Minireview

The role of the 'Rieske' iron sulfur protein in the hydroquinone oxidation (Q_P) site of the cytochrome bc_1 complex

The 'proton-gated affinity change' mechanism

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Abstract The essential reaction in the widely accepted protonmotive Q-cycle mechanism of the bc_1 complex is the bifurcation of the electron flow during hydroquinone oxidation at the hydroquinone oxidation (Q_P) site formed by the 'Rieske' iron sulfur protein and by the heme b_L domain of cytochrome *b*. The 'Rieske' [2Fe-2S] cluster has a unique structure containing two exposed histidine ligands, which are the binding site for quinones. The affinity of the 'Rieske' cluster for quinones increases several orders of magnitude upon reduction; this will stabilize semiquinone at the Q_P site. Based on this affinity change, a reaction scheme is presented which can explain the bifurcation of the electron flow without invoking highly unstable semiquinone species.

Key words: Cytochrome bc_1 complex; 'Rieske' iron sulfur protein; Hydroquinone oxidation; Q cycle; Semiquinone stability; Affinity change

1. Introduction: The Q-cycle mechanism and the bifurcation of electron flow at the Q_P site

The ubiquitous cytochrome bc_1 complexes form the middle part of the electron transfer chains of mitochondria and many bacteria. The related b_6f complexes are found in chloroplasts, algae, and some Gram-positive bacteria. Together they form the family of *bc* complexes; they all contain two heme *b* centers, a high-potential heme center (heme c_1 or heme *f*) and a 'Rieske' iron sulfur protein comprising a high-potential [2Fe-2S] cluster.

1.1. The Q-cycle mechanism and the bifurcation of electron flow at the Q_P site

The bc_1 complex oxidizes ubihydroquinone and transfers electrons to the water-soluble electron acceptor, cytochrome *c*. It is generally accepted that electron transfer is coupled to proton translocation by the protonmotive Q-cycle mechanism first proposed by Peter Mitchell [1]. The central reaction of the Q cycle is a bifurcation of the pathway of electrons upon oxidation of hydroquinone at the hydroquinone oxidation (Q_P) site which is in contact with the positive side of the membrane: one electron is transferred via the 'high-potential chain' ('Rieske' iron sulfur cluster and cytochrome c_1) to cytochrome *c*, while the second electron is transferred to heme b_L and from there across the membrane dielectric to heme b_H (recent reviews in [2–4]). Heme b_H is part of the quinone reduction (Q_N) site which is in contact with the negative N-side of the membrane; at the Q_N site, heme b_H reduces quinone to hydroquinone. In total, half of the electrons are recycled back to quinone; thus, the Q-cycle mechanism leads to a doubling of the protonmotive efficiency of the bc_1 complex.

The hydroquinone oxidation reaction at the Q_P site is strictly coupled: always, one electron is transferred to the 'Rieske' cluster and the second electron to heme b_L. Although transfer of the second electron to the 'Rieske' cluster ($E_m \approx +300$ mV) and cytochrome c_1 ($E_m \approx +250$ mV) is energetically favorable compared to electron transfer to heme b_L ($E_m \approx 0$ mV) by 25–30 kJ/mol, the second electron must not be allowed to go into the 'high-potential chain' after the first electron has been delivered to the 'Rieske' cluster, even under conditions where the second electron acceptor (heme b_L) is already reduced. Otherwise, uncoupling of electron transfer and proton translocation would occur which is not observed experimentally.

Several mechanisms have been suggested to explain this obligatory bifurcation of the electron flow. Brandt and von Jagow [5] have proposed different conformational states and a 'catalytic switch'. Crofts and Wang [6] and Ding et al. [7] have proposed kinetically controlled mechanisms involving a highly unstable semiquinone anion. The model proposed by Ding et al. [7] involves two quinone molecules (Q_{os} and Q_{ow}) bound simultaneously in the Q_P site in a linear edge-to-edge arrangement. Fast electron transfer from hydroquinone in the Q_{ow} site to semiquinone in the Q_{os} site (formed by the first electron transfer to the 'Rieske' cluster) should allow charge separation and move the semiquinone away from the 'Rieske' cluster, thus preventing transfer of the second electron to the 'high-potential chain'. Brandt [4] has suggested a 'proton-gated charge-transfer' mechanism which involves a stacked quinone/hydroquinone pair ($Q_{os}/Q_{ow}H_2$); after activation by deprotonation ($Q_{os}/Q_{ow}H^-$), symproportionation of the two quinones occurs and the double semiquinone ($Q_{os}^{-\bullet}/Q_{ow}^{-\bullet}$)

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Abbreviations: CD, circular dichroism; ENDOR, electron nuclear double resonance; EPR, electron paramagnetic resonance; ESEEM, electron spin echo envelope modulation; FT-IR, Fourier-transform infrared spectroscopy; ISF, 'Rieske' iron sulfur fragment of the bc_1 complex; Q_A, Q_B site, primary and secondary quinone sites of the bacterial photosynthetic reaction centers, respectively; Q_N, Q_P site, quinone reduction and hydroquinone oxidation sites of the bc_1 complex, respectively

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simultaneously transfers two electrons to the 'Rieske' cluster and to heme b_L. The symproportionation should be driven by the oxidized 'Rieske' cluster acting as a Lewis acid.

2. Structure and properties of the 'Rieske' protein

The 'Rieske' iron sulfur protein has first been described and isolated by Rieske et al. [8] and has later been identified as the 'oxidation factor' of the bc_1 complex [9,10]. After crystallization of a water-soluble fragment of the bovine 'Rieske' protein (ISF) containing the intact [2Fe-2S] cluster [11], the high-resolution structure of the catalytic domain could be determined by X-ray crystallography and refined at 1.5 Å [12]. The unique feature of the 'Rieske' cluster is that one iron atom (Fe-2) of the [2Fe-2S] cluster is coordinated by two histidine residues (His-141 and His-161) through their Nδ atoms. This coordination is in agreement with the interpretation of ENDOR and ESEEM spectra [13–15].

The [2Fe-2S] cluster is at the tip of the protein; the reduced iron atom of the localized mixed valence cluster (Fe-2) is close to the surface of the protein. The NεH groups of the coordinating histidines are accessible and exposed to the solvent within a hydrophobic surface of the protein; they are the primary binding site for quinones and for inhibitors like stigmatellin and 2-hydroxyquinones which show strong structural homology to the natural substrate, quinone. The suggested direct interaction between the proximal carbonyl oxygen of quinone and a histidine ligand of the 'Rieske' cluster is supported by the following evidence [16,17]:

- The analogy to the primary (Q_A) and secondary (Q_B) quinone binding sites of the photosynthetic reaction center where both quinones bind to a histidine residue which is coordinated to an iron (Fe(II)) ion [18]. In the Q_B site of the reaction center, stigmatellin binds in the same position as quinone via a hydrogen bond to His L-190 which is a ligand of the Fe(II) [19].
- The fact that the affinity of the bc_1 complex for stigmatellin is largely decreased when the 'Rieske' iron sulfur protein is depleted [20].
- The effect of binding of quinones and quinone-like inhibitors on the EPR spectrum of the reduced 'Rieske' cluster, which leads to a shift and a change of the line shape of the high-field (g_x) signal.
- The effect of a mutation of residue Leu-136 in *Rhodobacter capsulatus* (corresponding to Leu-142 in beef heart): this residue is part of this hydrophobic surface and is adjacent to one of the histidine ligands of the 'Rieske' cluster (His-141). Mutation of this residue to a charged residue completely blocked quinone binding while the properties of the cluster were apparently not perturbed [21].

2.1. Redox-dependent protonation/deprotonation

The redox potential of the 'Rieske' cluster is pH dependent [22]. From the analysis of data obtained by direct electrochemistry of the ISF, the existence of two groups with redox-dependent pK_a values could be concluded; the pK_a values were 7.6 and 9.2 on the oxidized protein and >11 on the reduced protein [23]. This result could be confirmed by the analysis of the pH dependence of the visible CD spectra of the oxidized ISF [24] and of the 'Rieske' protein in the bc_1 complex (T.A. Link and O.M. Hatzfeld, unpublished results). The residues undergoing redox-dependent protonation/deprotonation are the exposed histidine ligands of the 'Rieske' cluster. This assignment is supported by the following observations:

- Two redox-dependent pK_a values or at least a slope $-\Delta E_m/\Delta pH > 60$ mV which cannot be explained with a single deprotonation step have been found in all 'Rieske' proteins involved in hydroquinone oxidation [25–28]; therefore, they can be considered as a characteristic feature of hydroquinone oxidising 'Rieske' clusters which should be associated with conserved residues.
- The pH dependence of the CD spectra shows that the groups with redox-dependent pK_a values must be electronically coupled to the [2Fe-2S] core. Electrostatic interactions between the [2Fe-2S] cluster and protonatable groups in its vicinity could explain the redox dependence of the pK_a values but not the spectral shifts.
- From the results of FT-IR spectroscopy, we could exclude deprotonation of aspartate, glutamate, or tyrosine (F. Baymann, T.A. Link, D.E. Robertson and W. Mäntele, manuscript in preparation).
- In the structure, we could not identify residues in the vicinity of the cluster except the histidine ligands which are likely to undergo redox-dependent protonation/deprotonation. The conserved Tyr-165 is buried in the protein and hydrogen bonded to the Sγ of Cys-139; this residue is structurally important but cannot undergo redox-dependent deprotonation.
- Secondary structure CD and FT-IR spectroscopy showed that no structural change occurs upon deprotonation.

Upon deprotonation, the histidine side chains will change from the neutral imidazole to the negatively charged imidazolate form. The pK_a value of this transition which is above 14 in free histidine is lowered by the electron withdrawing effect of the Fe(III) on the oxidized cluster. In the reduced form of the cluster, the electron withdrawing effect is reduced and the pK_a values are closer to their 'normal' values.

Since the first pK_a value is at 7.6, redox-dependent proton uptake or release cannot be expected to be functionally relevant during hydroquinone oxidation since the histidine will be largely protonated both in the reduced and in the oxidized state. However, the shift of the pK_a values is a consequence and therefore an indicator of the different electron density at the imidazole rings in the oxidized and reduced state. The strong redox dependence of the electron density of a basic nitrogen (NεH) is a truly unique feature of the 'Rieske' cluster.

2.2. Redox dependence of ligand binding to the 'Rieske' cluster

Two classes of inhibitors which strongly resemble the natural substrate, quinone, bind directly to histidine ligands of the 'Rieske' [2Fe-2S] cluster: 2-hydroxyquinones and chromone inhibitors, i.e. stigmatellin [29]. These inhibitors also bind in the Q_B site of the photosynthetic reaction center in the same position as quinone (and probably semiquinone) to a histidine residue which is coordinated to an iron (Fe(II)) ion [18,19]. Binding of both classes of inhibitors to the reduced 'Rieske' cluster can be monitored by EPR spectroscopy since it induces shifts of the g values and line-shape changes. Upon

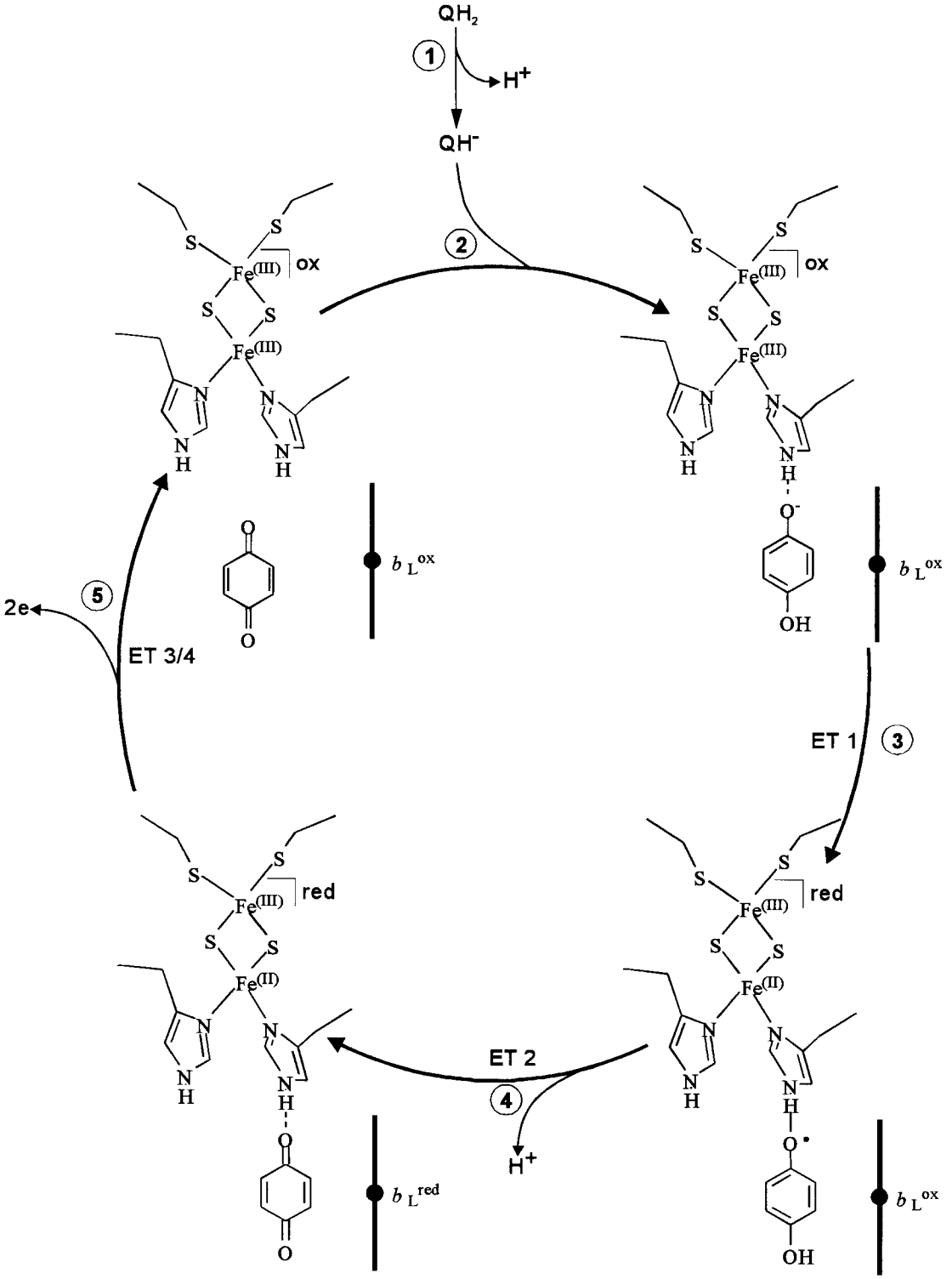

Fig. 1. 'Proton-gated affinity change' mechanism of hydroquinone oxidation in the Q_P site. Step 1: hydroquinone must first be deprotonated, before it can bind to the oxidized 'Rieske' cluster (Step 2). Step 3: the first electron is transferred to the 'Rieske' cluster (ET1); thereby, a semiquinone is formed which is bound and magnetically coupled to the 'Rieske' cluster (bottom right). Step 4: after the second deprotonation step, the second electron is transferred to heme b_L (ET2). Step 5: after electron transfer from the 'Rieske' cluster to cytochrome c_1 (ET3) and from heme b_L to heme b_H (ET4), quinone is released and the 'Rieske' cluster can bind the next molecule of deprotonated hydroquinone. The different line types between the imidazole of the 'Rieske' cluster and the quinone molecule indicate the change of the binding constant in different redox states: broken line, weak binding; full line, strong binding.

binding of the inhibitors, the redox potential of the 'Rieske' cluster is shifted towards more positive potentials; this is equivalent to the statement that the inhibitors bind several orders of magnitude tighter to the reduced than to the oxi-

dized cluster. Stigmatellin induces a potential shift of more than 250 mV corresponding to a binding constant of the 'Rieske' cluster which is almost 5 orders of magnitude higher in the reduced than in the oxidized state [30]; for 2-hydroxyquinone, the potential shift is 70–85 mV corresponding to an affinity which is 15 times higher in the reduced than in the oxidized state [31]. Recently, we have measured a 200 mV potential shift for 2-bromo-ubiquinone corresponding to an affinity which is 3 orders of magnitude higher in the reduced than in the oxidized state (O.M. Hatzfeld and T.A. Link, unpublished results). The change of the binding constant of the 'Rieske' cluster is induced by the pK_a shift of the histidine ligands which will increase the strength of the hydrogen bond between the histidine ligand and the proximal carbonyl group of the inhibitor; the same affinity changes should occur with the natural substrate, quinone.

Due to the affinity change of the 'Rieske' cluster upon reduction, a semiquinone formed by the electron transfer from hydroquinone to the 'Rieske' cluster will be bound more tightly to the reduced 'Rieske' cluster than the hydroquinone to the oxidized cluster; thus, the resulting semiquinone will be *stabilized* in the Q_P site. The stabilization of the semiquinone in the Q_P site which is a consequence of the electrochemical properties of the 'Rieske' cluster is in sharp contrast to the previous speculation that the semiquinone should be *destabilized* [6,7]. Since the semiquinone generated during the catalytic reaction is stabilized on the 'Rieske' cluster, we can rule out a destabilized semiquinone as a reaction intermediate and therefore the presence of a semiquinone barrier as postulated by Ding et al. [7].

The semiquinone in the Q_P site will be stabilized only when it is bound to the reduced 'Rieske' cluster; thus, the semiquinone will be magnetically coupled to the 'Rieske' cluster and will not be observed by EPR spectroscopy.

As the need to invoke highly unstable semiquinone intermediates has been eliminated, a mechanism can be formulated which is based solely on the established electrochemistry of the 'Rieske' cluster and takes advantage of the affinity change of the 'Rieske' cluster upon reduction as the key reaction step.

3. The 'proton-gated affinity change' mechanism

The 'proton-gated affinity change' mechanism shown in Fig. 1 consists of five reaction steps:

(1) In the first step, hydroquinone is deprotonated. The fact that hydroquinone has to be deprotonated before it can be oxidized is not only evident from the electrochemistry of quinone (the E_m of the couple $QH_2/QH_2^{+\bullet}$ has been estimated at >850 mV), but Brandt and Okun have also demonstrated that hydroquinone deprotonation forms the activation barrier of hydroquinone oxidation ([4,38]). Hydroquinone oxidation requires that a group on the bc_1 complex with a pK_a of 6.5 is deprotonated; when this group is protonated at low pH, the activity of the bc_1 complex is decreased ([32,38]). It is possible that the deprotonation occurs at a 'pre-binding' site so that only the deprotonated hydroquinone can enter the reaction site and bind to the 'Rieske' cluster (step 2).

(2) In the second step, the deprotonated hydroquinone binds to one of the histidine ligands of the oxidized 'Rieske' cluster. Apparently, this histidine is the one having a $pK_{a,ox}$ of 9.2; the pH dependence of the activity of the bc_1 complex shows a decrease at $pH > 9$ which can be modelled with a pK_a of 9.2 [38].

(3) In step 3, an electron is transferred from the bound, deprotonated hydroquinone to the 'Rieske' cluster; this is the key reaction step. Upon reduction of the 'Rieske' cluster, its affinity for semiquinone will increase by several orders of magnitude; thus, the semiquinone is now tightly bound to the reduced 'Rieske' cluster. Concomitantly, the redox potential of the 'Rieske' cluster will be increased which prevents electron transfer to cytochrome c_1. Therefore, the 'Rieske' cluster cannot accept the second electron from the semiquinone since it is held in the reduced state until the second electron has been transferred to heme b_L. Thus, the affinity change of the 'Rieske' cluster enables the obligatory bifurcation of electron flow since it prevents both types of wasteful side reactions, dissociation of semiquinone as well as transfer of the second electron into the 'high-potential chain'.

(4) In step 4, the bound semiquinone is deprotonated (4a) and the second electron is transferred to heme b_L (4b). The distance of 27–35 Å between the center of the 'Rieske' cluster and the iron atom of heme b_L determined by X-ray crystallography ([33], E.A. Berry, personal communication) is compatible with this mechanism: the distance between the center of the 'Rieske' cluster and the distal oxygen is 13 Å, the distance between a heme edge and the central iron atom 6 Å. Therefore, electron transfer between semiquinone bound to the 'Rieske' cluster and heme b_L would occur over a distance of 8–16 Å which is compatible with electron transfer rates $>10^6$ s^{-1}.

(5) In the last step, electrons are transferred from the 'Rieske' cluster to cytochrome c_1 and from heme b_L to heme b_H. Electron transfer from the 'Rieske' cluster to cytochrome c_1 is now possible since the redox potential of the 'Rieske' cluster will be decreased as the oxidized quinone binds with lower affinity than semiquinone to the reduced 'Rieske' cluster; thus, the block of the 'Rieske' cluster will be released after the second electron transfer step (4b). The oxidized quinone will dissociate from the oxidized 'Rieske' cluster, so that the reaction cycle can start again.

Fig. 2 shows a schematic energy profile of the reaction which has been drawn using standard free energies. For each of the five quinone species involved, the total energy of the system Q/'Rieske' has been drawn for the free oxidized and for the reduced 'Rieske' cluster and for the quinone bound to the oxidized and reduced 'Rieske' cluster (20 states in total). In (A), QH_2 binds weakly to the oxidized 'Rieske' cluster with a dissociation constant in the mM range. The transition state (‡) is similar to a deprotonated hydroquinone; the activation barrier is 33–44 kJ/mol at pH 7 [38]. The anionic deprotonated hydroquinone will bind more tightly than QH_2 (B). After the first electron transfer from the deprotonated hydroquinone to the 'Rieske' cluster, the resulting semiquinone will be tightly bound to the reduced 'Rieske' cluster (C); in state (C), both the binding energy of the semiquinone and the redox energy of the 'Rieske' cluster will be increased by approximately 20 kJ/mol (corresponding to a potential shift of 200 mV). Whether the semiquinone will be bound as neutral $QH^\bullet$ (C) or as anionic $Q^{-\bullet}$ (D) depends on their binding energies at the 'Rieske' cluster. After the second electron transfer, the binding energy of the semiquinone will be released (E).

The schematic energy profile shows that the intermediates

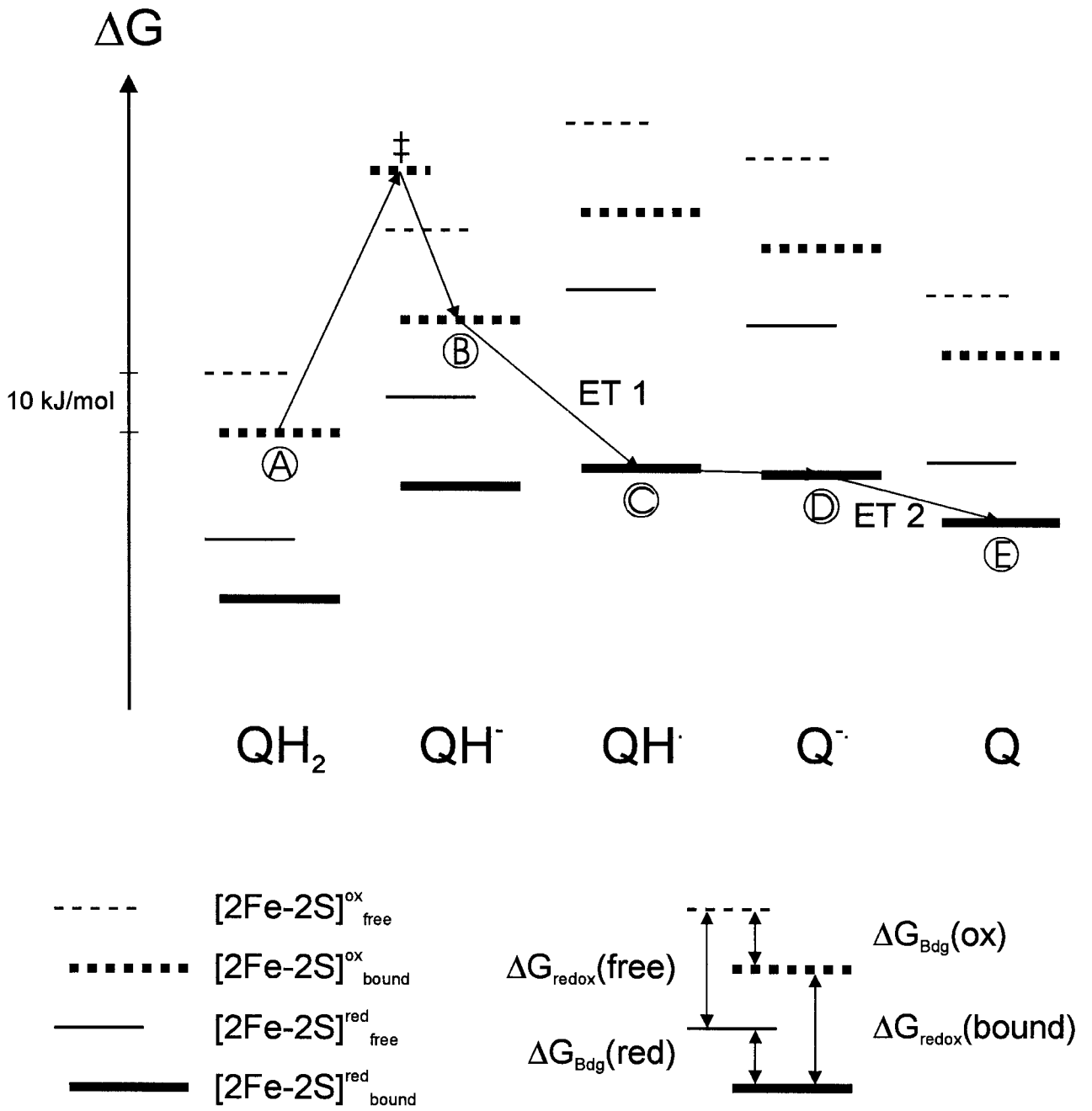

Fig. 2. Schematic energy profile according to the 'proton-gated affinity change' mechanism of hydroquinone oxidation in the Q_P site. The energies of the states were calculated from the following standard redox potentials: [2Fe-2S]: +290 mV; QH_2/Q: +70 mV. The difference between the bound and the free states represents the binding energy, the difference between the oxidized and the corresponding reduced state the redox energy ($\Delta G = -n \cdot F \cdot \Delta E$).

after the activation barrier have comparable energy; this is a general requirement for an optimized (enzymatic) reaction. Furthermore, after the first electron transfer all states containing the oxidized 'Rieske' cluster (which would result from electron transfer to cytochrome c_1) are energetically well separated from the reaction intermediates; the same is true for states containing free semiquinone.

The 'affinity change' mechanism provides a rationale for the observation that in all *bc* complexes the potential of the 'Rieske' cluster is higher than that of the high-potential heme (c_1 or *f*). This 'potential inversion' gives an additional energy barrier for electron transfer from the 'Rieske' cluster with bound semiquinone (state (C)) to cytochrome c_1 and thus prevents transfer of the second electron via the 'high-potential chain'.

3.1. Estimation of the semiquinone stability constant

From the redox potentials of the species, the stability constant of the semiquinone K_S can be estimated. If we assume that both electron transfer steps (hydroquinone to the 'Rieske' cluster = ET1 and semiquinone to heme b_L = ET2) should occur exergonically ($\Delta G \leq 0$), we obtain the following limiting

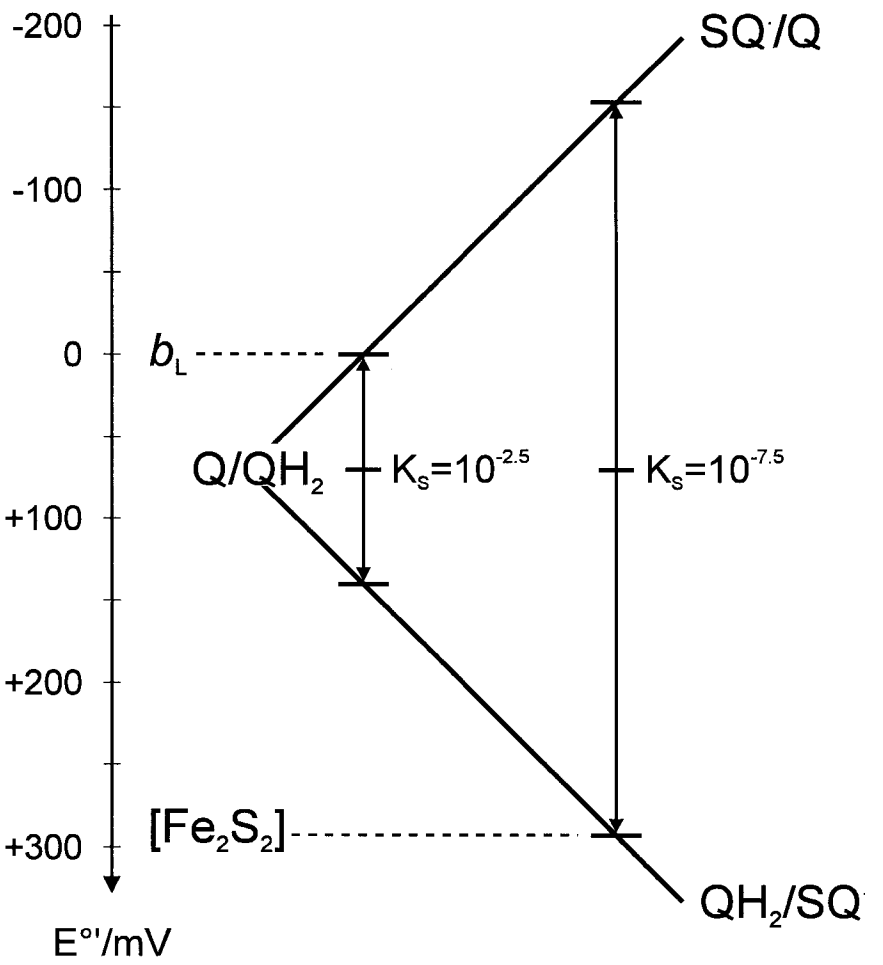

Fig. 3. Estimation of the stability constant for the semiquinone in the Q_P site. The stability constant is related to the difference between the redox potentials of the one-electron transfer steps of the quinone by the equation $K_S = \exp[(\Delta E\{QH_2/SQ^{\bullet}\} - \Delta E\{QH_2/SQ^{\bullet}\}) \cdot F/RT]$.

potentials for the one-electron transitions of quinone (Fig. 3; for simplicity, again the standard potentials have been used and not the actual potentials):

(1) $\Delta G(ET2) = 0$: $E^{o'}\{SQ^{\bullet}/Q\}$: 0 mV; $E^{o'}\{QH_2/SQ^{\bullet}\}$: +140 mV; therefore $K_S \approx 10^{-2.5}$;

(2) $\Delta G(ET1) = 0$: $E^{o'}\{QH_2/SQ^{\bullet}\}$: +290 mV; $E^{o'}\{SQ^{\bullet}/Q\}$: −150 mV; therefore $K_S \approx 10^{-7.5}$.

It is likely that the value of the stability constant K_S is between these extremes; therefore, a reasonable estimate is $K_S \approx 10^{-5}$. This value is 5 orders of magnitude above the value estimated for free semiquinone ($K_S \approx 10^{-10}$) and is well within the experimental limits ($< 10^{-3}$ in mitochondria, $< 2 \times 10^{-4}$ in chromatophores [6]) even if we disregard the possibility that the semiquinone might be EPR-silent.

3.2. *Comparison of the 'proton-gated affinity change' mechanism with other mechanistic models*

The 'proton-gated affinity change' mechanism presented here is the first proposed mechanism which fully incorporates the electrochemical properties of the 'Rieske' cluster, in particular the increased affinity of the reduced cluster for bound quinones. According to the semiquinone barrier mechanism, the electrochemical properties of the 'Rieske' cluster are by and large irrelevant [7]: the overall energetics of the reaction is governed by the potential of the terminal acceptor, cytochrome *c* (c_2), while the reaction rate is controlled by the postulated semiquinone barrier. According to the 'proton-gated charge-transfer' mechanism [4], the symproportionation of two quinones is driven by the Lewis acidity of the oxidized 'Rieske' cluster but no specific function is attributed to the reduced 'Rieske' cluster.

Due to the stabilization of quinonoid species on the reduced 'Rieske' cluster which is a consequence of the exposure of the histidine ligands of the 'Rieske' cluster at the tip of the 'Rieske' protein, the existence of highly destabilized semiquinone species as reaction intermediates can be ruled out. This is not in conflict to experimental reports since there is no compelling experimental evidence for the existence of such semiquinone species [4]. Hence, all models involving a highly destabilized semiquinone are incompatible with the electrochemical properties of the 'Rieske' cluster reviewed here. The fact that the semiquinone is stabilized at the Q_P site indicates that the activation barrier should be at the deprotonated hydroquinone state, which is prerequisite to the 'proton-gated charge-transfer' mechanism proposed by Brandt [4] and is also implied in the 'proton-gated affinity change' mechanism presented here. The results obtained by Brandt and Okun [38] support this notion.

Another important difference between different models is the number of quinone molecules which must be present in the Q_P site. From an analysis of the size and redox state of the quinone pool on the g_x signal of the EPR spectrum of the

reduced 'Rieske' cluster in membranes of *Rhodobacter capsulatus*, Ding et al. [34] have proposed that the Q_P site can accommodate two ubiquinone molecules: a strongly bound species (Q_{os}) and a weakly bound species (Q_{ow}). However, studies of mutants have failed to identify ligands specific to either Q_{os} or Q_{ow} [7,35,36]; the Q_P site appears to be one contiguous reaction site with respect to the binding of quinones while mutations in different parts of the Q_P site show specific effects on the binding of different inhibitors (reviewed in [37]). Therefore, the possibility must be considered that the effects studied by Ding et al. [7,34] are due to two different binding modes of quinone rather than to two quinone molecules bound simultaneously.

Both the semiquinone barrier mechanism and the 'proton-gated charge-transfer' mechanism operate on two quinone molecules in the Q_P site while the 'proton-gated affinity change' mechanism as described in this paper is functional with a single quinone molecule. Even if the emerging crystal structures indicate the presence of two molecules of quinone in the Q_P site, the principles of the 'affinity change' mechanism which follow directly from the electrochemical properties of the 'Rieske' cluster must be applied to the mechanism of hydroquinone oxidation; these are compatible in many aspects to the 'proton-gated charge-transfer' mechanism but not to the concept of a highly unstable semiquinone intermediate.

4. Conclusions

The 'Rieske' protein is known for a long time to be a key element in the Q_P site of the bc_1 complex; however, its electrochemical properties have so far been largely ignored in the discussion of the mechanism of hydroquinone oxidation. In particular, the different affinity of the oxidized and reduced 'Rieske' cluster towards bound quinonoid compounds has been reported but its mechanistic implications have not been recognized; therefore, the semiquinone intermediate has been assumed to be highly unstable despite the fact that semiquinone will be stabilised on the reduced 'Rieske' cluster. The occurrence of this stable semiquinone provides a route for hydroquinone oxidation to occur without involving energetically unfavorable intermediates.

The binding of quinone and hydroquinone at the Q_P site has been studied by looking at the effect on the EPR spectra of the reduced 'Rieske' cluster [7,16,34]. Using this approach, only the product complex of the reaction can be studied since hydroquinone reacts with the oxidized 'Rieske' cluster which is EPR silent. Although these studies have been performed with great expertise and care, the conclusions drawn should therefore be taken with caution.

From the electrochemistry of the 'Rieske' cluster, specific predictions can be made about the nature of the reaction intermediates of the hydroquinone oxidation reaction which will be tested experimentally. However, this cannot be done using EPR spectroscopy since most intermediates are EPR silent. Fourier-transform infrared (FT-IR) spectroscopy is an obvious choice for the experiments required to prove or disprove the mechanism presented here since it can provide local and specific structural information on each of the states. In particular, kinetically resolved vibrational spectroscopy should allow the identification of reaction intermediates on a realistic time scale.

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