4924 *Biochemistry* **2000,** *39,* 4924−4928

# Effects of Kinetic Coupling on Experimentally Determined Electron Transfer Parameters[†]

Victor L. Davidson*

*Department of Biochemistry, The University of Mississippi Medical Center, Jackson, Mississippi 39216-4505*

*Received November 19, 1999; Revised Manuscript Received February 8, 2000*

ABSTRACT: Coupled electron transfer (ET) occurs when a relatively slow nonadiabatic ET reaction is preceded by a rapid but unfavorable adiabatic reaction that is required to activate the system for ET. As a consequence of this, the observed ET rate constant ($k_{ET}$) is an apparent value equal to the product of the true $k_{ET}$ and the equilibrium constant for the preceding reaction step. Analysis of such reactions by ET theory may yield erroneous values for the reorganizational energy ($\lambda$), electronic coupling ($H_{AB}$), and ET distance that are associated with the true $k_{ET}$. If the $\Delta G°$ dependence of the rate of a coupled ET reaction is analyzed, an accurate value of $\lambda$ will be obtained but the experimentally determined $H_{AB}$ will be less than the true $H_{AB}$ and the ET distance will be greater than the true distance. If the temperature dependence of the rate of a coupled ET reaction is analyzed, the experimentally determined value of $\lambda$ will be greater than the true $\lambda$. The magnitude of this apparent $\lambda$ will depend on the magnitude of $\Delta H°$ for the unfavorable reaction step that precedes ET. The experimentally determined values of $H_{AB}$ and distance will be accurate if $\Delta S°$ for the preceding reaction is zero. If $\Delta S°$ is positive, then $H_{AB}$ will be greater than the true value and the distance will be less than the true value. If $\Delta S°$ is negative, then $H_{AB}$ will be less than the true value and the distance will be greater than the true value. Data sets for coupled ET reactions have been simulated and analyzed by ET theory to illustrate these points.

The theoretical basis for what physical parameters control the rates of nonadiabatic electron transfer (ET)[1] reactions is well-established. Unlike adiabatic chemical reactions which involve the making and breaking of bonds and proceed via a well-defined reaction coordinate, the substrates and products of a nonadiabatic protein ET reaction are often chemically indistinguishable. For an adiabatic reaction, the probability of the reaction occurring when the activation energy is achieved is approximately 1, but for a nonadiabatic reaction, the probability of the reaction occurring when the activation energy is achieved is much less than 1. Consequently, a modified form of transition state theory is required to adequately describe such reactions. Nonadiabatic ET theory (*1*) (eq 1) predicts

$$k_{ET} = \frac{4\pi^2 {H_{AB}}^2}{h\sqrt{4\pi\lambda RT}} \exp[-(\Delta G° + \lambda)^2/4\lambda RT] \qquad (1)$$

that the rate of an ET reaction will vary predictably with temperature ($T$) and $\Delta G°$ (which is related to the redox potential difference between reactants). $H_{AB}$ is the electronic coupling matrix element, and $\lambda$ is the reorganizational energy. The other terms in eq 1 are Planck's constant ($h$) and the gas constant ($R$; the Boltzmann constant may alternatively be used). Detailed discussions of the mathematical and physical meaning of $H_{AB}$ and $\lambda$ may be found in a number of reviews of ET theory (*1*−*5*). In simple systems, $H_{AB}$ will decrease exponentially with distance. This is reflected in eq 2, which is a simplified form of eq 1.

$$k_{ET} = k_o \exp[-\beta(r - r_o)] \exp[-(\Delta G° + \lambda)^2/4\lambda RT] \qquad (2)$$

where the donor to acceptor distance is $r$ and $r_o$ is the close contact distance (3 Å). The parameter $\beta$ is used to quantitate the nature of the intervening medium with respect to its efficiency to mediate ET, and $k_o$ is the characteristic frequency of the nuclei which is usually assigned a value of $10^{13}$ s$^{-1}$.

A significant problem in applying ET theory to long-range ET reactions that occur in and between proteins is that it is often difficult to ascertain whether the measured rate of the redox reaction is a true ET rate constant ($k_{ET}$). For a simple bimolecular protein ET reaction, the following three-step reaction scheme may be envisioned (eq 3).

$$A_{ox} + B_{red} \overset{K_d}{\rightleftharpoons} A_{ox}/B_{red} \underset{k_{-x}}{\overset{k_x}{\rightleftharpoons}} [A_{ox}/B_{red}]^* \underset{k_{-ET}}{\overset{k_{ET}}{\rightleftharpoons}} A_{red}/B_{ox} \qquad (3)$$

In this scheme, some adiabatic reaction step with a forward rate constant $k_x$ and an equilibrium constant $K_x$ ($k_x/k_{-x}$) occurs after binding of the two redox proteins and is required to activate the protein complex for ET. If $k_x < k_{ET}$, then the reaction is defined as being gated (*6*, *7*). Interpretation of the thermodynamic behavior of a gated reaction is relatively

---

[†] Supported by National Institutes of Health Grant GM-41574.

* To whom correspondence should be addressed: Department of Biochemistry, The University of Mississippi Medical Center, 2500 N. State St., Jackson, MS 39216-4505. Telephone: (601) 984-1516. Fax: (601) 984-1501. E-mail: vdavidson@biochem.umsmed.edu.

[1] Abbreviations: ET, electron transfer; $H_{AB}$, electronic coupling; $\lambda$, reorganizational energy; $E_m$, oxidation−reduction midpoint potential; $k_{ET}$, true electron transfer rate constant; $k_x$, rate constant for a non-ET reaction that precedes the ET reaction step; $K_x$, equilibrium constant for a non-ET reaction that precedes the ET reaction step.

10.1021/bi992671j CCC: $19.00

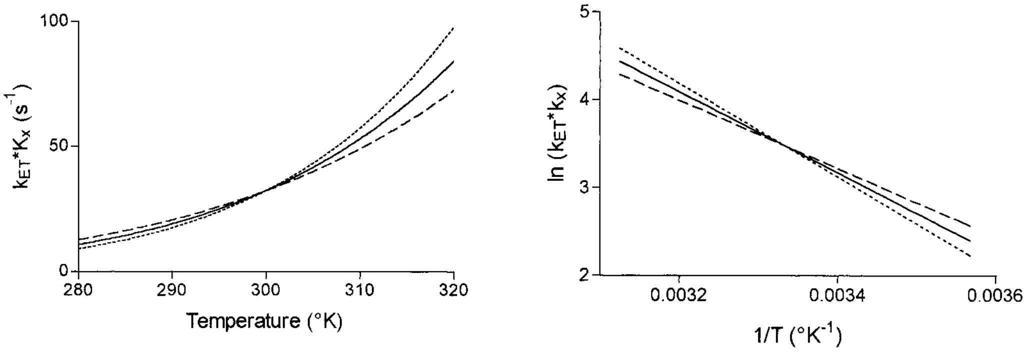

FIGURE 1: Temperature dependence of coupled ET reactions. Three coupled reactions are displayed. In each case, $K_x$ for the reaction is equal to $10^{-3}$ at 298 K, but each $K_x$ exhibits different values of $\Delta H°$ and $\Delta S°$: (solid lines) $\Delta H° = 17$ kJ/mol and $\Delta S° = 0$, (dotted lines) $\Delta H° = 23$ kJ/mol and $\Delta S° = 20$ J mol$^{-1}$ K$^{-1}$, and (dashed lines) $\Delta H° = 11$ kJ/mol and $\Delta S° = -20$ J mol$^{-1}$ K$^{-1}$. The values for the true $k_{ET}$ in each simulated coupled reaction were generated for a data set with a $\lambda$ of 1.0 eV, a $\Delta G°$ of $-5.0$ kJ/mol, and an $H_{AB}$ of 1.0 cm$^{-1}$. The values for ET parameters that are obtained by fitting these data sets of apparent $k_{ET}$ values (i.e., $K_x$*$k_{ET}$) to eqs 1 and 2 are listed in Table 1.

straightforward. The reaction rate will not depend on $\Delta G°$ for the redox reaction since $k_{ET}$ is not rate-determining. The temperature dependence of the reaction rate, in this case $k_x$, may be analyzed by transition state theory to obtain activation parameters (i.e., $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$) for the rate-limiting adiabatic non-ET reaction step.

An ET reaction is defined as kinetically coupled if $k_x > k_{ET}$, but $K_x \ll 1$ for the prerequisite adiabatic reaction step (*7, 8*). In this case, the rate constant for the conversion of $A_{ox}/B_{red}$ to $A_{red}/B_{ox}$ will not be $k_{ET}$, but $k_{ET}$*$K_x$. The characterization of ET reactions as being coupled is difficult. The reaction rate will still vary with $\Delta E_m$ for the redox centers since the limiting first-order rate constant is still proportional to $k_{ET}$. As such, it is important to understand the consequences of analyzing a kinetically coupled ET reaction by ET theory.

This paper describes exactly how the $K_x$ for the prerequisite adiabatic reaction step affects the values of $H_{AB}$, $\lambda$, and $r$ that are determined experimentally by analyzing the temperature or $\Delta G°$ dependence of a coupled ET reaction using eqs 1 and 2. In some cases, these experimentally determined parameters will be apparent values that are affected by the prerequisite step. Which parameters are affected, and to what extent, will depend both on the thermodynamic parameters ($\Delta H°$ and $\Delta S°$) for the prerequisite non-ET reaction and on whether one is analyzing the temperature or $\Delta G°$ dependence of the reaction rate. In this study, simulations have been performed to determine precisely and predictably how $K_x$ in a coupled ET reaction will influence the experimentally determined ET parameters for that reaction.

## EXPERIMENTAL PROCEDURES

Simulated sets of data for coupled ET reactions were generated as follows. A set of ET parameters (i.e., $H_{AB}$ and $\lambda$) for a true ET reaction was arbitrarily chosen. Using eqs 1 and 2, theoretical data sets were generated for both $k_{ET}$ versus temperature and $k_{ET}$ versus $\Delta G°$ for the true ET reaction. To simulate the effects of kinetic coupling, these values of $k_{ET}$ were each multiplied by values of $K_x$ for different hypothetical prerequisite adiabatic reactions. When data sets for temperature dependence studies were being generated, simulated values of $K_x$ were calculated for each temperature over the entire temperature range. This was done by assigning values of $\Delta H°$ and $\Delta S°$ for the theoretical prerequisite adiabatic reaction step. Using eq 4, the values of $K_x$ at each temperature were determined.

$$\ln K_{eq} = -\Delta H/RT + \Delta S/R \qquad (4)$$

For each temperature, the apparent $k_{ET}$ was calculated as $k_{ET}$*$K_x$. These simulated data sets of the apparent $k_{ET}$ versus temperature were then fit to eqs 1 and 2 to determine $H_{AB}$, $\lambda$, and $r$.

## RESULTS AND DISCUSSION

*Analysis of the Temperature Dependence of Coupled ET Reactions*. As seen in Figure 1, the temperature dependence of a coupled ET reaction will depend not only on the temperature dependence of $k_{ET}$ but also on the temperature dependence of $K_x$. The latter will be a consequence of the relative values of $\Delta H°$ and $\Delta S°$ which determine $K_x$. In this example, three coupled reactions were simulated with approximately equal $K_x$ magnitudes. In each case, $K_x$ equals $10^{-3}$ at 298 K. Because different values of $\Delta H°$ and $\Delta S°$ were used for each simulated reaction, each $K_x$ varies differently with temperature, yielding a distinct temperature dependence for each coupled reaction (Figure 1), even though the true $k_{ET}$ is the same in each. Consequently, the apparent values of $\lambda$, $H_{AB}$, and $r$ that are obtained from analysis of the temperature dependence of each coupled ET reaction by eqs 1 and 2 also each vary depending upon the $\Delta H°$ and $\Delta S°$ for each prerequisite adiabatic reaction step (Table 1). The apparent $\lambda$ value is primarily affected by the value of $\Delta H°$ for the preceding adiabatic reaction step. The apparent value of $H_{AB}$, and the calculated value of $r$, are affected primarily by the value of $\Delta S°$ for the preceding adiabatic reaction step. It should be noted that slightly lower values of $\lambda$ are obtained when the same data are analyzed by eq 2 compared with analysis by eq 1. That is because the minor contribution of $\lambda$ to the rate dependence in the prefactor in eq 1 is neglected in eq 2.

By definition, $K_x$ must be much less than 1 for the ET reaction to be classified as coupled. Since this prerequisite adiabatic reaction is very unfavorable, the $\Delta H°$ for this reaction will most likely be positive. A consequence of this positive $\Delta H°$ is that the apparent value of $\lambda$ that is obtained from the analysis of temperature dependence data will be greater than the true value of $\lambda$ for $k_{ET}$. The magnitude of

Table 1: Effects of Coupled ET on Parameters Determined from the Variation of Rate with Temperature

| | determinants of $K_x$ | | | fitted parameters | | |
|---|---|---|---|---|---|---|
| | $\Delta H°$ (kJ/mol) | $\Delta S°$ (J mol$^{-1}$ K$^{-1}$) | | $\lambda$ (kJ/mol) | $H_{AB}$ (cm$^{-1}$) | $r$ ($\beta = 1.0$) (Å) |
| true $k_{ET}$ | | | eq 1 | 100 | 1.0 | |
| | | | eq 2 | 92 | | 14.1 |
| coupled $k_{ET}$ | 17 | 0 | eq 1 | 168 | 1.1 | |
| | 17 | 0 | eq 2 | 157 | | 14.1 |
| | 23 | 20 | eq 1 | 192 | 3.9 | |
| | 23 | 20 | eq 2 | 180 | | 11.7 |
| | 11 | −20 | eq 1 | 144 | 0.33 | |
| | 11 | −20 | eq 2 | 134 | | 16.5 |

$\Delta H°$ for $K_x$ has no significant effect on the experimentally determined values of $H_{AB}$ and $r$. In contrast, the magnitude of $\Delta S°$ for $K_x$ has no significant effect on the experimentally determined value of $\lambda$, but primarily influences the experimentally determined values of $H_{AB}$ and $r$. If $\Delta S°$ for $K_x$ is zero, then only the experimentally determined $\lambda$ value will be affected (Table 1). The fitted values of $H_{AB}$ and $r$ will be those for the true ET reaction. If $\Delta S°$ for $K_x$ is negative, then the experimentally determined value of $H_{AB}$ will be smaller than the true value and the corresponding value of $r$ will be larger than the actual ET distance. If $\Delta S°$ for $K_x$ is positive, then the experimentally determined value of $H_{AB}$ will be larger than the true value and the corresponding value of $r$ will be smaller than the actual ET distance. The value of $\Delta S°$ for $K_x$ has no significant effect on the experimentally determined value of $\lambda$.

In practice, how significant the effects of $\Delta S°$ will be on experimentally determined values of $H_{AB}$ and $r$ will depend on the type of adiabatic, non-ET, reaction that is kinetically coupled to the ET event. If the prerequisite reaction is a rapid but unfavorable protein conformational change or rearrangement of proteins within a complex, with only subtle changes in the overall structure, one would intuitively expect these essentially unimolecular reactions to exhibit a relatively small $\Delta S°$. If so, then the effect of coupling would be manifested primarily in an artificially large value of $\lambda$ with a relatively accurate determination of $H_{AB}$ and $r$. However, if the rapid but unfavorable activation step is linked to the binding or release of water, protons, or other ligands, then a significant $\Delta S°$ could be associated with $K_x$. Since this reaction must by definition be thermodynamically unfavorable, binding reactions which would lead to a negative $\Delta S°$ are the more likely scenario. As seen in Table 1, this would cause an underestimation of $H_{AB}$ and an overestimation of $r$ from the analysis of temperature dependence data. It is important to note that an opposite trend has been reported (*9*−*13*) for the apparent ET parameters that are obtained from the analysis of the temperature dependence of gated ET reactions by eqs 1 and 2 (i.e., overestimation of $H_{AB}$ and underestimation of $r$). It should also be noted that even for a true ET reaction, the experimentally determined value of $r$ should be considered an estimate and not an absolute value, given the uncertainties in other parameters such as $\beta$. For use in eq 2, $\beta$ values ranging from 0.7 to 1.4 Å$^{-1}$ have been used to analyze protein ET reactions (*2*). In the example given in Table 1, a $\beta$ of 1.0 Å$^{-1}$ was used. With all other parameters unchanged, the use of a $\beta$ of 0.7 Å$^{-1}$ would yield an $r$ value of 18.8 Å and a $\beta$ of 1.4 Å$^{-1}$ would yield an $r$ value of 10.3 Å. As seen in Table 1, even with the error in the predicted distances which is caused by the influence of the given values of $\Delta S°$ for the coupled reactions, the calculated values of $r$ in these examples fall within the range of uncertainty (i.e., 10.3−18.8 Å) due to uncertainty in $\beta$. Therefore, even for a coupled ET reaction, it is possible to obtain reasonable estimates of the ET distance from analysis of the temperature dependence of the reaction rate, unless the absolute value of $\Delta S°$ for the prerequisite reaction is extremely large.

Table 2: Effects of Coupled ET on Parameters Determined from the Variation of Rate with $\Delta G°$

| | | | fitted parameters | | |
|---|---|---|---|---|---|
| | $K_x$ | | $\lambda$ (kJ/mol) | $H_{AB}$ (cm$^{-1}$) | $r$ ($\beta = 1.0$) (Å) |
| true $k_{ET}$ | | eq 1 | 100 | 1.0 | |
| | | eq 2 | 92 | | 14.1 |
| coupled $k_{ET}$ | $10^{-3}$ | eq 1 | 100 | 0.03 | |
| | $10^{-3}$ | eq 2 | 97 | | 20.5 |

*Analysis of the $\Delta G°$ Dependence of Coupled ET Reactions*. In some studies of protein ET reactions, it may be possible to obtain values for $\lambda$ and $H_{AB}$ from analysis of the dependence of the rate of the reaction on $\Delta G°$. Ideally, $\Delta G°$ is varied over a wide range which spans the Marcus "inverted" region (*1*) where $-\Delta G° > \lambda$. Two experimental approaches have primarily been used to modify $\Delta G°$ for protein ET reactions. Intramolecular ET through the protein matrix has been studied by covalently attaching ruthenium complexes with varied redox potentials to specific sites on the surface of a redox protein, thereby modulating $\Delta G°$ for the reaction between the ruthenium complex and the native redox center (*14*). Alternatively, redox potentials of the donor or acceptor have been modulated by substitution of metal cofactors (*15*, *16*), modification of organic prosthetic groups (*2*, *17*), and genetic modifications (*18*−*20*) of amino acid residues near the redox center.

When the $\Delta G°$ dependence for a coupled ET reaction is being simulated, the values of $\Delta H°$ and $\Delta S°$ for the preceding adiabatic reaction step are not relevant since the temperature is not varied. All that matters is the value of $K_x$ for this reaction step. As can be seen in Table 2, analysis by eqs 1 and 2 of the simulated data for the coupled reaction yields a value for $\lambda$ that is identical to the true $\lambda$. The experimentally determined value for $H_{AB}$, however, is less than the true $H_{AB}$ for the ET reaction. It is reduced by a factor equal to $K_x^{0.5}$. It follows that the analysis of the $\Delta G°$ dependence for a coupled ET reaction will predict a distance that is greater than the true value of $r$ for the ET. As seen by comparison of the values in Tables 1 and 2, the effect of coupling on the experimentally determined values of $H_{AB}$ and $r$ will be different depending on whether it is the temperature or $\Delta G°$ dependence of the rate that is being analyzed.

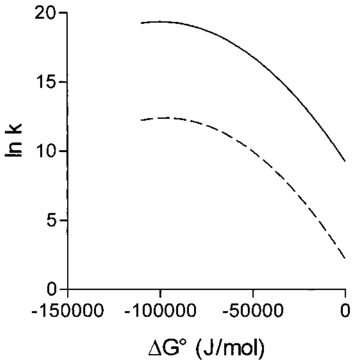

FIGURE 2: Free energy dependence of coupled ET reactions. Data sets were simulated for a true $k_{ET}$ (solid line) with a $\lambda$ of 1.0 eV, an $H_{AB}$ of 1.0 cm$^{-1}$, and a $T$ of 298 K; and a coupled ET reaction (dashed line) with a $K_x$ of $10^{-3}$. The values for ET parameters that are obtained by fitting these data sets to eqs 1 and 2 are listed in Table 2.

*Effect of Kinetic Coupling on Experimentally Determined $\beta$ Values*. The most appropriate value for $\beta$, and the issue of whether $r$ describes the direct donor−acceptor distance or is pathway-dependent, have been a matter of much debate (*2*, *3*, *21*). In most studies, a value for $\beta$ is assigned for use in eq 2 to experimentally determine the ET distance. Values of $\beta$ that are typically used range from 0.7 to 1.4 Å$^{-1}$. Analysis of an ET reaction of a known distance can yield an experimentally determined value for $\beta$. If the ET reaction is coupled, even in model systems, then the experimentally determined value of $\beta$ may be inaccurate depending on the method used to collect and analyze the data. As stated earlier, analysis of the $\Delta G°$ dependence for a coupled ET reaction will underestimate $H_{AB}$. Since $H_{AB}{}^2$ is related to $\beta r$ (see eq 2), if a value of $r$ is assigned, then the value of $\beta$ obtained from analysis of the data will be an apparent value that is larger than the true value. In the example shown in Figure 2 and Table 2, if the correct value of $r$ is used, analysis of the data with eq 2 will yield a $\beta$ value of 1.6. This is a significant overestimate of the value of 1.0 that is obtained from the analysis of the true values of $k_{ET}$.

In most studies, empirical values of $\beta$ for protein ET reactions have been obtained from the analysis of the dependence of rate on distance under conditions of activationless ET (i.e., $-\Delta G° = \lambda$) (*2*, *3*). Under these conditions, eq 2 reduces to eq 5.

$$k_{ET} = k_o \exp[-\beta(r - r_o)] \tag{5a}$$

$$\ln k_{ET} = \ln k_o - \beta(r - r_o) \tag{5b}$$

A plot of ln $k_{ET}$ versus $r - r_o$ for such data will be linear and yield a slope of $-\beta$. If a coupled ET reaction is analyzed in this manner, an accurate value for $\beta$ will be obtained since the slope will be unaffected. For a coupled ET reaction, the actual equation which describes the linear relationship is eq 6.

$$\ln k_{ET} = \ln k_o - \ln K_{eq} - \beta(r - r_o) \tag{6}$$

Since the measured rate is an apparent $k_{ET}$ that is equal to $k_{ET}K_x$, the intercept of this plot will not be equal to ln $k_o$ but will be less than the theoretical maximum rate for ET. If such data are plotted and yield an intercept significantly less than ln($10^{13}$), then this would be diagnostic of kinetic coupling. Furthermore, from these data, it should be possible to determine the value of $K_x$ for the preceding reaction from the fit of the data to eq 6.

*Distinguishing Coupled ET Reactions from Gated ET Reactions*. If a reaction is gated, then no dependence of the reaction rate on the $\Delta G°$ for ET should be observed. In practice, however, it is often not possible to experimentally vary $\Delta G°$ for protein ET reactions because one often cannot alter the $E_m$ values of the protein-bound redox centers without altering the protein. Thus, analysis of the temperature dependence of the reaction may be the only option available for distinguishing between coupled and gated ET reactions. It has been shown in at least some cases that when the analysis of an ET reaction with eqs 1 and 2 yields a value of $H_{AB}$ that exceeds the nonadiabatic limit, and a corresponding negative value of $r$, this is diagnostic of a gated ET reaction (*9*−*13*). As discussed above, analysis of a coupled ET reaction may also yield apparent values of $H_{AB}$ and $r$ which differ from the true values. However, the inaccuracies in the experimentally determined values of $H_{AB}$ and $r$ that are caused by coupling tend to push those values in the opposite direction as was reported for gating. As seen in Table 2, the effect of coupled ET was to cause the apparent value of $H_{AB}$ to be smaller than the actual value and $r$ to be larger than the true value. Even for a coupled ET reaction that is analyzed by varying the rate with temperature, it is unlikely that one would obtain the pattern of unreasonable values that are obtained from the corresponding analysis of a gated ET reaction. As can be seen from the results in Table 1, to obtain a negative value of $r$ for a coupled reaction, the preceding adiabatic reaction would have to exhibit an extremely large positive $\Delta S°$, which in turn would require an enormous positive $\Delta H°$ to maintain the requirement for the reaction to be very unfavorable.

*Distinguishing Coupled ET Reactions from True ET Reactions*. As seen in Table 1, if temperature dependence studies yield an unusually large value of $\lambda$ together with a value of $H_{AB}$ that is within the nonadiabatic limit, then this may be diagnostic, although not proof, of a coupled ET reaction. If it is possible to examine both the temperature and $\Delta G°$ dependence of a reaction, a reasonable inference may be made. If the same values of $\lambda$ and $H_{AB}$ are obtained from both temperature and $\Delta G°$ dependence analyses, then this strongly suggests that the reaction is true ET. If different values are obtained, then this would strongly suggest that the reaction is coupled (see Tables 1 and 2). If it is not possible to vary $\Delta G°$ and only the temperature dependence of the reaction can be monitored, then distinguishing coupled reactions from true ET reactions remains a difficult task and additional biochemical approaches will likely be required.

*Conclusions*. Even when it is possible to examine the $\Delta G°$ dependence of a protein ET reaction, these studies will yield apparent values for $H_{AB}$ and $r$ if the reaction is coupled. In practice, it is not usually possible to perform a $\Delta G°$ dependence analysis of the rate of a physiologic protein ET reaction. It is often not possible to modulate the redox potential of the native redox center without perturbing or destroying the protein matrix. Furthermore, if the redox potential of the center is altered by metal substitution or site-directed mutagenesis of nearby amino acid residues, one cannot know for certain whether this has changed the $\lambda$ for the reaction as well. Since temperature dependence studies will most likely be the only option available for the study of most natural protein ET reactions, consideration of the effects of kinetic coupling on the experimentally determined ET parameters will remain a critical issue when this experimental approach is used. The results presented here will provide a framework in which to interpret such data so that we can more fully elucidate the physical, chemical, and kinetic parameters that control rates of biological ET reactions.

## REFERENCES

1. Marcus, R. A., and Sutin, N. (1985) *Biochim. Biophys. Acta 811*, 265−322.
2. Moser, C. C., Keske, J. M., Warncke, K., Farid, R. S., and Dutton, P. L. (1992) *Nature 355*, 796−802.
3. Gray, H. B., and Winkler, J. R. (1996) *Annu. Rev. Biochem. 65*, 537−561.
4. McLendon, G. (1988) *Acc. Chem. Res. 21*, 160−167.
5. Davidson, V. L. (2000) *Acc. Chem. Res. 33*, 87−93.
6. Hoffman, B. M., and Ratner, M. A. (1987) *J. Am. Chem. Soc. 109*, 6237−6243; (1988) *J. Am. Chem. Soc. 110*, 8267 (Erratum).
7. Davidson, V. L. (1996) *Biochemistry 35*, 14035−14039.
8. Harris, T. K., Davidson, V. L., Chen, L., Mathews, F. S., and Xia, Z.-H. (1994) *Biochemistry 33*, 12600−12608.
9. Bishop, G. R., and Davidson, V. L. (1995) *Biochemistry 34*, 12082−12086.
10. Bishop, G. R., and Davidson, V. L. (1997) *Biochemistry 36*, 13586−13592.
11. Lanzilotta, W. N., Parker, V. D., and Seefeldt, L. C. (1998) *Biochemistry 37*, 399−407.
12. Lee, H. J., Basran, J., and Scrutton, N. S. (1998) *Biochemistry 37*, 15513−15522.
13. Hyun, Y.-L., Zhu, Z., and Davidson, V. L. (1999) *J. Biol. Chem. 274*, 29081−29086.
14. Winkler, J. R., and Gray, H. B. (1992) *Chem. Rev. 92*, 369−379.
15. Conklin, K. T., and McLendon, G. (1988) *J. Am. Chem. Soc. 110*, 3345−3350.
16. Zhou, J. S., and Kostic, N. M. (1993) *J. Am. Chem. Soc. 115*, 10796−10804.
17. Warncke, K., and Dutton, P. L. (1993) *Biochemistry 32*, 4769−4779.
18. Arlt, T., Penzkofer, H., Oesterhelt, D., and Zinth, W. (1996) *J. Phys. Chem. 100*, 12060−12065.
19. Nagarajan, V., Parson, W. W., David, D., and Schenck, C. C. (1993) *Biochemistry 32*, 12324−12336.
20. Ortega, J. M., Mathis, P., Williams, J. C., and Allen, J. P. (1996) *Biochemistry 35*, 3354−3361.
21. Regan, J. J., Risser, S. M., Beratan, D. N., and Onuchic, J. N. (1993) *J. Phys. Chem. 97*, 13083−13088.

BI992671J