Kinetic and Thermodynamic Analysis of a Physiologic Intermolecular Electron-Transfer Reaction between Methylamine Dehydrogenase and Amicyanin†

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ABSTRACT: The quinoprotein methylamine dehydrogenase (MADH) and a type I copper protein, amicyanin, form a physiologic complex in which electrons are transferred from tryptophan tryptophylquinone to copper. The reoxidation of MADH by amicyanin has been studied by stopped-flow spectroscopy. The rate constant for the electron-transfer (ET) reaction and the dissociation constant for the complex have been determined at different temperatures. Marcus theory was used to calculate the distance, reorganizational energy, and electronic coupling for the intermolecular ET reaction. The ET reaction exhibited a large apparent reorganizational energy of approximately 225 kJ mol^{-1} (2.3 eV) and a coupling of approximately 11.7 cm^{-1}. From X-ray crystallographic studies of an actual complex of these proteins from *Paracoccus denitrificans* [Chen, L., et al. (1992) *Biochemistry 31*, 4959–4964], it was possible to infer putative pathways of ET. The ET distance predicted by Marcus theory from kinetic data correlated reasonably well with the structural information. Thus, it has been possible to correlate ET theories with data from solution studies and a known structure for a naturally occurring ET reaction between soluble proteins.

The methylamine dehydrogenase (MADH)[1]–amicyanin complex (Chen et al., 1992) and the cytochrome *c*–cytochrome *c* peroxidase complex (Pelletier & Kraut, 1992) are the only complexes of soluble redox proteins for which a detailed crystal structure is known. This paper reports the kinetic and thermodynamic analysis of binding and intermolecular electron-transfer (ET) reactions between the former pair of soluble proteins which form a physiologic complex of known structure. This study has provided a rare opportunity to apply Marcus theory to an intermolecular ET reaction. Good correlations were observed between theory, structure, and experimental data.

MADH is a periplasmic enzyme which has been purified from several Gram-negative bacteria and catalyzes the oxidation of methylamine to formaldehyde and ammonia (Davidson, 1993). It exhibits an $\alpha_2\beta_2$ structure and possesses the novel redox prosthetic group, tryptophan tryptophylquinone (TTQ) (McIntire et al., 1991; Chen et al., 1991). The primary electron acceptor for MADH is a type I copper protein, amicyanin (Husain & Davidson, 1985). The specificity of the interaction between amicyanin and MADH is best demonstrated by studies of the isolated proteins from *Paracoccus denitrificans*. Each of these proteins is induced in this bacterium only during growth on methylamine as a carbon source (Husain & Davidson, 1985, 1987). Furthermore, the amicyanin gene is located immediately downstream of that for MADH, and inactivation of the former by means of gene replacement resulted in complete loss of the ability to grow on methylamine (van Spanning et al., 1990). In *P. denitrificans*, amicyanin mediates the transfer of electrons from MADH to soluble periplasmic *c*-type cytochromes, of which cytochrome *c*-551i is the most efficient electron acceptor in vitro (Husain & Davidson, 1986). The interaction between MADH and amicyanin has been characterized by absorption spectroscopy (Gray et al., 1988), potentiometric studies (Gray et al., 1988), steady-state kinetics (Davidson & Jones, 1991; Brooks et al., 1993), chemical cross-linking (Kumar & Davidson, 1990), resonance Raman spectroscopy (Backes et al., 1991) and X-ray crystallography (Chen et al., 1992). Considered together, the results of these studies suggest that some combination of electrostatic and hydrophobic interactions is required for efficient complex formation and for facilitating the intermolecular ET reaction. Actual crystals of the complex of MADH and amicyanin have been obtained, and the structure of this complex has been solved (Chen et al., 1992). From this structure it is possible to predict the likely pathways of ET from TTQ to copper. This provides an excellent opportunity to correlate kinetic, thermodynamic, and structural data for a long-range intermolecular ET reaction between proteins and to evaluate the ability of existing theories to describe these data.

Marcus theory (Marcus & Sutin, 1985) successfully predicts the ET rates of inorganic reactants using the relationships

$$k_{ET} = \frac{4\pi^2 H_{AB}^{\ 2}}{h\sqrt{4\pi\lambda RT}} e^{-(\Delta G^\circ+\lambda)^2/4\lambda RT} \quad (1)$$

$$k_{ET} = k_0 e^{-\beta(r-r_0)} e^{-(\Delta G^\circ+\lambda)^2/4\lambda RT} \quad (2)$$

H_{AB} is the electronic coupling between redox centers, λ is the reorganizational energy, h is Planck's constant, R is the gas constant, T is temperature, ΔG° is the standard free energy difference, k_0 is the characteristic frequency of the nuclei, which is usually assigned a value of 10^{13} s^{-1}, r is the distance between donor and acceptor, r_0 is the close contact distance (3 Å), and β is the electronic decay factor (Marcus & Sutin, 1985). The ability of classical and quantum mechanical Marcus theory to predict long-range ET rates in proteins has been examined in systems with known structures, such as

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[1] Abbreviations: ET, electron transfer; E_m, oxidation–reduction midpoint potential; H_{AB}, electronic coupling; λ, reorganizational energy; MADH, methylamine dehydrogenase; TTQ, tryptophan tryptophylquinone; PQQ, pyrroloquinoline quinone.

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bacterial photosynthetic reaction centers (Moser et al., 1992), ruthenium-modified proteins (Onuchic et al., 1992), and synthetic peptides (Isied et al., 1992). It has been suggested that ET rates depend upon the nature of the actual path that electrons traverse since the ET rates in the ruthenium-modified proteins did not correlate with the direct edge to edge donor to acceptor distances (Wuttke et al., 1992; Beratan et al., 1992). Because β depends upon the nature of the intervening medium between the donor and the acceptor, a controversy has arisen as to which β value is most appropriate for ET reactions in proteins. Should one use a β value of 1.4 $Å^{-1}$, which is a value intermediate between the value for ET through a vacuum and that for covalently bridged donor–acceptor molecules, or 0.7 $Å^{-1}$, which corresponds to ET through triptycene, pentiptycene, spirocyclobutanes, and other synthetically coupled redox centers, i.e., through bonds (Moser et al., 1992)? On the basis of the notion that no single β is appropriate for protein ET reactions but that H_{AB} depends upon the nature of each pathway, the Pathways algorithm was developed to search a crystal structure for pathways. The Pathways II v2.01 algorithm predicts the optimal ET path(s) by maximizing H_{AB} according to

$$H_{AB} \propto \prod_{i=1} \epsilon_i \tag{3}$$

where i ranges over the pathway steps and ϵ_i is a wave-function decay factor for step i defined by $\epsilon_i = 0.6e^{-\beta_0(r-1.4)}$. β_0 equals 0 for steps through covalent bonds, and β_0 equals 1.7 $Å^{-1}$ for through-space jumps. The distance between the localization sites on the path is r (Regan et al., 1993).

We present here a kinetic and thermodynamic analysis of the binding and ET reactions between MADH and amicyanin. These solution studies are analyzed by Marcus theory, and the parameters for the ET reaction are discussed in the context of the known structure of the complex.

EXPERIMENTAL PROCEDURES

Purifications of MADH (Davidson, 1990) and amicyanin (Husain & Davidson, 1985) from *P. denitrificans* were as previously described. Protein concentrations were calculated from known extinction coefficients (Husain & Davidson, 1985; Husain et al., 1987). Reduced MADH was prepared by anaerobic spectrophotometric titration with sodium dithionite. An On-Line Instrument Systems (OLIS, Bogart, GA) stopped-flow sample handling unit coupled to Durrum optics was used for all stopped-flow experiments. Data were collected and analyzed using a 486-class computer controlled by OLIS software. All reactions were performed in 10 mM potassium phosphate, pH 7.5. The reaction of reduced MADH and oxidized amicyanin was monitored at either 443 or 330 nm. Typically three to four data sets, each containing 1000 data points, were averaged. The spectral changes at these wavelengths correspond to the interconversion of MADH between the dithionite-reduced and the semiquinone forms (Husain et al., 1987). As suggested in previous steady-state kinetic studies (Brooks et al., 1993), the reaction between reduced MADH and oxidized amicyanin was slow enough to be studied by stopped-flow spectroscopy. The observed amplitudes for the reactions were consistent with the known extinction coefficients for the dithionite-reduced and semiquinone forms of MADH ($\Delta\epsilon_{330}$ = 31 200 M^{-1} cm^{-1} and $\Delta\epsilon_{443}$ = 25 000 M^{-1} cm^{-1}). The $\Delta\epsilon$'s showed no significant temperature dependence over the ranges studied. At saturating amicyanin concentrations the data for the oxidation of reduced MADH were best described by a single exponential (Figure 1A). At less than saturating concentrations this was not true (Figure 1B) because the reaction was no longer pseudo first order (Hiromi, 1979; Strickland et al., 1979). For the oxidation of reduced MADH by amicyanin, the initial rate of the stopped-flow trace (v) was determined from the slope of the initial linear region of the reaction and was used to determine k_3 and K_d (Brooks & Davidson, 1993; discussed below):

$$v/[E_o] = k_3[S_o]/(K_d + [S_o]) \tag{4}$$

Nonlinear curve fitting of data was performed with the Enzfitter (Elsevier-BIOSOFT, Cambridge) and SigmaPlot 5.0 (Jandel Scientific, San Raphael, CA) computer programs.

RESULTS AND DISCUSSION

The oxidative half-reaction of MADH is believed to proceed through two sequential one-electron reductions as described by the minimal scheme below in which M_r is reduced MADH, M_s is semiquinone MADH, M_o is oxidized MADH, A_o is oxidized amicyanin, and A_r is reduced amicyanin.

$$M_r + A_o \underset{k_2}{\overset{k_1}{\rightleftharpoons}} M_rA_o \underset{k_4}{\overset{k_3}{\rightleftharpoons}} M_sA_r \underset{k_6}{\overset{k_5}{\rightleftharpoons}} M_s + A_r$$

$$M_s + A_o \underset{k_8}{\overset{k_7}{\rightleftharpoons}} M_sA_o \underset{k_{10}}{\overset{k_9}{\rightleftharpoons}} M_oA_r \underset{k_{12}}{\overset{k_{11}}{\rightleftharpoons}} M_o + A_r$$

The ET reaction described by k_3 (k_{ET}) is associated with conversion of MADH from the reduced to the semiquinone form. Because the reaction is monitored at wavelengths which are isosbestic for M_s and and M_o, all steps beyond k_3/k_4 are spectroscopically invisible. Thus, the rate equation for the appearance of M_sA_r is described by eq 4, where $[E_o]$ corresponds to the initial $[M_r]$ and $[S_o]$ corresponds to initial $[A_o]$. Equation 4 has been shown to be valid for a steady-state approximation for which $K_d = (k_2 + k_3)/k_1$ provided that $K_d \geq [E_o]$ (Brooks & Davidson, 1993). The identical equation may also be derived assuming that $M_r + A_o$ and M_rA_o are in rapid equilibrium ($K_d = k_2/k_1$). It was necessary to use the initial rates rather than k_{obs} because the K_d for complex formation is not much greater than the amount of protein required to obtain a detectable absorbance change. Traditional methods such as those of Strickland et al. (1979) and Hiromi (1979), which determine k_3 and K_d from k_{obs}, require $[S_o]$ to be much greater than $[E_o]$, and therefore these methods are not applicable to this system. It can be seen that when reduced MADH and oxidized amicyanin were mixed under conditions where $[E_o] \sim [S_o]$, $d[M_r]/dt$ was not described by a single exponential (Figure 1B). Furthermore, the k_{obs} obtained from such data did not show any direct correlation with $[S_o]$ at less than saturating amicyanin concentrations. Analysis of $v/[E_o]$, however, provided a reasonable correlation in this concentration range (Figure 2).

The $v/[E_o]$ was measured over a range of amicyanin concentrations from 0.2 to 32 μM at temperatures from 10 to 50 °C. Eleven data sets were collected at different temperatures, and the values of k_3 and K_d were determined from fits of each set to eq 4. These data were obtained at either 330 or 443 nm, and essentially identical values of k_3 and K_d were obtained irrespective of which wavelength was used to collect the data. Furthermore, several different MADH and amicyanin preparations were used in these studies, and no difference in either k_3 or K_d was observed between protein preparations.

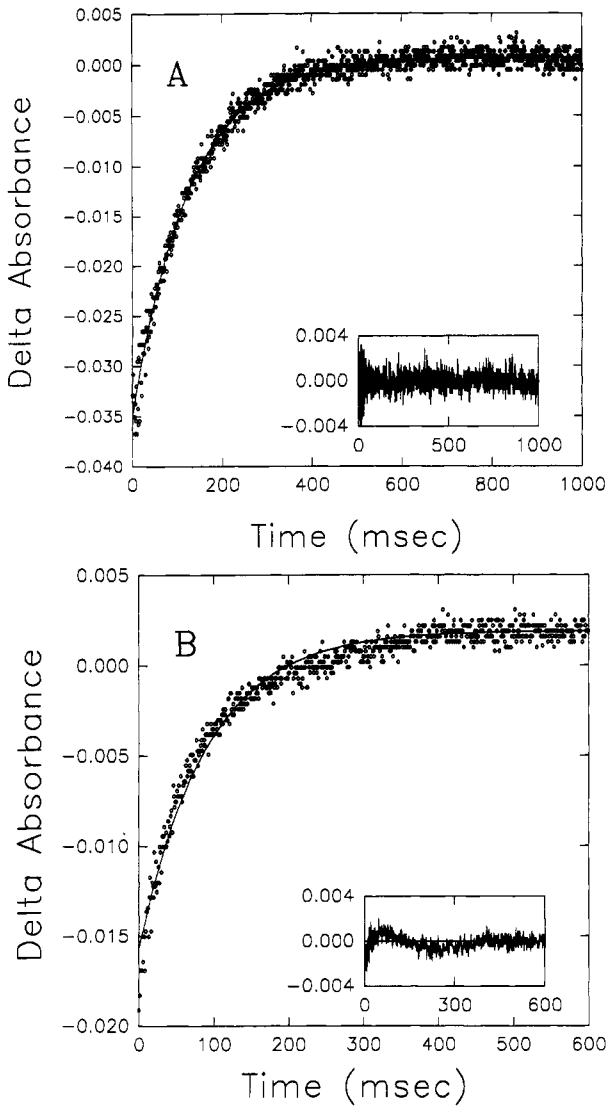

FIGURE 1: (A) Oxidation of MADH by amicyanin. In this representative experiment dithionite-reduced MADH (1 μM) was mixed with oxidized amicyanin (32 μM) in 10 mM potassium phosphate, pH 7.5, at 25.0 °C. The conversion of MADH from reduced to semiquinone was monitored at 443 nm. The solid line represents a fit to a single exponential with a k_{obs} of 8 s^{-1}. The inset shows the residuals for this fit. (B) Oxidation of MADH by amicyanin. In this representative experiment dithionite-reduced MADH (1 μM) was mixed with oxidized amicyanin (2 μM) in 10 mM potassium phosphate, pH 7.5, at 35.0 °C. The conversion of MADH from reduced to semiquinone was monitored at 443 nm. The solid line represents a fit to a single exponential with a k_{obs} of 11 s^{-1}. The inset shows the residuals for this fit and the failure of the single exponential to adequately describe the data.

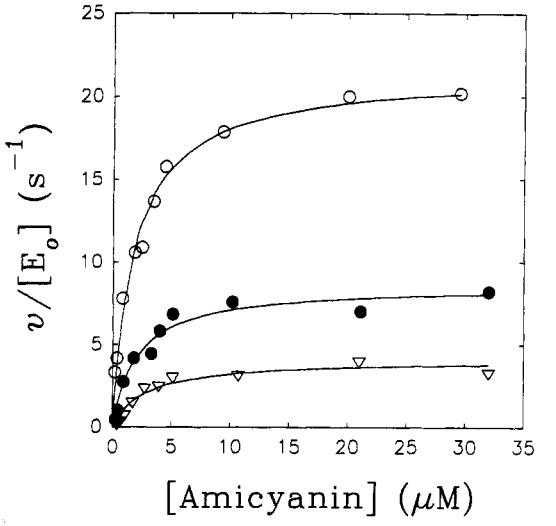

FIGURE 2: Rate of oxidation of reduced MADH as a function of amicyanin concentration and temperature. For clarity only three data sets are shown of the eleven which are further analyzed in Figures 3 and 4. Stopped-flow measurements were made as described under Experimental Procedures at 35.0 °C (○), 25.0 °C (●), and 15.0 °C (▽).

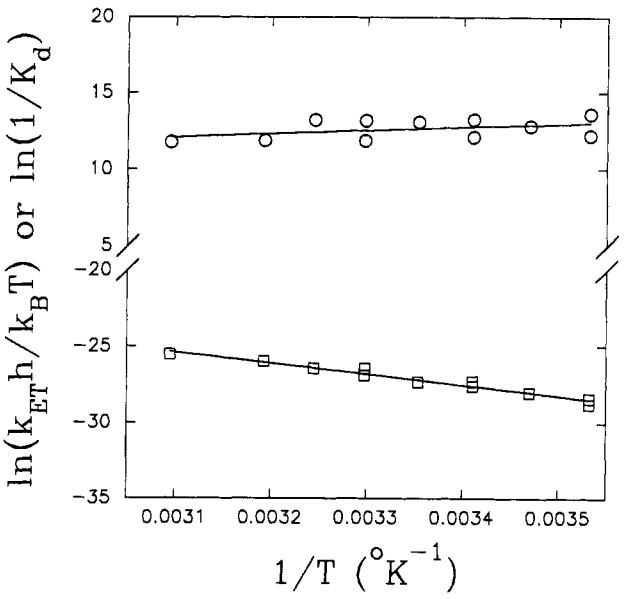

FIGURE 3: Thermodynamic analysis of binding and ET rate constants. Values of the association constant, $1/K_d$, for oxidized amicyanin with reduced MADH (○) were fit to the van't Hoff equation [$\ln(1/K_d) = -\Delta H°/RT + \Delta S°/R$]. Values of k_3 for reduced MADH (□) were fit to the Eyring equation [$\ln(k_{ET}h/k_BT) = -\Delta H^*/RT + \Delta S^*/R$].

The K_d exhibited very little temperature dependence ($\Delta H°$ = −18 ± 12 kJ mol^{-1}) and a mean value of 4.0 ± 2.5 μM (Figure 3). This value correlates very well with the K_d of 4.5 ± 0.5 μM, which was determined in a direct binding assay with oxidized MADH and amicyanin (Davidson et al., 1993). A van't Hoff plot of $\ln[1/K_d]$ versus $1/T$ (Figure 3) shows that the $\Delta S°$ is 47 ± 41 J mol^{-1} K^{-1}. Several solution studies (Gray et al., 1988; Davidson & Jones, 1991; Backes et al., 1991) have suggested a role for electrostatic interactions in stabilizing complex formation between MADH and amicyanin. The interface between MADH and amicyanin in complex has been shown to be comprised of primarily hydrophobic amino acid residues (Chen et al., 1992). The positive $\Delta S°$ observed in this study would be consistent with either desolvation of hydrophobic amino acid residues or ionic interactions at the interface between the two proteins or both providing the thermodynamic driving force for association (Ross & Subramanian, 1981).

Eyring plots (Figure 3) of the ET rate constants k_3 (k_{ET}) yielded a ΔH^* of 59.2 ± 3.8 kJ mol^{-1} and a ΔS^* of −27.5 ± 12.9 J mol^{-1} K^{-1}. Interpretation of these parameters for an ET reaction is not straightforward as this is not a binding or catalytic reaction where the reaction coordinate is well-defined. Marcus theory (eq 1) was used to analyze the temperature dependence of k_{ET}. To use this equation, $\Delta G°$ must be known. The E_m value for the two-electron oxidized/reduced couple of MADH is known to be +100 mV (Husain et al., 1987). The semiquinone form of MADH was not observed during that redox titration. The E_m value for the semiquinone/reduced couple of the structurally similar cofactor, pyrroloquinoline quinone (PQQ), is +190 mV (Oshiro & Itoh, 1993). The E_m

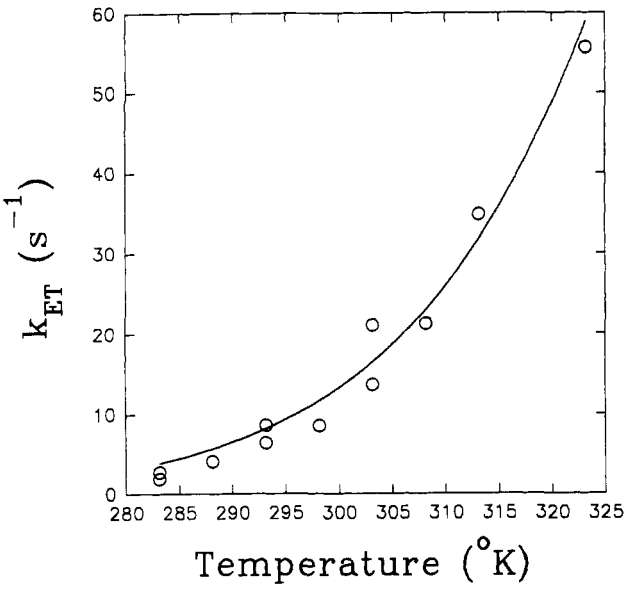

FIGURE 4: Analysis of the temperature dependence of the ET rate constant by Marcus theory. Values of k_3 were determined from sets of data such as those shown in Figure 2 which were fit to eq 4. The solid line represents the fit of the data to eq 1.

value for amicyanin when bound to MADH is +221 mV (Gray et al., 1988). Using these values, the ΔE_m for the ET reaction can be estimated to be in the range of 31–121 mV (–3.0 to –11.7 kJ mol^{-1}). Using these values of ΔE_m to calculate $\Delta G°$ and fitting the data to eq 1 (Figure 4), values of H_{AB} of 11.6–11.8 cm^{-1} and λ of 218–235 kJ mol^{-1} (2.2–2.4 eV) are obtained. It can be seen that for this ET reaction $\Delta G°$ is relatively insignificant compared to λ and that $\Delta G°$ has relatively little effect upon the H_{AB}.

It must be noted that alternatives to the Marcus equation exist (Jortner, 1976; Hopfield, 1974), which introduce an additional term, the characteristic frequency ($h\omega/2\pi$), to account for quantum mechanical effects which are neglected in eq 1. However, this correction is only required when $h\omega/2\pi > k_BT$ (Moser et al., 1992; Jortner, 1976). Fitting our data to the Hopfield equation [eq 8 in Hopfield (1974)] gave an estimate of H_{AB} of 10.9–11.8 cm^{-1}, λ of 217–235 kJ mol^{-1}, and $h\omega/2\pi$ of 0.0017–0.00002 eV. Since $h\omega/2\pi$ is much less than k_BT (0.026 eV), the application of eq 1 is valid for these data.

For protein ET reactions in which the $\Delta G°$ dependence has been used to determine λ, values of λ between 0.7 and 1.4 eV have been obtained. The reorganizational energy for ET through a protein has been reported for the bacterial photosynthetic reaction center (λ = 0.7 eV; Moser et al., 1992), ruthenated azurins (λ = 0.9 eV; Winkler & Gray, 1992), ruthenated cytochromes (λ = 1.2 eV; Winkler & Gray, 1992), ruthenated myoglobin (λ = 1.3 eV; Winkler & Gray, 1992), the cytochrome *c*–cytochrome b_5 complex (λ = 0.8 eV; McLendon & Hake, 1992), the hemoglobin–cytochrome b_5 complex (λ = 0.9 eV; Simmons et al., 1993), and the cytochrome *c*–cytochrome *c* peroxidase complex (λ = 1.4 eV; McLendon & Hake, 1992). According to Marcus and Sutin (1985), $\lambda = \lambda_i + \lambda_o$ in which λ_i represents changes in the bond lengths of the reactants and λ_o represents changes in solvent orientation. Marcus theory was derived for simple inorganic molecules. As the reactant molecules become large, as are proteins, the λ_o term is likely to be negligible. Furthermore, with protein complexes λ_i not only represents changes in bond lengths of residues directly linked to the redox center but also will reflect any changes in the bond lengths in the portion of the protein through which ET occurs, as well as any reorientation of the protein complex from the ground state. For intermolecular protein ET reactions a change in orientation of the protein complex from the ground state may be required to achieve the optimal coupling between donor and acceptor. As the energy barrier for such a reorientation becomes increasingly large, it becomes more likely that an intermediate species with an activation energy λ_c will be required to attain that optimal configuration. For any conformational change preceding ET, λ_c will be reflected in the experimentally determined λ. Under conditions where the rate of the conformational change is slower than the rate of ET, the reaction is gated (Hoffman & Ratner, 1987; Brunschwig & Sutin, 1989). In such a situation k_3 is actually the rate of the conformational change and $\lambda = f(\lambda_c)$. If, however, the rate of the conformational change is not rate limiting for the ET reaction, then k_{ET} will reflect both the rate of ET and the equilibrium constant for that conformational change. This will not be a gated reaction but a reaction in which $\lambda = f(\lambda_i,\lambda_c)$. For the reaction between MADH and amicyanin with an experimentally determined λ of 2.3 eV, the additional contribution to λ beyond the typically reported values of 0.7–1.4 eV is likely due to a relatively large λ_c. This suggests that the large λ observed for this reaction is truly an *apparent* λ containing components from both the ET reaction and a conformational change, λ_c. The λ observed in other protein complexes also likely contains a λ_c component. It is unclear at this time what gives rise to the unusually large λ in the MADH–amicyanin complex. Further experimental approaches which address the role of protein dynamics in ET reactions in both the MADH–amicyanin complex and other systems will be required to resolve this important issue.

It is tempting to speculate as to the nature of λ_c which results in the large λ for this ET reaction. Although several explanations are possible, one could be a requirement for conformational flexibility. This was demonstrated by covalent cross-linking of either cytochrome *c* or cytochrome *f* to plastocyanin, which dramatically lowered the rate of ET between these proteins (Peerey et al., 1991; Qin & Kostic, 1993). The requirement for dynamic interactions between proteins has also been suggested on the basis of studies of the cytochrome *c*–cytochrome *c* peroxidase complex (McLendon et al., 1993). It is also noteworthy that the crystal structures of MADH and the MADH–amicyanin complex indicate that the angle between the two indole rings of TTQ is approximately 45° (Chen et al., 1991, 1992). In bis(porphyrins), it has been shown that ET reaction rates are minimal when the angle between conjugated ring systems is 45° and maximal at 0° and 90° (Helms et al., 1991). Hence, another possibility is that the large λ is due to a reorientation of TTQ which reduces or increases the torsion angle between ring systems to allow electrons access to the surface-exposed ring. Thus, either a reorientation of the two proteins once in complex or a change in the torsion angle between ring systems of TTQ or both may contribute to the large experimentally determined λ. With this pair of physiologic redox partners we are *not* examining an activationless ET reaction (one in which $-\Delta G°$ is approximately equal to λ) but an ET reaction which requires a transition of the complex from the ground state in order to achieve optimal electronic coupling. As such, the magnitude of λ strongly influences the rate of this physiologic ET reaction.

While crystal structures are widely accepted to accurately represent the structures of individual proteins, for protein complexes it is important that crystal structures be correlated with solution studies to be certain that the structures seen in

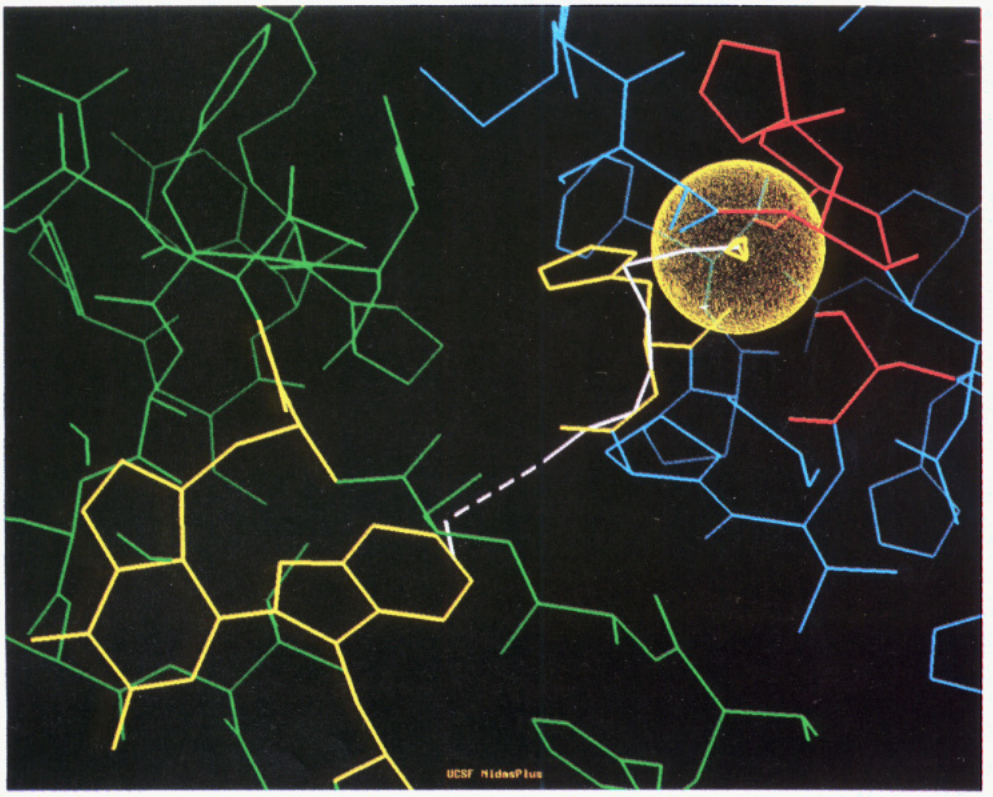

FIGURE 5: Possible ET pathway from TTQ to copper in the MADH–amicyanin complex. This structure was determined from crystals of an actual complex of these proteins (Chen et al., 1992). The α-subunit of MADH which possesses TTQ is green. Amicyanin is blue. TTQ, copper, the surface-exposed His 95 ligand to copper, and the carbonyl oxygen and carbon of Pro 94 are yellow. The His, Met, and Cys, which also are ligands to copper, are red. The most probable pathway for ET is indicated in white. It includes a 3.6-Å jump through space from TTQ to the carbonyl oxygen of Pro 94 (dashed line) and pasaage through six covalent bonds to the copper atom.

the crystal are not mere artifacts of the crystallization conditions. Some verification of the crystal structure of the MADH–amicyanin complex comes from chemical cross-linking studies (Kumar & Davidson, 1990) which confirm that both the α- and β-subunits of MADH are in contact with amicyanin as observed in the crystal structure (Chen et al., 1992). Additional validity for the complex structure may be inferred from the studies reported herein when eqs 1 and 2 are used to correlate solution studies with the crystal structure. Furthermore, the crystal structure of a complex of MADH, amicyanin, and cytochrome *c*-551i has recently been solved (Chen et al., 1993, 1994), and the orientation and interface between MADH and amicyanin are essentially the same as in the binary MADH–amicyanin complex.

If the use of a single β value in eq 2 is appropriate, then Marcus theory should be able to predict the direct distance between redox centers for an ET reaction. Conklin et al. (1988) assumed an exponential dependence of the electronic coupling upon the distance between donor and acceptor, as in eq 2, to obtain an estimate for λ of 1.5 eV, which agreed well with the λ of 1.4 eV determined from the $\Delta G°$ dependence for the cytochrome *c*–cytochrome *c* peroxidase complex (McLendon & Hake, 1992). Moser et al. (1992) have shown an excellent correlation between the distance between donor and acceptor redox centers and the rate of ET in proteins using a β of 1.4 Å^{-1}. Using the β value of 1.4 Å^{-1} for a protein ET reaction (Beratan et al., 1991; Onuchic et al., 1992; Moser et al., 1992), eq 2 predicts a distance (r) of 7.9 Å between the TTQ of MADH and the center of the copper atom of amicyanin. This is quite close to the 9.4-Å distance from atom CH2 of Trp 108 of TTQ to the center of the copper atom in the crystallized complex (Chen et al., 1992).

It has also been suggested that β values vary depending upon the nature of the intervening media, i.e., the path(s) of electron transfer (Beratan et al., 1991). Using the crystal structure of the MADH–amicyanin complex, eq 3 was used to predict dominant pathways for ET. Assuming that the electrons are delocalized over both ring systems of TTQ and starting from the exposed edge of Trp 108 within TTQ, the Pathways II program (Regan et al., 1993) predicts from the crystal structure of the MADH–amicyanin complex (Chen et al., 1992) the best pathway for ET (Figure 5). While there are several potential pathways, the one shown in Figure 5 is predicted to be at least 10-fold more efficient than any pathway involving other residues. This pathway has a length of 14.0 Å and includes a 3.6-Å jump through space from TTQ to the carbonyl oxygen of Pro 94, followed by passage through six covalent bonds. The distance from the His ligand to copper (2.0 Å; Durley et al., 1993) has been included in the calculation of the path distance. It is interesting to note that using a β value of 0.7 Å^{-1}, which is applicable for triptycene, pentiptycene, spirocyclobutanes, and other covalently coupled synthetic systems (Moser et al., 1992), a distance of 12.4 Å is predicted by eq 2. This also correlates well with the distance for the pathway described above. Furthermore, the experimentally determined H_{AB} of 11.7 cm^{-1}, which was obtained with eq 1, may be considered reasonable for ET over either the direct distance between redox centers of 9.4 Å (Meade et al., 1989) or the pathway distance of 14.0 Å (Wuttke et al., 1992). The close correlation between the donor and acceptor distance predicted by eq 2 and the known structure and the H_{AB} predicted by eq 1 provide support for the relevance of the crystal structure of this complex and suggest that we are indeed observing an ET reaction, not solely a conformational change that is gating an ET reaction. It should be noted that an alternative pathway involving an intracomplex water molecule was observed in the ternary complex of MADH, amicyanin, and cytochrome *c*-551i (Chen et al., 1994). This pathway is

moderately more efficient than the one shown in Figure 5 but depends critically on the presence of the water molecule which may not always be occupied (Chen et al., 1994).

Intermolecular electron transfer is currently an active area of research, and the role of protein dynamics in the regulation of these reactions is an open question. The only complexes of soluble redox proteins for which a detailed crystal structure is known are the MADH–amicyanin complex (Chen et al., 1992) and cytochrome *c*–cytochrome *c* peroxidase complex (Pelletier & Kraut, 1992). A third physiologic ET complex has also recently been crystallized containing MADH, amicyanin, and cytochrome *c*-551i (Chen et al., 1993, 1994). It is still not clear whether rates of ET reactions can be correlated directly with distance or whether they are highly dependent upon the nature of the heterogeneous intervening media. These data suggest that, over the relatively short distances observed between redox centers in this complex, either the β value of 1.4 $Å^{-1}$ to be used with the direct distance between redox centers or the β value of 0.7 $Å^{-1}$ to be used with the through-path distance is adequate for predicting the ET distance. It will be interesting to see if this conclusion holds true for intermolecular ET reactions over longer distances. Furthermore, the large apparent λ observed for this reaction suggests the importance of protein dynamics in regulating the intermolecular ET reaction within the MADH–amicyanin complex. Relatively few studies of physiologic ET reactions between proteins have been reported. Further studies of such intermolecular ET reactions will be required in order to accumulate a sufficient data base to allow the formulation of general rules regarding the process of long-range ET between proteins.

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