Role of the Rieske Iron–Sulfur Protein Midpoint Potential in the Protonmotive Q-Cycle Mechanism of the Cytochrome bc_1 Complex

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The midpoint potential of the [2Fe–2S] cluster of the Rieske iron–sulfur protein ($E_{m7} = +280$ mV) is the primary determinant of the rate of electron transfer from ubiquinol to cytochrome c catalyzed by the cytochrome bc_1 complex. As the midpoint potential of the Rieske cluster is lowered by altering the electronic environment surrounding the cluster, the ubiquinol-cytochrome c reductase activity of the bc_1 complex decreases; between 220 and 280 mV the rate changes 2.5-fold. The midpoint potential of the Rieske cluster also affects the presteady-state kinetics of cytochrome b and c_1 reduction. When the midpoint potential of the Rieske cluster is more positive than that of the heme of cytochrome c_1, reduction of cytochrome b is biphasic. The fast phase of b reduction is linked to the optically invisible reduction of the Rieske center, while the rate of the second, slow phase matches that of c_1 reduction. The rates of b and c_1 reduction become slower as the potential of the Rieske cluster decreases and change from biphasic to monophasic as the Rieske potential approaches that of the ubiquinone/ubiquinol couple. Reduction of b and c_1 remain kinetically linked as the midpoint potential of the Rieske cluster is varied by 180 mV and under conditions where the presteady state reduction is biphasic or monophasic. The persistent linkage of the rates of b and c_1 reduction is accounted for by the bifurcated oxidation of ubiquinol that is unique to the Q-cycle mechanism.

KEY WORDS: Iron–sulfur protein; midpoint potential; cytochrome bc_1 complex; ubiquinol; cytochrome c_1; hydrogen bonds.

INTRODUCTION

The protonmotive Q cycle, originally proposed by Peter Mitchell (1976) and shown in Fig. 1, accounts for the electron transfer and protonmotive activities of the cytochrome bc_1 complex (Trumpower, 1990). The Rieske iron–sulfur protein fulfills several functions in the Q-cycle mechanism (Edwards *et al.*, 1982). The iron–sulfur protein is the primary electron acceptor in the bc_1 complex and drives the Q cycle by oxidizing ubiquinol and divergently transferring one electron to cytochrome c_1 and forming a ubisemiquinone anion that reduces b_L. The Rieske protein catalyzes the electron transfer reaction with the largest potential change in the Q cycle and which releases both protons from ubiquinol at the electropositive surface of the bc_1 complex in the inner mitochondrial membrane. Mutations to the Rieske protein affect the activity of the bc_1 complex (Denke *et al.*, 1998). Mutations that impair the fidelity of the divergent oxidation of ubiquinol could also lower the H^+/e^- stoichiometry and/or contribute to formation of reactive oxygen species through aberrant reactivity of the intermediate ubisemiquinone (Brandt and Trumpower, 1994).

The midpoint potential of the [2Fe–2S] cluster of the Rieske iron–sulfur protein is unusually positive. Whereas the midpoint potentials of [2Fe–2S] ferredoxins are typically −400 mV, the midpoint potential of

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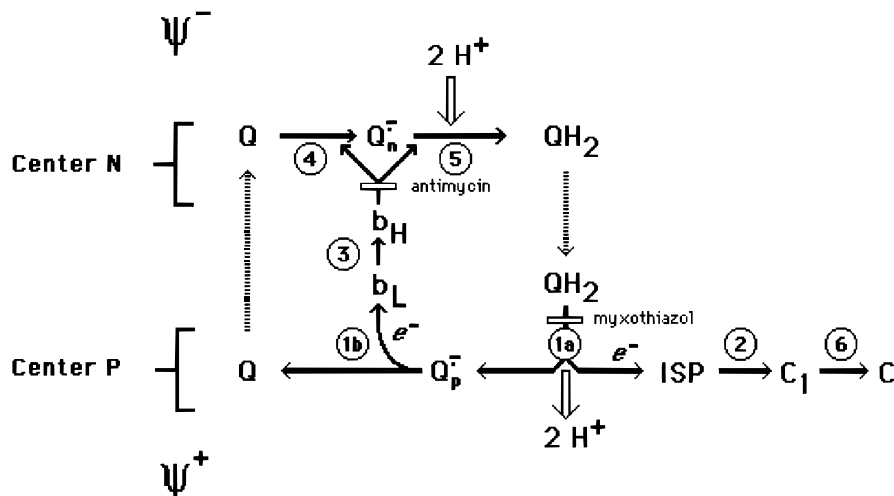[3] Author to whom correspondence should be sent.

Fig. 1. Protonmotive Q-cycle mechanism of electron transfer through the cytochrome bc_1 complex showing individual electron transfer reactions. The scheme shows the branched, cyclic pathway of electron transfer from ubiquinol (QH$_2$) to cytochrome c(C) through the four redox centers of the cytochrome bc_1 complex. The circled numbers designate electron transfer reactions. Dashed arrows represent movement of ubiquinol and ubiquinone (Q) between the site where ubiquinol is oxidized at center P on the positive side of the membrane and the site where ubiquinone and ubisemiquinone are reduced at center N on the negative side of the membrane. In step 1, ubiquinol is oxidized at center P in an essentially concerted reaction, in which one electron is transferred from ubiquinol to iron–sulfur protein (step 1a), generating a ubisemiquinone anion ($Q_p^{\cdot-}$), which immediately reduces the b_L heme group (step 1b). In step 2, the electron transferred to iron–sulfur protein is transferred to cytochrome c_1 and then to cytochrome c (step 6). In step 3, the electron transferred to the b_L heme group is transferred to the b_H heme. In step 4, the b_H heme reduces ubiquinone to the relatively stable ubisemiquinone anion ($Q_n^{\cdot-}$). When b_H is rereduced by repetition of the preceding reactions, b_H reduces ubisemiquinone anion to ubiquinol (step 5). The divergent oxidation of ubiquinol at center P and electron transfer through the two b hemes to the Q/QH$_2$ couple at center N occurs twice during one complete Q cycle. One complete Q cycle thus deposits four protons on the positive side of the membrane, reduces two cytochrome c molecules, and consumes two protons from the negative side of the membrane. The open boxes show the sites at which myxothiazol and antimycin inhibit electron transfer reactions within the complex.

the mitochondrial Rieske iron–sulfur protein is +280 mV. The parameters that contribute to the high midpoint potential of the Rieske iron–sulfur cluster are not fully understood. It is also not fully appreciated how the high midpoint potential of the iron–sulfur cluster affects the function of the Rieske iron–sulfur protein in the Q-cycle mechanism.

In this review we have focused on the thermodynamic constraints placed on the microscopic rates in the Q cycle by the midpoint potentials and apparent potentials of the redox components. We describe how the midpoint potential of the Rieske iron–sulfur cluster is influenced by amino acids that form hydrogen bonds to the iron–sulfur cluster and discuss how the Rieske midpoint potential affects the catalytic activity and presteady-state reduction kinetics of the bc_1 complex.

THERMODYNAMIC PROFILE OF THE CYTOCHROME bc_1 COMPLEX

The redox centers of the mitochondrial bc_1 complex have oxidation–reduction midpoint potentials that span >350 mV. The midpoint potentials of the cytochromes and iron–sulfur protein and their relationship to that of the ubiquinone/ubiquinol couple are shown in Fig. 2. The figure illustrates the potentials in the bc_1 complex of wild-type yeast and in the bc_1 complex of yeast with a S183A point mutation in the Rieske iron–sulfur protein.

The relationship between the potentials of the redox centers illustrates the large change in energy associated with transfer of an electron from ubiquinol to the [2Fe–2S] cluster of the Rieske protein and also
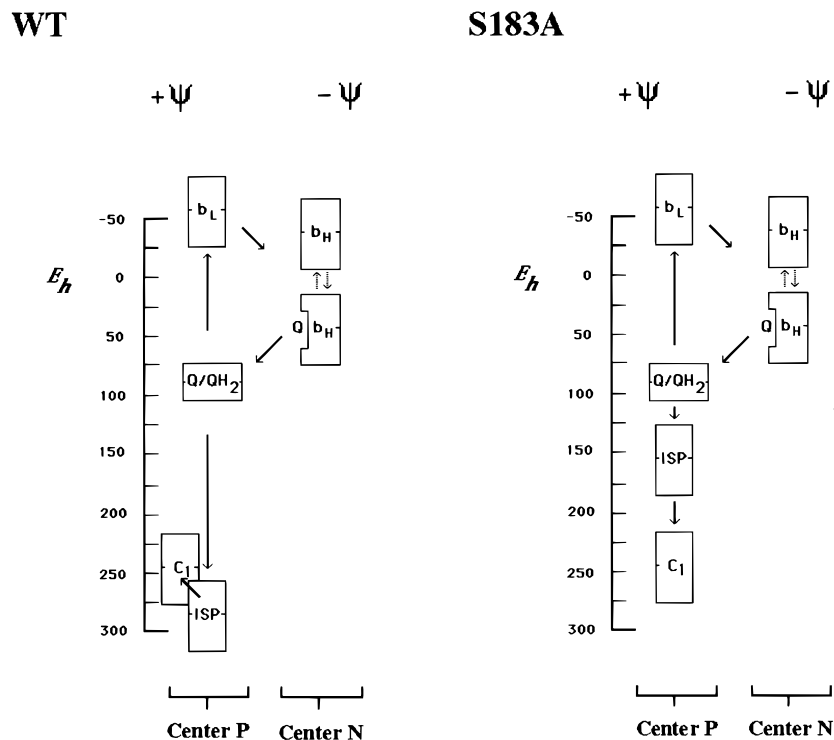
WT **S183A**

Fig. 2. Thermodynamic profile of the Q cycle. The profile on the left is of the bc_1 complex in a wild-type yeast at pH 7 and that on the right is of the bc_1 complex with a S183A mutation in the Rieske iron–sulfur protein. The redox groups are arranged vertically according to their oxidation–reduction potentials and horizontally according to their disposition across the inner mitochondrial membrane. The open boxes delineate the approximate range of potentials spanned by the redox components as their oxidation–reduction status varies in response to changes in rates of electron transfer through the cytochrome bc_1 complex. Cytochrome b_H is a mixture of two potentiometric species, in which ubiquinone is bound proximal to heme b_H and reduction of the quinone to quinol lowers the potential of a portion of the b_H from approximately $+40$ to -50 mV (Salerno *et al.*, 1989). The ubisemiquinone couples at center N and center P are not shown. The midpoint potential of the Rieske protein is pH dependent (Link, 1994). At pH 6, the midpoint potential of the Rieske protein in the wild-type yeast is $+305$ mV and that of the Rieske protein with the S183A mutation is $+185$ mV (T. Merbitz-Zahradnik, unpublished results).

shows the significant thermodynamic barrier to reduction of the heme b_L ($E_{m,7} = \sim -60$ mV) by ubiquinol ($E_{m,7} = \sim +90$ mV). These two reactions are mechanistically coupled in the Q cycle so that the energetically favorable oxidation of ubiquinol by the Rieske cluster drives the otherwise unfavorable reduction of heme b_L (Mitchell, 1976). This coupling is possible because the oxidation–reduction potential of the ubiquinone/ubiquinol couple, (Q/QH_2), is the arithmetic average of the oxidation–reduction potentials of the ubiquinone/ubisemiquinone couple, ($Q/Q\cdot$), and the ubisemiquinone/ubiquinol couple, ($Q\cdot/QH_2$).

$$E_m\ (Q/QH_2) = 1/2[E_m\ (Q/Q\cdot) + E_m(Q\cdot/QH_2)]$$

If the first electron from ubiquinol is transferred to iron–sulfur protein at a potential of $+280 \pm 60$ mV, the ubisemiquinone formed at center P may attain a potential as low as -160 mV, which is strongly reducing, even in comparison to heme b_L.

One consequence of the coupling between reduction of the Rieske cluster and reduction of the heme b_L is that anything that slows one electron transfer reaction will obligatorily slow the other. In this manner the midpoint potential of the Rieske cluster exerts a thermodynamic control on the presteady-state kinetics

of cytochrome b reduction, as shown below. A related form of this coupling is the controlled reduction of the b cytochromes exerted by the apparent potential of the Rieske cluster, in which reduction of cytochrome b through center P is completely blocked when the Rieske cluster is reduced (Trumpower and Katki, 1975).

The reduction of heme b_L linked to oxidation of ubiquinol by the Rieske cluster has received much attention and forms the basis of numerous mechanistic proposals for the bifurcated nature of the Q_p site reaction (Brandt, 1998; Crofts and Wang, 1989; Ding *et al.*, 1995; Link, 1997). The reciprocal relationship implied by this mechanistic coupling, whereby the redox status of the b cytochromes controls reduction of the Rieske cluster and cytochrome c_1 has received much less attention. If the ubisemiquinone at center P is sufficiently unstable such that the potential of the $Q\cdot/QH_2$ couple is more positive than that of the Rieske cluster, reduction of the iron–sulfur cluster (Reaction 1a, Fig. 1) will be thermodynamically unfavorable unless the semiquinone is removed by reduction of heme b_L (Reaction 1b). Junemann and co-workers (1998) have pointed out that this "intermediate controlled bifurcation mechanism" could explain how antimycin inhibits oxidation of ubiquinol, although the iron–sulfur cluster is oxidized by electron transfer through the c cytochromes and oxidase to oxygen. If cytochrome b is trapped in the reduced form by antimycin, the semiquinone can not be removed, and the high potential of the $Q\cdot/QH_2$ couple prevents reduction of the Rieske cluster. They also noted that this thermodynamic-based mechanism is consistent with the lack of an EPR detectable semiquinone under conditions of oxidant-induced reduction of cytochrome b.

The lack of EPR detectable semiquinone at center P and the simultaneous lack of an EPR signal from the Rieske center can also be explained by an alternative thermodynamic based mechanism in which the potential of the Rieske cluster plays a prominent role (Link, 1997) The key feature of the "proton-gated affinity change" mechanism is that when an electron is transferred from ubiquinol to the Rieske cluster, the affinity of the iron–sulfur protein for the resulting ubisemiquinone increases several orders of magnitude. The binding of stigmatellin proximal to the Rieske cluster (Zhang *et al.*, 1998) is a paradigm for this interaction, and stigmatellin is considered a mimetic of ubisemiquinone. The inhibitor binds five orders of magnitude more tightly when the cluster is reduced and causes a 250 mV increase in the potential of the [2Fe–2S] clus-

ter (von Jagow and Ohnishi, 1985). The effect of this affinity change is that the potential of the [2Fe–2S] cluster becomes sufficiently positive that electron transfer from the cluster to the heme of c_1 can not occur until heme b_L oxidizes the semiquinone, thereby lowering the potential of the cluster. Notably, this mechanism requires that ubisemiquinone is stable enough to attain concentrations approximately equal to that of the Rieske center. The inability to detect this stable ubisemiquinone may be attributable to antiferromagnetic coupling between the semiquinone and the reduced Rieske cluster, which would render both EPR silent, consistent with the observed absence of either species in the EPR experiments of Junemann and co-workers (1998).

Although it has been proposed that ubiquinol ionization precedes binding to one of the histidine ligands to the Rieske cluster (Link, 1997), deprotonation of ubiquinol is not necessary. The fully protonated ubiquinol may displace a proton from the imidazole of the histidine to form a quinol–imidazolate complex, with the result that the latter is the electron donor to the cluster. The formation of such a quinol–imidazolate complex would explain the pH dependence of the ubiquinol: cytochrome c reductase activity (Brandt and Okun, 1997) and would obviate the necessity of an extremely alkaline local environment on the iron–sulfur protein to promote ionization of ubiquinol, which has an estimated $pK_a = 11.25$ (Rich, 1984).

PARAMETERS AFFECTING THE MIDPOINT POTENTIAL OF THE RIESKE IRON–SULFUR PROTEIN

Anything that preferentially stabilizes the reduced or oxidized form of the iron–sulfur protein will increase or decrease the midpoint potential of the [2Fe–2S] cluster, respectively. Stigmatellin (von Jagow and Ohnishi, 1985) and hydroxyquinones (Bowyer *et al.*, 1982) bind more tightly to the bc_1 complex when the Rieske center is reduced and thus increase the midpoint potential. Notably, neither of these inhibitors binds to the isolated Rieske protein, but bind at center P proximal to the Rieske protein. Thus the effects of these ligands on the midpoint potential of the Rieske cluster is indirect.

In the crystal structure of the cytochrome bc_1 complex the Rieske protein is proximal to cytochrome c_1 in the native enzyme and proximal to cytochrome b when stigmatellin is bound at center P (Zhang *et al.*,

1998). If the peripheral domain of the Rieske protein moves as the cluster is oxidized and reduced, as suggested by these crystal structures, mutations that change the interactions between the Rieske protein and the neighboring proteins are likely to affect the midpoint potential of the [2Fe–2S] cluster. Such mutations need not be at the interacting interfaces between proteins, since mutation-induced structural changes can be transmitted significant distances through proteins.

The most obvious parameter affecting the midpoint potential of the [2Fe–2S] cluster is the electronic environment surrounding the redox active iron. The crystal structure of the peripheral domain of the bovine Rieske iron–sulfur protein has been elucidated at 1.5 A (Iwata *et al.*, 1996) and is shown in Fig. 3A. From the crystal structure, several factors were identified that contribute to the positive midpoint potential of the Rieske protein, including a net charge of 0/-1 for the oxidized and reduced iron–sulfur cluster, the elec-

tronegativity of the histidine ligands, and the proximity of Fe-2 to the surface of the protein.

The crystal structure also indicates an extensive hydrogen bond network links the iron–sulfur cluster to the surrounding protein. As shown in Fig. 3B, the hydroxyl group of Ser183 is hydrogen bonded to S-1 of the [2Fe–2S] cluster, at 3.2 A. The hydroxyl group of Tyr185 is hydrogen bonded to the S_g of Cys159, a ligand of Fe-1, at 3.1 A. We predicted that these residues contribute to the positive midpoint potential of the Rieske protein by decreasing the negative charge density around the sulfur atoms through their respective hydrogen bonds.

We altered Ser183 and Tyr185 in the *Saccharomyces cerevisiae* Rieske iron–sulfur protein by site-directed mutagenesis of the iron–sulfur protein gene to examine how these hydrogen bonds affect the midpoint potential of the iron–sulfur cluster (Denke *et al.*, 1998). Eliminating the hydrogen bond from the hydroxyl group of Ser183 to S-1 of the cluster lowered the
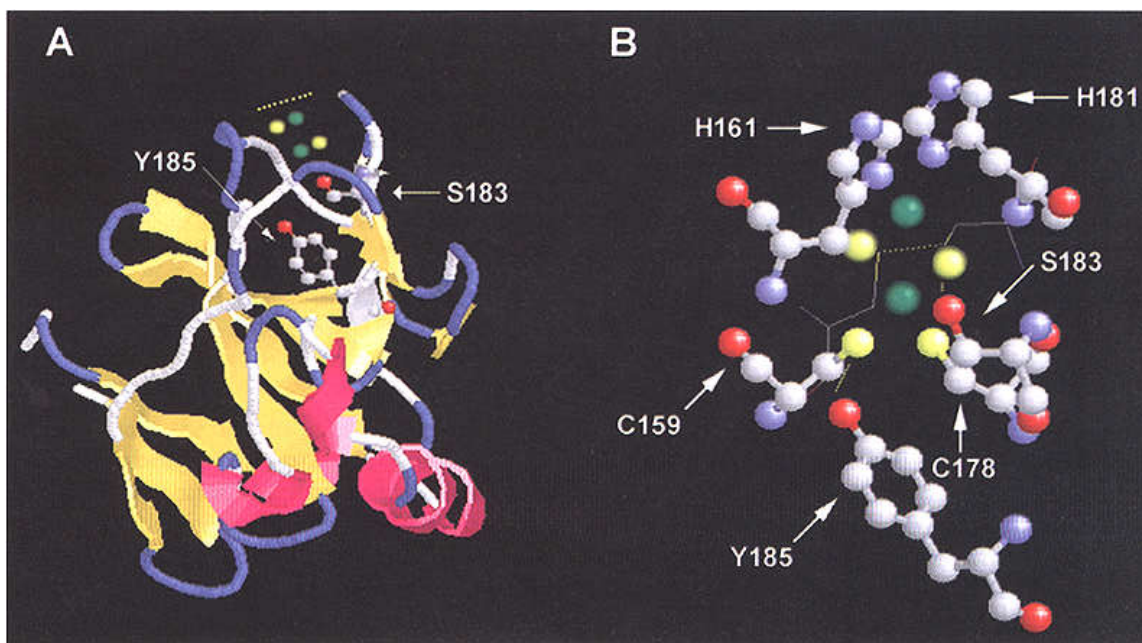

Fig. 3. Structure of the Rieske iron–sulfur protein, showing the location of Tyr185 and Ser183 in relation to the [2Fe–2S] cluster. The crystal structure in panel A is of the bovine iron–sulfur protein (Iwata *et al.*, 1996) and shows the location of Tyr185 and Ser183 in the yeast protein, equivalent to Tyr165 and Ser163 in the bovine protein, and the [2Fe–2S] cluster. The disulfide bridge that stabilizes the cluster binding fold is shown as yellow dots across the top of the fold. Iron and sulfur atoms of the cluster are in green and yellow, respectively. Helices are red and b-strands are yellow. The redox active Fe-2 is proximal to the disulfide bridge. The C-terminus of the protein and the N-terminus, where the protein is anchored to the membrane, are to the left of the protein. Panel (B) shows an expanded view of the protein in proximity to the [2Fe–2S] cluster, including ball-and-stick representations of His161 and His181, which are ligands to the redox active Fe-2, Cys159 and Cys178, which are ligands to Fe-1, Tyr185, and Ser183. Also shown are the hydrogen bond between the OH group of Tyr185 and the S_g of Cys159 and the hydrogen bond between the OH group of Ser183 and S-1 of the [2Fe–2S] cluster. The disulfide bridge between Cys164 and Cys180 is shown in chain representation in back of the cluster.
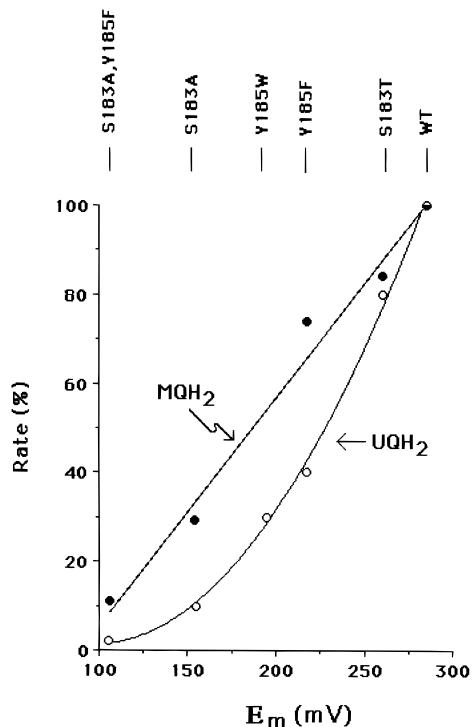
Fig. 4. Cytochrome *c* reductase activities versus midpoint potential of the Rieske iron–sulfur protein in mitochondrial membranes from yeast expressing mutated forms of the iron–sulfur protein. The curve labeled UQH$_2$ shows the cytochrome *c* reductase activities obtained with ubiquinol as substrate and that labeled MQH$_2$ shows the activities with menaquinol as substrate. Mitochondrial membranes were isolated from wild-type yeast (WT) and from yeast containing mutated forms of the Rieske iron–sulfur protein. The iron–sulfur protein mutations are identified by the number of the amino acid residue that was mutated and the amino acid change made at that position and are indicated across the top of the figure.

$$k_e = Ae^{-\frac{(\Delta G + \lambda)^2}{4\,KT\lambda}}$$

$$\text{Log } k_e = \text{Log } A - \frac{(\Delta G + \lambda)^2}{(2.3\,KT)\,(4\lambda)}$$

$$\text{Log } k_e = \text{Log } A - \frac{(\Delta G^2 + 2\,\Delta G\lambda + \lambda^2)}{(2.3\,KT)\,(4\lambda)}$$

If $\Delta G \ll \lambda$

$$\text{Log } k_e = \text{Log } A - \frac{(\Delta G)}{2\,(2.3\,KT)} - \frac{\lambda}{4(2.3\,KT)}$$

$$\Delta \text{Log } k_e = -\frac{\Delta\,(\Delta G)}{2\,(2.3\,KT)} \quad , \text{where } 2.3\,KT = .06 \text{ eV}$$

If $\Delta(\Delta G) = -.06$ eV

$$\Delta \text{Log } k_e = \frac{1}{2}$$

$$\Delta k_e = 3.16$$

Fig. 5. Relationship between electron transfer rate (k_e) and change in oxidation–reduction potential (ΔG) of the electron transfer reaction. Although the potential of the Q·/QH$_2$ couple at center P is not known for electron transfer from ubiquinol to the iron–sulfur cluster $\Delta G = \sim 0.19$ V. If the reorganization energy (λ) is approximately 1 V (Moser *et al.*, 1992), the equation can be simplified by assuming that $\Delta G \ll \gamma$, as shown. For a treatment of the thermodynamics and kinetics of electron transfer reactions, including a description of reorganization energy, see the textbook by Cramer and Knaff (1990).

midpoint potential of the cluster by 130 mV and eliminating the hydrogen bond from the hydroxyl group of Tyr185 to S$_\gamma$ of Cys159 lowered the midpoint potential by 65 mV. Eliminating both hydrogen bonds had an approximately additive effect, lowering the midpoint potential by 180 mV. Thus, these hydrogen bonds contribute significantly to the positive midpoint potential of the cluster, but are not essential for its assembly.

EFFECT OF IRON–SULFUR PROTEIN MIDPOINT POTENTIAL ON THE KINETICS OF ELECTRON TRANSFER IN THE *bc*$_1$ COMPLEX

To examine how the midpoint potential of the iron–sulfur cluster affects the catalytic activity of the *bc*$_1$ complex we measured the cytochrome *c* reductase activities of *bc*$_1$ complexes in which the midpoint potential of the cluster was altered. As shown in Fig. 4, the ubiquinol:cytochrome *c* reductase activity of the *bc*$_1$ complex decreases with the decrease in midpoint potential of the iron–sulfur cluster. This result suggests that oxidation of ubiquinol by the iron–sulfur protein is the rate-limiting partial reaction in the *bc*$_1$ complex and that the rate of this reaction is extensively influenced by the increment between the potential of ubiquinol and that of the iron–sulfur cluster.

If the latter conclusion is correct, the cytochrome *c* reductase rate should change less as the midpoint potential of the iron–sulfur protein changes if menaquinol is used as substrate. The increment between the potential of menaquinol ($E_{m7} = -74$ mV; Kroger and
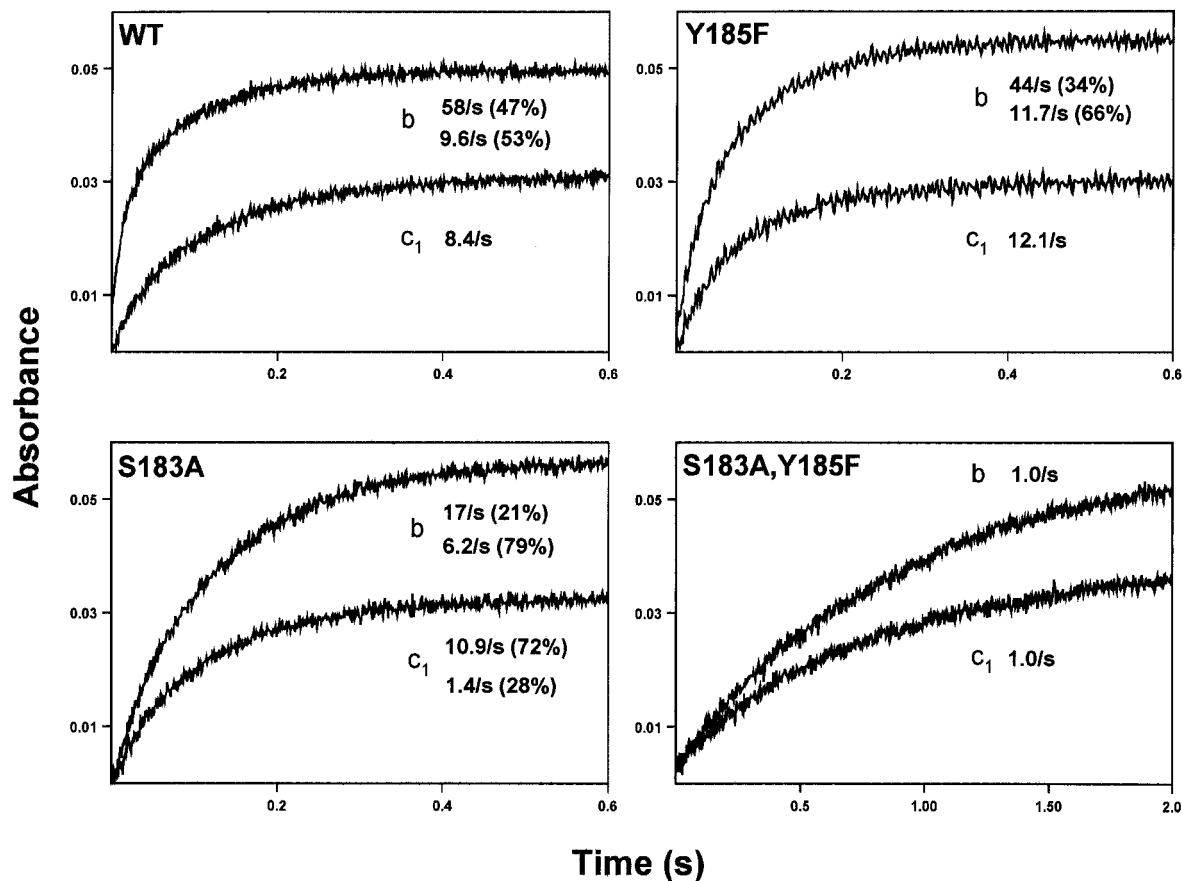
Fig. 6. Effect of the midpoint potential of the Rieske iron–sulfur protein on the presteady-state rates of reduction of cytochromes b and c_1. The traces show reduction of cytochrome b and c_1 when 1 μM cytochrome bc_1 complex is reduced by 50 μM menaquinol in the presence of antimycin at pH 6.0. Cytochrome bc_1 complexes were purified from wild-type yeast (WT) and from yeast containing mutated forms of the Rieske iron–sulfur protein. The iron–sulfur protein mutations are identified by the number of the amino acid residue that was changed and the amino acid change that was made at that position.

Unden, 1985) and the Rieske cluster is ~160 mV greater than that between ubiquinol (E_{m7} = +90 mV) and the cluster. Consequently, changes in the midpoint potential of the Rieske cluster should impact less on the menaquinol:cytochrome c reductase than on the ubiquinol:cytochrome c reductase activity. As can be seen in Fig. 4, a 60 mV decrease in midpoint potential of the iron–sulfur cluster causes the ubiquinol:cytochrome c reductase activity to drop by 60%, while the menaquinol:cytochrome c reductase activity only drops by ~30 %.

The equation relating electron transfer rate (k_e) to the free energy of the electron transfer reaction (ΔG), is shown in Fig. 5. If the rate of electron transfer from ubiquinol to cytochrome c is determined solely by the energy of electron transfer from ubiquinol to the Rieske cluster, a change of 60 mV in midpoint potential of the Rieske cluster should change the electron transfer rate 3.16-fold. The results in Fig. 4 show that as the midpoint potential of the Rieske cluster increases 60 mV, from 220 to 280 mV, the ubiquinol:cytochrome c reductase activity changes 2.5-fold. This calculation indicates that the increment between the potential of ubiquinol and that of the iron–sulfur cluster is not the sole rate determinant. However, the magnitude of the change in ubiquinol:cytochrome c reductase activity as the midpoint potential of the Rieske cluster changes indicates that this potential increment is quantitatively the most significant parameter affecting this rate.

The midpoint potential of the Rieske cluster also affects the presteady-state kinetics of cytochrome b and c_1 reduction. The presteady state reduction of cytochrome b and cytochrome c_1 in the bc_1 complex from a wild-

type yeast and in the bc_1 complexes from three yeast mutants in which the midpoint potential of the Rieske cluster was changed by site-directed mutagenesis of the cloned gene is shown in Fig. 6. In the bc_1 complex from the wild-type yeast, the midpoint potential of the Rieske cluster is more positive than that of the heme of cytochrome c_1 (Fig. 2). Under these conditions, reduction of cytochrome b is biphasic, and reduction of c_1 is monophasic. The explanation for the apparently different kinetics of b and c_1 reduction is that the fast phase of b reduction is linked to the optically invisible reduction of the Rieske center, while the rate of the second, slow phase matches that of c_1 reduction. The first electron from menaquinol equilibrates between the Rieske center and c_1 and only when the electron is localized to c_1 can the second molecule of menaquinol be oxidized and transfer electrons to the iron–sulfur protein and cytochrome b. This same explanation applies to the presteady-state kinetics in the bc_1 complex with the Y185F mutation in the Rieske protein in which the midpoint potential of the Rieske cluster at pH 6 (+250 mV) is slightly greater than that of c_1.

In the bc_1 complex with the S183A mutation in the Rieske protein, the midpoint potential of the Rieske cluster at pH 6 (+185 mV) is lower than that of c_1, but the first electron from menaquinol can still equilibrate between the Rieske center and c_1. The relative potentials in this mutant are such that 72% of the c_1 is rapidly reduced and the reduction of cytochrome b and the reduction of c_1 are both biphasic. During both phases reduction of c_1 is slower than reduction of cytochrome b. This lag may reflect the time required for the peripheral domain of the Rieske protein to move between cytochrome b and c_1 subsequent to oxidation of the semiquinone by heme b_H.

In the bc_1 complex with the S183A, Y185F double mutation in the Rieske protein, the midpoint potential of the Rieske cluster approaches that of the ubiquinone/ubiquinol couple. The potential increment between menaquinol and the Rieske cluster is decreased to a degree that oxidation of menaquinol is much slower than reduction of cytochrome b by the semiquinone and much slower than electron transfer from the Rieske cluster to c_1. Consequently, the kinetics of reduction of both cytochrome b and cytochrome c_1 changes from biphasic to monophasic and reflects the slow oxidation of the menaquinol.

The presteady state kinetics illustrate the importance of the high midpoint potential of the Rieske cluster to the Q-cycle mechanism. The catalytic activity of the bc_1 complex decreases and the presteady state rates of b and c_1 reduction become slower as the midpoint potential of the Rieske cluster decreases. Notably, reduction of b and c_1 remain kinetically linked as the midpoint potential of the Rieske cluster is varied by 180 mV and under conditions where the presteady state reduction is biphasic or monophasic. The persistent linkage of the rates of b and c_1 reduction is accounted for by the bifurcated oxidation of ubiquinol that is unique to the Q-cycle mechanism.

ACKNOWLEDGMENTS

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