Biochemistry

 Volume 35, Number 18 May 7, 1996

New Concepts in Biochemistry

Internal Electron-Transfer Reactions in Cytochrome *c* Oxidase†

Peter Brzezinski*

Department of Biochemistry and Biophysics, Göteborg University and Chalmers University of Technology, Medicinaregatan 9C, S-413 90 Göteborg, Sweden

Received February 2, 1996; Revised Manuscript Received March 11, 1996

In the catalytic cycle of cytochrome *c* oxidase (EC 1.9.3.1), electrons are transferred sequentially from water-soluble cytochrome *c* to Cu_A and heme *a* and to the binuclear center heme a_3/Cu_B, where dioxygen is reduced to water. The electron current through the enzyme, across the membrane dielectrics, drives a transmembrane proton current in the opposite direction. This coupling requires careful control of the pathways and rates of the internal electron- and proton-transfer reactions in order to prevent dissipation of the electronic energy into heat. The basic principles of this coupling have been summarized in the so-called cubic scheme by Wikström et al. (1981) as well as by Malmström (1985) in the transition-state model.

When an electron is injected into Cu_A of the fully oxidized enzyme, it equilibrates first with heme *a* with a rate constant of about 10^4 s^{-1} [see the review by Winkler et al. (1995)]. The reduction rate of heme a_3 is much slower, and rate constants in the range $1-100$ s^{-1} have been observed, depending on the experimental conditions [Verkhovsky et al., 1995; Sarti et al., 1990; Antalis & Palmer, 1982; reviewed by Winkler et al. (1995)]. Thus, the electron-transfer rates from Cu_A to hemes *a* (k_{Aa})[1] and a_3 (k_{Ab}), respectively, differ by 2−4 orders of magnitude.

Recently, two high-resolution three-dimensional structures of cytochrome *c* oxidases from *Paracoccus denitrificans* (Iwata et al., 1995) and bovine heart (Tsukihara et al., 1995) were determined. An analysis of these structures shows that the distances from Cu_A to hemes *a* and a_3 are 19.5 and 22.1 Å, respectively[2] [given in Iwata et al. (1995)]. The relatively small difference in distances cannot alone account for the large difference in the electron-transfer rates between Cu_A and the two hemes. In this work, two models (not mutually exclusive) are proposed which explain this apparent inconsistency.

Rapid internal electron-transfer reactions in cytochrome *c* oxidase have been studied following flash photolysis of the mixed-valence CO complex [e.g. Ädelroth et al. (1995a), Verkhovsky et al. (1992), and Boelens et al. (1982)]. In this complex, reduced heme a_3/Cu_B is stabilized by the binding of CO, whereas heme *a* and Cu_A are oxidized. Upon pulsed illumination, CO dissociates, which results in a decrease of the apparent reduction potential of heme a_3 and electron transfer from this site to heme *a* (Figure 1A). In bovine cytochrome *c* oxidase, the observed rate constant of this reaction is 3×10^5 s^{-1} and the driving force (from heme *a* to a_3) is about −40 meV (Verkhovsky et al., 1992; Ädelroth et al., 1995a). This equilibration is followed by equilibration with Cu_A with an observed rate constant of about 2×10^4 s^{-1}, about the same as that observed in the three-electron reduced enzyme (Morgan et al., 1989).

These reactions are followed by additional electron transfer from heme a_3 to *a* on a millisecond time scale (Figure 1B). Both the rate constant and the extent of this reaction display complex pH dependencies (Ädelroth et al., 1995b; Hallén et al., 1994; Brzezinski & Malmström, 1987). Recently, our research group showed that this electron transfer is kinetically coupled to proton release (Figure 1C) from a protonatable

† This study has been supported by grants from the Swedish Natural Science Research Council, the Magn. Bergvalls Foundation, and the Helge Ax:son Johnsons Foundation.

* Author to whom correspondence should be addressed. Fax: (+46)-31-773 3910. E-mail: PETER.BRZEZINSKI@BCBP.GU.SE.

[1] Abbreviations: a, heme *a*; b, heme a_3; A, copper A; B, copper B. These abbreviations are used as indexes of the different variables. For example, k_{ab} represents the electron-transfer rate from heme *a* to a_3 and k_{Ab} from Cu_A to heme a_3. k_B is the Boltzmann constant, p*K* is used for pK_a.

[2] All distances are center to center.

S0006-2960(96)00260-7 CCC: $12.00

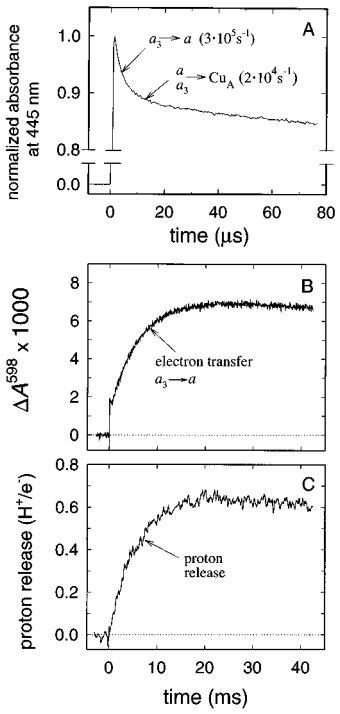

FIGURE 1: (A) Transient absorbance changes at 445 nm following dissociation of CO from mixed-valence bovine cytochrome *c* oxidase, normalized to the CO-dissociation change. (B) Absorbance changes at 598 nm on a longer time scale than in panel A measured simultaneously with proton-conductance changes (C) associated with proton release (pH 10). A change in conductivity associated with heating of the sample by the laser flash (by $<10^{-3}$ °C) has been truncated. The slow signal decrease in panels B and C is associated with CO recombination and proton uptake, respectively, with a rate constant of $\sim$50 s^{-1}. Data from Ädelroth et al. (1995a,b).

group in the vicinity of heme a_3. This group interacts more strongly with heme a_3 than with heme *a*, and is buried inside the protein, in contact with the bulk protons through a proton-conducting pathway (Ädelroth et al., 1995b). The p*K*s of this group with heme a_3 oxidized and reduced are 8.5 and 9.7, respectively. The interaction energy was determined to be about 70 meV, and the maximum proton release/uptake was found to be $\sim$0.7 H^+/e^-. Thus, the mixed-valence experimental system provides a unique opportunity to study the intrinsic[3] internal electron transfers and, in the same experiment, the proton-transfer coupled electron transfers.

The above-discussed results are consistent with the three-dimensional *P. denitrificans* structure which shows that a larger number of protonatable groups are found around heme a_3 than near heme *a*. This situation is similar to that on the acceptor side of the photosynthetic reaction center (e.g. from *Rhodobacter sphaeroides*) in which reduction of the primary acceptor quinone, Q_A (compare to heme *a*), results in a smaller fractional proton uptake ($\leq$0.4 H^+/Q_A^-, in the pH range 6−10) than reduction of Q_B (compare to heme a_3) (0.4−1.0 H^+/Q_B^-) (McPherson et al., 1988; Maróti & Wraight, 1988).

Recently, Verkhovsky et al. (1995) presented a model which suggests that, during reduction of the fully oxidized enzyme, the observed reduction rate of heme a_3 is not determined by the intrinsic heme *a*-to-a_3 electron-transfer

[3] The "intrinsic rate" is defined as the rate of the electron transfer alone, not limited by other events such as protonation reactions or structural changes.

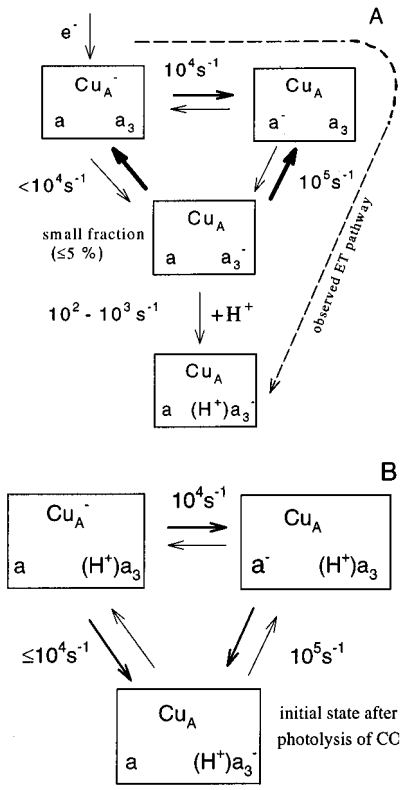

FIGURE 2: (A) Model for internal electron-transfer reactions following electron injection into the fully oxidized enzyme. The intrinsic electron-transfer rates between Cu_A, heme *a*, and heme a_3 are all rapid, but the apparent rate to heme a_3 is slow because the electron must be stabilized by a proton [proposed by Verkhovsky et al. (1995)]. A limit is given for the Cu_A-to-heme a_3 transfer (see panel B). (B) Electron-transfer reactions in mixed-valence cytochrome *c* oxidase following flash photolysis of CO. On the time scale of these measurements, groups interacting with heme a_3 are protonated. Experimentally, it is *observed* that heme a_3 and heme *a* equilibrate first, followed by equilibration with Cu_A. It is not possible to determine if heme a_3 equilibrates directly with Cu_A or through heme *a*. Only a limit of $\leq 10^4$ s^{-1} can be given for the Cu_A−heme a_3 equilibrium. Bold arrows indicate larger rate constants.

rate, which is always rapid, but rather by proton uptake associated with the electron transfer to heme a_3. These electron−proton coupled reactions are also likely to determine the route of intramolecular electron transfer.

As discussed above, electron transfer from Cu_A to heme *a* is accompanied by a smaller proton-charge compensation than that associated with electron transfer to heme a_3 (Mitchell & Rich, 1994). This may explain why electron transfer from Cu_A to heme *a* is always rapid ($\sim 10^4$ s^{-1}), whereas the intrinsic direct electron-transfer rate from Cu_A to heme a_3 may be rapid, but since it is limited by a larger extent of proton uptake, the apparent rate is slow (Figure 2A). This is the same model as that proposed by Verkhovsky et al. (1995) for the electron transfer from heme *a* to a_3, but here applied to the Cu_A-to-heme a_3 electron transfer. In the mixed-valence system, the intrinsic electron-transfer rates are observed (see Comment) because the measurement time scale is shorter than that of proton transfer. From these experiments, it is not possible to conclude whether electrons equilibrate directly between heme a_3 and Cu_A or through heme *a*, and it is only possible to give a limit for the direct heme a_3-to-Cu_A rate constant of $\leq 10^4$ s^{-1}, i.e. the observed reduction rate of Cu_A (see Figure 2B). Thus, the intrinsic electron-transfer rate between Cu_A and *both* hemes *a* and a_3

may be rapid, but since the rate of electron transfer to heme a_3 is limited by proton uptake (Verkhovsky et al., 1995; Ädelroth et al., 1995b), *kinetically*, reduction of Cu_A is always followed by rapid reduction of heme *a* before heme a_3 is reduced.

The electron-transfer rate, k_{ET}, from a donor (D) to a weakly coupled acceptor (A) is [first investigated by Marcus (1956); for reviews see e.g. Marcus and Sutin (1985) and Moser et al. (1992)]

$$k_{ET} = \frac{2\pi}{\hbar}|T_{DA}(r)|^2(FC) \quad (1)$$

where (FC) is the Franck−Condon factor and $|T_{DA}(r)|^2$ is the electronic-tunneling matrix element, coupling the donor and acceptor localized states. The latter factor generally decreases exponentially with increasing D-to-A distance, *r*:

$$|T_{DA}(r)|^2 = T_0{}^2 \exp[-\beta(r - r_0)] \quad (2)$$

where $T_0{}^2$ is the maximum electronic coupling at van der Waals contact ($r = r_0 = 3$ Å) and $\beta = 1$ Å^{-1} has earlier been found to be applicable for the heme *a*-to-a_3 transfer (Ädelroth et al., 1995a) and is used for the other electron-transfer reactions (see below). Assuming the classical limit where the vibrational energy is much smaller than the thermal energy (k_BT), (FC) is

$$(FC) = \frac{1}{\sqrt{4\pi\lambda k_B T}} \exp[-(-\Delta G^\circ - \lambda)^2/4\lambda k_B T] \quad (3)$$

where $-\Delta G^\circ$ is the free energy difference between D and A and λ is the reorganization energy. It is the magnitude of the electronuclear coupling, i.e. the energy required to change the nuclear configuration around the redox site in response to the change in charge. It generally increases with increased polarity of the protein environment around the site.

The parameters of eqs 2 and 3 for the electron transfer from heme *a* to a_3 in the mixed-valence enzyme are as follows: (Ädelroth et al., 1995a) (see Comment): $\lambda_{ab} \cong 0.76$ eV, $|T_{ab}(r_{ab})| \cong 9.9 \times 10^{-5}$ eV, $\Delta G^\circ_{ab} \cong -40$ meV, and $k_{ab} \cong 2 \times 10^5$ s^{-1} (at pH 7 and 22°C). In Figure 3 is shown the driving-force dependence of the electron-transfer rate from heme *a* to heme a_3. Note that under these conditions groups interacting with heme a_3 are protonated; i.e. the heme a_3/Cu_B "charge environment" is different than in the oxidized enzyme. Upon electron injection into the fully oxidized enzyme, the intrinsic rate may therefore be slower due to a smaller driving force. It should also be noted that, under the conditions when the highest $k_{ab} \cong 2 \times 10^5$ s^{-1} is *measured* (in the mixed-valence enzyme), $|\Delta G^\circ_{ab}| \ll \lambda_{ab}$. Consequently, this measured rate constant is 3 orders of magnitude smaller than the maximum value of $\sim 2 \times 10^8$ s^{-1}, which would be obtained at $-\Delta G^\circ_{ab} = \lambda_{ab}$.

The observed electron-transfer rate between Cu_A and heme *a* (i.e. $k_{Aa} + k_{aA}$) in the mixed-valence enzyme is about 2×10^4 s^{-1} [e.g. Ädelroth et al. (1995a) and Morgan et al. (1989)]. In this enzyme form, the equilibrium constant between Cu_A and heme *a* is ∼1.5 (Morgan et al., 1989), which gives $k_{Aa} \cong 1.2 \times 10^4$ s^{-1}. The reorganization energy associated with the Cu_A-to-heme *a* transfer can be calculated from the ratio of k_{Aa} and k_{ab} using eqs 1−3:

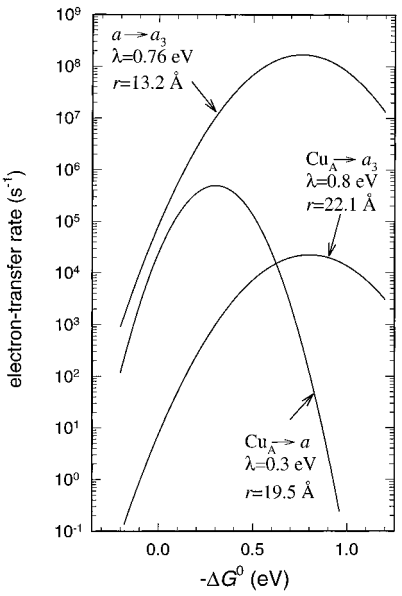

FIGURE 3: Electron-transfer rates as a function of driving force for three different internal electron-transfer reactions. Also see Comment.

$$\frac{k_{Aa}}{k_{ab}} = \exp[-\beta(r_{Aa} - r_{ab})]\frac{\sqrt{\lambda_{ab}}}{\sqrt{\lambda_{Aa}}} \exp\left[-\left[\frac{(-\Delta G^\circ_{Aa} - \lambda_{Aa})^2}{4k_B T\lambda_{Aa}} - \frac{(-\Delta G^\circ_{ab} - \lambda_{ab})^2}{4k_B T\lambda_{ab}}\right]\right] \quad (4)$$

Using the distances determined from the three-dimensional cytochrome *c* oxidase structures (Iwata et al., 1995; Tsukihara et al., 1995), $\Delta G^\circ_{Aa} = -12$ meV, and the same parameters as for the heme *a*-to-heme a_3 transfer (see above and Table 1), a λ_{Aa} value of ∼0.4 eV is obtained.

The temperature dependence of the observed electron-transfer rate between Cu_A and heme *a* in the mixed-valence enzyme is small; the "activation enthalpy" is 20−60 meV (Ädelroth et al., 1995a; Brzezinski & Malmström, 1987). A value in that range was also obtained by Morgan et al. (1989) in studies of the perturbation of the Cu_A−heme *a* equilibrium in the three-electron reduced enzyme. Assuming the extreme case where the temperature dependence of the observed rate constant originates only from that of the forward rate constant, i.e. k_{Aa}, a limiting value for λ_{Aa} of ≤0.2 eV was estimated. Ramirez et al. (1995) have estimated this value to fall in the range 0.15−0.5 eV. Theoretical calculations of the reorganization energy of the binuclear Cu_A site alone have given a value of ∼0.2 eV (Larsson et al., 1995). In the calculations below, an average value $\lambda_{Aa} = 0.3$ eV will be used.

To estimate a λ-value for the direct Cu_A-to-heme a_3 transfer, it is assumed that it is possible to ascribe a reorganization energy to each of the redox sites, that these values are linearly additive, and that all individual λ-values are ≥0.1 eV (values are in electronvolts):

$$\lambda_a + \lambda_b = 0.8, \quad \lambda_A + \lambda_a = 0.3$$

$$\Rightarrow \lambda_A \text{ and } \lambda_a \le 0.2, \quad 0.6 \le \lambda_b \le 0.7,$$
$$0.7 \le \lambda_A + \lambda_b \le 0.9 \quad (5)$$

Using eqs 1−3 with an average value $\lambda_{Ab} = 0.8$ eV and keeping other parameters the same as those determined

Table 1: Summary of Parameters of Internal Electron-Transfer Reactions in Cytochrome *c* Oxidase[a]

electron-transfer reaction	distance (Å)	λ (eV)	$\lvert T_{DA}(r)\rvert$ (eV)	maximum rate (at $-\Delta G^\circ = \lambda$) ($s^{-1}$)
$a \rightarrow a_3$ (k_{ab})	13.2	0.76	9.9×10^{-5}	2×10^8
$Cu_A \rightarrow a$ (k_{Aa})	19.5	0.3	3.8×10^{-6}	5×10^5
$Cu_A \rightarrow a_3$ (k_{Ab})	22.1	0.8	1.0×10^{-6}	2×10^4

[a] A β-value of 1 $Å^{-1}$ was assumed for all reactions (see text). The value $T_0^2 = 2.2 \times 10^{-4}$ eV^2, determined from measurements on k_{ab}, was used for all reactions. Also see Comment at the end of the paper.

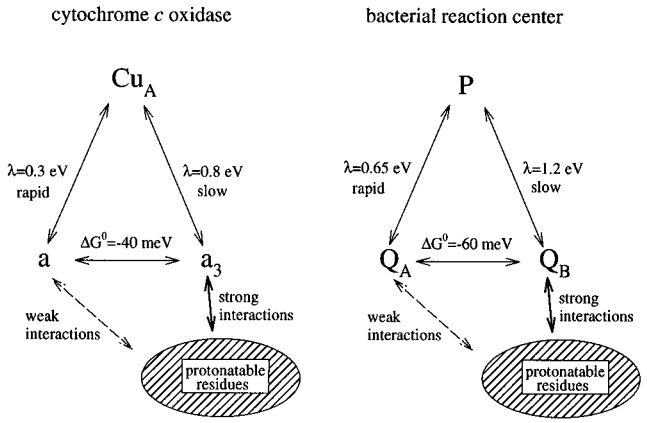

FIGURE 4: Comparison of internal electron-transfer reactions in bacterial photosynthetic reaction centers (from *Rb. sphaeroides*) and in cytochrome *c* oxidase. In reaction centers, the *direct* charge-recombination rate from Q_B^- to P^+ is slower than that from Q_A^- to P^+ because the reorganization energy (λ) associated with the Q_B site is larger than that associated with the Q_A site [λ values are from Labahn et al. (1995) and Gunner and Dutton (1989)]. Similarly, in cytochrome *c* oxidase, the reorganization energy associated with heme a_3 is much larger than that associated with heme *a*. In addition, heme a_3/Q_B interacts more strongly than heme a/Q_A with protonatable groups, resulting in a net proton uptake upon electron transfer from heme *a* to a_3 in cytochrome *c* oxidase and from Q_A to Q_B in reaction centers. The driving force for electron transfer from Q_A to Q_B is given at pH 8. The similarities between the two systems discussed in this figure are only on a functional level. In cytochrome *c* oxidase, they refer only to electron-transfer reactions in the absence of O_2.

experimentally from measurements on k_{ab} (Table 1), it is also possible to estimate the driving-force dependence of k_{Aa} and k_{Ab}, respectively (Figure 3).

Because of the larger reorganization energy associated with heme a_3 than with heme *a*, for driving forces in the range $-200 < -\Delta G^\circ < +500$ meV, the direct electron transfer from Cu_A to heme a_3 is much slower than that to heme *a* (Figure 3). The larger reorganization energy associated with heme a_3 rather than with heme *a* is consistent with the fact that reactions involving protons and water take place at heme a_3/Cu_B, and therefore this center is likely to be in a more polar environment than heme *a*. This situation is similar to that found in bacterial photosynthetic reaction centers (Figure 4) where the *direct* charge-recombination rate from Q_B^- to the oxidized donor (P^+) is much slower than that from Q_A^- to P^+, respectively, even though both distances and driving forces are similar (Takahashi & Wraight, 1992; Labahn et al., 1995). This difference in rates was found to be due to a difference in the reorganization energies associated with these two reactions (Labahn et al., 1995), the Q_A-reorganization energy being smaller than that associated with Q_B because the latter is in a more polar environment.

In the above discussion, the details of the medium between Cu_A and the two hemes have not been considered; i.e. it is assumed that $T_{DA}^2(r)$ only depends on the site-to-site distance, and possible pathways for electron transfer have not been considered [see e.g. Wuttke et al. (1992) and Beratan et al. (1991)]. However, this type of model is not expected to change the conclusions of the above discussion [see coupling distances in Fig 1 in Ramirez et al. (1995)].

Figure 3 also shows that, for large driving forces (>500 meV), e.g. when partly reduced oxygen intermediates are bound to heme a_3 or in mutant bacterial cytochrome *c* oxidase [e.g. Calhoun et al. (1994)] in which reduction potentials and/or reorganization energies are altered, k_{Ab} may become comparable to or larger than k_{Aa} and a direct transfer from Cu_A to heme a_3 may be observed. However, this refers only to the intrinsic electron-transfer rates. If slow protonation reactions limit the reduction rate of heme a_3 [c.f. Verkhovsky et al. (1995)], heme *a* would still become reduced before heme a_3 during electron input into the enzyme. In the mixed-valence enzyme, on the other hand, where the rapid electron-transfer reactions on time scales of <100 μs are not coupled to protonation events, a direct electron transfer from heme a_3 to Cu_A may be possible.

COMMENT

The Marcus parameters for the heme *a*-to-heme a_3 (3 μs) electron transfer were determined from the temperature dependence of the electron-transfer rate. These parameters may be *incorrect* if the electron transfer is rate-limited by other events such as e.g. structural changes [cf. photosynthetic reaction centers (Brzezinski & Andréasson, 1995)]. An indication against such rate-limiting structural changes around heme a_3 is that in the closely related cytochrome bo_3 the inter-heme rate slows upon replacement of heme *b* by heme *o* (Morgan et al., 1993). A more complete study should include systematic variation of $-\Delta G^\circ$, as has been done in bacterial photosynthetic reaction centers (Gunner & Dutton, 1989; Feher et al., 1988; Lin et al., 1994). Future mutant studies may provide more accurate values [cf. Lin et al. (1994)].

ACKNOWLEDGMENT

I thank Pia Ädelroth, Håkan Sigurdson, and Margareta Svensson-Ek for helpful discussions.

REFERENCES

Ädelroth, P., Brzezinski, P., & Malmström, B. G. (1995a) *Biochemistry 34*, 2844−2849.
Ädelroth, P., Sigurdson, H., Hallén, S., & Brzezinski, P. (1995b) *Proc. Natl. Acad. Sci. U.S.A.* (submitted for publication).
Antalis, T. M., & Palmer, G. (1982) *J. Biol. Chem. 257*, 6194−6206.
Beratan, D. N., Betts, J. N., & Onuchic, J. N. (1991) *Science 252*, 1285−1288.
Boelens R., Wever, R., & Van Gelder, B. F. (1982) *Biochim. Biophys. Acta 682*, 264−272.
Brzezinski, P., & Malmström, B. G. (1987) *Biochim. Biophys. Acta 894*, 29−38.
Brzezinski, P., & Andréasson, L.-E. (1995) *Biochemistry 34*, 7498−7506.
Calhoun, M. W., Thomas, J. W., & Gennis, R. B. (1994) *Trends Biochem. Sci. 19*, 325−330.
Feher, G., Arno, T. R., & Okamura, M. Y. (1988) in *The photosynthetic bacterial reaction center* (Breton, J., & Vermeglio, A. Eds.) pp 271−287, Plenum Publishing Corp., New York.

Gunner, M. R., & Dutton, P. L. (1989) *J. Am. Chem. Soc. 111*, 3400−3412.
Hallén, S., Brzezinski, P., & Malmström, B. G (1994) *Biochemistry 33*, 1467−1472.
Iwata, S., Ostermeier, C., Ludwig, B., & Michel, H. (1995) *Nature 376*, 660−669.
Labahn, A., Paddock, M. L., McPherson, P. H., Okamura, M. Y., & Feher, G. (1994) *J. Phys. Chem. 98*, 3417−3423.
Larsson, S., Källebring, B., Wittung, P., & Malmström, B. G. (1995) *Proc. Natl. Acad. Sci. U.S.A. 92*, 7167−7171.
Lin, X., Murchison, H. A., Nagarajan, V., Parson, W. W., Allen, J. P., & Williams, J. C. (1994) *Proc. Natl. Acad. Sci. U.S.A. 91*, 10265−10269.
Malmström, B. G. (1985) *Biochim. Biophys. Acta 811*, 1−12.
Marcus, R. A. (1956) *J. Chem. Phys. 24*, 966−978.
Marcus, R. A., & Sutin, N. (1985) *Biochim. Biophys. Acta 811*, 265−322.
Maróti, P., & Wraight, C. A. (1988) *Biochim. Biophys. Acta 934*, 329−347.
McPherson, P. H., Okamura, M. Y., & Feher, G. (1988) *Biochim. Biophys. Acta 934*, 348−368.
Mitchell, R., & Rich, P. R. (1994) *Biochim. Biophys. Acta 1186*, 19−26.
Morgan, J. E., Li, P. M., Jang, D.-J., El-Sayed, M. A., & Chan, S. I. (1989) *Biochemistry 28*, 6975−6983.
Morgan, J. E., Verkhovsky, M. I., Puustinen, A., & Wikström, M. (1993) *Biochemistry 32*, 11413−11418.
Moser, C. C., Keske, J. M., Warncke, K., Farid, R. S., & Dutton, P. L. (1992) *Nature 355*, 796−802.
Ramirez, B. E., Malmström, B. G., Winkler, J. R., & Gray, H. B. (1995) *Proc. Natl. Acad. Sci. U.S.A. 92*, 11949−11951.
Sarti, P., Antonini, G., Malatesta, F., Vallone, B., & Brunori, M. (1990) *Ann. N. Y. Acad. Sci. 550*, 161−166.
Takahashi, E., & Wraight, C. A. (1992) *Biochemistry 31*, 855−866.
Tsukihara, T., Aoyama, H., Yamashita, E., Tomizaki, T., Yamaguchi, H., Shinzava-Itoh, K., Nakashima, R., Yaono, R., & Yoshikawa, S. (1995) *Science 269*, 1069−1074.
Verkhovsky, M. I., Morgan, J. E., & Wikström, M. (1992) *Biochemistry 31*, 11860−11863.
Verkhovsky, M. I., Morgan, J. E., & Wikström, M. (1995) *Biochemistry 34*, 7483−7491.
Wikström, M., Krab, K., & Saraste, M. (1981) *Annu. Rev. Biochem. 50*, 623−655.
Winkler, J. R., Malmström, B. G., & Gray, H. B. (1995) *Biophys. Chem. 54*, 199−209.
Wuttke, D. S., Bjerrum, M. J., Winkler, J. R., & Gray, H. B. (1992) *Science 256*, 1007−1009.

BI960260M