Interprotein electron transfer (ET) occurs between the tryptophan tryptophylquinone (TTQ) prosthetic group of aromatic amine dehydrogenase (AADH) and copper of azurin. The ET reactions from two chemically distinct reduced forms of TTQ were studied: an O-quinol form that was generated by reduction by dithionite, and an N-quinol form that was generated by reduction by substrate. It was previously shown that on reduction by substrate, an amino group displaces a carbonyl oxygen on TTQ, and that this significantly alters the rate of its oxidation by azurin (Hyun, Y.-L., and Davidson, V. L. (1995) Biochemistry 34, 12249–12254). To determine the basis for this change in reactivity, comparative kinetic and thermodynamic analyses of the ET reactions from the O-quinol and N-quinol forms of TTQ in AADH to the copper of azurin were performed. The reaction of the O-quinol exhibited values of electronic coupling ($H_{AB}$) of 0.13 cm$^{-1}$ and reorganizational energy ($\lambda$) of 1.6 eV, and predicted an ET distance of approximately 15 Å. These results are consistent with the ET event being the rate-determining step for the redox reaction. Analysis of the reaction of the N-quinol by Marcus theory yielded an $H_{AB}$ which exceeded the nonadiabatic limit and predicted a negative ET distance. These results are diagnostic of a gated ET reaction. Solvent deuterium kinetic isotope effects of 1.5 and 3.2 were obtained, respectively, for the ET reactions from O-quinol and N-quinol AADH indicating that transfer of an exchangeable proton was involved in the rate-limiting reaction step which gates ET from the N-quinol, but not the O-quinol. These results are compared with those for the ET reactions from another TTQ enzyme, methylamine dehydrogenase, to amicyanin. The mechanism by which the ET reaction of the N-quinol is gated is also related to mechanisms of other gated interprotein ET reactions.

Aromatic amine dehydrogenase (AADH)$^1$ from Alcaligenes faecalis catalyzes the oxidation of a wide range of primary amines to their corresponding aldehyde plus ammonia (1, 2). AADH exhibits an $\alpha_2\beta_2$ structure with subunit molecular weights of 39,000 and 18,000. Each small subunit contains a covalently bound tryptophan tryptophylquinone (TTQ) (3) prosthetic group, which is involved both in catalysis and in subsequent electron transfer (ET) to its physiologic electron acceptor. The physical, spectral, and structural properties of AADH are very similar to those of methylamine dehydrogenase (MADH) (4) which is the only other known TTQ-containing enzyme. No structural information is yet available for AADH, however, the crystal structures of MADH alone (5) and in complex with its protein electron acceptor (6, 7) have been determined. Each TTQ enzyme uses a type 1 copper protein as its physiologic electron acceptor: azurin for AADH (8) and amicyanin for MADH (4, 7). However, azurin does not function as an effective electron acceptor for MADH, and amicyanin does not function as an effective electron acceptor for AADH (9). Thus, despite the fact AADH-azurin and MADH-amicyanin are analogous sets of ET reaction partners, in that they use the same redox cofactors, there is a strong specificity for which copper protein serves as the electron acceptor for each TTQ enzyme.

Transient kinetic studies yielded significantly different values for the limiting first-order rate constant for the oxidation of reduced AADH by azurin depending upon whether AADH had been reduced chemically with dithionite, or with the substrate tyramine (9). These data suggested that two chemically distinct reduced forms of TTQ in AADH could be formed, respectively, by dithionite or substrate (Fig. 1). $^{15}$N-NMR studies proved that the substrate-derived amino group remains covalently bound to the TTQ prosthetic group of AADH, and is only released after oxidation (10). Thus, the incorporation of the substrate-derived amino group into the reduced TTQ of AADH significantly affects its reactivity with azurin. A similar phenomenon was observed for the ET reactions from different reduced forms of MADH to oxidized amicyanin. Significantly different reaction rate constants were obtained depending upon whether MADH was reduced chemically with dithionite, or with the substrate methylamine (11, 12). Thermodynamic analysis of these redox reactions indicated that the oxidation of dithionite-reduced quinol (O-quinol) TTQ by amicyanin was rate-limited by the ET event (13, 14), but that the oxidation of the substrate-reduced aminooquinol (N-quinol) TTQ by amicyanin was a gated ET reaction (11, 12). Furthermore, kinetic solvent isotope effect (KSIE) studies proved that proton transfer from the substrate-derived amino nitrogen on TTQ in MADH was the rate-limiting reaction step that gates ET in the latter reaction (11, 12).

We present comparative kinetic and thermodynamic analyses of the ET reactions from the O-quinol and N-quinol forms of TTQ in AADH to the copper in azurin (Fig. 1). These results...
A

**AADH-Azurin Electron Transfer**

**Fig. 1. One-electron oxidations of TTQ in AADH.** The different forms of TTQ that are generated by reduction of AADH by dithionite (A) and substrate (B) are shown. The protonation states of the quinol and semiquinone forms of TTQ in AADH were determined in previous redox studies (27). In the semiquinone forms, the electron spin density is probably asymmetrically distributed throughout the prosthetic group (40) and so the exact distribution of spin density should not be inferred from this figure.

A

**Experimental Procedures**

Purifications of AADH (1) and azurin (8) from *A. faecalis* (IFO 14479) were as described previously, and protein concentrations were calculated from previously determined extinction coefficients (1, 15). D$_2$O (99.9%) was obtained from C/D N Isotopes. The chemicals that were used in this study were obtained from either Aldrich or Sigma.

Transient kinetic experiments were performed using an On-Line Instrument Systems (OLIS, Bogart GA) RSM1000 rapid-scanning stopped-flow spectrophotometer. The experimental procedures for the rapid mixing experiments were as described previously (9) for the reactions of each reduced AADH form with azurin. The relevance of the significant difference in the reorganization energies for the ET reactions from the O-quinol forms of TTQ to copper in the AADH-azurin and MADH-amicyanin systems is also discussed.

**Results**

**Kinetic Analysis**—The concentration dependence of the rate of oxidation of reduced AADH forms by azurin was determined over a range of temperatures from 12 to 35 °C. In each case, the data were well fit by Equation 2 (Fig. 2) and yielded a limiting first-order rate constant ($k_0$) for the redox reaction. Rates varied with temperature from approximately 30–180 s$^{-1}$ for the reactions of the N-quinol, and from approximately 2–5 s$^{-1}$ for the reactions of the O-quinol. The temperature dependence of $k_0$ for the reactions of each reduced AADH form with azurin was analyzed by both transition state theory and ET theory (23).

**Determination of Thermodynamic Activation Parameters**—For analysis by transition state theory, data were fit to the Eyring equation (Equation 3),

$$
\ln(k_0/k_B T) = (-\Delta H^*/RT) + (\Delta S^*/R)
$$

which describes the temperature dependence of a reaction rate in which $h$ is Planck's constant, $R$ is the gas constant, $T$ is temperature, $k_B$ is the Boltzmann constant, $\Delta H^*$ is activation enthalpy, and $\Delta S^*$ is activation entropy. Analysis of the temperature dependence of the $k_0$ for each reaction by Equation 3 yielded linear plots (Fig. 3). The fitted parameters are listed in Table 1 and compared with each other, and with the previously reported parameters from the analogous reactions of reduced forms of MADH with amicyanin.

**Determination of ET Parameters**—For analysis of the temperature dependence of $k_3$ by ET theory, data were fit to Equations 4 and 5 (Fig. 4),

$$
k_{ET} = \frac{4\pi H_{AB}^2}{h\sqrt{4\pi k_B T}} \exp\left[-(\Delta G^0 + \lambda)^2/4RT\right]
$$

where $\lambda$ is the reorganization energy, $H_{AB}$ is the electronic coupling matrix element, $h$ is Planck's constant, and $R$ is the gas constant. In Equation 5, $H_{AB}$ is alternatively described in terms of the ET distance between redox centers ($r$ is the center to center distance and $r_o$ is the close contact distance which is
Distinguish between Gated and Ungated ET Reactions—In the simple kinetic model used to analyze these data (Equation 1), the limiting first-order rate constant \( k_3 \) may not necessarily be a true ET rate constant \( k_{ET} \) (28–31). In the absence of additional data, \( k_3 \) should be considered an apparent ET rate constant, because any spectroscopically invisible reaction steps that may occur after binding and before ET may be reflected in \( k_3 \). For a hypothetical three-step reaction (Equation 6),

\[
\text{AADH}_{\text{red}} + \text{azurin}_{\text{ox}} \overset{k_d}{\rightarrow} \text{AADH}_{\text{red}}/\text{azurin}_{\text{ox}} \quad \overset{k_x}{\rightarrow} \quad \text{AADH}_{\text{red}}/\text{azurin}_{\text{ox}}^0 \quad \overset{k_{ET}}{\rightarrow} \quad \text{AADH}_{\text{red}}/\text{azurin}_{\text{ox}}^0
\]

in which some adiabatic reaction step \( k_x \) occurs after binding and is required, to activate the system for ET, \( k_3 \) will be a true \( k_{ET} \) only if \( k_{ET} \) is rate-limiting for the three-step reaction mechanism. If \( k_x \) is rate-limiting instead, then it will be a gated ET reaction and \( k_3 \) in Equation 1 will be equal to \( k_x \).



FIG. 2. Concentration and temperature dependence of \( k_{obs} \) for the oxidation by azurin of dithionite-reduced (A) O-quinol AADH and substrate-reduced (B) N-quinol AADH. The temperature at which each set of experiments was performed are from top to bottom in A, 35, 30, 25, 20, and 12 °C; and in B, 34, 30, 25, 21, and 14 °C. The solid lines represent the fits of each set of data to Equation 2.

FIG. 3. Analysis by transition state theory of the temperature dependence of limiting first-order rate constants for the oxidation by azurin of dithionite-reduced (A) O-quinol AADH and substrate-reduced (B) N-quinol AADH. Values of \( k_x \) were determined from the data shown in Fig. 2, which were fit to Equation 2. The solid lines represent the fits of each set of data to Equation 3.

The \( \Delta G^0 \) used in Equations 4 and 5 was calculated from the difference of the \( E_m \) values of the TTQ and copper redox centers. We measured the \( E_m \) value of azurin to be +275 mV under our experimental conditions (data not shown). It should be noted that in the MADH-amiocyanin system, the \( E_m \) value of amicyanin changes on complex formation with MADH. This is because a histidine residue that provides one of the ligands for the copper of amicyanin undergoes a pH-dependent conformational change in the reduced state that removes it from the copper coordination sphere when protonated, and this “histidine flip” is sterically restricted when amicyanin is in complex with MADH (22). There is no evidence that azurin undergoes such a process under our experimental conditions, and so no correction for complex-dependent changes was made for the \( E_m \) value of azurin. The \( E_m \) value of the one-electron AADH$_{\text{red}}$/AADH$_{\text{semi}}$ couple cannot be measured directly, but a good approximation was inferred from previous redox studies of AADH and MADH (27). The two-electron AADH$_{\text{red}}$/AADH$_{\text{ox}}$ redox couple is 20 mV less positive than the AADH$_{\text{red}}$/AADH$_{\text{ox}}$ couple, and each exhibits exactly the same dependence on pH. The relative \( E_m \) values for the two one-electron couples of each enzyme also exhibit the same pH dependence (27). The \( E_m \) value of the one-electron MADH$_{\text{red}}$/MADH$_{\text{semi}}$ couple, which could be determined, is +190 mV (14). As such, we used a 20 mV less positive value of +170 mV for the AADH$_{\text{red}}$/AADH$_{\text{semi}}$ couple in this analysis. This yields a \( \Delta E_m \) for the ET reaction from reduced AADH to oxidized azurin of +105 mV, which corresponds to a \( \Delta G^0 \) of -10.131 J/mol. As discussed previously (28), when \( \lambda \) is very large compared with \( \Delta G^0 \), any variation in the value of \( \Delta G^0 \) used in Equations 4 and 5 will have a negligible effect on the fitted values of \( H_{AB} \) and \( r \), and an effect on the fitted value of \( \lambda \) that is proportional to the error in \( \Delta G^0 \). The parameters that are obtained from the fits of the data to Equations 4 and 5 are listed in Table I and compared with each other, and with the previously reported parameters from the analogous reactions of reduced forms of MADH with amicyanin.

Kinetic Solvent Isotope Effects—For the reaction of O-quinol AADH with azurin, a secondary KSIE on \( k_3 \) of 1.5 ± 0.1 was observed (Fig. 5A). This is consistent with \( k_3 \) describing an ET event, which would not be expected to exhibit a primary KSIE. Under the same conditions, the reaction of N-quinol AADH with azurin exhibited a significantly larger KSIE on \( k_3 \) of 3.2 ± 0.2 (Fig. 5B), which suggests that the rate-limiting step for the observed redox reactions involves the transfer of an exchangeable proton (discussed later).

**DISCUSSION**

For example, if one assumes that \( \Delta E_m \) for the reaction of O-quinol AADH with azurin is 0 mV rather than +105 mV, fits of the temperature dependence of the rate to Equations 4 and 5 yield identical values of \( H_{AB} \) and \( r \), and a value of \( \lambda \) of 1.4 eV rather than 1.6 eV. Alternatively, if one assumes that this \( E_m \) value is +200 mV rather than +105 mV, fits of the temperature dependence of the rate to Equations 4 and 5 again yield identical values of \( H_{AB} \) and \( r \), and a value of \( \lambda \) of 1.8 eV.

\[ \text{AADH}_{\text{red}} + \text{azurin}_{\text{ox}} \overset{k_d}{\rightarrow} \text{AADH}_{\text{red}}/\text{azurin}_{\text{ox}} \quad \overset{k_x}{\rightarrow} \quad \text{AADH}_{\text{red}}/\text{azurin}_{\text{ox}}^0 \quad \overset{k_{ET}}{\rightarrow} \quad \text{AADH}_{\text{red}}/\text{azurin}_{\text{ox}}^0 \]
For the reactions of O-quinol and N-quinol AADH with azurin, different reaction steps must be rate-limiting for each of the two redox reactions (Table I). The activation parameters, $\Delta H^*$ and $\Delta S^*$, are given primarily for comparison. Interpretation of these parameters for a nonadiabatic ET reaction is not straightforward, as this reaction does not involve the formation or breakage of bonds. However, it is evident from the very different values for the reactions of O-quinol and N-quinol AADH that these sets of thermodynamic activation parameters must be describing different types of reactions. A qualitatively similar trend was previously reported for the redox reactions between MADH with amicyanin (11, 12).

Comparison of the values for the ET parameters for the reactions of O-quinol and N-quinol AADH provides an explanation for the differences in the temperature dependence and activation parameters of these reactions that are caused by the modification by substrate of the reduced TTQ prosthetic group. The values of $\lambda$, $H_{AB}$, and $r$ that describe the reaction of O-quinol AADH with azurin are consistent with $k_3$ describing an ET event (28). In contrast, the values of $\lambda$, $H_{AB}$, and $r$ that describe the reactions of N-quinol AADH with azurin are diagnostic of a gated ET reaction (28). Nonadiabatic ET reactions, by definition, exhibit weak electronic coupling between redox centers and values of $H_{AB}$ that are within the nonadiabatic limit, if the ET event is rate-limiting for the redox reaction. It has been proposed that nonadiabatic ET reactions should exhibit an $H_{AB}$ value of less than 80 cm$^{-1}$ (32). The $H_{AB}$ for the reaction of O-quinol AADH with azurin is well within this limit. The reaction of N-quinol AADH with azurin, however, exhibited an $H_{AB}$ of 820 cm$^{-1}$ which indicates that $k_3$ for this reaction does not describe an ET event. Furthermore, the value of 15 Å that is obtained for the reaction of O-quinol AADH with azurin is reasonable (discussed later), whereas the analysis of the $\xi_3$ for the reaction of N-quinol AADH with azurin yields a negative value for ET distance. This absurd value is obtained because Equations 4 and 5 are only appropriate for the analysis of nonadiabatic reactions, and this result is further evidence that $k_3$ actually describes the rate of a non-ET reaction step (i.e., $k_i$ in Equation 6) that precedes the true ET. The much larger value of $\lambda$ obtained for the reaction of the N-quinol is also consistent with the conclusion that this is a gated ET reaction which is not appropriately described by ET theory.

Mechanism of Gated ET from AADH—Since it is known that the ET reaction from N-quinol MADH to amicyanin is gated by

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**Table I**

| Parameters                      | AADH | MADH
|---------------------------------|------|------|
| $\Delta S^*$ (J/mol K)          | -116 ± 4 | -6 ± 7 |
| $\Delta H^*$ (kJ/mol)           | +16 ± 1 | +64 ± 2 |
| $\lambda$ (eV)                 | 1.6 ± 0.1 | 3.1 ± 0.1 |
| $H_{AB}$ (cm$^{-1}$)            | 0.13 ± 0.02 | 820 ± 480 |
| $r$ (Å, for $\beta = 1.0$)     | 15.2 ± 0.4 | -16 ± 1 |
| $k_{3}^{0}k_{5}^{0}$           | 1.5 ± 0.1 | 3.2 ± 0.2 |
| Rate-limiting reaction step     | Electron transfer | Proton transfer |
|                                 | Electron transfer | Proton transfer |

*Values were taken from Refs. 11–13. Standard errors are not included for these values since they were not listed in the references.

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**Fig. 4.** Analysis by electron transfer theory of the temperature dependence of limiting first-order rate constants for the oxidation by azurin of dithionite-reduced (A) O-quinol AADH and substrate-reduced (B) N-quinol AADH. Values of $k_3$ were determined from the data shown in Fig. 2, which were fit to Equation 2. The solid lines represent the fits of each set of data to Equations 4 and 5. The fits to the two equations are superimposable.

**Fig. 5.** Concentration dependence of $k_{obs}$ for the oxidation by azurin of dithionite-reduced (A) O-quinol AADH and substrate-reduced (B) N-quinol AADH in buffered H$_2$O (■) and D$_2$O (●). The solid lines represent the fits of each set of data to Equation 2.
the deprotonation of the substrate-derived amino group on TTQ (11, 12). KSIE studies were performed with AADH and azurin to see whether the same is true in this case. Incubation of an enzyme in D₂O leads to a multitude of isotopic exchanges within the enzyme framework. By performing the reactions in H₂O and D₂O, a KSIE \( \delta^{H}(k_{\text{ET}}) \) can be determined. A significant KSIE is only observed when an exchangeable proton is transferred in the rate-limiting step. Secondary effects, defined as those involving conformational changes in the enzyme caused by changing the properties of hydrogen bonds, hydrophobic bonds, and other factors will yield a KSIE less than 2.0 and do not involve hydrogen transfer in the rate-limiting step (20). The analysis of data for the reactions of O-quinol AADH with oxidized azurin yielded a KSIE of 1.5, which likely describes such secondary effects. This result is consistent with \( k_3 \) describing an ET event, which would not exhibit a large KSIE since it does not involve proton transfer. In contrast, the reaction of N-quinol AADH with azurin yielded a KSIE of 3.2, which is consistent with the \( k_3 \) for that reaction describing a proton transfer step, involving an exchangeable proton, that is gating the ET. The modification by substrate of the reduced TTQ cofactor of AADH causes the ET reaction to azurin to become gated by proton transfer. Thus, this previously reported novel feature in the MADH-amicyanin system is not an isolated event, but apparently a common feature of TTQ-dependent enzymes.

How a Gated ET Reaction Can Be Faster Than an Ungated ET Reaction—These results raise the question of why the gated ET reaction from N-quinol AADH to azurin is faster than the ungated ET reaction from the O-quinol to azurin. The phenomenon was observed for the ET reactions from TTQ in MADH to copper in amicyanin (11, 12). The most likely explanation for this is that incorporation of N into the C-6 position of TTQ raises its \( E_m \) value relative to the O-quinol (33) such that ET to azurin becomes thermodynamically much less favorable and now requires a prerequisite activation step to occur to a significant extent. For AADH, this activation step appears to be the same as that for MADH (12), deprotonation of the N-quinol to yield a highly reactive species which will transfer electrons much faster than either the unactivated N-quinol or the O-quinol. Thus, the unactivated and ungated ET reaction of the O-quinol is slower than the gated but activated ET reaction of the N-quinol.

Protein-dependent Differences in Reorganizational Energy—The \( \lambda \) value of 1.6 eV for the ET reaction from O-quinol AADH to azurin, while relatively large, is much less than the value of 2.3 eV that was obtained for the ET reaction from O-quinol MADH and amicyanin. The latter value was obtained from the \( \Delta G^0 \) dependence of ET reactions of different redox forms of MADH (14), and from temperature dependence studies of the O-quinol (13). It is important to try to understand the basis for these relatively large \( \lambda \) values. One possibility is that they reflect the kinetic complexity of this reaction (28). These ET reactions may be representative of kinetically coupled ET. If for the three-step model in Equation 6, \( k_3 \) is relatively fast but very unfavorable \((i.e. K_3 \ll k_{-3})\) then \( k_3 \) will be influenced by the equilibrium constant for that adiabatic process such that \( k_3 = k_{\text{ET}} K_3 \). It follows that the experimentally derived \( \lambda \) will contain contributions from both the ET event and the preceding reaction step \( (i.e. \lambda_{\text{obs}} = [\Delta G^0, \lambda_i]) \). At least two possible non-ET reaction steps may be required to activate the system for ET. A conformational rearrangement of the proteins within the ET complex may be needed to orient the proteins from a geometry which is optimal for binding to one which is optimal for ET. Alternatively, a conformational reorientation of the two indole rings \( (e.g. \text{change in dihedral angle between rings}) \) which comprise the TTQ cofactor may be needed to optimize the system for ET. The need for such a perturbation of the angle between the TTQ rings for ET reactions has also been suggested on the basis of studies with TTQ model compounds (34). It is also possible that this is a true ET reaction with a large intrinsic \( \lambda \) value. While much is known about the \( \lambda \) values associated with redox changes of metal redox centers, such as type 1 copper and heme, these systems are very different from TTQ which contains no metal and is comprised of two unfused indole ring systems joined by a single bond. The \( \lambda \) value for the reaction of AADH with azurin is approximately 0.8 eV less than that for the corresponding reaction of MADH with amicyanin. This suggests that the \( \lambda \) value of 2.3 eV for the latter reaction cannot be attributed solely to it being an inherent property of TTQ. It is more likely related to the relative flexibility in the orientation of the two TTQ rings with respect to each other in the respective quinoproteins, or differences in the structural features at the respective protein-protein interfaces that may affect the energetics of a conformational rearrangement of protein partners.

Relevance of \( H_{AB} \) Values—The \( H_{AB} \) value for the reaction of O-quinol AADH with azurin is approximately 100-fold less than that for the reaction of O-quinol MADH with amicyanin. This appears to be due to a greater ET distance for the AADH-azurin reaction. The O-quinol reaction, which is rate-limited by ET, the value which was obtained for the MADH-amicyanin reaction closely matches the actual distance seen in the crystal structure of MADH-amicyanin complex where the redox centers are about 9.4 Å apart (6, 7). In that structure, the edge of the unquinolated indole ring of TTQ is exposed at the MADH surface and interacts with amicyanin at the so-called hydrophobic patch surrounding a histidine which serves as a copper ligand. If one assumes that AADH and azurin interact in an orientation similar to that of MADH and amicyanin, then the decreased \( H_{AB} \) could be a result of the TTQ or copper centers being somewhat more buried in their respective structures, or an increased interprotein distance between AADH and azurin relative to that which separates MADH and amicyanin. A relatively small increase in interprotein distance can cause a large decrease in \( H_{AB} \) and large apparent increase in overall distance when a single \( \beta \) value is used (35). As stated earlier, the negative values for the reactions of the N-quinols reflect the fact that these are gated reactions which cannot be described by ET theory.

Correlation with Other Gated Interprotein ET Reactions—Interprotein ET reactions are often suggested or suspected to be gated by a non-ET reaction step. However, there are very few instances in which such reactions have been analyzed by \( \Delta G^0 \) or temperature dependence studies to determine ET parameters, or studied over a range of reaction conditions to clearly distinguish whether or not the ET is really gated. In addition to the N-quinol AADH-azurin reaction described here, three physiologic interprotein ET reactions have been shown to be gated based on analyses by Equations 4 and 5. The ET reactions from N-quinol MADH to amicyanin (11, 12) have been discussed earlier. The ET between the iron protein and molybdenum-iron protein of the nitrogenase complex has been shown to be gated by conformational events associated with either MgATP binding or hydrolysis (36). ET from flavin to iron in the rubredoxin reductase-rubredoxin complex is gated by an as yet undetermined process (37). As is seen for the reaction of N-quinol AADH with azurin, analyses of each of these other three gated reactions by Equations 4 and 5 also yielded negative values for ET distance and \( H_{AB} \) values which exceed the nonadiabatic limit by orders of magnitude (36, 37). Kostic and co-workers (38, 39) have also provided strong evidence from
viscosity dependence studies and site-directed mutagenesis that non-physiologic ET reactions between cytochrome c and plastocyanin are gated by a conformational rearrangement between proteins after binding. The reactions of N-quinol AADH with azurin and N-quinol MADH with amicyanin stand out as examples of interprotein ET reactions that are gated by proton transfer events, as indicated by a significant KSIE for the apparent $k_{ET}$.

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