performed the energy minimization by the package PRESTO[21] with a suitable combination of steepest descent and conjugate gradient techniques, and then we performed the normal mode analysis.[12] The minimization and the normal mode analysis were performed with the reduced (Fe^{II}/Ru^{III}) protein charge set.

3.1. Estimation of the Pressure Dependence of Reaction Free Energy. In this section, we discuss numerically the protein part of the reaction volume. To do so, we use eq 53. In this relation, there are two sets of parameters, C_i and $\partial V/\partial q_i$, and to estimate these values, normal mode analysis can be used. The coefficients C_i can be calculated from the relation eq 37 (NMRES model).[11] To consider the volume dependences, we defined the effective volume $V(q)$ as the excluded volume, and then the differentials $\partial V/\partial q_i$ can be estimated by a numerical method.[22−24] When we estimated $^1/_2 q_i^2 \partial^2 V/\partial q_i^2$ (a part of eq 52) as well as $\tilde{q}_i \partial V/\partial q_i$ (a part of eq 51) numerically for all i, we found the former is always less than 4% of the latter. This suggests that the second derivative of the system volume is negligible against the linear term.

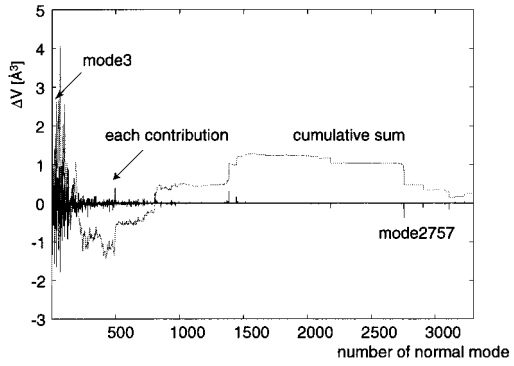

Figure 4. Reaction volume of $Ru(NH_3)_5$(His33)−cytochrome *c*. Contributions from low-frequency modes (shown by bars) are individually large in absolute values, but with both positive and negative signs, causing their cumulative sum (shown by a dotted line) to stay small. On the other hand, there are a few high-frequency modes with some contributions to the reaction volume.

Figure 4 shows the result of simulation. As can be expected from eq 53, the contributions of low-frequency modes are individually large. However, their cumulative sum is still small, because each contribution does not have a uniform sign. This result should be compared with calculation of the reorganization energy λ,[11,12] in which the signs of the contributions from normal modes are uniform, and thus the contributions from low-frequency modes are dominant and high-frequency modes have no significant contributions to a total parameter. In other words, only low-frequency modes determine these characteristics of protein. However, the reaction volume cannot be determined only by global motions of protein for the case of protein electron transfer. Figure 5a shows an example of such a mode of global motion.

On the other hand, there are some contributions from a few higher frequency modes. The absolute values of these contributions are not particularly large, but look significant, because the cumulative contribution from low-frequency modes happens to be very small. The high-frequency modes with some contributions involve motions around the ruthenium moiety. Figure 5b shows an example of mode of such a local motion. This result implies that the local environment of ruthenium site affects the reaction volume.

3.2. Estimation of The Pressure Dependence of Coupling Constant. We have done a simulation to estimate the pressure dependence of coupling constant or $\Delta V_{cpl}^{\ddagger}$. To do so, we simulate structural change of protein under a pressure environment by normal mode analysis,[23,24] and then estimate the effect of structural changes on the coupling constant.

If we ignore the second order of volume expansion, the shift of each normal mode coordinate under the pressure p is given by[23,24]

$$\Delta q_i(p) = -\frac{p}{\omega_i^2}\frac{\partial V}{\partial q_i} \tag{60}$$

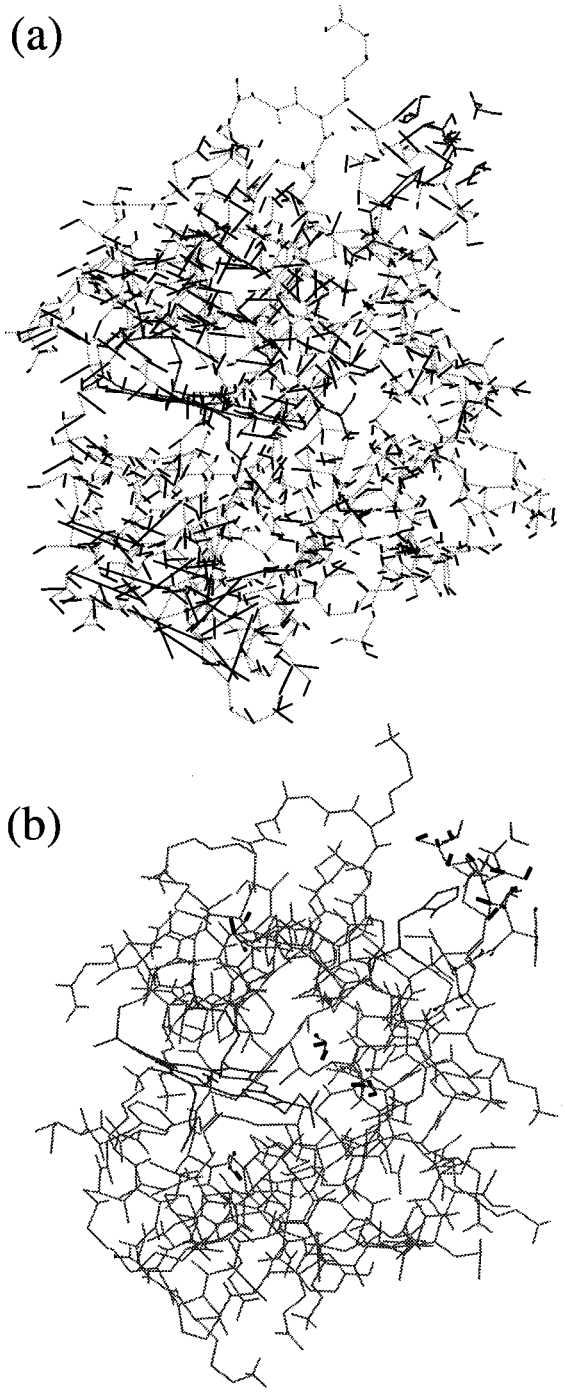

Figure 5. Examples of normal modes. (a) Mode 3. A low-frequency mode, involving a global motion which couples to both donor and acceptor sites. (b) Mode 2757. A high-frequency mode, which has some contributions to the total reaction volume. This mode involves a local motion around the ruthenium site.

This relation is the multidimensional case of eq 26. With this relation, we can estimate structural change of protein under a pressure environment,[23,24] and then, as we saw in section 2.2, we can estimate the pressure dependence of coupling constant through the changes of the structure.

Figure 6 shows the original structure of cytochrome *c* and the changed structure affected by 1000 atm pressure. It can be seen that the structural changes caused by exerting 1000 atm pressure are very slight, but through-space gaps in the tunneling pathways do shrink. In the pathway from $Ru(NH_3)_5$(His33) to HEME, there is one through-space gap[2] (P30 to H18) and it shrinks from 1.720 to 1.709 Å. From eq 8, this shrinkage

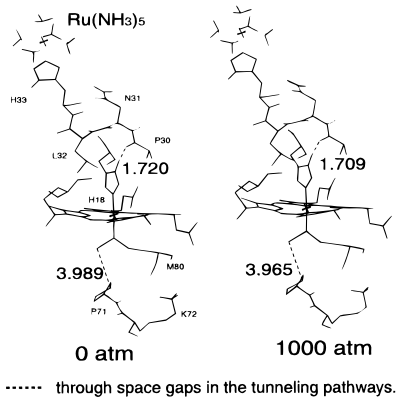

Figure 6. Structural changes caused by applied 1000 atm pressure. The changes are very small, but through-space gaps in the tunneling pathways do shrink. The gap in the pathways His33 to HEME shrinks from 1.720 to 1.709 Å and that of His72 to HEME shrinks from 3.989 to 3.965 Å. Each shortening corresponds, respectively, to −0.91 and −1.99 cm^3/mol of the activation volume.

corresponds to −0.91 cm^3/mol of the activation volume. In the pathway of the electron transfer reaction of $Ru(NH_3)_5$(His72), there is also one through-space gap (P71 to M80).[2] The shrinkage of this gap was from 3.989 to 3.965 Å, which corresponds to −1.99 cm^3/mol of activation volume. As a result, there is a contribution from gap shortening, but it is only a small part of usually observable $\Delta V^{\ddagger}$. Therefore $\Delta V^{\ddagger}_{fc}$ should usually be a dominant part. Only when $\Delta V^{\ddagger}_{fc}$ vanishes, $\Delta V^{\ddagger}_{cpl}$ becomes dominant (see section 4).

4. Review of Experiments

Until now, we have discussed the pressure dependence of protein electron transfer reaction. In these discussions, it has been shown that the activation volume is a sum of two contributions arising from dependence of the coupling constant and that of the Franck−Condon factor. Following these theoretical discussions, we review in this section various experimental studies to form a quantitative picture regarding the two contributions. Such a picture is applied to reveal the relative causes of the two contributions in each reaction condition.

The results of early studies on ruthenium-modified cytochrome *c* are listed in Table 1, reactions 1−5. In these reactions magnitude of activation volume is in the range of 10−20 cm^3/mol. Contributions from pressure dependence of the coupling constant $\Delta V^{\ddagger}_{cpl}$ are found in section 3.2 to be in the range of 1−2 cm^3/mol, and therefore are only a small part of $\Delta V^{\ddagger}$. By neglecting the latter in these cases, we obtain from eqs 5 and 55 a simple relation

$$\Delta V^{\ddagger} = \frac{\lambda + \Delta G}{2\lambda}\Delta V \tag{61}$$

The obtained equation can now be applied to further analysis of reaction 1, for which data[1] of that $\lambda = 1.2$ eV and $\Delta G = -0.18$ eV are available. From $\Delta V^{\ddagger} = -17.7$ cm^3/mol and eq 61, we have the value of reaction volume $\Delta V = -42$ cm^3/mol. As indicated in eq 56, this quantity should be a sum of ΔV_{prot} and ΔV_{wat}. If we use the value of $\Delta V_{wat} \sim -30$ cm^3/mol obtained from reaction 10, we are led to the value of $\Delta V_{prot} \sim -10$ cm^3/mol. However, this value should not be taken too literally but should be understood to indicate that ΔV_{prot} is smaller in magnitude than ΔV_{wat}.

M. Meier and co-workers[2] have investigated the intramolecular electron transfer reactions of $(NH_3)_5$Ru(His33)Zn−cyt

TABLE 1: Intra- and Intermolecular Electron Transfer Reactions

	reaction	$\Delta V^{\ddagger}$ (cm^3/mol)	Ru	λ(eV)	$-\Delta G$(eV)
1	$(NH_3)_5Ru^{2+}$(His33) → Fe^{3+}	-17.7 ± 0.9^b	II → III	1.2[a]	0.18[a]
2	$(NH_3)_5Ru^{2+}$(His39) → Fe^{3+}	-18.3 ± 0.7^b	II → III		
3	$Ru(NH_3)_6^{2+} \xrightarrow{inter} Fe^{3+}$−cyt *c*	-15.6 ± 0.6^b	II → III		
4	$Ru(NH_3)_5isn^{2+} \xrightarrow{inter} Fe^{3+}$−cyt *c*	-17.2 ± 1.5^c	II → III		
5	the reverse process of above	$+16.0 \pm 1.5^c$	III → II		
6	ZnP* → $(NH_3)_5Ru^{3+}$(His33)	nearly 0[d]	III → II	1.10[a]	0.7[a]
7	$(NH_3)_5Ru^{2+}$(His33) → ZnP^+	-12 ± 1^d	II → III	1.19[a]	1.01[a]
8	Fe^{2+} → $(bpy)_2(im)Ru^{3+}$(His33)	nearly 0[d]	III → II		0.74[a]
9	Fe^{2+} → $(bpy)_2(im)Ru^{3+}$(His72)	-6 ± 2^d	III → II		0.74[a]
		ΔV (cm^{3}mol)			
10	$Ru(NH_3)_6^{2+}$ → $Ru(NH_3)_6^{3+}$	-28.7 ± 0.8^d	II → III		

[a] Reference 1. [b] Reference 3. [c] Reference 14. [d] Reference 2.

c at ambient pressure, reaction 6 and 7 of Table 1. In the reaction ZnP* → $(NH_3)_5Ru^{3+}$(His33), reaction 6, the activation volume is nearly 0. From this fact Meier suggested on the basis of assuming that $\Delta V^{\ddagger}$ is somehow proportional to ΔV that the reaction volume ΔV should also be nearly vanishing. However, the proportional relation of eq 61 was derived by neglecting another contribution of $\Delta V^{\ddagger}_{cpl}$. When $\Delta V^{\ddagger}$ itself is small, $\Delta V^{\ddagger}_{cpl}$ is no longer negligible. We have to consider both of the two factors, i.e.,

$$\Delta V^{\ddagger} = \Delta V^{\ddagger}_{cpl} + \Delta V^{\ddagger}_{fc}(\text{small}) \tag{62}$$

If we use the result of simulation, $\Delta V^{\ddagger}_{cpl} = -0.91$ cm^3/mol and $\Delta V^{\ddagger} \sim 0$, then we have $\Delta V^{\ddagger}_{fc} \sim 1$ cm^3/mol. By substituting this value together with $\lambda = 1.10$ eV and $\Delta G = -0.7$ eV known for this reaction,[1] we have from eq 55 $\Delta V \sim 5.0$ cm^3/mol. These results, however, should be understood only to indicate the order of magnitude.

When we use eq 61 to an analysis of reaction 7, we obtain $\Delta V \sim 150$ cm^3/mol, an unacceptably large value. Such a large value is obtained because $\lambda = 1.19$ eV and $\Delta G = -1.01$ eV nearly cancel each other in the coefficient of eq 61. We have not been able to resolve the difficulty caused by this unacceptable value.

Meier and co-workers also examined[2] the reactions of Ru(bpy)$_2$(im)-modified His33 and His72 cytochrome *c* (bpy = 2,2′-bipyridine; im = imidazole), reactions 8 and 9. These reactions are reported as activationless[2] ($-\Delta G \sim \lambda$), and in addition, the reaction volume will be small since the ruthenium complexes contain the larger molecules bpy and im. In such a case, we see from eq 55 that the pressure dependence of Franck−Condon factor $\Delta V^{\ddagger}_{fc}$ will be smaller than the usual cases. As a result, the pressure dependence of the coupling constant should turn out to have a significant contribution to the activation volume.

To explain the result of the small negative $\Delta V^{\ddagger}$ for His72, Meier and co-workers have suggested that it is a result of an increase of electronic coupling through structural changes due to the pressure environment (not mentioned for the reaction of His33). Our simulation in section 3.2 is in fact a demonstration this hypothesis. Though their hypothesis agrees with our analysis, the results of simulation conflict with their conclusions. In both of the two reactions there are contributions for $\Delta V^{\ddagger}_{cpl}$, but the estimated $\Delta V^{\ddagger}_{cpl}$ for His72 is smaller than the experimental $\Delta V^{\ddagger}$. It means that we cannot neglect the pressure dependence of the Franck−Condon factor even though it may be small. Existence of such small contributions from $\Delta V^{\ddagger}_{fc}$ suggests that the condition of activationless reaction ($-\Delta G \sim \lambda$) is not satisfied precisely.

Let us summarize our discussion. The activation volume can be divided into two terms, $\Delta V^{\ddagger}_{cpl}$ and $\Delta V^{\ddagger}_{fc}$. In usual cases, $\Delta V^{\ddagger}_{fc}$ is the dominant part and we can estimate this value with three quantities, ΔV, λ, and ΔG by eq 61. In two cases of an activationless limit $\lambda \sim -\Delta G$ and no reaction volume limit $\Delta V \sim 0$, the pressure dependence of the Franck−Condon factor becomes small, and thus the pressure dependence of coupling constant $\Delta V^{\ddagger}_{cpl}$ can be a major part. The activation volume in such reactions should be small and negative.

One of the consequence of eq 61 is that in the Marcus' inverted region,[6] $-\Delta G > \lambda$, $\Delta V^{\ddagger}$ should have an opposite sign from ΔV. This curious phenomenon is one of the prediction from our analysis.

The first step toward elucidation of the mechanism of pressure effect is to measure the three quantities, ΔV, λ, and ΔG. In either of the two cases of $\lambda \sim -\Delta G$ and $\Delta V \sim 0$, another quantity $\Delta V^{\ddagger}_{cpl}$, should also be considered. In Table 1, we see that, in many cases, values of these quantities are not available. These missing quantities must be measured for further clarification of the mechanism of reactions.

Once experimental data for reaction volume ΔV become available for various reaction systems, then it should be understood in the way of eq 59. By doing so, the transferability of components of ΔV among various reaction systems may be achieved, thus enabling us to construct an overall view of the phenomenon of protein electron transfer reaction.

5. Summary

The aim of this paper is to provide a comprehensive framework for understanding the pressure dependence of protein electron transfer reaction. The rate constant of protein electron transfer reaction is determined by various characteristics of the reaction system, and since the rate constant is given by a product of the coupling constant and the Franck−Condon factor, the effects of changing the system appear to each of the two factors. Thus, we can understand the pressure dependence by studying how both of the two factors are affected by pressure environment. Thus we have divided the activation volume into two parts, $\Delta V^{\ddagger}_{cpl}$ and $\Delta V^{\ddagger}_{fc}$, and then analyzed each of them.

We used the pathway model to discuss $\Delta V^{\ddagger}_{cpl}$ and considered the pressure dependence of coupling constant as the pressure effect on the through-space gaps. We demonstrated this gap shortening effect by a simulation. The effects do exist, but the contribution to the activation volume is small. Thus, only for cases in which the other term, $\Delta V^{\ddagger}_{fc}$, happens to be small, this shortening effect manifest itself significantly in the total rate constant.

We have performed a detailed analysis of the pressure dependence of the Franck−Condon factor, $\Delta V^{\ddagger}_{fc}$. Since the Franck−Condon factor is given in terms of λ and ΔG, its pressure dependence should appears through the dependence of each of two factors. However, our analysis indicates that the pressure dependence of λ is generally small. By neglecting this dependence, we obtain eq 55 in which ΔV is the reaction volume of the system. Unlike $\Delta V^{\ddagger}_{fc}$, ΔV can be expressed as a sum of several terms, each of which reflects chemical characteristics of various sites.

We can understand results of various reactions in experimental studies in this framework. In usual cases, $\Delta V^{\ddagger}_{fc}$ is the dominant part. However, in two limiting cases ($\Delta V \sim 0$ or $\lambda \sim -\Delta G$), this part becomes small and the effect of $\Delta V^{\ddagger}_{cpl}$ becomes significant in the total activation volume $\Delta V^{\ddagger}$.

Acknowledgment. The authors thank H. Kashiwagi for providing the charge set of porphine, S.-H. Chong for helpful discussion on the electron transfer theory, I. Morishima and Y. Furukawa for discussion about experimental studies, and G. Basu and A. Kitao for simulation. This study has been supported by Grants-in Aid to N.G. from Ministry of Education, Japan. Computation has been done in Computer Centers in Kyoto University, in the Institute for Molecular Science, and in the Japan Atomic Energy Research Institute.

Appendix A. Free Energy Profile and the Marcus Relation

The general theory of the Franck−Condon factor of nonadiabatic electron transfer reaction is reformulated[6,25,26] so as to be referred to in the text. To discuss the pressure dependence, we consider the system under the PT-constant environment. The Gibbs free energy of reactant and product states, $G_R(\Delta e)$ and $G_P(\Delta e)$, respectively, are defined as

$$G_{R(P)}(\Delta e) = -k_B T \log \int \mathrm{d}V \int_V \mathrm{d}q \; e^{-\beta(U_{R(P)}+pV)} \delta(\Delta e - \Delta E(q)) \quad \text{(A1)}$$

where $\Delta E(q) = U_P(q) - U_R(q)$ and this parameter has the role of the reaction coordinate. In this definition, the coordinates q pertain both to protein and all water molecules, and therefore in our discussion, on the basis of the volume defined by eq 11, the effects of pressure on hydrophobic interactions, are automatically included.

There is an exact relation[25]

$$G_P(\Delta e) - G_R(\Delta e) = \Delta e \quad \text{(A2)}$$

If the free energy of the system has a quadratic dependence on the reaction coordinate Δe, then $G_R(\Delta e)$ and $G_P(\Delta e)$ must have the following form with the same curvature

$$G_R(\Delta e) = \frac{1}{4\lambda}(\Delta e - \Delta G - \lambda)^2 + G_0 \quad \text{(A3)}$$

$$G_P(\Delta e) = \frac{1}{4\lambda}(\Delta e - \Delta G + \lambda)^2 + \Delta G + G_0 \quad \text{(A4)}$$

The parameter G_0 is independent of Δe, but may be dependent on p. These two parabolas are characterized by the two parameters λ and ΔG. The former, λ, has the meaning of reorganization energy as is clear from Figure 1. The latter, ΔG, is reaction free energy. However, λ can also be regarded as a quantity related to the curvature of the parabola. From this fact, λ is also related to the magnitude of fluctuation of the reaction coordinate in equilibrium as[26]

$$\lambda \equiv \frac{1}{2}\beta\langle \Delta e^2 \rangle_c \quad \text{(A5)}$$

where $\langle\ \rangle_c$ indicates the cumulant average. The Franck−Condon factor, FC, which is the probability that the system is at the intersection region, is given by

$$\mathrm{FC} = e^{-\beta G_R(0)} / \int \mathrm{d}\Delta e \; e^{-\beta G_R(\Delta e)} \quad \text{(A6)}$$

This expression reduces to eq 2.

Appendix B. Enthalpic Potential

We consider a system in which one protein (large molecule) q_p and N bulk water molecules (small molecules) q_w are in a box of volume V and we define its potential as $U(q_p,q_w)$.

The partition function of this system is given by

$$Z(p, T) = \int \mathrm{d}V \int^V \mathrm{d}q_p \, \mathrm{d}q_w \, e^{-(U+pV)} \quad \text{(B1)}$$

where we set that $\beta = 1$. We divide the coordinate of large molecule q_p into the center of mass coordinate q_0 and other relative coordinates q'. We divide the interaction energy into the repulsive energy and attractive energy. If we define *the effective volume of the large molecule* $v(q')$ as the region that bulk water molecules cannot intrude due to the repulsive effect, we can rewrite the partition function Z as

$$Z(p, T) \sim \int \mathrm{d}V \, e^{-pV} \int^{\infty} \mathrm{d}q' \int^{V-v(q')} \mathrm{d}q_0 \, \mathrm{d}q_w \, e^{-(U-U_{rep})} \quad \text{(B2)}$$

If we integrate $\mathrm{d}q_w$, it can be written as

$$Z(p, T) \sim \int \mathrm{d}V \, e^{-pV} \int^{\infty} \mathrm{d}q' (V - v(q'))^{N+1} e^{-f(q')} \quad \text{(B3)}$$

where $f(q')$ is the free energy of the large molecule. Changing the order of integrations, we obtain

$$Z(p, T) \sim \int^{\infty} \mathrm{d}q' e^{-f(q')} \int_{v(q')}^{\infty} \mathrm{d}V \, e^{-pV} (V - v(q'))^{N+1} \quad \text{(B4)}$$

Note that there is no contribution from the range $0 \sim v(q')$ of the volume integral. As the result, the partition function is rewritten as

$$Z(p, T) = \frac{(N+1)!}{p^{N+2}} \int \mathrm{d}q' \, \mathrm{e}^{-(f(q')+pv(q'))} \quad \text{(B5)}$$

where $h(q') = f(q') + pv(q')$ is *the enthalpic potential*. The concept of enthalpic potential has already been employed to discuss pressure effect of protein conformation.[23,24] The above derivation provides justification for this concept. With this potential, we can discuss the pressure dependence of systems through the microscopic volumes of large molecules.

Supporting Information Available: A file of potential parameters for Im(His33−Ru$(NH_3)_3$. Supporting information is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Winkler, J. R.; Gray, H. B. *Chem. Rev.* **1992**, *92*, 369−379.
(2) Meier, M.; van Eldik, R.; Chang, I.-J.; Mines, G. A.; Wuttke, D. S.; Winkler, J. R.; Gray, H. B. *J. Am. Chem. Soc.* **1994**, *116*, 1577−1578.
(3) Wishart, J. F.; van Eldik, R.; Sun, J.; Su, C.; Isied, S. S. *Inorg. Chem.* **1992**, *31*, 3986−3989.

(4) Scott, J. R.; Fairris, J. L.; McLean, M.; Wang, K.; Sligar, S. G. *Inorg. Chim. Acta* **1996**, *243*, 193−200.
(5) Sugiyama, Y.; Takahashi, S.; Ishimori, K.; Morishima, I. *J. Am. Chem. Soc.* **1997**, *119*, 9582−9583.
(6) Marcus, R. A.; Sutin, N. *Biochim. Biophys. Acta* **1985**, *811*, 265−322.
(7) Beratan, D. N.; Betts, J. N.; Onuchic, J. N. *Science* **1991**, *252*, 1285−1288.
(8) Onuchic, J. N.; Beratan, D. N. *J. Chem. Phys.* **1990**, *92*, 722−733.
(9) Beratan, D. N.; Onuchic, J. N.; Hopfield, J. J. *J. Chem. Phys.* **1987**, *86*, 4488−4498.
(10) Beratan, D. N.; Onuchic, J. N.; Betts, J. N.; Bowler, B. E.; Gray, H. B. *J. Am. Chem. Soc.* **1990**, *112*, 7915−7921.
(11) Basu, G.; Kitao, A.; Kuki, A.; Go, N. *J. Phys. Chem. B* **1998**, *102*, 2076−2084.
(12) Basu, G.; Kitao, A.; Kuki, A.; Go, N. *J. Phys. Chem. B* **1998**, *102*, 2085−2094.
(13) Cruañes, M. T.; Rodgers, K. K.; Sligar, S. G. *J. Am. Chem. Soc.* **1992**, *114*, 9660−9661.
(14) Bansch, B.; Meier, M.; Martinez, P.; van Eldik, R.; Su, C.; Sun, J.; Isied, S. S.; Wishart, J. F. *Inorg. Chem.* **1994**, *22*, 4744−4749.
(15) Bushnell, G. W.; Louie, G. V.; Brayer, G. D. *J. Mol. Biol.* **1990**, *214*, 585−595.
(16) Northrup, S. H.; Pear, M. R.; Morgan, J. D.; McCammon, J. A.; Karplus, M. *J. Mol. Biol.* **1981**, *153*, 1087−1109.
(17) Kashiwagi, H.; Obara, S. *Int. J. Quantum Chem.* **1981**, *XX*, 843−859.
(18) Gruschus, J. Ph.D. Thesis, Cornell University, 1993.
(19) Jorgensen, W. L.; Tirado-Rives, J. *J. Am. Chem. Soc.* **1988**, *110*, 1657−1666.
(20) Weiner, S. J.; Kollman, P. A. *J. Comput. Chem.* **1986**, *7*, 230−252.
(21) Morikami, K.; Nakai, T.; Kidera, A.; Saito, A.; Nakamura, A. *Comput. Chem.* **1992**, *16*, 243−248.
(22) Higo, J.; Go, N. *J. Comput. Chem.* **1989**, *10*, 376−379.
(23) Yamato, T.; Higo, J.; Seno, Y.; Go, N. *Proteins* **1993**, *16*, 327−340.
(24) Kobayashi, N.; Yamato, T.; Go, N. *Proteins* **1997**, *28*, 109−116.
(25) Tachiya, M. *J. Phys. Chem.* **1989**, *93*, 7050−7052.
(26) Chong, S.-H.; Hirata, F. *Chem. Phys. Lett.* **1998**, *293*, 119−126.