Ionic Strength Dependence of the Reaction between Methanol Dehydrogenase and Cytochrome *c*-551i: Evidence of Conformationally Coupled Electron Transfer†

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Received March 3, 1994; Revised Manuscript Received August 8, 1994®

ABSTRACT: The quinoprotein methanol dehydrogenase and cytochrome *c*-551i are two soluble acidic proteins that form a physiological complex in which electrons are transferred from pyrroloquinoline quinone to heme. The oxidation of methanol dehydrogenase by the cytochrome was studied as a function of ionic strength using stopped-flow spectroscopy. The dissociation constant (K_d) for complex formation decreased 2-fold with increasing ionic strength from 0.21 to 1.3 M and increased at higher ionic strengths. The rate constant for the electron transfer reaction (k_{ET}) increased 2-fold with increasing ionic strength from 0.21 to 1.3 M and decreased at higher ionic strengths. The variation of K_d and k_{ET} over this range of ionic strengths was described by Van Leeuwen theory, which takes into account monopole–dipole and dipole–dipole forces, in addition to the monopole–monopole force, to predict the interactions between large molecules. Analysis of the kinetic results in terms of these electrostatic interactions indicated the probable orientations for protein–protein binding and electron transfer. To explain the ionic strength dependence of the observed k_{ET}, a model is presented in which the true k_{ET} is reduced by a factor K_c, an equilibrium constant that describes some rearrangement of the proteins after a nonoptimal collision to produce the most efficient orientation for electron transfer. This model is consistent with the notion that the large reorganizational energy obtained from temperature-dependence studies of this electron transfer reaction [Harris, T. K., & Davidson, V. L. (1993) *Biochemistry 32,* 14145–14150] is due to such an intracomplex rearrangement. Kinetic schemes are presented that distinguish between an electron transfer reaction that is absolutely gated and one that is conformationally coupled, such as that between methanol dehydrogenase and cytochrome *c*-551i.

This paper reports the first analyses by Van Leeuwen theory of the ionic strength dependencies of both the equilibrium binding constant and the limiting first-order rate constant for an intermolecular electron transfer reaction. Studies of the reaction between the physiologic protein partners, methanol dehydrogenase and cytochrome *c*-551i, (1) reveal that dipolar interactions strongly influence the interactions between these proteins, (2) allow prediction of the sites of protein–protein interaction, and (3) suggest a kinetic mechanism that includes a conformational preequilibrium process, which occurs after the initial binding step and precedes the electron transfer step.

Paracoccus denitrificans provides a powerful and convenient system for studying the mechanisms of long range intermolecular electron transfer. Regulation of the synthesis of a variety of soluble electron transport proteins in this bacterium is mediated by growth conditions. For example, when methanol is used as the sole source of carbon and energy, it initially is oxidized by an inducible periplasmic quinoprotein, methanol dehydrogenase (Bamford & Quail, 1979). The electrons derived from the oxidation of methanol to formaldehyde are then transferred to a membrane-bound cytochrome oxidase by *c*-type cytochromes. The periplasmic cytochromes of *P. denitrificans* include a constitutive class I cytochrome *c*-550 and two other *c*-type cytochromes, designated cytochrome *c*-551i and cytochrome *c*-553i, which are induced during growth on methanol (Husain & Davidson, 1986). The three cytochromes and methanol dehydrogenase are each acidic proteins. It has been shown that cytochrome *c*-551i is the physiologic electron acceptor for methanol dehydrogenase (Long & Anthony, 1991). The p*I* values for methanol dehydrogenase (Bamford & Quail, 1979) and cytochrome *c*-551i (Husain & Davidson, 1986) are 3.7 and 3.5, respectively. It has also been demonstrated *in vitro* that cytochrome *c*-550 mediates electron transfer from cytochrome *c*-551i to the membrane-bound oxidase (Davidson & Kumar, 1989). Except for the membrane-bound oxidase, these periplasmic redox enzymes and proteins are soluble and provide an opportunity to study physiologic electron transfer reactions between proteins in solution. The fact that the components of this soluble electron transfer chain are each acidic raises questions as to the role of electrostatic interactions in the binding and electron transfer reactions between these proteins.

Methanol dehydrogenase possesses an $\alpha_2\beta_2$ structure with subunits of 67 and 9.5 kDa (Nunn et al., 1989; Davidson et al., 1992), noncovalently bound pyrroloquinoline quinone (PQQ) (Salisbury et al., 1979), and tightly bound Ca^{2+} (White et al., 1993). Methanol dehydrogenase is isolated in a stable semiquinone redox state (Frank et al., 1989). We have previously performed stopped-flow kinetic studies of the temperature dependence of the protein–protein association and the single-electron transfer reactions from the semiquinone

† This work was supported by National Institutes of Health Grants GM-41574 and GM-31611 and NSF Grant No. MCB-9342992.

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® Abstract published in *Advance ACS Abstracts,* September 15, 1994.

0006-2960/94/0433-12600$04.50/0

of methanol dehydrogenase to the oxidized form of cytochrome c-551i (Harris & Davidson, 1993). Analysis of the temperature dependence of the electron transfer reaction by Marcus theory (Marcus & Sutin, 1985) yielded values of 1.9 eV for the reorganizational energy and 0.071 cm^{-1} for the electronic coupling associated with this reaction and predicted a distance between redox centers of 15 Å. A K_d[1] value for complex formation of 375 μM was determined kinetically and by a direct ultrafiltration binding assay. These data were obtained at an ionic strength of 0.29 M. In this paper, we characterize the ionic strength dependence of the binding and electron transfer reactions between methanol dehydrogenase and cytochrome c-551i and discuss these data in the context of the known structures of these proteins. These results form the basis for a hypothesis regarding kinetic mechanisms of intramolecular electron transfer between proteins.

EXPERIMENTAL PROCEDURES

Proteins. Purifications of methanol dehydrogenase (Davidson et al., 1992) and cytochrome c-551i (Husain & Davidson, 1986) from *P. denitrificans* were as described previously. Methanol dehydrogenase was isolated as the semiquinone form of the enzyme (Frank et al., 1989). Methanol dehydrogenase concentrations were calculated from an $\epsilon_{1mg/mL}$ at 280 nm of 3.70 under native conditions, as given by a dry weight determination. Cytochrome c-551i concentrations were calculated from the known extinction coefficient (Husain & Davidson, 1986).

Kinetics. Stopped-flow experiments were performed with an On-Line Instrument Systems (OLIS, Bogart, GA) stopped-flow sample-handling unit coupled to Durrum optics. Data were collected and analyzed using OLIS software on an IBM-compatible 486 personal computer. Experiments were carried out by mixing methanol dehydrogenase in 0.1 M potassium phosphate buffer (pH 7.5) at varying NaCl concentrations, with an equal volume of cytochrome c-551i in the same buffer. Ionic strength was adjusted by the addition of NaCl, and in calculating ionic strength, correction was made for the association between monohydrogen phosphate and sodium and potassium (Smith & Alberty, 1956). Reduction of oxidized cytochrome c-551i by methanol dehydrogenase was monitored at 551 nm ($\Delta\epsilon = 13.3\ mM^{-1}\ cm^{-1}$) where absorbance by methanol dehydrogenase is minimal. Under conditions where the varied reactant, methanol dehydrogenase, was in excess of the concentration of the fixed reactant, cytochrome c-551i, the observed rate constants (k_{obs}) at each temperature could be determined from the data fit to the equation for a single-exponential rise:

$$\Delta A_{551nm} = C(1 - e^{-k_{obs}t}) + b \quad (1)$$

where C is a constant related to the initial absorbance and b represents an offset value to account for a non-zero baseline (Figure 1). All observed absorbance changes were monophasic, indicating that the reduction of cytochrome c-551i was pseudo-first-order under these conditions. The k_{obs} for each reaction was determined from the average of at least three measurements. Nonlinear curve fitting of data was performed with the Enzfitter (Elsevier-BIOSOFT, Cambridge, U.K.)

[1] Abbreviations: H_{AB}, electronic coupling; K, equilibrium binding constant for the protein–protein association reaction; K_d, dissociation constant; k_{ET}, electron transfer rate constant; λ, reorganizational energy; μ, ionic strength; P, dipole moment; PQQ, pyrroloquinoline quinone; θ, angle between the dipole moment vector and the vector from the center of mass to the reaction site on the surface for each protein reactant.

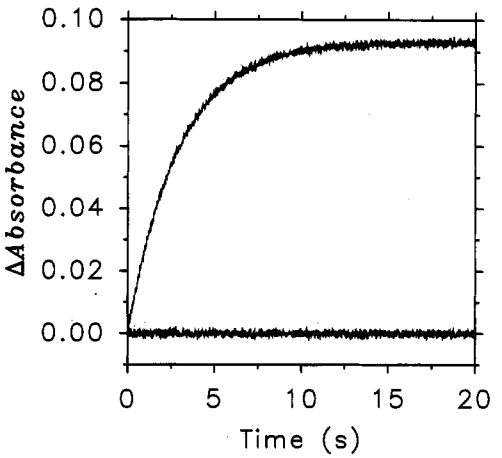

FIGURE 1: Reduction of cytochrome c-551i by methanol dehydrogenase. Oxidized cytochrome c-551i (3.5 μM) was mixed with methanol dehydrogenase semiquinone (74.2 μM) in 0.1 M potassium phosphate (pH 7.5) with 0.1 M NaCl at 30 °C. The conversion of cytochrome c-551i from oxidized to reduced was monitored at 551 nm with a 2 cm path length. The solid line is a fit of the experimental data to eq 1. The residuals (experimental curve – calculated curve) are plotted near the origin of the y axis.

and Sigma Plot 5.0 (Jandel Scientific, San Raphael, CA) computer programs.

Electrostatic Theory. The ionic strength of a solution determines the distance over which the electrostatic field of an ion extends with appreciable strength, and the reciprocal of this ionic atmosphere radius (κ) is given by

$$\kappa = \sqrt{e^2 N_A (2 \times 10^{-27})/\epsilon_0 \epsilon k_B T}\sqrt{\mu} \quad (2)$$

where e is the elementary charge on a proton (1.602×10^{-19} C), N_A is Avogadro's number ($6.022 \times 10^{23}\ mol^{-1}$), ϵ_0 is the permittivity constant ($8.854 \times 10^{-22}\ C^2\ J^{-1}\ Å^{-1}$), ϵ is the dielectric constant of water (76.54 at 303 K), k_B is the Boltzmann constant ($1.3807 \times 10^{-23}\ J\ K^{-1}$), T is the absolute temperature (K), and the ionic strength (μ) is in terms of molarity. The units of κ reduce to $Å^{-1}$ by including the factor 2×10^{-27}.

The ionic strength dependence of the association constant for two large dipolar proteins is described according to Van Leeuwen theory (1983):

$$\ln K_\mu = \ln K_{inf} - [Z_1Z_2 + (\mathbf{ZP})(1 + \kappa R) + (\mathbf{PP})(1 + \kappa R)^2]\frac{e^2}{4\pi\epsilon_0\epsilon k_B T R} f(\kappa) \quad (3)$$

$$f(\kappa) = \frac{1 - \exp(-2\kappa R_2)}{2\kappa R_2(1 + \kappa R_1)} \quad (4)$$

where K_μ and K_{inf} are association constants at a given ionic strength μ and at infinite ionic strength, respectively; Z_1 and Z_2 are net charges; R_1 and R_2 are the radii of the two reacting proteins, with $R = R_1 + R_2$ as defined by Van Leeuwen (1983); and κ is defined by eq 2. The term Z_1Z_2 describes the isotropic monopole–monopole interaction and depends on the magnitude and sign of the net charge of each of the two proteins. Most proteins contain an anisotropic distribution of charges and therefore have a dipole moment (vectors **P** with magnitudes P). In such cases, the association constant also becomes dependent on the relative orientation of the reactants given by the monopole–dipole interaction (**ZP**) (eq 5) and the dipole–dipole interaction (**PP**) (eq 6):

$$(ZP) = \frac{Z_1P_2 \cos\theta_2 + Z_2P_1 \cos\theta_1}{eR} \quad (5)$$

$$(PP) = \frac{P_1P_2 \cos\theta_1 \cos\theta_2}{(eR)^2} \quad (6)$$

P_1 and P_2 are the dipole moments of the two reactants and θ_1 and θ_2 correspond to the angle between the dipole moment vector and the vector from the center of mass to the reaction site on the surface for each protein reactant. The angles θ_1 and θ_2 do not specify unique sites, but rather a band of sites equatorial to the dipole vector of the molecule. For ionic strengths below 0.1 M, the dipole moment does not significantly contribute to the ionic strength dependence, which is dominated by the monopole–monopole interaction (Z_1Z_2). However, at ionic strengths above 0.1 M, dipolar interactions are the dominant contributing force (Van Leeuwen, 1983). Under physiological conditions, the ionic strength is about $\mu = 0.1$–0.2 M, and the orientation of the dipole moments of the two reacting proteins relative to the active site is very important and may explain the specificity of many protein–protein interactions.

Calculation of Dipoles. Dipoles were calculated using the programs QUANTA and CHARMm (Molecular Simulations, Burlington, MA) using

$$P = \sum q_i r_i$$

where q_i is the partial charge on the ith atom and r_i is the position of that atom relative to the center of mass on that molecule. The sum is over all atoms. The pseudocharges at the ends of an α-helix to account for the parallel orientation of the carbonyl groups, therefore, are not needed since the dipoles arising from the carbonyls are included explicitly, along with those from all other atoms.[2] The partial charges are assigned by the program according to Momany and Rome (1992) by first assigning atom types to each atom. This assignment is based on a consideration of the bonding environment for each atom, its element type, and the element types of those atoms bonded to it. Partial charges are then assigned by using a charge template for that atom type and smoothed as necessary to give the molecule as a whole an integral charge that will depend on the number and type of charged residues.

RESULTS

Stopped-Flow Kinetic Studies. Values of k_{obs} were obtained at different ionic strengths by varying the methanol dehydrogenase concentration (36–375 μM) with a fixed concentration of cytochrome *c*-551i (3.5 μM) (Figure 2). It was not possible to obtain data at higher concentrations due to the requirement of excessively high concentrations of methanol dehydrogenase (>750 μM before mixing). The reduction of cytochrome *c*-551i by methanol dehydrogenase at ionic strengths less than 0.1 M was undetectably slow. Monopole–monopole interactions are predominant at low ionic strengths, and therefore the reaction may be inhibited by a strong repulsion between these two highly acidic proteins. Detectable reactions occurred at higher ionic strengths, and k_{obs} showed a hyperbolic concentration dependence with respect to methanol dehydrogenase (Figure 2). This saturation effect is evidence for a process involving a minimum of two steps in

[2] Personal communication from Dr. Robert B. Funchess, senior support scientist, Molecular Simulations.

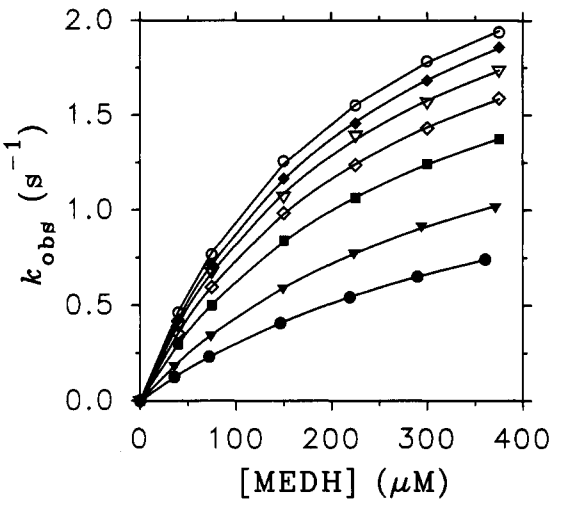

FIGURE 2: Dependence of the observed rate constants (k_{obs}) for the reduction of cytochrome *c*-551i as a function of methanol dehydrogenase concentration and ionic strength. Stopped-flow measurements were made as described under Experimental Procedures at ionic strengths 0.21 (●), 0.29 (▼), 0.34 (■), 0.80 (◆), 1.3 (○), 1.9 (▽), and 2.9 M (◇). Solid lines are fits of each data set to eq 9.

which an initial binding step equilibrates much faster than the following electron transfer step.

The simplest kinetic mechanism for the electron transfer reaction, which includes a complex formation step, is given by eq 7, where oxidized cytochrome *c*-551i and methanol dehydrogenase semiquinone [$MEDH_{sem}$] react to form reduced cytochrome *c*-551i and oxidized methanol dehydrogenase [$MEDH_{ox}$]. In this scheme, k_3 is the forward rate constant

$$MEDH_{sem} + \text{cyt } c\text{-}551i_{ox} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} [MEDH_{sem}{}^{*}\text{cyt } c\text{-}551i_{ox}] \underset{k_4}{\overset{k_3}{\rightleftharpoons}} [MEDH_{ox}{}^{*}\text{cyt } c551i_{red}] \quad (7)$$

of the electron transfer reaction and k_4 is the rate of the reverse reaction, if there is one. The ratio of the rate constants, k_1/k_2, describes the equilibrium binding constant for protein–protein complex association. Thus, k_3 may be considered k_{ET}, and K_d is equal to k_2/k_1. As discussed in Harris and Davidson (1993), the data were analyzed according to eq 8. The rapid

$$k_{obs} = \frac{k_3[MEDH]}{[MEDH] + K_d} + k_4 \quad (8)$$

equilibrium assumption used to derive eq 8 will be true for many cases where complex formation of the reacting protein occurs in a fast, diffusion-controlled process followed by a slow, rate-determining electron transfer step.

At each ionic strength, the y-intercepts for plots of k_{obs} versus the concentration of methanol dehydrogenase passed through the origin (Figure 2), indicating that k_4 is essentially zero (Strickland et al., 1975). The finding that this electron transfer reaction is irreversible is consistent with the redox potentials of the reactants (Harris & Davidson, 1993). When the reverse reaction for the electron transfer is negligible (*i.e.*, $k_4 = 0$), eq 8 is reduced to eq 9, which was used to fit the data shown in Figure 2. The kinetic parameters for the reduction

$$k_{obs} = \frac{k_3[MEDH]}{[MEDH] + K_d} \quad (9)$$

of cytochrome *c*-551i by methanol dehydrogenase are given in Table 1.

Table 1: Kinetic Parameters for the Reduction of Cytochrome c-551i by Methanol Dehydrogenase[a]

ionic strength (M)	K_d (μM)	k_{ET} (s^{-1})
0.21	451 ± 7	1.67 ± 0.02
0.29	359 ± 13	2.01 ± 0.04
0.34	287 ± 4	2.43 ± 0.02
0.80	249 ± 4	3.08 ± 0.03
1.3	226 ± 6	3.12 ± 0.04
1.9	247 ± 11	2.88 ± 0.06
2.9	264 ± 4	2.70 ± 0.02

[a] Experiments were performed as described under Experimental Procedures. Kinetic parameters were obtained from a fit of the data in Figure 2 to eq 9.

The K_d value for complex formation decreased 2-fold as the ionic strength was increased from 0.21 to 1.3 M and then increased slightly as the ionic strength was further increased to 2.9 M (Table 1). Likewise, the value of k_3 increased 2-fold with increasing ionic strength, reaching a maximum at 1.3 M, and decreased slightly at higher ionic strengths. It is possible that the deviation at high ionic strengths is due to some nonspecific effect, such as a conformational change unrelated to the reaction of interest. However, in subsequent analyses of the ionic strength dependence of both K_d and k_3 according to the Van Leeuwen equation (eq 3), essentially the same fits were obtained with or without inclusion of the data points for the two highest ionic strengths. Thus, this behavior appears to be relevant. Tighter complex formation with increasing ionic strength is indicative of electrostatic interactions between two reactants of like charges, as discussed earlier.

Ionic Strength Dependence of the Association Constant. In order to evaluate eq 3, the parameters R_1, R_2, Z_1, Z_2, P_1, P_2, θ_1, θ_2, and ln K_{inf} must be known or solved for. For a protein of molecular weight M_r, the radius may be estimated from eq 10 (Tanford, 1961; Wherland & Gray, 1976; Brothers et al., 1993):

$$R = 0.717 M_r^{1/3} \quad (10)$$

which yields values of $R_1 = 38.3$ Å for methanol dehydrogenase and $R_2 = 18.6$ Å for cytochrome c-551i. The charge on the protein may be estimated from the amino acid compositions of methanol dehydrogenase (Harms et al., 1987; Van Spanning et al., 1991) and cytochrome c-551i (Van Spanning et al., 1991). At pH 7.5, it was assumed that all glutamates and aspartates were deprotonated, all lysines were protonated, arginine was in its monopositive form, the terminal amino group was protonated, the terminal carboxyl group was deprotonated, and half of the histidines were protonated. For cytochrome c-551i, it was assumed that the propionic side chains of the heme moiety were deprotonated and that the charge of the iron atom in its oxidized state was +1. For methanol dehydrogenase, the charges associated with the carboxyl groups of the PQQ prosthetic group and Ca^{2+} were included. These calculations estimated a net charge of $Z_1 = -43$ for methanol dehydrogenase and a net charge of $Z_2 = -18$ for cytochrome c-551i. The net charge estimated for cytochrome c-551i in this manner was essentially the same as that computed by QUANTA from the structure after the assignment of partial charges to each atom. It should be noted that, because of the relatively large charge on each protein, small errors in calculating the net charge do not significantly affect fits of the data to eq 3.

The orientation of the dipole moment vector for cytochrome c-551i (Figure 3A) was determined as described earlier from the crystal structure (Chen et al., 1994), and the magnitude of the dipole moment (P_2) was calculated to be 367 D.

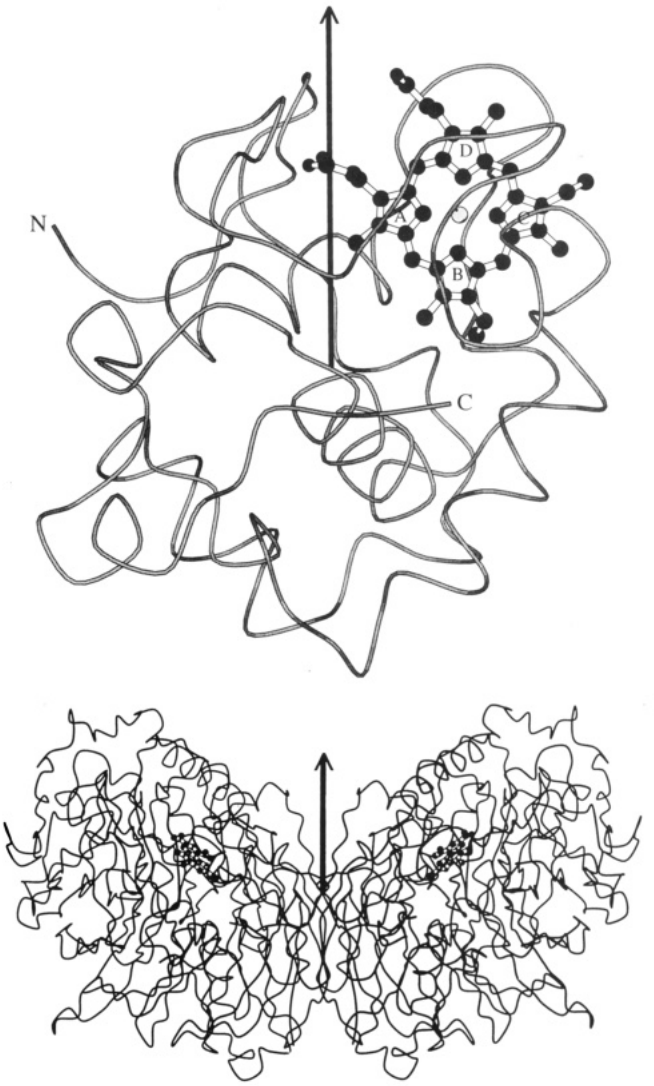

FIGURE 3: (A, top) Backbone ribbon and heme group of cytochrome c-551i from *P. denitrificans* (Chen et al., 1994) oriented with the positive dipole vector verticle (arrow). The rings of the heme group are labeled. (B, bottom) Backbone ribbon of the methanol dehydrogenase dimer from *Methylophilus* W3A1 (Xia et al., 1992) with the PQQ prosthetic group included. The positive direction of the dipole vector estimated using the sequence from *P. denitrificans* (see text) is indicated by the vertical arrow. The figure was made using the program MOLSCRIPT (Kraulis, 1991).

Although the three-dimensional structure of methanol dehydrogenase from *P. denitrificans* is not known, the crystal structure of a homologous methanol dehydrogenase from *Methylophilus* W3A1 has been determined (Xia et al., 1992). In this structure, the pair of heterodimers is related by a molecular 2-fold axis of symmetry. Because of this symmetry, the dipole moment vector will lie along this axis, with its direction dependent on the distribution of charges. The published sequence for methanol dehydrogenase from *P. denitrificans* (Harms et al., 1987; Van Spanning et al., 1991) gave a satisfactory match to the electron density maps in this analysis (Xia et al., 1992), and this model indicates that the dipole moment vector is oriented along the molecular 2-fold axis, as shown in Figure 3B.[3] The magnitude of the dipole moment for methanol dehydrogenase from *P. denitrificans* (P_1) cannot be determined precisely because this methanol dehydrogenase contains extensions of amino acid residues in the tail regions of both the large and small subunits that are not present in the W3A1 structure. However, we have assumed that the direction of the dipole vector is the same.

The PQQ prosthetic group of methanol dehydrogenase is located in a funnel-shaped central channel that lies along a pseudo-8-fold axis of symmetry of the large subunit, which is inclined by approximately 45° to the molecular 2-fold axis. The nearest surface side chains are located within the top of this funnel approximately 10 Å from PQQ. It has been estimated by Marcus theory (Harris & Davidson, 1993) and by preliminary modeling (Chen et al., unpublished results) that the nearest that the surface of a globular protein such as the cytochrome could be positioned to the PQQ prosthetic group would be approximately 15 Å. This suggests that the interaction site for the cytochrome on methanol dehydrogenase consists of a ring of surface residues at the top of the central channel that makes an average angle θ_1 of about 45° to the dipole moment vector. This is a reasonable estimate because there is very little accessible surface at angles less than 45°, as this includes merely the conical space between the two heterodimers. Methanol dehydrogenase surfaces located at angles greater than 45° are sterically accessible only at regions that are between 25 and 40 Å from PQQ due to the loss of surface area of each subunit at the interface between the two heterodimers. This distance would be inconsistent with the distance predicted from solution studies.

In cytochrome *c*-551i, the methyl and part of the propionate side chains on pyrrole ring D (Figure 3A) are exposed. The methyl group corresponds to an angle of approximately 29°, while the propionate group corresponds to an angle of approximately 14° relative to the dipole vector. The vinyl and methyl carbon atoms on pyrrole ring C of the heme are also exposed. The vinyl group corresponds to an angle of approximately 50°, while the methyl group corresponds to an angle of approximately 57° relative to the dipole vector. A reaction site at another position on the surface outside these ranges of θ_2, where the heme is not exposed, would require distances too long for the electron transfer reaction from PQQ, based on predictions from solution studies (Harris & Davidson, 1993). Given the possible errors inherent in the calculation of the dipole vector, it is reasonable to assume that these angles should be considered crude approximations.

The relationship between K_d and ionic strength is shown in Figure 4A. The unknown values of P_1 (dipole moment of methanol dehydrogenase), cos θ_2 (angle associated with the cytochrome), and ln K_{inf} in eq 3, into which eqs 5 and 6 are substituted, cannot be uniquely solved for because the data can be fit successfully by any number of combinations of these three values. The relationship between P_1 and θ_2, which is illustrated in Figure 5, was obtained by inputting into eq 3 different *fixed* theoretical values of P_1 ranging from 0 to 2000 D. This covers the range of dipole moments that have been observed for most proteins. Similarly, the relationship between P_1 and ln K_{inf} is illustrated in Figure 6A. Fits of the data to eq 3 at P_1 values ranging from 0 to 700 D were unacceptable because they yielded very large standard errors in θ_2. Solutions for θ_2 in eq 3 with standard errors <10% were obtained at P_1 values ranging from 700 to 2000 D. It can be seen from Figure 5 that the minimum value with reasonable error for the angle θ_2 associated with the cytochrome is about 35°. As the value of the dipole moment of methanol dehydrogenase (P_1) increases, the value for θ_2 also increases. These simulations suggest that neither the exposed propionate nor the methyl group on pyrrole ring D of the heme, which are located

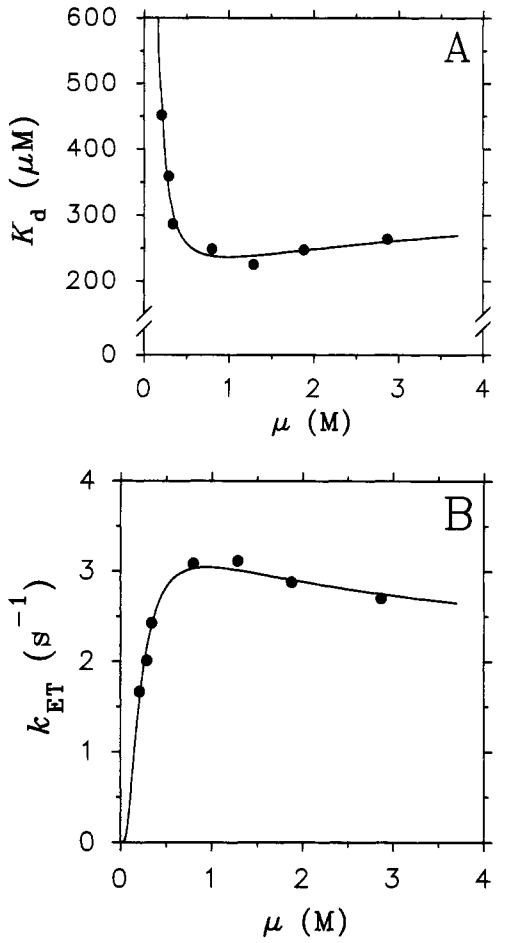

FIGURE 4: (A) Dependence of the dissociation constant on the ionic strength. The solid line represents the fits of the values of K_d to the Van Leeuwen equation (eq 3). (B) Dependence of the apparent transfer rate constant (k_3) on the ionic strength. The solid line represents the fits of the values of k_3 to the Van Leeuwen equation (eq 3).

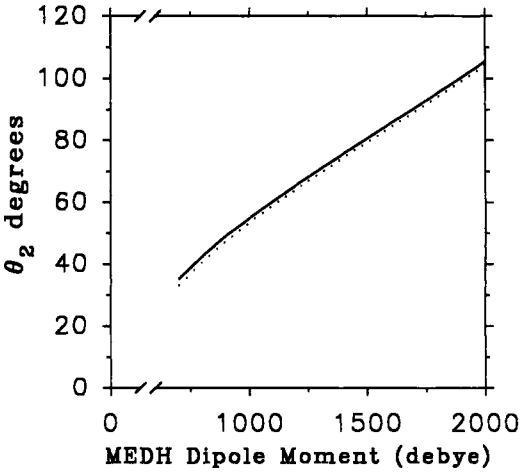

FIGURE 5: Dependence of the fitted parameter, θ_2 (angle associated with cytochrome *c*-551i), on given fixed values of the magnitude of the methanol dehydrogenase dipole moment (P_1) for the ionic strength dependence of K (—) and k_{ET} (···). The solid line represents values of θ_2 obtained from a fit to eq 3 of the data shown in Figure 4A using fixed values of P_1 (dipole moment of methanol dehydrogenase) over the range 700–2000 D. The dotted line represents values of θ_2 obtained from a fit of the data shown in Figure 4B using fixed values of P_1 over the range 700–2000 D.

[3] Structural conservation among methanol dehydrogenases is further supported by the fact that the structure of methanol dehydrogenase from *Methylobacterium organophilum* XX could be readily solved by molecular replacement using the W3A1 structure as a search molecule. This work is described in an abstract submitted to the 1994 American Crystallographic Association meeting in Atlanta, GA. The authors are R. Radhakrishan, C. A. Earhart, W. A. Froland, D. H. Dyer, J. D. Lipscomb, D. H. Ohlendorf, Z.-X. Xia, and F. S. Mathews.

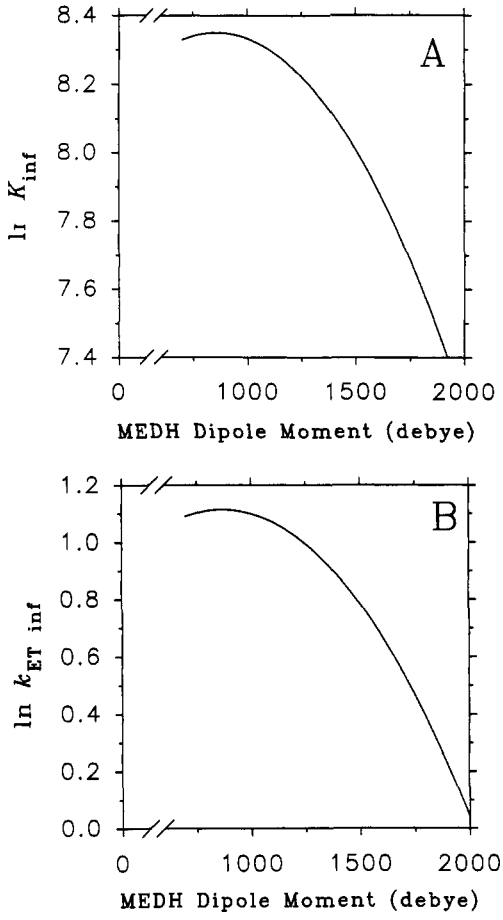

FIGURE 6: (A) Dependence of the fitted parameter, ln K_{inf}, on given fixed values of the magnitude of the methanol dehydrogenase dipole moment (P_1) for the ionic strength dependence of ln K. The solid line represents values of ln K_{inf} obtained from a fit to eq 3 of the data shown in Figure 4A, using fixed values of P_1 (dipole moment of methanol dehydrogenase) over the range 700–2000 D. (B) Dependence of the fitted parameter, ln $k_{ET,inf}$, on given fixed values of the magnitude of the methanol dehydrogenase dipole moment (P_1) for the ionic strength dependence of k_{ET}. The solid line represents values of ln $k_{ET,inf}$ obtained from a fit to eq 3 of the data shown in Figure 4B using fixed values of P_1 over the range 700–2000 D.

approximately 14° and 29°, respectively, from the dipole vector, is likely to be the reacting site. However, the exposed vinyl group at θ = 50° and the methyl group at θ = 57° on pyrrole ring C are strong candidates for the reactive site.

It can be seen from Figure 6A that, for the P_1 range between 700 and 2000 D, the value of ln K_{inf} increases to a maximum at P_1 = 850 D and then decreases. The binding constant that was actually determined at the highest ionic strength is probably a good approximation of ln K_{inf}. By assuming ln K_{inf} = 8.24 (Table 1, μ = 2.9 M), Figure 6A gives the value P_1 = 1225 D, which corresponds to a θ_2 value of approximately 67° according to Figure 5. Given the potential error associated with these measurements and calculations, this value correlates reasonably closely to the value of θ_2 associated with the methyl group on pyrrole ring C of the heme. It has been generally assumed that electron transfer between an external redox partner and horse heart cytochrome c occurs via the exposed methyl group of pyrrole ring C of the heme where the ring of positive surface charges around the heme edge matches the negative charges on the recognition site of the partner protein (Zhou & Kostic, 1993; Hahm et al., 1992; Corin et al., 1991).

Ionic Strength Dependence of the Electron Transfer Rate Constant. The rate constant for the electron transfer reaction (k_{ET}) between methanol dehydrogenase and cytochrome c-551i was also found to be ionic strength dependent (Table 1). Van Leeuwen (1983) suggested that k_{ET} will be ionic strength dependent if dipole moments are important. These data were analyzed exactly as described earlier for the ionic strength dependence of the association constants. The values for the angle associated with the cytochrome (θ_2) and ln $k_{ET,inf}$ were determined from a fit of the data for the ionic strength dependence of k_{ET} to eq 3 (Figure 4B) at different fixed values of P_1. The relationship between P_1 and θ_2 is shown in Figure 5 and that between P_1 and ln $k_{ET,inf}$ in Figure 6B. The relative magnitudes of the ionic strength dependence of both K and k_{ET} were very similar. This suggests that the optimal orientation for electron transfer is similar to that for binding. Any difference may be attributed to some rearrangement of the proteins after binding to produce the most efficient orientation for electron transfer.

DISCUSSION

Analysis of the ionic strength dependence of a reaction between proteins may potentially provide interesting information about protein orientations necessary for their interaction and function. When the structure of each reactant is known, the observed ionic strength dependence may be analyzed with respect to monopole–monopole, monopole–dipole, and dipole–dipole interactions (Van Leeuwen, 1983). Such an analysis considers the overall charge distribution of the reactants. Kostic and co-workers have applied Van Leeuwen theory to investigate the ionic strength dependence of bimolecular rate constants for electron transfer reactions between cytochromes c and plastocyanin (Zhou & Kostic, 1992, 1993a; Brothers et al., 1993). Alternatively, it has been shown that for the reactions between flavodoxin and c-type cytochromes, electrostatic interactions may be governed more by the local charges of the interacting surface domains than by the overall charge (Tollin et al., 1984). Eltis et al. (1991) have combined electrostatic analysis and Brownian dynamics simulation to describe the reaction between cytochrome c and cytochrome b_5. In most of these studies, the electron transfer reactions were inhibited by increasing ionic strength, and in each case bimolecular electron transfer rate constants were analyzed. The reaction between the two acidic proteins, methanol dehydrogenase and cytochrome c-551i from *P. denitrificans*, however, was detectable only at ionic strengths greater than 0.1 M and approached limiting values for k_{obs}, so that the binding and electron transfer reactions could be separated.

One might intuitively expect that a true electron transfer reaction should be independent of ionic strength. There are a number of other reports of the ionic strength dependencies of electron transfer rates within protein complexes measured by direct transient state techniques. A slight decrease in the electron transfer rate constant from 85 s^{-1} at μ = 48 mM to 66 s^{-1} at μ = 76 mM was reported in the flavodoxin–cytochrome c complex (Simondsen et al., 1982), while a substantial decrease in the rate constant from 4000 s^{-1} at μ = 310 mM to 1600 s^{-1} at μ = 460 mM was reported for the ferredoxin–ferrodoxin-$NADP^+$ reductase complex (Bhattacharyya et al., 1986). The first-order rate-limiting process for the reduction of cytochrome oxidase by cytochrome c showed a marked ionic strength effect, with a maximum rate constant of 1470 s^{-1} occurring at μ = 110 mM and decreased rate constants of 630 s^{-1} at μ = 10 mM and 45 s^{-1} at μ = 510 mM (Hazzard et al., 1991). The rate constant for electron transfer within the cytochrome peroxidase–cytochrome c complex is also highly ionic strength dependent (Hazzard et al., 1988).

The limiting first-order rate constant could be a measure of the actual electron transfer step (which should be influenced

Scheme 1

$$A + B \underset{k_2}{\overset{k_1}{\rightleftharpoons}} C \underset{k_{4a}}{\overset{k_{3a}}{\rightleftharpoons}} D \underset{k_{4b}}{\overset{k_{3b}}{\rightleftharpoons}} E$$

Case 1a: $k_1, k_2, k_{3a}, k_{4a} >> k_{3b}, k_{4b}$

Case 1b: $k_1, k_2 >> k_{3a}, k_{4a} << k_{3b}, k_{4b}$

by redox potential), as well as any conformational steps that may exist along the reaction pathway (discussed later). Likewise, these previously reported ionic strength dependencies of electron transfer rate constants have been attributed to the formation of nonoptimal complexes that require geometrical rearrangement prior to electron transfer. When cytochrome *c*–plastocyanin and the physiological cytochrome *f*–plastocyanin electron transfer reaction partners were covalently cross-linked, the electron transfer reactivity of the proteins was abolished (Peerey et al., 1991; Qin & Kostic, 1993). These authors suggested that the cross-links prevented conformational fluctuations or protein rearrangements that take place in the electrostatic complex and accompany electron transfer. The conformation associated with the most stable complex, in many cases, therefore may not be the most favorable conformation for electron transfer to occur. If the k_{ET} for the reaction between methanol dehydrogenase and cytochrome *c*-551i contains a component that reflects such a conformational rearrangement, this would explain why k_{ET} is dependent on ionic strength.

The coupling between a conformational change and the electron transfer following complex formation can be described by a three-step kinetic model (Scheme 1). In this model, the ratio of the rate constants, k_2/k_1, describes the dissociation constant (K_d) for the initial binding exactly as was described for the first step of the two-step model in eq 7. The forward and reverse reactions for the electron transfer step described by k_3 and k_4 in eq 7 are now described by a two-step process, where the ratio of the rate constants, k_{3a}/k_{4a}, describes the conformational equilibrium (K_c) between the initial binding complex and the complex from which electron transfer occurs, while k_{3b} and k_{4b} are the forward and reverse electron transfer rate constants.

Consider the case where the first step (binding) and the second step (conformational change) equilibrate much faster than the third step (electron transfer), so that the rate of electron transfer is indeed the rate-limiting step (Scheme 1, case 1a). For this example, the rate of the reverse electron transfer reaction is neglible ($k_{4b} = 0$). The dependence of k_{obs} associated with the rate-limiting electron transfer step on the initial concentration of the varied reactant, $[S]_0$, when it is held in excess of the fixed reactant, is then given by (see Appendix)

$$k_{obs} = \frac{k_{3b}K_c[S]_0}{K_d + (1 + K_c)[S]_0} \qquad (11)$$

If the initial binding complex is much more stable than the complex necessary for electron transfer to occur, then the equilibrium for the conformational change or geometrical rearrangement between two proteins lies far to the left, so that $K_c \ll 1$. In such a situation, K_c drops out of the denominator in eq 11, giving the same form as the equation derived for the two-step process (eq 9) where rapid equilibration precedes a rate-determining electron transfer step. In such a case, the value for K_d is unaffected, but the rate-determining *apparent* electron transfer rate constant, k_3, is now equal to $k_{3b}K_c$ (Scheme 1, case 1a). Thus, the apparent electron transfer rate constant, k_{ETapp} (k_3) (eq 9), is reduced from the actual electron transfer rate constant, k_{3b}, by the factor K_c. The reported ionic strength dependencies of electron transfer rate constants may thus be a consequence of a preequilibrium process, defined by K_c, that is ionic strength dependent.

Now consider the alternative case where the first step (binding) equilibrates much faster than the slow, rate-determining second step (conformational change) followed by a fast electron transfer step (Scheme 1, case 1b). The dependence of k_{obs} associated with the apparent electron transfer on the initial concentration of the varied reactant $[S]_0$, when it is held in excess of the fixed reactant, is then given by eq 8, where k_3 and k_4 are actually the forward and reverse rate constants for the conformational change (k_{3a}, k_{4a}) and k_2/k_1 is still the dissociation constant, K_d. If this were the case, then the rate-determining rate constant would contain no contribution from the actual electron transfer reaction.

For the reaction between methanol dehydrogenase and cytochrome *c*-551i, the temperature dependence of this apparent electron transfer rate constant analyzed by Marcus theory (Marcus & Sutin, 1985) yielded values of 1.9 eV for the reorganizational energy and 0.07 cm^{-1} for the electronic coupling associated with this reaction and predicted a distance between redox centers of 15 Å (Harris & Davidson, 1993). The value for H_{AB} of 0.07 cm^{-1} is within the range of values observed in model systems (Onuchic et al., 1992). In addition, the value of 15 Å correlates closely with the minimum possible distance between redox centers for these two proteins. Although possible, it is highly unlikely that an analysis of the temperature dependence of a conformational change would yield such reasonable values for the Marcus electron transfer parameters. Therefore, it is reasonable to rule out the possibility of case 1b and assume that this reaction may be described as case 1a in Scheme 1. The actual electron transfer rate constant, k_{3b}, *does* contribute to the expression for the rate-determining apparent k_{ET} in eq 9 ($k_{ETapp} = k_{3b}K_c$). The relatively large value for the reorganizational energy, $\lambda = 1.9$ eV, in this system is consistent with the notion that the apparent k_{ET} may contain a contribution from some rearrangement of the proteins after a nonoptimal collision to produce the most efficient orientation for electron transfer.

Hoffman and Ratner (1987) described the coupling of a conformational equilibrium with an electron transfer reaction as a gated electron transfer, and they classify the case when conformational equilibrium is fast compared to the electron transfer rate as a fast gate and the case when conformational equilibrium is slow compared to the electron transfer rate as a slow gate. In contrast to Hoffman and Ratner (1987), Brunschwig and Sutin (1989) reserve the term gating only for cases where the observed rate constant is controlled by a *slow* conformational change, as in Scheme 1, case 1b. They explain that the conformational preequilibrium, when $K_c \ll 1$, contributes to the overall reaction barrier for the electron transfer reaction and is related by $K_c = \exp(-\lambda_c/RT)$. Therefore, the value for the overall reorganizational parameter, λ, contains contributions from the *actual* electron transfer step, λ_{3b}, as well as the conformational preequilibrium step, λ_c, so that $\lambda = \lambda_{3b} + \lambda_c$. Thus, the large apparent λ for this reaction is likely a consequence of the contribution from λ_c.

The contribution of a conformational preequilibrium that accompanies an electron transfer reaction between proteins appears to be common to both nonphysiological and physiological electron transfer partners. Zhou and Kostic (1993b) have recently described an apparent driving force dependency of when and if a reaction becomes gated. This has to do with whether the rate of electron transfer is faster or slower than the preceding conformational change. If the driving force is high, so that the electron transfer rate constant is maximized (when $\Delta G°$ approaches the value of λ), then the conformational step may become rate limiting and the reaction is gated. However, the electron transfer rate constant is more likely to be rate limiting as the driving force is decreased ($\Delta G° \ll \lambda$).

When the preceding conformational change occurs much faster than the following electron transfer step, the reaction is *not* gated, but is conformationally coupled. Conformationally coupled electron transfer may be a property of many electron transfer reactions between protein partners.

ACKNOWLEDGMENT

We thank Harold B. Brooks for helpful discussions, assistance in performing dipole calculations, and providing a computer program to facilitate calculations for ionic strength corrections.

APPENDIX

The time-dependent concentrations, c_A, c_B, c_C, c_D, and c_E, in Scheme 1 can be expressed in terms of their new equilibrium values and their time-dependent deviations from these equilibrium values (Eigen & DeMaeyer, 1963; Czerlinski, 1966; Bernasconi, 1976). The bar over the concentration symbols denotes the final equilibrium concentrations of each species:

$$c_A = \bar{c}_A + \Delta c_A \quad \text{(A.1a)}$$

$$c_B = \bar{c}_B + \Delta c_B \quad \text{(A.1b)}$$

$$c_C = \bar{c}_C + \Delta c_C \quad \text{(A.1c)}$$

$$c_D = \bar{c}_D + \Delta c_D \quad \text{(A.1d)}$$

$$c_E = \bar{c}_E + \Delta c_E \quad \text{(A.1e)}$$

According to the principle of mass conservation,

$$c_A + c_B = \bar{c}_A + \bar{c}_B \quad \text{(A.2a)}$$

$$c_A + c_C + c_D + c_E = \bar{c}_A + \bar{c}_C + \bar{c}_D + \bar{c}_E \quad \text{(A.2b)}$$

Substitution of eqs A.1 for c_A, c_B, c_C, c_D, and c_E in eqs A.2 leads to the following stoichiometric relations:

$$\Delta c_A = \Delta c_B \quad \text{(A.3)}$$

$$\Delta c_A + \Delta c_C + \Delta c_D + \Delta c_E = 0 \quad \text{(A.4)}$$

In order to derive the relaxation time associated with the rate-limiting third step, the rate equation must first be set up using a concentration variable that participates only in the slowest reaction. If it is assumed that the reverse reaction for the third step is negligible, i.e., $k_{4b} = 0$, and as long as the slower of either the binding step (k_1, k_2) or the conformational change step (k_{3a}, k_{4a}) equilibrates more rapidly than the final electron transfer step (k_{3b}, k_{4b}), the linearized rate equation for the species that participates only in the slowest reaction is given by

$$\frac{d\Delta c_E}{dt} = k_{3b}\Delta c_D \quad \text{(A.5)}$$

The next step is to transform eq A.5 into the characteristic form for chemical relaxations where the perturbation is small, given by

$$\frac{d\Delta c_E}{dt} = -\left(\frac{1}{\tau_3}\right)\Delta c_E \quad \text{(A.6)}$$

In eq A.6, $1/\tau_3$ is the reciprocal of the relaxation time associated with the third step, which is equivalent to the observed rate constant (k_{obs}) for that step. For this transformation, the term Δc_D in eq A.5 must be expressed in terms of Δc_E. This can be obtained by rearranging eq A.4 from mass balance considerations and finding suitable expressions for Δc_A and Δc_C in terms of Δc_D:

$$-\Delta c_E = \Delta c_A + \Delta c_C + \Delta c_D \quad \text{(A.7)}$$

The assumption that the first two steps are at equilibrium while the rate-limiting electron transfer process is relaxing allows further equations relating Δc_A, Δc_C, Δc_D, and Δc_E to be written. Following a perturbation, the equilibrium position for the first step (binding) (K_1) and second step (conformational change) (K_c) is reached very rapidly, before any significant equilibration of the third step takes place. This leads to a set of time-dependent pseudoequilibrium concentrations, $c_A^{1,2}$, $c_B^{1,2}$, $c_C^{1,2}$, and $c_D^{1,2}$, that have not yet reached the final equilibrium concentrations. Under pseudo-first-order conditions, the difference between the two is typically small, as $K_1^{1,2} = K_1$ and $K_c^{1,2} = K_c$ are related through

$$\frac{c_C^{1,2}}{c_A^{1,2}c_B^{1,2}} = \frac{\bar{c}_C}{\bar{c}_A\bar{c}_B} = K_1 = \frac{k_1}{k_2} \quad \text{(A.8a)}$$

$$\frac{c_D^{1,2}}{c_C^{1,2}} = \frac{\bar{c}_D}{\bar{c}_C} = K_c = \frac{k_{3a}}{k_{4a}} \quad \text{(A.8b)}$$

In other words, $c_A^{1,2}$, $c_B^{1,2}$, $c_C^{1,2}$, and $c_D^{1,2}$ exist after the first two steps have reached equilibrium, but equilibration of the third step has not yet occurred. Final equilibrium concentrations exist after all three steps have reached equilibrium. By expressing $c_A^{1,2} = \bar{c}_A + \Delta c_A$, $c_B^{1,2} = \bar{c}_B + \Delta c_B = \bar{c}_B + \Delta c_A$, $c_C^{1,2} = \bar{c}_C + \Delta c_C$, and $c_D^{1,2} = \bar{c}_D + \Delta c_D$, eqs A.8a and A.8b can be written as

$$K_1 = \frac{\bar{c}_C}{\bar{c}_A\bar{c}_B} = \frac{(\bar{c}_C + \Delta c_C)}{(\bar{c}_A + \Delta c_A)(\bar{c}_B + \Delta c_B)} \quad \text{(A.9a)}$$

$$K_c = \frac{\bar{c}_D}{\bar{c}_C} = \frac{(\bar{c}_D + \Delta c_D)}{(\bar{c}_C + \Delta c_C)} \quad \text{(A.9b)}$$

Expansion of eq A.9a results in

$$\bar{c}_A\bar{c}_B\bar{c}_C + \bar{c}_A\bar{c}_C\Delta c_A + \bar{c}_B\bar{c}_C\Delta c_A + \bar{c}_C\Delta c_A^2 = \bar{c}_A\bar{c}_B\bar{c}_C + \bar{c}_A\bar{c}_B\Delta c_C \quad \text{(A.10)}$$

By crossing out like terms and dividing by c_Ac_B, eq A.10 is simplified and expressed in terms of Δc_C:

$$\Delta c_C = K_1\bar{c}_A\Delta c_A + K_1\bar{c}_B\Delta c_A + K_1\Delta c_A^2 \quad \text{(A.11)}$$

If only small equilibrium perturbations are considered, such that $\Delta c_A \ll c_A$, then the squared term can be neglected and

eq A.11 becomes

$$\Delta c_C = K_1(\bar{c}_A + \bar{c}_B)\Delta c_A \quad (A.12)$$

and finally in terms of Δc_A:

$$\Delta c_A = \frac{\Delta c_C}{K_1(\bar{c}_A + \bar{c}_B)} \quad (A.13)$$

Likewise, eq A.9b reduces to

$$\Delta c_C = \frac{\Delta c_D}{K_c} \quad (A.14)$$

Substitution of eq A.14 into eq A.13 gives Δc_A in terms of Δc_D:

$$\Delta c_A = \frac{\Delta c_D}{K_1 K_c(\bar{c}_A + \bar{c}_B)} \quad (A.15)$$

Substitution of eqs A.14 and A.15 into eq A.4 gives eq A.16 in terms of Δc_D and Δc_E:

$$-\Delta c_E = \frac{\Delta c_D}{K_1 K_c(\bar{c}_A + \bar{c}_B)} + \frac{\Delta c_D}{K_c} + \Delta c_D \quad (A.16)$$

Simplification of eq A.16 in terms of Δc_D yields

$$\Delta c_D = -\Delta c_E\left[\frac{K_1 K_c(\bar{c}_A + \bar{c}_B)}{1 + K_1(\bar{c}_A + \bar{c}_B) + K_1 K_c(\bar{c}_A + \bar{c}_B)}\right] \quad (A.17)$$

Substitution of eq A.17 for Δc_D in the original linearized rate equation (eq A.5) leads to an equation in the form of eq A.6, where

$$k_{obs} = \frac{1}{\tau_3} = \frac{k_{3b}K_1 K_c(\bar{c}_A + \bar{c}_B)}{1 + K_1(\bar{c}_A + \bar{c}_B) + K_1 K_c(\bar{c}_A + \bar{c}_B)} \quad (A.18)$$

In stopped-flow experiments, the varied reactant is often held in vast excess of the fixed reactant in order to maintain pseudo-first order conditions, and therefore, it is convenient to use the initial concentration of the reactant in vast excess in place of the equilibrium concentration terms. If eq A.18 is divided by K_1, then it becomes (see eq 11 in Discussion)

$$k_{obs} = \frac{k_{3b}K_c[S]_0}{K_d + (1 + K_c)[S]_0}$$

REFERENCES

Bamford, C. W., & Quayle, J. R. (1979) *Arch. Microbiol. 119*, 91–97.

Bhattacharyya, A. K., Meyer, T. E., & Tollin, G. (1986) *Biochemistry 25*, 4655–4661.

Brothers, H. M., II, Zhou, J. S., & Kostic, N. M. (1993) *J. Inorg. Organomet. Polym. 3*, 59–76.

Brunschwig, B. S., & Sutin, N. (1989) *J. Am. Chem. Soc. 111*, 7454–7465.

Chen, L., Durley, R., Mathews, F. S., & Davidson, V. L. (1994) *Science 264*, 86–89.

Corrin, A. F., McLendon, G., Zhang, Q., Hake, R. A., Falvo, J., Lu, K. S., Ciccarelli, R. B., & Holzschu, D. (1991) *Biochemistry 30*, 11585–11595.

Davidson, V. L., & Kumar, M. A. (1989) *FEBS Lett. 245*, 271–273.

Davidson, V. L., Wu, J., Miller, B., & Jones, L. H. (1992) *FEMS Microbiol. Lett. 94*, 53–58.

Eltis, L. D., Herbert, R. G., Baker, P. D., Mauk, A. G., Northrop, S. H. (1991) *Biochemistry 30*, 3663–3674.

Frank, J., Dijkstra, M., Balny, C., Verwiel, P. E. J., & Duine, J. A., (1987) in *PQQ and Quinoproteins* (Jongejan, J. A., & Duine, J. A., Eds.) pp 239–241, Kluwer Academic Publishers, Dordrecht, The Netherlands.

Hahm, S., Durham, B., & Millet, F. (1992) *Biochemistry 31*, 3472–3477.

Harms, N., deVries, G. E., Maurer, K., Hoogen dijk, J. & Stouthamer, A. H. (1987) *J. Bacteriol. 169*, 3969–3975.

Harris, T. K., & Davidson, V. L. (1993) *Biochemistry 32*, 14145–14150.

Hazzard, J. T.; Moench, S. J.; Erman, J. E., Satterlee, J. D., & Tollin, G. (1988) *Biochemistry 27*, 2002–2008.

Hazzard, J. T., Rong, S.-Y., & Tollin, G. (1991) *Biochemistry 30*, 213–222.

Hoffman, B. M., & Ratner, M. A. (1987) *J. Am. Chem. Soc. 109*, 6237–6243.

Husain, M., & Davidson, V. L. (1986) *J. Biol. Chem. 261*, 8577–8580.

Kraulis, P. J. (1991) *J. Appl. Crystallogr. 24*, 946.

Long, A. R., & Anthony, C. (1991) *J. Gen. Microbiol. 137*, 415–425.

Marcus, R. A., & Sutin, N. (1985) *Biochim. Biophys. Acta 811*, 265–322.

Momany, F. A., & Rone, R. (1992) *J. Comput. Chem. 13*, 888–900.

Nunn, D. N., Day, D., & Anthony, C. (1989) *Biochem. J. 260*, 857–862.

Onuchic, J. N., Beratan, D. N., Wrinkler, J. R., & Gray, H. B. (1992) *Annu. Rev. Biophys. Biomol. Struct. 21*, 349–377.

Peerey, L. M., Brothers, H. M., II, Hazzard, J. T., Tollin, G., & Kostic, N. M. (1991) *Biochemistry 30*, 9297–9304.

Qin, L., & Kostic, N. M. (1993) *Biochemistry 32*, 6073–6080.

Salisbury, H. A., Forrest, H. A., Cruse, W. B. T., & Kennard, O. (1979) *Nature 280*, 843–844.

Simondsen, R. P., Weber, P. C., Salemme, F. R., & Tollin, G. (1982) *Biochemistry 21*, 6366–6375.

Smith, R. M., & Alberty, R. A. (1956) *J. Phys. Chem. 60*, 180–184.

Strickland, S., Palmer, G., & Massey, V. (1975) *J. Biol. Chem. 250*, 4048–4052.

Tanford, C. (1961) *Physical Chemistry of Macromolecules*, pp 336–341, John Wiley, New York.

Tollin, G., Cheddar, G., Watkins, J. A., Meyer, T. E., & Cusanovich, M. A. (1984) *Biochemistry 23*, 6345–6349.

van Leeuwen, J. W. (1983) *Biochim. Biophys. Acta 743*, 408–421.

van Spanning, R. J. M., Wansell, C. W., de Boer, T., Hazelaar, M. J., Anazawa, H., Harms, N., Oltmann, L. F., & Stouthamer A. H. (1991) *J. Bacteriol. 173*, 6948–6961.

Wherland, S., & Gray, H. B. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 2950–2954.

White, S., Boyd, G., Mathews, F. S., Xia, Z.-X., Dai, W.-W., Zhang, Y.-F., & Davidson, V. L. (1993) *Biochemistry 32*, 12955–12958.

Xia, Z., Dai, W., Xiong, J., Hao, Z., Davidson, V. L., & Mathews, F. S. (1992) *J. Biol. Chem. 267*, 22289–22297.

Zhou, J. S., & Kostic, N. M. (1992) *Biochemistry 31*, 7543–7550.

Zhou, J. S., & Kostic, N. M. (1993a) *Biochemistry 32*, 4539–4546.

Zhou, J. S., & Kostic, N. M. (1993b) *J. Am. Chem. Soc. 115*, 10796–10804.