Review

Proton pumping in the bc1 complex: A new gating mechanism that prevents short circuits

Antony R. Crofts a,b,⁎, Sangmoon Lhee b, Stephanie B. Crofts b, Jerry Cheng b, Stuart Rose a

a Department of Biochemistry, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA
b Center for Biophysics and Computational Biology, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA

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Abstract

The Q-cycle mechanism of the bc1 complex explains how the electron transfer from ubihydroquinone (quinol, QH2) to cytochrome (cyt) c (or c2 in bacteria) is coupled to the pumping of protons across the membrane. The efficiency of proton pumping depends on the effectiveness of the bifurcated reaction at the Qo-site of the complex. This directs the two electrons from QH2 down two different pathways, one to the high potential chain for delivery to an electron acceptor, and the other across the membrane through a chain containing heme bL and bH to the Qi-site, to provide the vectorial charge transfer contributing to the proton gradient. In this review, we discuss problems associated with the turnover of the bc1 complex that center around rates calculated for the normal forward and reverse reactions, and for bypass (or short-circuit) reactions. Based on rate constants given by distances between redox centers in known structures, these appeared to preclude conventional electron transfer mechanisms involving an intermediate semiquinone (SQ) in the Qo-site reaction. However, previous research has strongly suggested that SQ is the reductant for O2 in generation of superoxide at the Qo-site, introducing an apparent paradox. A simple gating mechanism, in which an intermediate SQ mobile in the volume of the Qo-site is a necessary component, can readily account for the observed data through a coulombic interaction that prevents SQ anion from close approach to heme bL when the latter is reduced. This allows rapid and reversible QH2 oxidation, but prevents rapid bypass reactions. The mechanism is quite natural, and is well supported by experiments in which the role of a key residue, Glu-295, which facilitates proton transfer from the site through a rotational displacement, has been tested by mutation.

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1. Introduction

The bc1 complex (Complex III of the mitochondrial respiratory chain) operates through a Q-cycle mechanism (reviewed in [1–4]). The key feature of this mechanism is the reaction at the Qo-site in which ubihydroquinone (quinol, QH2) is oxidized. The two electrons from QH2 are passed to two different electron transport chains — the first to a high potential chain of the Rieske iron–sulfur protein (ISP), cyt c1, cyt c (or c2 in bacteria), and a terminal oxidant. The second electron is transferred to a low potential chain of hemes bL,bH and the Qi-site, and crosses the membrane, to contribute to both the electrical and chemical work of the proton gradient. It has generally been considered that the reaction proceeds through separate 1-electron steps, with generation of an intermediate SQ. However, as discussed further below, the viability of schemes involving
significant occupancy of the SQ state has recently been challenged.

Since the bifurcation is essential for proton pumping, a perennial question has been how the site operates to prevent both electrons passing down the more favored pathway, to the high potential chain, to decouple this process. A second question relates to a design failure of the bc1 complex. When electrons back-up in the low potential chain, the Qo-site functions to reduce O2 to superoxide, the precursor of reactive oxygen species that lead to DNA and protein damage and cause cellular aging and death [5]. How does the mechanism minimize this deleterious reaction and other potential bypass reactions?

In this brief review, we will first provide an overview of the Q-cycle and its operation as a proton-pumping machinery, and address recent advances in our understanding of the critical Qo-site reaction. This will entail a brief survey of the kinetic, spectroscopic, structural, and theoretical tools available, and the use of these to set constraints on viable mechanisms, especially with respect to transfer of the first electron, which seems to be the rate determining process. We will then discuss the properties of the second electron transfer, the role of the SQ intermediate, and the different strategies that have been suggested to explain how the bypass reactions are minimized. Finally, we will discuss a mechanism incorporating coulombic gating that accounts for the peculiarities of the Qo-site reaction in the context of a model previously proposed [6].

2. The modified Q-cycle

The bifurcation of QH2 oxidation, the reaction at the heart of the Q-cycle, was first recognized by Wikstron and Berden [7] as an explanation for the phenomenon of “oxidant-induced reduction of cytochrome b” [8]. It was Mitchell’s genius to incorporate this reaction into the proton-motive Q-cycle [9,10] by directing one electron across the membrane to reduce ubiquinone (quinone, Q) on the other side. He named the two quinone-processing sites Qo and Qt, to differentiate whether the H+ came out on oxidation of QH2, or in on reduction of Q. Garland [11] recognized that a “stand-alone” enzyme would need to deliver two electrons to the Qo-site, requiring two turnovers of the Qo-site for net oxidation of 1 QH2, and called this a modified Q-cycle. Mitchell [10] discussed many variants of the Q-cycle, and laid down some of the thermodynamic constraints, but the experimental evidence available did not allow discrimination between these until the early 1980s. Detailed kinetic studies in chromatophores (the re-sealed invaginations of the inner bacterial membranes of photosynthetic bacteria, produced by mechanical disruption) showed that the modified Q-cycle could account for the paradoxical behavior observed in this system [12,13] (reviewed in [4]). The version shown in Fig. 1 is a consensus Q-cycle adopted by most workers in the field, and is supported by an extensive set of kinetic and thermodynamic data for the partial processes [1,14]. The mechanism is also now strongly supported by structures [15,16] (reviewed in [17]), all of which show the redox centers, and their organization with respect to membrane topology, predicted from these earlier experiments.

Proton pumping is achieved indirectly by movement of two negative charges across the membrane, carried by the electrons passing through the b-heme chain from Qo to Qo, and by release or uptake of H+, respectively, on oxidation or reduction of quinone, to give an overall yield of 2H+ pumped for each QH2 oxidized:

$$\text{QH}_2 + 2\text{cyt} \, e^+ + 2\text{H}_2\text{O}^+ \rightleftharpoons \text{Q} + 2\text{cyt} \, c + 2\text{H}_2\text{O}^+ + 2\text{H}_2\text{O}^+ \text{ (scalar)}$$

In chromatophores, the electrogenetic processes can be followed through the electrochromic carotenoid changes, which provide a “membrane voltmeter” function [18], and this convenience has allowed a detailed matching of electrogenic events to partial processes [12,14,19,20]. By careful correction of absorbance changes in the cytochrome α-band region for contributions from carotenoid changes [21], this approach was extended to measurement of driving forces from electron transfer during development of the proton gradient in the coupled steady-state [22–24]. Under static head conditions, the poise of the electron transfer chain was close to that expected from the modified Q-cycle in equilibrium with the proton gradient,
indicating a tight control. These results were in line with results from mitochondrial studies [25] in which the proton gradient was varied through poising of the ATPase reaction. In the chromatophore experiments, the development of both electron transfer poise and proton gradient could be resolved kinetically.

In chromatophores, where superior kinetic resolution and redox poising make it possible to separate out the partial processes contributing to the Q-cycle, activation energies can be measured or estimated for each of these within well-defined constraints [26]. From such experiments, it was established that the reaction at the Qo-site is rate limiting under conditions of substrate saturation, and that it is responsible for the high activation energy of the overall reaction. However, this reaction is itself complex, and under non-saturating conditions, rates are also in part determined by reactions involved in formation of the ES-complex, and dissociation of products. A full understanding of mechanistic detail therefore requires analysis of the partial processes contributing to the Qo-site reaction [1,13,27], and their structural context [26].

3. Movement of the ISP extrinsic domain—a dancer in the Qo-site ballet

A surprise from the structures was the large-scale movement of the extrinsic domain of the ISP [16], which is now recognized as essential for delivery of electrons from the Qo-site to cyt c1 [28–31]. The role of this movement in control of the Qo-site reaction has been the topic of much speculation. It seems clear from molecular dynamics simulation [34] and from kinetic measurements [14,35] that the movement itself is stochastic, and so rapid as not to be rate limiting for the overall forward reaction [32,33]. The critical issues for discussion therefore center on the involvement of the ISP mobile domain in formation of transient complexes with its reaction partners—with cyt b and the occupants of the Qo-site (inhibitors, Q and QH2), or with cyt c1 and its heme [6,36–38]. Structures showing the reduced ISP (ISPH) forming H-bonded complexes in situ with several Qo-site inhibitors (stigmatellin, NQNO, HHDBT and UHDBT) occupying the domain of the Qo-site distal from heme b2 (the distal domain), have been solved crystallographically [15,16,38–41].

The existence of such complexes was known from earlier biochemical evidence, and EPR spectroscopy [42,43]. In addition, it had been early speculated that the EPR line at \( g_S = 1.80 \) reflected an interaction of ISPH with quinone (Q) bound at the Qo-site, and that QH2 also likely might bind in close association with ISP in formation of the ES-complex [44]. Of these complexes, the reactions involved in formation of the ES-complex at the Qo-site are the most important in understanding mechanism [36,45–47], but because these occur only under the metastable condition in which oxidized ISP and ubihydroquinone are both present, these are also the least accessible to direct observation and thermodynamic characterization.

4. Configuration of the ES-complex

Because the ISP extrinsic domain is mobile, it acts as a diffusible second substrate (albeit, a tethered one) in the reaction at the b-interface, so that the binding of two substrates, ISPox and QH2, is needed for formation of the ES-complex. Since an electron is transferred from QH2 to ISPox, the ES-complex is expected to form with QH2 close to the ISP docking interface. The PDB file 1ntz has coordinates (with high B-factors) for a quinone occupant, but the experimental basis for these has not been discussed by the authors [48]. None of the other structures currently available shows any quinone species bound at the Qo-site. Modeling of quinone or quinol occupancy has therefore been based on occupancy of inhibitors. Because the rate-limiting reaction involves reduction of the oxidized ISP (ISPox), requiring a relatively short electron transfer path, the most obvious choice has been the stigmatellin structure, which shows a direct H-bond between Nε of His-161 of the reduced ISP and a carbonyl group of the occupant. A quinol modeled with H-bonds to the same ligands as stigmatellin can replace the inhibitor in the structure without strain, and fits within the electron density of the inhibitor [41,49]; the quinone species modeled in 1ntz is in a similar configuration. Models of this sort have been the starting point for most discussions of the ES-complex (Fig. 2) [36,49,50].

If a H-bond similar to that seen with stigmatellin, but from the quinol –OH to His-161 of ISPox, is involved in stabilization of the ES-complex, it would likely be to the imidazolate form [51,52]. The relative pK values for quinol and ISPox would favor an H-bond with the Nε of His-161 of ISPox in the dissociated form as H-bond acceptor, and the quinol –OH as donor. This configuration was suggested on the basis of the thermodynamic properties, but required the assumption that the pK on the oxidized form at 7.6 observed through redox titration of ISP was associated with the histidine involved in H-bonding [51].

Recently, Iwaki et al. [53] have shown that well-characterized bands in the FTIR difference spectrum represent “the loss of the imidazolate form of one or both of the Nε-ligated imidazole histidine ligands to the [2Fe–2S] center”, which are “in the imidazolate form in the oxidized protein above pH 7.6, and ...
The binding constant for ISPox in the absence of quinol could be estimated from crystallographic data [33]. The relatively weak occupancy of ISP, as estimated from the electron densities relative to the rest of the protein in structures of the native complex from different groups, was used to suggest that the affinity was relatively weak for either the b-position, or the c-position [33]. These estimates provide constraints on the fourth value needed for the thermodynamic binding square. The four values provide the same overall $\Delta G_0^\circ$, in the range $\sim -8$ kJ/mol (or 83 mV in electrical units) from the sum of separate $\Delta G_0^\circ$ terms, for formation of the ES-complex by either pathway from vacant site (left) to ES complex (right) of the thermodynamic square (see Fig. 3).

5. The EP-complex

The EP-complex in which quinone interacts with ISP has been studied through several different approaches. As noted above, the line at $g_x = 1.80$ in X-band CW-EPR depends on the presence of both Q and ISP. With the recognition from ENDOR and crystallographic data that the histidine ligands to the [2Fe–2S] cluster were exposed [60,61], several groups noted the possibility, based on the involvement of histidines in ligation of Q in bacterial reaction centers, that one of the histidines might also serve as a ligand to Q in the Qo-site [62–64]. From more detailed ESEEM studies, the hyperfine interactions of the N_e of a histidine (likely H-161 in bovine numbering) were shown to be similar in the $g_x = 1.80$ and stigmatellin complexes, and different from those seen when the complex was dissociated on addition of myxothiazol [65]. Addition of myxothiazol or MOA-type inhibitors, eliminated this band, presumably by displacing Q, and also led to a change in $E_m$ of $\sim 30$ mV of ISP [45,66–69], suggested to reflect the loss of the H-bond with quinone. Interestingly, when similar experiments were performed using mutant strains in which the linking sequence through which the extrinsic head is attached to the anchoring N-terminal tail was modified [37], the myxothiazol-induced change in $E_m$ was much greater, suggesting that additional forces had come into play [69]. The simplest interpretation was a change in strength of the H-bond arising from the closer proximity allowed by the lengthened link [45,68], but orientation-selective EPR measurements have suggested additional complications [67].

6. Forward electron transfer — the oxidation of QH$_2$

Much discussion of the efficiency of the bifurcated reaction has centered on “gating mechanisms” to prevent passage of both electrons to the high potential chain. Two natural gating processes are intrinsic to all Q-cycle mechanisms.

(i) The formation of the ES-complex will occur only under the metastable conditions in which the QH$_2$ substrate is available, and the high potential chain is oxidized. Normally, this non-equilibrium state is generated only under conditions appropriate for net forward electron transfer.

(ii) Under normal operation (“state-3” conditions in mitochondria), the quinone pool is maintained at least partly
oxidized. This is necessarily the case in chromatophores since otherwise, the photochemistry would fail; similarly, the dehydrogenases would be turned off if no Q was available in respiratory chains. As a consequence, an acceptor is always available at the Qₐ-site, so that the low potential chain is partly oxidized, and hence can rapidly remove the intermediate SQ product. Even under static head (“state 4”) conditions, when the backpressure from Δp is maximal, heme bₗ is about 50% oxidized [22–24,72,82].

It is not widely appreciated that, because net forward electron transfer can occur only when both conditions (ISP and heme bₗ both oxidized) are satisfied, and net reverse electron transfer when both ISP and bₗ are reduced, these constraints represent an intrinsic double-gating in both directions built into the Q-cycle. Under normal forward conditions, the efficiency of bifurcation is high, and bypass or short-circuit reactions [1,70–72,74] are negligible. Under static head conditions, heme bₗ becomes partly reduced because of “back-pressure” from the proton gradient, and a physiologically important bypass reaction occurs, leading, in an aerobic system, to reduction of O₂ to form superoxide [72,82,83]. Bypass reactions of this sort are also intrinsic to all Q-cycle mechanisms that involve formation of a SQ intermediate. However, the bypass rate is much greater when electron transfer to the Qₐ-site is blocked, and these inhibited conditions are normally used to study the reactions. It is estimated that the rate of superoxide generation in antimycin-inhibited mitochondria is ~80% of the total cellular production of reactive oxygen species [70–72,82], and such rates might apply in mutant strains, or myopathies with mutations in the Qₐ-site. The debate over how the complex has evolved to minimize these bypass reactions can be framed in terms of the following questions.

(iii) Are the rates of bypass reactions explicable in terms of the intrinsic electron transfer mechanisms, or
(iv) are there are additional special gating mechanisms that involve, for example, allostERIC conformational coupling, etc.

In order to explore these questions, it is necessary to establish a minimal model for the mechanism of the site at the molecular level.

7. Kinetic constraints on the reaction mechanism at the Qₐ-site

Distances between redox centers involved in catalysis can be measured from the structures. Empirical evidence and theoretical considerations have suggested that distances determine the “intrinsic” rate constant, kᵣ, in the Arrhenius–Randall–Wilkins treatment, kᵣ = kₑe⁻ΔG/RΔT. When expressed in log₁₀ form, and expanded to include a Marcus term for the activation energy, and a Moser–Dutton [73] treatment of kᵣ, this gives:

\[ \log_{10} k_{\text{cat}} = 13 - 0.6(R - 3.6) - \frac{\gamma(\Delta G' - \lambda)^2}{\lambda} \]

where R is the electron transfer distance (the closest distance between conjugate rings for cytochromes, chlorophylls, etc.), λ is the reorganization energy. The factor γ is 4.23 (F/(4×2.303 RT) at 298 K in the classical Marcus treatment, or 3.1 in the Moser–Dutton equation. This latter value reflects the contribution of quantum mechanical corrections that were applied to generate fits to experimental data. This useful equation can be applied to discussion of the mechanism of the Qₐ-site in the context of the structure, since it provides constraints determined by distances. For the Qₐ-site reaction, application depends on the answers to three questions:

(i) What is the structure from which the reaction proceeds?

(ii) What is the nature of the reaction?

(iii) If the reaction is sequential, which of the two 1-electron transfer steps is rate determining?

In the discussion of rates that follows, we will use the Moser–Dutton equation (with γ = 3.1), which gives increasingly higher rate constants than the classical treatment as λ and −ΔG° diverge. However, in most cases, plausible rate constants can be calculated using either approach. In the discussion of bypass rates below, the calculated SQ occupancy would vary depending on choice of γ. Since subsequent calculations are “normalized” to this occupancy, relative rates and occupancies will for the most part show the same pattern, and lead to similar conclusions, with either choice. Where anomalies occur they will be pointed out.

From the considerations in the section above, the location of the ES-complex with QH₂ in the distal domain seems firmly based, and this will provide the starting point for the remaining discussion. In our view, and not withstanding recent suggestions to the contrary [74,75], the evidence in favor of a sequential electron transfer with an intermediate semiquinone product is also overwhelming. Perhaps, most compelling is the demonstration that the overall rate is determined by the driving force for the first electron transfer, and is relatively independent of the driving force for the second electron transfer. The experimental basis for the first conclusion comes from several different groups who have reported mutations of ISP in which the redox potential of the [2Fe–2S] cluster has been modified [26,52,59,76,77]. The driving force is determined by the redox difference:

\[ \Delta G'' = -zF(E_{m(ISP)} - E_{m(QH_2)}) \]

It seems unlikely that mutations in the ISP cluster-binding domain would affect the Eₘ of the semiquinone couple. If Eₘ(QH₂) is unaffected, changes in Eₘ(ISP) would be directly reflected in changes in the driving force. After normalization to the wild-type rate, the results from separate studies crossing the bacterial/mitochondrial divide, and different genera of bacteria, have shown the same behavior, all data falling on the same curve of log₁₀k₀ v. ΔG°, which can be well fitted by a classical Marcus curve [47]. In contrast, no such relationship has been reported for mutations changing Eₘ(heme bₗ) [36,78]. Although mutants have been reported from several labs (cf. [26,36,78]). Similarly, the ~60 mV change in Eₘ of heme bₗ on reduction of heme bₜ
does not appear to have a significant effect on the rate or activation energy.

Hong et al. [26] suggested that determination of $k_{\text{cat}}$ from distances in the structures could be used to put constraints on reactions at the $Q_{\alpha}$-site, based on the simple kinetic relationship:

$$v = k_{\text{cat}} \cdot \text{occupancy}$$

They evaluated a number of different mechanisms, and concluded that, in the context of an ES-complex formed at the distal end of the pocket, viable schemes could be limited to three types:

(i) The intermediate SQ product was constrained to the distal domain, for example by a relatively stable ISP.HSQ intermediate product [41,79]. In this case, a relatively high occupancy was required for overall forward chemistry at the observed rate because of the limitation on the rate constant for the second electron transfer arising from the long distance to the acceptor, heme $b_1$.

(ii) The SQ product was liberated by dissociation of the ISP.H after the first electron transfer, and could move within the volume of the $Q_{\alpha}$-site [36,49]. Hong et al. [26] favored this mechanism because of the need to minimize harmful short-circuit reactions by keeping [SQ] occupancy to a minimum. Because the rate constant is higher at shorter distances to the acceptor heme $b_1$, the observed rate could be attained at much lower SQ occupancy.

(iii) Double occupancy of the $Q_{\alpha}$-site by two quinones [44,80], arranged so as to bridge the distance between ISP and heme $b_1$, would also provide a mechanism allowing a higher rate constant for transfer of the second electron, and hence allow a lower occupancy. However, as discussed at length elsewhere, the failure to find in the kinetic behavior or the structures the properties expected was seen as problematical [26,36].

Among mechanisms rejected were all those in which the two electron transfers occurred from a common activated state, since only one of the two steps, the first electron transfer, showed control by driving force.

8. The first electron transfer from $QH_2$ to $ISP_{\alpha}$

Identification of the first electron transfer as the limiting process raised the question of why this reaction was so slow. As shown in Fig. 2, the electron transfer distance is $<7.0$ Å, which, with conventional parameters for driving force and reorganization energy ($\lambda$) in the Moser–Dutton equation, would be consistent with a rate in the sub $\mu$s range rather than the $1.3 \times 10^3$ s$^{-1}$ observed. The “theoretical rate” could be “slowed” by assuming both a strongly endergonic first electron transfer, and a large value for $\lambda$, consistent with the high activation barrier [26]. However, the reaction interface provided no obvious explanation for the high activation barrier. From the nature of the proposed ES-complex, and the redox properties of the reaction partners, it was apparent that a proton transfer must be coupled to the electron transfer reaction, and this insight leads to a more satisfactory explanation. The $pK$ values for $QH_2$ and $ISP_{\alpha}$ determine the probability for proton transfer along the H-bond joining them, through a Brønsted term, $\Delta G^\circ /RT = 2.303$ (p$pK_{\text{donor}} - pK_{\text{acceptor}}$). Use of the measured $pK$ values, together with the overall free-energy, allowed construction of a thermodynamic cycle from which the probability of different pathways could be assessed. The likely sequence in the reaction is the pathway involving the transfer of a proton first, then the electron; the electron transfer occurs from a proton configuration that is kinetically favorable, but thermodynamically improbable [46,81]. The proton transfer step is strongly uphill ($\Delta G^\circ = 21 – 27$ kJ/mol), and contributes about half the activation barrier. The remaining barrier is that for the electron transfer itself. This has a driving force closer to the isopotential range, and can occur with conventional reorganization energy and rate constant. The observed rate is slow because transfer occurs from a very low occupancy of the kinetically favorable proton configuration [47].

Rates of proton transfer along H-bonds can be extremely rapid (in the range $10^{11}$ to $10^{13}$ s$^{-1}$). Even allowing for the strongly unfavorable equilibrium constant, rate constants in both directions will be much more rapid than the overall electron transfer rate, so the contribution of the proton barrier can be treated using a simple kinetic expression, $k_{\text{app}} = k_{ET}k_{\text{proto}}$. Expansion of the Moser–Dutton equation to include this term gives the following [47].

$$\log_{10}k = 13 - \frac{\beta}{2.303} (R - 3.6) - \frac{\gamma (\Delta G^\circ_{ET} + \lambda_{ET})^2}{\lambda_{ET}} - (pK_{QH_2} - pK_{ISP_{\alpha}}).$$

9. Role of the $pK$ of $ISP_{\alpha}$ in determining rate

An interesting consequence of this treatment is that the $pK$ of $ISP_{\alpha}$ has two roles to play in determining the rate of reaction. One is through the Brønsted term in the above equation. An additional contribution, as noted above, arises from the role of the dissociated form of $ISP_{\alpha}$ as the substrate involved in formation of the ES-complex, and dependence of the concentration of this form on pH and $pK$. The probability of forming ES is dependent on $pK$. These different roles can be explored through use of mutant strains in which the $pK$ is modified. Raising the $pK$, as in strain Y156W [47,59], with $pK$ changed from 7.6 to 8.5, leads to a change in the pH dependence of electron transfer to the higher value expected from the role in formation of ES. However, the higher $pK$ would be expected to lower the Brønsted barrier, and therefore lead to a higher rate constant. This effect is also observed, though convoluted with the change linked to the change in driving force arising from the changed $E_m$. Because the two changes have opposing effects on rate, the net result is that at any fixed pH below the $pK$, the rate appears to depend only on the $E_m$ value, as observed [59]. Further exploration of this relationship with new mutant strains in which Ser-154 is modified support these conclusions (SangMoon Lhee and ARC, unpublished).
The second electron transfer involves oxidation of the SQ by heme b$_{L}$. Examination of the available structures showed several features that provided clues to the mechanism [36,49].

(i) The volume of the site was larger than necessary to accommodate the QH$_2$ substrate.

(ii) Inhibitors were bound in different but overlapping domains; the H-bonding of stigmatellin by ISP constrained it to the domain distal from heme b$_{L}$, but myxothiazol and MOA-type inhibitors were bound more proximal to heme b$_{L}$.

(iii) Glu-272 was in different positions in different structures, either pointed towards the distal domain to act as a second ligand to stigmatellin, or rotated by $>130^\circ$, to point towards heme b$_{L}$ in the native structure, or those with proximal domain inhibitors.

(iv) Residues changed in strains resistant to myxothiazol but not to stigmatellin lined the myxothiazol binding domain as expected, but changes at these positions correlated with two additional properties of mechanistic interest; (a) electron transfer was inhibited, but (b) the binding of Q to ISPH indicated by EPR signal at $g_x = 1.80$ was not. These effects were interpreted as showing that in these strains, binding of quinone species (including QH$_2$) at the distal domain was unimpeded, but that occupancy of the proximal domain was blocked, and that such an occupancy by some intermediate was necessary during turnover.

On the basis of these properties, and of changes on mutagenesis of Glu-295 (the equivalent of Glu-272 in Rb. sphaeroides), Crofts et al. suggested a mechanism in which the SQ could move in the site closer to heme b$_{L}$ (Fig. 4) [34,36,49]. Molecular dynamics simulations had revealed a buried water chain to which the glutamate connected in the rotated position. The rotation of Glu-272 therefore provided a mechanism for exit of the second H$^+$ from the site. An additional point was that, with a shorter distance to heme b$_{L}$, the necessary rate could still be achieved at a lower occupancy of SQ.

As Hong et al. [26] pointed out, several critical parameters needed to assess this mechanism were unknown, in particular the SQ occupancy, and the distance over which electron transfer occurs. Osyczka et al. [75] introduced the idea that the occupancy of SQ could be estimated from the rate of short-circuit reactions, since all of them involve SQ as an intermediate. This approach, and the earlier arguments of Hong et al., now make it possible to determine important constraints on mechanism that allow a choice between the options previously discussed [26].

The problems can be summarized through the terms in Eq. (2) – occupancy, rate, and rate constant – the latter provided by the distances through which electron transfer reactions involving SQ might occur (Fig. 5). Starting with a SQ in the distal position (Fig. 5A), where it would be formed from an ES-complex of the sort discussed above, evolution of the state of the system under “bypass-conditions” (forward electron transfer inhibited because heme b$_{L}$ is reduced), can occur through several pathways that lead to loss of the SQ [5,22,75]. Muller et al. [5] showed that the dominant pathway ($\sim 70\%$) was by oxidation, either by O$_2$ under aerobic conditions, or through the high potential chain (ISP, heme c$_{1}$, cyt c) under anaerobic conditions. Because cyt c can rapidly oxidize the superoxide generated under aerobic conditions, both pathways could be assayed through the reduction of cyt c in the presence of antimycin. Oxidation under anaerobic conditions necessarily requires the dissociation of the intermediate product (ISPH.SQ) after the first electron transfer, because movement of the ISPH is necessary for reduction of heme c$_{1}$ [5]. The immediate oxidant for SQ is ISP$_{ox}$ on its return from delivery of an electron to cyt c$_{1}$. It is assumed that ISP$_{ox}$ accepts electrons from the same position as in the ES-complex, through a distance of $\sim 7.0$ Å. It is likely that O$_2$ reacts through a similar distance, since the docking site for the ISP would provide an

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**Fig. 4. Configurations of the Q$_{o}$-site involved in electron transfer.** In A, the configuration before transfer of the first electron is shown. The first electron transfer occurs through the 7 Å distance shown between QH$_2$ and the 2Fe–2S cluster of ISP, with the constraints discussed in the text. Immediately after the electron transfer, the intermediate QH$^{+}$ would be in a similar configuration, but with the reduced 2Fe–2S cluster dissociated so as to liberate both ISPH and SQ for subsequent movement. In B, the mobilized SQ has passed a proton to the –PEWY– glutamate (Glu-272 in chicken numbering), the side chain of which has rotate so that the carboxylic acid group contacts a water chain leading to the P-side aqueous phase. The Q$^{+}$ is now close enough to heme b$_{L}$ to deliver an electron rapidly, even at the low occupancy of SQ suggested (see text).
access port when the domain was not there. These assumptions provide a value for the distance in the Moser–Dutton equation, allowing calculation of a plausible rate constant. From the measured rate and Eq. (2), we should now be in a position to calculate occupancy. Osyczka et al. [75] discuss the problem in terms of the stability constant for SQ formation under equilibrium conditions, $K_{\text{stab}}$. This provides a measure of occupancy/bc1 complex through $[\text{SQ}] = (0.25 K_{\text{stab}})^{1/2}$, but is otherwise not relevant to the Q$_o$-site mechanism. Using the ~7.0 Å distance, a rate for bypass of ~0.3 s$^{-1}$ measured from the decay of reduced heme b$_{L}$, a driving force for electron transfer from SQ to ISP$_{\text{ox}}$ of ~0.5 V, and a conventional value for $\lambda$ ~1 V, they calculated an occupancy in the range $5 \times 10^{-8}$ SQ/bc1 complex. The rate of electron transfer from SQ in the distal domain to heme b$_{L}$ at this occupancy, which would determine the net rate of forward electron transfer, would be ~0.25 s$^{-1}$, clearly inconsistent with the measured forward rate of $1.3 \times 10^{3}$ s$^{-1}$ [26]. The rate of bypass measured by Osyczka et al. (~0.3 s$^{-1}$) was 10-fold slower than rates commonly reported in the literature [71]. The latter are measured under “oxidant-induced reduction” conditions, with the two b-hemes completely reduced, which might be expected to maximize SQ occupancy. In estimation of the operational SQ occupancy under conditions of normal forward electron transfer, we have to keep in mind the possibility of gating effects from the heme. If we ignore such effects, the SQ occupancy would likely be lower than that calculated from maximal bypass rates because the rate constant for SQ removal is higher than for formation. However, using a bypass rate of 3.0 s$^{-1}$ [5], in the range commonly reported, giving an occupancy $\sim 4 \times 10^{-8}$, the calculated rate for transfer of the second electron would still be 3 orders of magnitude slower than the observed forward rate (Fig. 5A a, b). In order to achieve the measured rate, an occupancy in the range $>10^{-5}$ SQ/(bc1 complex) would be needed. However, and here’s the problem, this would be expected to allow rates for bypass reactions similar to those for forward chemistry, and would lead to massive inefficiency in proton pumping. We can endorse the conclusion reached by Osyczka et al. “Thus, there is no single value of $K_{\text{stab}}$ that will permit rapid sub-millisecond forward and reverse electron tunneling and simultaneously limit short-circuit tunneling to the timescale of seconds” [75], and recognize the need for some gating mechanism.

The suggestion that the SQ might move in the site [26,36,49] provides some escape from this paradox. Using the SQ occupancy of $4 \times 10^{-8}$ calculated from the observed bypass rate, the change in rate constant for the electron transfer from SQ to heme b$_{L}$ obtained by changing the distance from 12.4 to 6.3 Å, allows for a ~1000-fold increase in rate, to a value greater than the observed rate (Fig. 5B(c)). However, we are now forced to consider possible consequences of allowing the SQ close to heme b$_{L}$, and the potential for short-circuit by other bypass reactions, such as those involving direct reduction of SQ by reduced heme b$_{L}$ [1,5,22,74]. Given the $E_{\text{m}}$ values of heme b$_{L}$ (~90 mV) and the SQ/QH$_2$ couple (likely >400 mV), the reaction would be strongly exergonic, and would occur over a short distance, leading, at the occupancy proposed, to a calculated bypass rate similar to the forward rate (Fig. 5(d)). Similar scenarios can be demonstrated for other positions because no matter what particular position we choose, rates for forward, back, and bypass reactions are in the same range, leading to prediction of crippled proton pumping efficiencies.

11. Mechanisms that prevent accumulation of SQ

In view of these difficulties, it is not surprising that Osyczka et al. [74,75] would suggest that mechanisms allowing accumulation of SQ should be discarded. However, this may be a case of throwing the (SQ) baby out with the bath-water — a
11.1. Concerted mechanisms

Hong et al. [26] had previously considered the evidence against some concerted mechanisms, and the points they raised are still valid. These and other difficulties can be summarized as follows:

(i) In a concerted reaction of the sort proposed, both electron transfers would have to occur at the same rate. If both electron transfers occur from a common activated state, then both would have to have the same intrinsic rate constant, \( k_a \), and the same control by driving force. From the lack of control in the second electron transfer, this is clearly not the case.

(ii) Similarly, the distance for transfer of both electrons to their separate acceptors would have to be about the same, with similar structure intervening between the QH\(_2\) donor and the two different acceptors. Neither the location for the reaction complex roughly midway between ISP\(_{ox}\) and heme b\(_1\), nor the structural similarity, has any justification from crystallographic data.

(iii) Separation of two electron charges from QH\(_2\) in a phase of relatively low dielectric would entail obvious physical problems. It is therefore necessary to address the question of the co-transfer of protons required by the overall reaction equation, and the synchronization of these processes with the electron transfers. No consideration was given to these aspects.

(iv) The exponential term in the Arrhenius equation provides an estimate of the occupancy of the activated state. Under conditions of substrate saturation, \( k_{cat}=k_a \exp(-\Delta G^0/RT) \approx k_a[ES]^\gamma \). This is because, in the standard treatment, \(-\Delta G^0/RT=\ln K^\gamma\), and \( K^\gamma=[ES^n]/[ES] \), so that \([ES]^{\gamma}\) becomes equal to the fractional occupancy of the activated state if \([ES]^{\gamma}<<[E_{cat}] \). For an activation energy of \(-600\) mV, occupancy would be \(10^{-10} \). Applying Eq. (2) to this occupancy shows that a rate constant \(10^{12} \) s\(^{-1}\) is required to give the observed rate. This is the value expected when the reactants are at van der Waals contact. No plausible configuration of the Q\(_a\)-site would allow both electron transfers to occur from such a distance.

(v) It is clear in any scenario that if the activated state is generated in the distal domain, electron transfer to heme b\(_1\) could not occur at a realistic rate at this occupancy [26].

(vi) Recognizing that the constraint suggested by this simplistic treatment might be too harsh, there are still problems even with the most permissive scenario. For a reaction complex midway between ISP\(_{ox}\) and heme b\(_1\), the rate constant calculated using the Moser–Dutton equation with \( \lambda=2.0, R=9.6 \) \( \AA \), and \( \Delta G^0=0 \), is \( k_{cat} \sim 1.4 \times 10^5 \) s\(^{-1}\). This is just adequate for concerted electron transfer from a fully occupied ES-complex in this position. However, such a scenario is difficult to justify. None of the structural or kinetic evidence discussed above is compatible with such a position, and the plausibility of the calculated rate constant depends on quantum mechanical corrections (implicit in the \( \gamma=3.1 \) of the Moser–Dutton treatment) to “lower” the barrier. With a classical Marcus barrier, this configuration gives rate constants 100-fold too slow.

(vii) An earlier mechanism discussed by Crofts and Wang [27] involved a SQ intermediate at the occupancy of the activated state, and is formally equivalent to a concerted reaction, since the second electron transfer occurred from this state. A similar mechanism was more recently discussed by Kim et al. [90]. The slope of the Marcus curve expected is much steeper than observed, and on this basis, mechanisms of this class were excluded [26].

11.2. Double-gated mechanisms

The discussion of how double-gated mechanisms might function [74,75] has been framed in rather general terms so as to allow formation of SQ only if both chains were oxidized and QH\(_2\) was available as substrate for the forward reaction, or both chains were reduced and Q available as substrate for the reverse reaction (see above). In either case, the SQ would always be rapidly removed on formation, so as to keep the occupancy low. There is no objection in principle to double-gating, since it is intrinsic to the Q-cycle mechanism. However, the mechanisms proposed by Osyczka et al. [74] were rather lacking in detail, and it was not clear what additional mechanistic components would be necessary to forestall bypass reactions, for instance under “oxidant-induced reduction” conditions. Rich [95] has discussed some specific “logic-gated” mechanisms, including gating functions linked to the redox state of the b-hemes, that would prevent binding of QH\(_2\) or formation of SQ [84,96], but Osyczka et al. [75] preferred the more general framework. In neither case were particulars included that might be tested experimentally. The structural context was weakly defined, and the evidence discussed above for specific features (such as the configuration of the ES-complex with a direct H-bond from His-161 of ISP, and a direct H-bonding role for Glu-272) was ignored. Instead, participation of waters as H-bonded bridges between the quinone species and unidentified ligands was proposed [75]. No evidence for such a configuration is available. The authors did not suggest experimental tests, except in
the general sense of accounting for the low rates of short-circuit reactions they measured. This argument was weakened by the fact that the rates they reported were 10-fold lower than those measured elsewhere.

The types of mechanism proposed by Osyczka et al. [74,75] would be expected to prevent significant occupancy of SQ, so neither accounts for the bypass rates that are observed, or the properties of these reactions that have led others to recognize that a SQ is involved. A plausible scheme in either the concerted or the double-gated scenario would therefore need to be supplemented by additional hypotheses.

12. Coulombic gating

An alternative approach is to look for explanations in the context of mechanisms in which SQ is a natural intermediate. A promising starting point is the mechanism involving mobility of the SQ in the site [36,49]. If the SQ could be prevented from getting close to reduced heme bL, the troubling short-circuit reactions associated with that configuration would be avoided. The mechanism summarized in Figs. 4 and 5 shows a gating process that is a natural consequence of the chemistry. It involves the other dancer in the Qo-site ballet — Glu-272 — previously proposed as a proton-carrying group involved in getting the second proton out of the Qo-site [36,49]. The pirouette of Glu-272 was apparent in early structures from Berry’s group [16,32]. In the stigmatellin structure PDB 2bcc, it was involved as a ligand to the inhibitor. In contrast, in the native structure PDB 1bcc, or in the presence of myxothiazol [36], the side chain was rotate around by >130° to connect to a chain of H2O molecules leading to the aqueous phase. This water chain was first identified in a molecular dynamics simulation of ISP movement [34], but has now been seen in higher resolution structures of bc1 complex from yeast [50] and bovine mitochondria [41].

The initial configuration in the mechanism suggested started with the ES-complex discussed above (Fig. 4A). After the first electron transfer and dissociation of ISP, a neutral SQ, QH+, was left at the distal end of the site, H-bonded to the Glu-272 carboxylate side chain. After H+ transfer from QH+ to glutamate, the side chain rotated to release the H+ to the water chain, leaving a SQ anion, Q−, free in the site. We assumed that with heme bL oxidized, Q− could diffuse to the proximal position, and transfer the electron to heme bH (Fig. 4B). However, as noted in [36], if heme bL were reduced, the net change in local negative change would have coulombic consequences—the Q− would be repelled. This would have two effects, (a) the reaction leading to formation of the anionic form (on transfer of the second H+ out of the site) would by discouraged, and (b) coulombic repulsion would prevented any Q− formed from getting close to heme bL.

The SQ would then be restricted to the neutral QH+ form, constrained to the distal domain (Fig. 5C)—just what is needed to prevent the rapid short-circuit reactions. These coulombic effects therefore provide the gating necessary to minimize bypass reactions while allowing rapid forward and reverse chemistry. The coulombic gating we have proposed functions in the context of a mechanism that requires formation of SQ, albeit at very low occupancy. This is in contrast to the suggestion of Osyczka et al. [75], where the gating was proposed to prevent formation of SQ.

13. Mutational experiments to test the role of the PEWY glutamate

In the mechanism proposed, Glu-272 obviously plays an important role. We have previously reported experiments on strains in which the equivalent residue (Glu-295) in Rb. sphaeroides was mutated to glutamine, glycine, or aspartate in strains E295Q, E295G and E295D, respectively [36,49]. All strains showed substantially inhibited electron transfer through the Qo-site, resistance to stigmatellin, and a modest increase in Km for QH2. The E295Q, E295G strains (the E295D strain was not tested) also retained a normal g∧ = 1.80 EPR signal, diagnostic of an uninhibited reaction of Q with ISPH at the b-interface. These properties were consistent with the mechanistic role for the ES-complex proposed. We have recently regenerated these and other mutations in a His-tagged background to facilitate spectroscopic studies. We have compared the bypass reactions in these strains with wild type under conditions designed to minimize artifacts, and we show preliminary results and example traces in Table 1 and Fig. 6. Several features of the results are noteworthy, and provide strong support for the coulombic gating mechanism proposed.

(i) It is clear from Table 1 that all strains showed bypass rates similar to or greater than those seen in wild type.

(ii) It will also be obvious that all mutant strains showed a strongly inhibited forward electron transfer. However, just as important is the fact that none showed the complete inhibition seen with mutations at other essential residues.

Each of these features is interesting. The bypass rates show clearly that SQ was being produced in all cases, at occupancies similar to or greater than in wild type under bypass conditions. Yet the inhibited rates show that the SQ was unable to reduce heme bL at the wild type rate. It is not clear what extent of inhibition the measured rates represent, because, as argued at length by Hong et al. [26], the rates for the second electron transfer in the absence of Qo-site inhibitor are not rate limiting. In the presence of antimycin, the rate of reduction of heme bL, seen when heme bH is pre-reduced is essentially the same as the rate of reduction of heme bH [97], despite that fact that the driving force differs due to the coulombic effect, which lowers the Em of heme bL by ~60 mV. In addition, the activation barrier for both
reactions was the same [26]. Because no control by driving force was apparent from this step, the rate must be fast enough that any slowing due to the change in driving force is not reflected in the observed rate [26]. Under the conditions of the experiments in Table 1, the reaction was limited by the relatively oxidized state of the quinone pool, but the intrinsic limitation was in the first electron transfer step, as discussed above. In wild type, with an unconstrained second electron transfer, a rate (determined by rate constant and occupancy as discussed above) in the range >10-fold higher than that for the first step seems reasonable, or >10⁴ s⁻¹. The observed rates in the mutant strains therefore likely represent a much more substantial inhibition than is at first apparent. Remarkably, the rates observed are in the range expected from electron transfer from the distal domain at the occupancy calculated from the bypass rate (see Fig. 5), as discussed more extensively below.

(iii) In Fig. 6, example traces of the kinetics of reaction center (RC) and cytochrome changes following flash excitation of chromatophores from a strain with wild type sequence, and in E295W, one of the more severely inhibited strains, are shown. The kinetics observed show clearly that, in both mutant and wild type strains, the distribution of electrons in the high and low potential chains is controlled by the equilibrium constants, as previously demonstrated [12]. That for the Qₒ-site reaction controls the overall distribution with respect to the quinone pool. In all cases, the Qₒ-site reaction is observed through the "oxidant-induced reduction of cyt b" [8], which is initiated when, with QH₂ available in the pool, oxidizing equivalents from the RC, generated on flash illumination, reach the Qₒ-site via ISPₒx. In Fig. 6A and B, the strongly inhibited rate in the mutant is apparent from the slow rate
of reduction of heme b11 (green trace) observed in the presence of antimycin following a single flash. In wild type, the reduction is substantially complete at 40 ms but in the mutant, barely detectable. If six flashes are given to fully oxidize the reaction center and maximize the driving force available in the high potential chain (Fig. 6C and D), and the time of measurement is extended, additional features are apparent, and a more complete reduction of heme b11 can be observed in the mutant. Note that, in the rise kinetics for both strains, the reduction of heme b11 lags that of b11, as expected from the difference in $E_m$ values. This lag is apparent in the wild type strain in Fig. 6A, where no reduction of heme b11 was seen after the first flash, but heme b11 was rapidly reduced. The reduction of heme b11 on the second and subsequent flashes can be readily seen if the time scale in Fig. 6C is expanded (cf. [97]). In the E295W mutant (Fig. 6D), the turnover of the Qo-site is so slow that the reaction has not yet come to equilibrium at the time of the last flash, and the traces showing reduction of both heme b11 and b11 continue to rise, the former even out until 4 s. However, as the high potential chain relaxes, with RC and then cyt c going re-reduced, the controlling effect of the Qo-site equilibrium constant can be seen, leading to the re-oxidation of heme b11 before any relaxation of heme b11. Essentially, the same relaxation effects can be seen in the wild type (Fig. 6C), but they start from the higher level of reduction of the b-heme chain achieved by the end of the flash sequence.

It is clear from these results that in both wild type and mutant, the electron transfer constants controlling the kinetic effects are similar, but that both forward and reverse rate constants are inhibited in the mutant strain. Similar effects were observed for all mutant strains [1].

(iv) The bypass rates were measured from the flux of electrons into the terminal acceptor of the high potential chain, the oxidized reaction center, immediately after the last flash of the sequence. At this point in the kinetic trace, heme b11 and heme b11 were essentially completely reduced in the wild type; in the mutant, heme b11 was ~80% oxidized and heme b11 was almost completely oxidized. Intermediate levels of reduction of heme b11 were seen in less severely inhibited strains [1]. Since the SQ was likely the source of electrons in all cases, we can say that the redox state of heme b11 determines neither the binding of QH2, nor the formation of SQ. From this, it seems likely that neither the docking of, nor the electron transfer to, ISPox are modified by the redox state of either b-heme, and that we can excluded any specific dependence on redox state of the b-hemes in gating of these partial processes.

The behavior summarized above is readily explained in terms of the hypothesis proposed here. The mutation of Glu-295 frustrates the proton exit mechanism for the second proton. This constrains the SQ to the neutral form, QH*, and to the distal domain, and likely also constrains Q to the distal domain. The electron transfer rates from the distal domain are limited by the greater distance to heme b11. From the distances, we might expect the rate constants to change by a factor of ~1000, as discussed above, but both forward and reverse rate constants would be changed by the same ratio, since the determining factor will be the same difference in distance between the occupant in distal and proximal locations and heme b11.

Table 2

<table>
<thead>
<tr>
<th>Reaction</th>
<th>$\Delta G^†F$ (mV)</th>
<th>$k_{\text{forward}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>First electron transfer</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>QH2 + ISPox + H+ + E b11 = ISPox + QH2 + E b11</td>
<td>−80 a</td>
<td>1.4 × 10^6 M^−1 s^−1</td>
</tr>
<tr>
<td>ISPox + QH2 + E b11 = ISPox + QH2 + E b11</td>
<td>300 b</td>
<td>10^10 s^−1</td>
</tr>
<tr>
<td>ISPox + QH2 + E b11 = ISPox + QH2 + E b11</td>
<td>360 c</td>
<td>10^9 s^−1</td>
</tr>
<tr>
<td>ISPox + QH2 + E b11 = ISPox + QH2 + E b11</td>
<td>−300</td>
<td>10^15 s^−1</td>
</tr>
<tr>
<td>QH2 + ISPox + H+ + E b11 = ISPox + (QH2) + E b11</td>
<td>280 to 440 d</td>
<td>1.3 × 10^3 s^−1</td>
</tr>
<tr>
<td><strong>Second electron transfer</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ISPox + QH2 + H+ + OOC-glu + E b11 = ISPox + QH2 + H+ + OOC-glu + E b11</td>
<td>−30 c</td>
<td>&gt;10^4 s^−1</td>
</tr>
<tr>
<td>ISPox + QH2 + H+ + OOC-glu + E b11 = ISPox + QH2 + H+ + OOC-glu + E b11</td>
<td>30 f</td>
<td>10^10 s^−1</td>
</tr>
<tr>
<td>ISPox + QH2 + H+ + OOC-glu + E b11 = ISPox + QH2 + H+ + OOC-glu + E b11</td>
<td>−30 g</td>
<td>&gt;10^8 s^−1</td>
</tr>
<tr>
<td>ISPox + QH2 + H+ + OOC-glu + E b11 = ISPox + QH2 + H+ + OOC-glu + E b11</td>
<td>−280</td>
<td>&gt;10^4 s^−1</td>
</tr>
<tr>
<td>ISPox + QH2 + H+ + OOC-glu + E b11 = ISPox + QH2 + H+ + OOC-glu + E b11</td>
<td>−310</td>
<td>&gt;10^4 s^−1</td>
</tr>
</tbody>
</table>

In all reaction equations, E stands for enzyme, and represents the Qo-site in cytochrome b.

A Visual Basic program, Marcus–Bronsted, which allows the user to explore how the Marcus curves for the two electron transfer reactions at the Qo-site vary with different parameters for driving force, SQ occupancy, pK of ISPox, etc., is available for downloading from the URL below. Files available include the program in executable (exe) form, and all source code files needed for compilation, together with an illustrated user manual.

http://www.life.uiuc.edu/crofts/Marcus_Bronsted/.

a The value in electrical units is derived from apparent binding constants.

b Several possible ways for normalization to concentration are discussed in [47].

c From $\Delta G^\circ = 2.303(RT/F)$ (pK_donor − pK_acceptor), using 11.3 for pK_donor and 6.3 for pK_acceptor, this latter value is appropriate for the pK of the form bound in the ES-complex [47].

d Assuming $\Delta G^\circ$ overall ~660 mV from the ES-complex [26].

e The energy level of the intermediate product is uncertain. The main constraint is the value used for rate constant, determined by the position from which the SQ transfers its electron. From myxothiazol to the nearest conjugate atom of heme b11 is ~6.3 Å, and a quinone modeled to occupy this position has a similar distance. A closer position for Q can be found (with a similar energy, a similar distance). A closer position for Q can be found (with a similar energy, a similar distance).

f The sum of free energies for the first and second electron transfers is constrained by the $\Delta G^*$ for the overall reaction, given by the $E_m$ values, using $\Delta G^*/F = (E_m + ISPox + E_m(heme_b11) − 2E_m(QH2)) ∼ −30$ mV [12].
14. Conclusions

In this review, we have discussed the evidence that supports a simple mechanism for the Qo-site of the bc1 complex, summarized in the partial reactions of Table 2, for which all necessary thermodynamic and kinetic values are given. The mechanism accounts for the experimentally observed properties of the Qo-site as it functions in the bc1 complex of Rhodobacter sphaeroides. All components of the mechanism are natural, and have been derived from measured properties of the system. In addition to the reaction properties, the mechanism requires certain structural features, which have all been based on known structures. The same mechanism accounts for most of the data from mitochondrial studies.

This simple mechanism is by no means generally accepted. Other groups have interpreted their data as showing mechanisms that are more complex. These include interactions between redox groups over substantial distances through postulated conformational changes. For many of these mechanisms, some features have been shown to be untenable in the context of the Rh. sphaeroides complex [26,75], and our experiments with Glu-295 mutants provide additional constraints. However, evidence for long-range interactions between Qo- and Qi-sites in both mitochondrial and bacterial complexes has come from structures [39,91], and from a recent EPR investigation of the configuration of the ISP mobile domain at the Qo-site on inhibition or mutagenesis at the Qi-site [92]. Similarly, in our own hands, mutations at the Qo-site designed to make the Rh. sphaeroides complex sensitive to funiculosin (V209A and V209A-1213L mutant strains), generated sensitivity, but at the Qi-site (Sangjin Hong and ARC, unpublished results). The mechanistic significance of these long-range effects is uncertain, since no kinetic control arising from such interactions has been demonstrated. On the other hand, it has been demonstrated that the rate of oxidation of QH2 on a single turnover is unaffected by binding of antimycin [14], and that binding of antimycin has no effect on the configuration of the Qo-site detectable in the highest resolution structure (2.1 Å) yet reported [93].

It is as well to recognize that the evolutionary divide between bacteria and mitochondria represents ~2 billion years of separate evolution, and is evidenced on the mitochondrial side by a substantial increase in structural complexity following incorporation into the euakaryote cell. This has presumably reflected some functional purpose. It is tempting, therefore, to ascribe the differences between mitochondrial and bacterial complexes to the greater structural complexity of the mitochondrial complexes, and the fine-tuning of mechanism to fit the complex to its euakaryotic role. On the other hand, some of the apparent differences between bacterial and mitochondrial systems might reflect difficulties in working with the latter. Most work reported has been using the solubilized mitochondrial complexes in stopped-flow experiments using unnatural quinone substrates. The time resolution is not as rapid as that available through photoactivation in chromatophores, and in most work, no attempt has been made to measure the separate kinetic contributions of the two b-hemes. The use of unnatural quinones, especially apparent in earlier work, introduces different driving forces, and may also lead to different contributions of bypass reactions [94]. Even when a good analog such as decyl ubiquinone is used, limitations are introduced by the low critical micellar concentration (~20 µM, [98]). These deficiencies, and the uncertainty of some of the values for redox potentials of centers in the yeast complex, mean that it has not been possible to analyze the data with the precision needed to discriminate between hypotheses. It might be as well to defer argument until constraints from data are better established. On the other hand, there is an old adage, “The heat of the controversy is inversely proportional to the hardness of the data”; despite the caveat, 45 years for one of us (ARC) in the bioenergetics field has shown that the controversy is half the fun.

Acknowledgement

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