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Review

A structural model for across membrane coupling between the Q_o and Q_i active sites of cytochrome bc_1

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ABSTRACT

The two spatially distant quinone-binding sites of the ubihydroquinone: cytochrome *c* oxidoreductase (cyt bc_1) complex have been shown to influence one another in some fashion. This transmembrane communication alters cofactor and redox partner binding interactions and could potentially influence the timing or 'concerted' steps involved in the steady-state turnover of the homodimeric enzymes. Yet, despite several lines of evidence corroborating the coupling of the quinone binding active sites to one another, little to no testable hypothesis has been offered to explain how such a "signal" might be transmitted across the presumably rigid hydrophobic domain of the enzyme. Recently, it has been shown that this interquinone binding sites communication influences the steady-state position of the mobile [2Fe–2S] cluster containing iron sulfur protein (Sarewicz M., Dutka M., Froncisz W., Osyczka A. (2009) *Biochemistry* 48, 5708–5720) as mediated by at least one transmembrane helix of the *b*-type cyt containing subunit (Cooley, J. W., Lee, D. W., and Daldal, F. (2009) *Biochemistry* 48, 1988–1999). Here we provide an overview of the evidence supporting the structural coupling of these sites and provide a theoretical framework for how the redox state of a quinone at one cofactor binding site might influence the cofactor–, inhibitor–, and/or protein–protein interactions at the structurally distant opposing Q binding site.

1. Introduction

The ubihydroquinone (QH_2): cytochrome (cyt) *c* oxidoreductases (cyt bc_1) are vital enzymes for most membrane-associated electron transport pathways that facilitate ATP production, exemplified by those found in mitochondria, chloroplasts, and most bacteria [1,2]. The enzymes in the family are homodimeric protein complexes, with each monomer minimally formed by at least three catalytically active subunits: the cyt *b* (with two *b*-type hemes b_H and b_L), the iron sulfur (FeS) protein with a high-potential [2Fe–2S] cluster and the cyt c_1 (with a *c*-type covalently attached heme) [1,2]. The cyt *b* subunits also contain two distinct niches for the binding of a quinone molecule, which are located on opposite sides of the membrane from one another. Specifically, one binding niche is located near the positive (p) or outside (o) membrane face and another is located near the negative (n) or inside (i) face. For this reason, the two Q binding niches within the cyt *b* subunit are termed the Q_o or Q_p and Q_i or Q_n binding sites.

The catalytic reaction performed by the cyt bc_1 enzymes is best understood by considering two consecutive QH_2 oxidations performed at the Q_o site of the enzyme [1–5]. The oxidation of QH_2 is initiated by the oxidized [2Fe–2S] cluster housed within the FeS protein subunit. Following QH_2 oxidation, this subunit then physically moves via a tethered rotational displacement from the cyt *b* to the cyt c_1 surface, where the reduced [2Fe–2S] cluster rapidly equilibrates with the cyt c_1. Thus, a high-potential electron carrier chain, formed of the FeS protein subunit and the cyt c_1, ultimately conveys one of the two electrons that originate from a QH_2 oxidation to an electron carrier cyt *c* (cyt c_2 or cyt c_y in *Rhodobacter capsulatus*) on the p side of the membrane. The second electron derived from each QH_2 oxidation is deposited into a lower potential electron transport chain formed by the b_L and b_H (for low and high potentials with respect to one another) hemes of cyt *b*. One such electron first converts a quinone (Q) to a semiquinone (SQ), and then a subsequent QH2-derived electron converts the temporally stable SQ to a QH_2 at the Q_i site of the enzyme. Thus, the two electrons derived from each QH_2 oxidation reaction are sent in opposing directions down two separate electron transfer chains with different overall thermodynamic properties [6,7]. While it is accepted that the cyt bc_1 catalyzes this unusual "bifurcated" QH_2 oxidation reaction, how it does so with remarkable efficiency in the presence of molecular oxygen is still a matter of debate [3,6–9].

Recently, the investigative eyes of several groups have turned towards the events that occur following the first turnover and before the second turnover at the Q_o site cyt bc_1 (i.e., between the first and the second QH_2 oxidations at the Q_o site). What is known is that the newly formed SQ at the Q_i site must be stabilized in anticipation of the arrival of a second electron via the low-potential chain. This intermediate is stabilized as an SQ anion radical, but how the protein achieves this remarkably stable and long-lived species is not wholly

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understood. EPR spectroscopic data have led to opposing mechanisms of SQ binding, with the only differences being the organismal origin of the purified enzyme [10–12].

Regardless of how SQ stabilization is achieved at the Q_i site, the oxidized Q molecule must diffuse out of the Q_o site and be replaced with a new QH_2 molecule. In addition, the now oxidized Fe/S subunit must diffuse back to the cyt *b* surface and associate with this new QH_2 at the Q_o site niche. Whether this latter process is a tethered free diffusion, essentially an oscillation of the rotational displacement that is thought to occur following the oxidation reaction, or the motion is somehow steered or controlled via its interaction with surface-exposed portions (i.e., the *cd* and *ef* loops of cyt *b*) of the enzyme lying along this path is unclear [13]. Earlier works clearly indicated that tight interactions between specific residues of the cyt *b* and the extrinsic domain of the FeS protein are important for the function of the enzyme [13,14]. Indeed, if the mobility of the latter subunit is compromised by lengthening the hinge, neck, or tether region, then the enzyme becomes nonfunctional despite the seemingly normal efficiency of the bifurcated QH_2 oxidation reaction [15]. To add cloudiness to an already murky subject, the binding of the second QH_2 and the reoxidized FeS protein subunit to the cyt *b* surface, which are two co-occurring dynamic binding processes at the Q_o site, is not mutually exclusive and may be dependent on one another.

Despite the number of events that must occur to reform the active Q_o site complex, cyt b–QH_2–$FeS^{oxidized}$, this process can be achieved relatively quickly because the rate-limiting step for the enzymes turnover, known to be the oxidation of the QH_2, is in the range of a millisecond [6]. It has been observed that the rate of this oxidation reaction can change as a function of alterations of the Q_o site itself as evidenced by several mutations in this region [16] and also that the efficacy of this reaction complex formation is additionally influenced by events or mutations that may occur at atomically distant locations from this Q binding niche [17–22]. The most striking of these observations has dealt with the anomalous behavior of the enzyme when Q reduction is competitively inhibited at the Q_i site by the Q analog antimycin A (Ant). It is known that, in the presence of Ant, the total yield of c reduction is nearly 50% of that observed in the uninhibited complex, as though only a single turnover of the Q_o site were allowed despite an oxidized b_L heme and [2Fe–2S] cluster [32]. In theory, upon oxidation of the [2Fe–2S] cluster and exchange of the oxidized Q for a new QH_2 molecule, the ability of the reaction to proceed should only be dictated by recoordination of the cyt $b^{oxidized}$–QH_2–$FeS^{oxidized}$ tertiary complex and the availability of the terminal electron sink, the oxidized soluble electron shuttle (usually a *c*-type cyt). Subsequent QH_2 oxidation reactions not in the reduction of a cyt *b* heme, but rather in bypass reactions where the second electron does not proceed down the low-potential pathway, but rather to another acceptor molecule such as molecular oxygen, have only been observed to occur at rates equivalent to <2% of the uninhibited Q_o site catalysis [34].

These observations and those looking at the steady-state dynamics of the enzyme structure in the presence of Ant and other Q_i site inhibitors appeared to hint that some type of regulation of the Q_o site QH_2 oxidation reaction is built into the enzyme's catalytic turnover [19,20,22,23]. The first observation that a Q_i site inhibitor might influence the overall cyt bc_1 structure in some manner came from the early work of John Rieske, outlining an enhancement of bile salt-mediated cleavage of the FeS subunit in the presence of Ant. Of course, it was not until more was understood about the structural arrangement of the enzyme complex that it became clear that the cleavage took place at a site distant from the inhibitor binding site. Subsequent approaches, carried out much later and after a more detailed understanding of the architecture of the protein complex, have run the gambit technique-wise from alteration of the kinetics or binding constants of Q_o site inhibitors using substoichiometric Q_i site inhibitor concentrations (or vice versa) [18,24,25], indicating a cooperative binding influence of one site over the other, to proteolytic cleavage assays probing changes in the secondary structure of the periplasmic portion of the FeS subunit [22,26], indicating a long-range influence of the Q_i site occupancy on the enzyme's structure at the opposing side of the membrane, to studies utilizing changes in the line shape and orientation dependence of the EPR spectra of the [2Fe–2S] cluster and *b* hemes in Langmuir-type ordered membrane arrays [23,27,28]. It is interesting to note that, in the two latter studies, the interaction of the inhibitor at the Q_i site significantly influenced the periplasmic portion of the FeS subunit independent of the intactness of the low-potential redox chain. In fact, analysis of the environment of the reduced [2Fe–2S] cluster by orientationally dependent EPR, which gives an idea about the heterogeneity of the FeS position with respect to the membrane proper, outlined two discrete structure-related effects of Q_i site mediation of the Q_o site environment. The first was a dramatic decrease in an ability to acquire orientationally dependent EPR spectra of the reduced [2Fe–2S] cluster in equivalently ordered membrane samples following the addition of Ant. Thus, Ant binding at the Q_i site caused a change in the propensity of the FeS subunit to be bound at the Q_o site. It remains unclear whether this phenomenon was caused by a change in the FeS subunit's structure position by a molecule bound over 30 Å away on another subunit or whether the Q_i site inhibitor had changed the interaction of FeS with the Q at the Q_o site by causing a change in the structure of the cyt *b* surface. The second observed Q_i site-related phenomenon occurred in the presence of another Q_i site inhibitor (HQNO). This inhibitor differs in its interaction with the Q_i site niche from Ant in that it lacks linkage (via either an indirect or a direct hydrogen bond) with a conserved histidine (H217 in *R. capsulatus* numbering) at this site. Introduction of HQNO to the ordered membrane samples significantly altered the EPR line shape, but unlike Ant, it appeared to have little effect on the mobility of the FeS subunit. Because this second effect of the Q_i site on the environment of the FeS was wholly distinct from that observed with native Q or Ant occupancy at the Q_i site, this was postulated to be an effect possibly associated with the formation of the SQ anion at the Q_i site.

Here, these oriented EPR-based results and complementary findings from other groups are discussed with an emphasis on how the Q_i site inhabitant might affect a change in the cyt *b*/Q/FeS interaction in the context of what we know about the enzyme's structure. In particular, for the first time, we were able to propose a testable hypothesis for how a long-range communication between the two Q binding sites might occur.

2. Materials and methods

2.1. Bacterial growth conditions

All *R. capsulatus* strains were grown in mineral–peptone–yeast–extract-enriched media (MPYE) under semiaerobic conditions in the dark at 35 °C, as described previously.

2.2. Preparation and spectroscopic analysis of ordered membrane samples

Chromatophore membrane isolation was carried out as described previously in Atta-Asafo-Adjei and Daldal [29]. Ordered membrane sample preparation was modified from those outlined in Roberts et al. [30] and Brugna et al. [31] and described in detail in Cooley et al. [27]. Using membrane samples, EPR spectra were recorded between angles 0° and 180° from the magnetic field vector using 10° rotational steps. EPR spectroscopy was carried out at a sample temperature of 20 K on a Bruker ESP 300E spectrometer (Bruker Biosciences), fitted with an Oxford instruments ESR-9 helium cryostat (Oxford Instrumentation Inc.). For orientation-dependent spectral acquisition, a manual goniometer accessory was utilized (Oxford instruments). The antimycin A (purchased from Sigma, Inc.) concentration was 10 μM (from

stock solutions where the inhibitor was initially dissolved in DMSO) per ~30 mg/ml total chromatophore membrane proteins, respectively, before drying the embranes. Chemical reduction of the samples was achieved by addition of Na-ascorbate (Sigma) to 5 mM final concentrations. EPR samples were stored in liquid N_2 until spectra were recorded.

3. Results and discussion

3.1. Changes seen in the opposing Q site as a function of a Q sites occupancy, hypotheses?

The evidences gathered regarding the cyt bc_1 Q sites coupling are of two types. The first line of evidence involving substoichiometric inhibitor concentrations indicated that binding of a molecule at one Q site of a monomer affects the binding affinity of inhibitors at the other Q site (presumably of the same monomer) [18,24,25,32]. Despite concerns raised that the equilibration times of the inhibitors are faster than the limits of the experimental analyses, meaning the inhibitors are free to alternate between monomers or between sites [35], these substoichiometric inhibitor studies gave rise to several hypotheses about potential cooperative or anticooperative binding phenomena within the complex, in turn giving rise to half the sites' concerted mechanism describing how electrons may flow and equilibrate within the dimeric enzyme during steady-state turnover. Initially, these results generated a hypothesis that, as the Q_i site inhibitors imparted a blockage of the catalytic electron transfer through the low-potential chain, the blockage would in turn lead to a backup of reducing equivalents at each *b*-type heme. Eventually, this increased reduction of the *b* hemes would influence the binding events at the Q_o site. Presumably, more reduced b_L hemes would alter their interaction with residues (in particular, the glutamic acid of the conserved *ef* loop PEWY motif), which in turn would influence the binding constant of the Q/QH_2 at the b_L adjacent Q_o binding site.

Several of the measurements indicating Q site interactions were taken on steady-state samples, which are not actively turning over [19,22,23]. Thus, the state that was observed by the investigators was one in which the Q sites had equilibrated to a new structural environment based on nothing other than the introduction of the inhibitor, i.e., no reducing equivalents present to back up within the chain. Separately, the Q_i inhibitor-mediated Q_o site interactions in the absence of an intact low-potential chain have been examined, i.e., those lacking the b_H heme, and have still seen Q site cooperative effects even in the absence of a backup of electrons through the low-potential chain [19,22,23]. Lastly, all of the solved structures with Ant present have been solved in the presence of the Q_o site inhibitor stigmatellin, whose presence is known to make observation of the Q_o–Q_i site's communication phenomenon nonfeasible [27]. In effect, the rigidity imparted by the extremely tight stigmatellin binding at the Q_o site that has been utilized so effectively to garner quality crystals for diffraction also negates the observed influence of the Ant binding event at the Q_i site on the *b*-type heme moieties, meaning that one would not expect to see an Ant-instigated structural change in the current inventory of solved atomic structures.

An alternate hypothesis to explain the influence of the Q_i site's occupancy over the Q_o site is that the two sites might be linked by a gross structural change, akin to a switch or lever. This hypothesis requires that the manner of interaction of particular moieties at the Q_i site, i.e., different hydrogen bonding, water coordination networks, and/or electrostatic interactions, somehow influences a portion of the global structure of the cyt bc_1 complex in such a way that either the binding of the Q_o site Q and/or the steady-state position of the mobile FeS subunit is altered, i.e., that the two Q sites of given cyt bc_1 monomer talk to one another regarding their respective occupancies as a function of change in the structure of the enzyme somehow. This type of coupling via a structural change was clearly documented by changes in the proteolytic activity towards the FeS subunit as a function of the identity of the inhabitant of the Q_i site [22]. Since these samples were assayed under conditions where there is no source of reducing equivalents, the turnover of the enzyme before protease exposure was negligible, and the authors went one step further and demonstrated a similar phenomenon in detergent-solubilized purified protein samples[22]. The proteolytic cleavability of the FeS in cyt bc_1 derived from strains with mutations of the cyt bc_1 complex conveying impairment of the electron transfer through the low-potential pathway, specifically the Q_i site's SQ stabilization-impaired *b*H217x mutants or the heme b_H lacking *b*H212N, was also examined. Remarkably, despite the impairment in Q to QH_2 reduction ability or SQ stability in the H217x mutant complexes, the increased cleavage associated with the binding of Ant persisted in these mutants. Since proteolytic cleavage of the FeS is dependent on the secondary structure of the "hinge" region, and the hinge structure differs when the FeS is at or away from the Q_o site, the proteolytic differences upon inhibitor binding were attributed to a change in the propensity of the FeS subunit to be found at the Q_o site niche as a function of the Q_i site inhibitor binding. These findings have since been corroborated using orientationally dependent CW-EPR [23,27] and, more recently, using pulsed EPR methodologies [17]. Since the CW-EPR of the oriented sample's observed effects of inhibitor binding was distinct for inhibitors with overlapping but discrete binding niches as defined by published atomic coordinates in either case, the authors were able to make the first reasonable hypotheses for what specific interactions might be influencing the global cyt bc_1 complex structure. Thus, these orientationally dependent CW-EPR studies were the first to offer potential pathways within the structure for how the two Q sites might be linked. Furthermore, the latter study also provided evidence that not only did Q_i site inhibitor binding influence the FeS–Q interaction but also the structural perturbations caused by mutations or inhibitor binding at the Q_i site were negated by the tight binding of the inhibitor stigmatellin at the Q_o site. Therefore, this study was the first to imply that the Q_o–Q_i site interaction requires some extent of structural flexibility or at least the potential thereof.

3.2. The cyt b E helix: a strong candidate for the Q sites transmission within cyt b

The first good candidate for the transmission mechanism of the "signal" from one Q site was finally put forth in Cooley et al. [19] based on the known differences in the manner in which the inhibitor molecules, HQNO and Ant, interacted with the Q_i site and correlated to the two overlapped but distinct FeS EPR spectral phenomena they observed. Of the three Q_i site-related cyt *b* transmembrane helices (helices E, D, and A), the E helix was deemed to be the strongest candidate. Specifically, the authors noted that altering the interaction of the inhibitor with the D helix residue H217 by mutagenesis had an intrinsic effect on the EPR spectra of the [2Fe–2S] cluster by broadening and shifting the ensemble resonant position in the magnetic field of the g_x transition. However, Ant still had a dramatic effect on the spectral shape as well as on the orientational dependence of the signal amplitude in these samples. In short, the loss of the histidine interaction with Q affected the FeS environment somehow but did not abolish the Q_o–Q_i "communication." Furthermore, the addition of HQNO, which appears to lack the H217 interaction seen with Ant, had little effect on either the spectral shape or the distribution of FeS orientations or positions. From this, the authors proposed that the Ant-mediated communication must take place through the use of the E and A helices as structural conduits. In a subsequent study, Cooley et al. [23] went on to show that, when the mobility of the FeS-soluble domain is limited, EPR spectra of ordered membrane samples contain two distinct g_x transitions. Therefore, Ant binding at the Q_i site causes two populations of FeS positional

distributions as if a barrier at the P side of the membrane had been created, trapping FeS subunits either at or away from the Q_o site proper. The analysis of double mutants containing the linker region insertion mutations coupled with mutations in the *ef* loop, the presumed structural barrier in the translation/rotational pathway of the FeS from the cyt *b* surface to the cyt c_1 position, abolished this partitioning of the FeS subunits into two distinct populations. Thus, it was apparent that the mechanism of the Q_i to Q_o site communication must involve the E helix and requires an intact extrinsic *ef* loop region.

3.3. A new proposal for transmembrane signal transmission

Examining the existing data, several simple models can be ruled out as less likely candidates. The first models that can be ruled out are those that claim that the communication involves a disequilibration in electrons within the cyt *b* (low-potential chain) as a function of competitive inhibition as Ant-induced phenomena (changed EPR spectra and altered equilibrium position of the bulk FeS) with and are the same regardless of the intactness of the low-potential ET chains. A second model that has been attractive is one in which the energy of the binding event (or the stabilization of Q at the Q_i site) could cause a structural rearrangement significant enough to alter the gross structure of the *ef* loop or cyt *b* surface at the opposite side of the membrane. Such a gross change in the structure would almost certainly be visible in crystal structures, even despite the necessary presence of stigmatellin to fix the FeS position. Yet, no discernible difference in the structure (in particular, in the E helix) is seen with Ant bound at the Q_i site when stigmatellin is bound at the Q_o site. Additionally, it appears that only modest changes might be present in the cyt *b* E helix order parameters in crystals, with antimycin lacking stigmatellin (F. Daldal and E. Berry, personal communications). A third model that has not been offered as yet is that the binding events may impart a structural rigidity to one of the sites and its interacting helices, which would alter the structural rigidity of the opposing binding site. This type of coupling has been touched on now in fields of long-range allostery in soluble proteins [33]. However, even in these few cases, no rational mechanism has been proven to explain the coupling in the absence of interconnecting structural alterations. One thought that has permeated these scientific circles is that the intrinsic motion of the connecting peptide regions could be altered, thereby implying that control of thermal motion of the protein (the extent of "breathing" as it were). In the case of the cyt bc_1 Q sites coupling, the binding site's rigidity could be translated by a changed intrinsic dynamic of the E helix to the opposite side of the membrane. This type of thermal or entropic communication would require not result in structural change in crystal structure and would perhaps only require on the order of a few stable hydrogen bonds to initiate it. Additionally, the limiting of intrinsic motion (thermal?) might be propagated cooperatively, as side chain interaction strength between

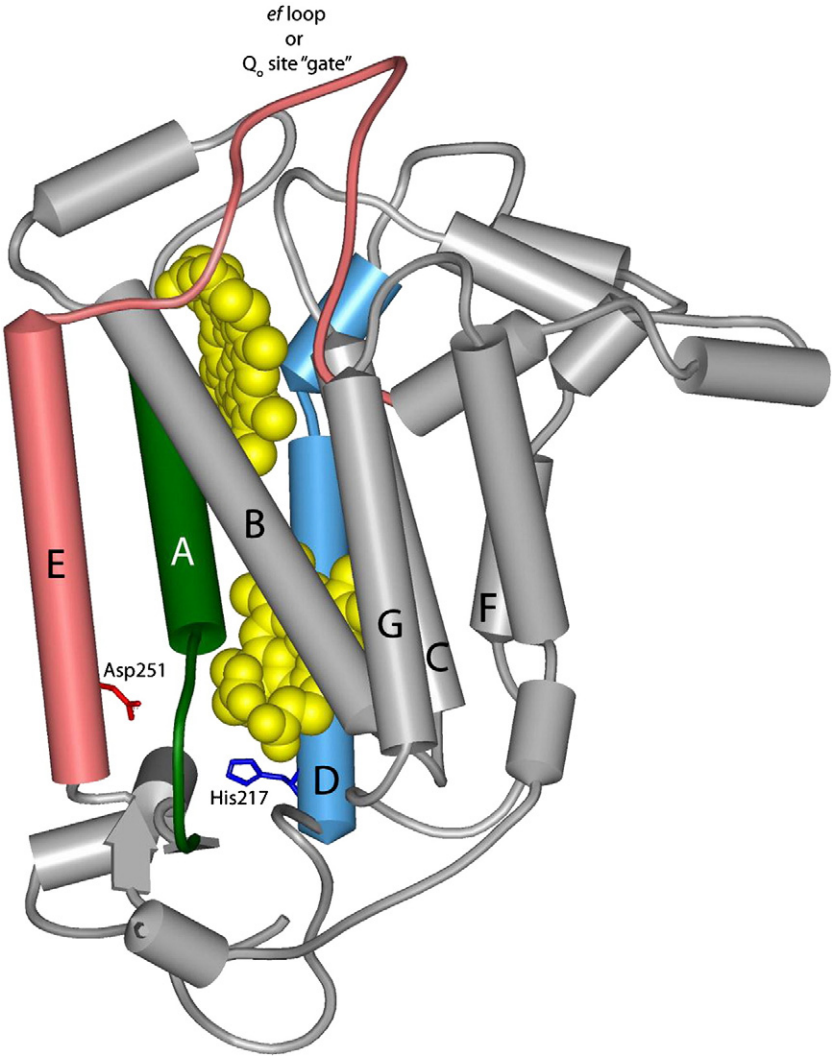

Fig. 1. Cartoon depiction of the cyt *b* subunit monomer (1ZRT.pdb) depicting the relative structural isolation of the E helix from the remainder of the *b* helices. The three Q_i site associated helices E, D, and A are colored red, blue, and green, respectively, for clarity as well as the Q binding residues bH217 and *b*D251 (*R. capsulatus* numbering).

helices would be greatly enhanced upon the diminished enthalpic barrier.

Logically, all that would be required for limiting of helical movement would be a helix with few, if any, hydrogen bonding or stearic clash interactions with adjacent helices (i.e., a helix that has a high degree of rotational freedom). Examination of the cyt *b* structure, the E helix that supplies the primary carboxylic acid residue necessary for all ligand interactions at the Q_i site, appears to be a prime candidate (Fig. 1). Secondly, the helix would need to be hindered in its motion as a function of the binding event at the Q_i site (or more specifically as a function of the redox status of the Q at the Q_i site). Such a tethering could very easily be accomplished for the E helix through the Q residue coordinated at its base by a conserved aspartate residue. A transient (or even temporally stable) interaction of the opposing carbonyl oxygen of the Q with the D helix-conserved histidine residue upon SQ formation as well as by the formation of a stable water mediated hydrogen bonding network at the site would then tether the E helix to the static D helix. Simply put, the stabilization of the SQ by the Q_i site might impart more rigidity to the molecule and/or hydrogen bonding network at the Q_i site as well as to the E helix.

This hypothesis is fully supported by the existing evidence in that the full extent of the effect induced by Ant requires the interaction of Ant with the D helix *b*H217 residue. The D helix, which is normally limited dynamically in this region by its coordination of the b_H heme at *b*H212, would presumably be less rigid when the b_H is removed by mutation. In fact, analysis of the b_H-less mutants revealed an intrinsic (independent of the ligand bound at the Q_i site) effect upon the interaction of the [2Fe–2S] cluster with the Q_o site inhabitant, as evidenced by the EPR spectral shape. However, despite this intrinsic alteration of the FeS/Q/cyt *b* interaction, the binding of Ant still causes a change in the FeS steady-state position. In effect, even in a now more disordered Q_i site, one can envision that Ant binding would still be able to tether the E helix to the less rigid D helix through the native *b*H217–ANT interaction potentially causing the motion of the *ef* loop to less easily allow for the steered diffusion of the FeS from the cyt *b* to the cyt c_1 surface or in the reverse direction (Fig. 2). Thus, the *ef* loop would act like a gate where greater motion might diminish the passage of the bulky FeS head domain to effectively equilibrate with the Q_o site niche. This argument is very feasible, as very small changes in this loop region have been shown to have dramatic effects on the path of the FeS.

To consider whether stability of the molecule bound at the Q_i site might be perturbed in other subtle ways, thereby conveying a similar change in the Q_o site (or [2Fe–2S] cluster signal as the case may be), other conserved potential Q or inhibitor H-bonding residues at this

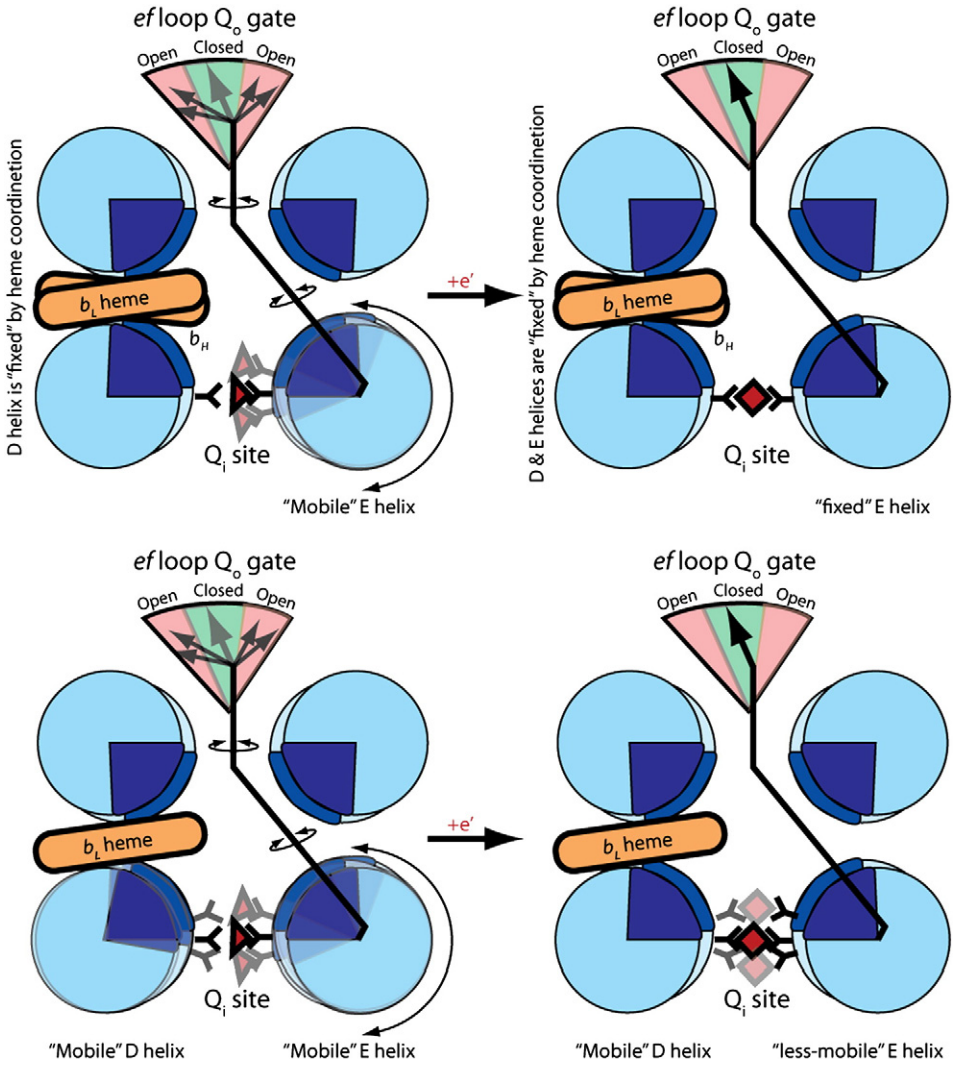

Fig. 2. Hypothetical model for the potential coupling of the Q_i site to the Q_o site via limitation of cyt *b* E helix dynamics. Models depicting potential coupling mechanism in native (upper panel) and less-static D helix N terminal in the absence of the b_H heme (lower panel) are envisioned where an oxidized Q molecule or HQNO molecule (red triangle) binds to the E helix aspartate residue black branched stick. Subsequently, following reduction, the SQ through its interaction with the D helix or when HQNO is replaced with Ant (red diamond), "fixes" the E helix, or limits its rotation. This diminished rotation causes the *ef* loop (or Q_o site gate) to inhibit effective passage of the FeS between the cyt *b* and c_1 surfaces.

site might influence were examined. The hypothesis being that removal of these "stabilizing" H-bonding interactions would impart a greater fluidity to bound molecules motions and result in an altered FeS position or Q_o site environment. If we consider two of these potential hydrogen bonding candidates, *b*I213 and *b*S233 (located at the base of the D helix and within the *de* loop making up the floor of the Q_i site, respectively), and the consequences of their mutations on the [2Fe–2S] cluster EPR spectra in ordered membranes (Fig. 3), it can be seen that the EPR spectra of the g_y and g_x transitions are altered from the wild type spectra. These alterations are two-fold: first, the g_y transitions are shifted towards values more correlative of a [2Fe–2S] cluster not interacting with a molecule at the Q_o site; and second, the g_x transition is likewise correlated to diminished Q_o interactions as it is broadened towards a higher magnetic field (lower g factor) values. It is important to bear in mind that this broadening represents a change in the heterogeneity of environments that the total population of [2Fe–2S] clusters are experiencing and thus represents a subtle shift in the equilibrium position of the FeS head domain with respect to the Q_o site niche proper. Therefore, it is clear that a similar phenotype is seen to that published previously for the *b*H212N and *b*H217x series of mutations, where a more promiscuously positioned FeS results from the legion at the Q_i site. The spectra in Fig. 3 imply that hydrogen bonding is important for Q_i to Q_o site communication similar to the loss of the b_H heme and mutations of the *b*H217. Therefore, in summary, a testable hypothesis can be made for how energetically small binding interactions at the Q_i site of the cyt bc_1 complex might enact potentially catalytically relevant changes at the alternate side of the membrane, based simply upon the limitation of one transmembrane helix's intrinsic motion.

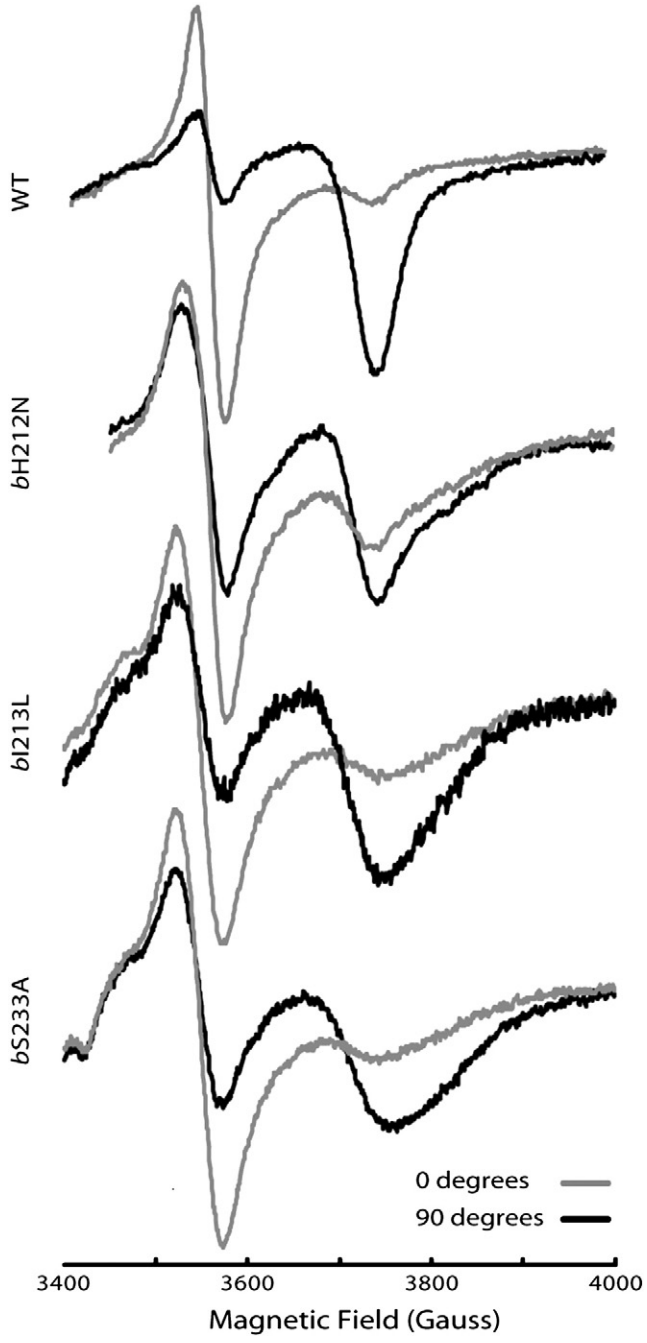

Fig. 3. Orientation-dependent CW–EPR of the reduced [2Fe–2S] clusters in ordered membrane samples derived from wild type and strains harboring mutations at or near the Q_i site. Orientation-dependent spectral extremes representing 0 and 90 degree rotations versus the magnetic field axis are shown for each strain. Both the transition maxima positions are altered in the mutant spectra versus the wild type spectra as well as the homogeneity of [2Fe–2S] cluster ensemble tensor versus the magnetic field (evidence by diminished orientation dependence).

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