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Control of Ubiquinol Oxidation at Center P (Q_O) of the Cytochrome bc_1 Complex

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The unique bifurcated oxidation of ubiquinol at center P (Q_o) of the cytochrome bc_1 complex is the reaction within the Q-cycle reaction scheme that is most critical for the link between electron transfer and vectorial proton translocation. While there is a general consensus about the overall reaction at center P, the nature of the intermediates and the way the reaction is controlled to ensure obligatory bifurcation is still controversial. By reducing the reaction to its essential steps, a kinetic net rate model is developed in which the activation barrier is associated with the deprotonation of ubiquinol, but the steady state rate is kinetically controlled by the occupancy of the ubiquinol anion and the semiquinone state. This concept is used to interpret experimental data and is discussed in terms of various mechanistic models that are under discussion. It is outlined how other aspects of the center P mechanism like the proposed "prosthetic" ubiquinone and the moving domain of the "Rieske" protein could be incorporated in the kinetic framework.

KEY WORDS: bc_1 complex; ubiquinone; Rieske protein; Qcycle; mechanism; deprotonation; catalytic switch.

INTRODUCTION

The cytochrome bc_1 complex forms the middle part of the mitochondrial and many bacterial respiratory chains (Brandt and Trumpower, 1994; Schagger *et al.*, 1995). In purple bacteria, it is part of the photosynthetic electron transfer chain (Gennis *et al.*, 1993). The larger family of *bc*-type complexes also includes the cytochrome b_6f complex found in chloroplasts, algae, and some Gram-positive bacteria (Cramer *et al.*, 1994).

The cytochrome bc_1 complex transfers electrons from ubiquinol to cytochrome *c* and links this electron transfer to the formation of a proton gradient across the inner mitochondrial or bacterial plasma membrane. The overall reaction is described by the following equation, in which subscripts *n* and *p* designate negative and positive sides of the membrane and ox and red refer to oxidized and reduced species:

$$QH_2 + 2\,\text{cyt}\,c^{ox} + 2\,H_n^+ \rightarrow Q + 2\,\text{cyt}\,c^{red} + 4\,H_p^+ \qquad (1)$$

The four redox prosthetic groups of the cytochrome bc_1 complex are located in three subunits, namely cytochrome *b* with two heme groups, cytochrome c_1, and the Rieske iron–sulfur protein. No significant functional differences have been found between the mitochondrial and the bacterial enzyme in which only the three redox proteins are present (Trumpower, 1991). Thus, the up to eight additional subunits, which lack prosthetic groups (Schagger *et al.*, 1995; Trumpower, 1990) found in cytochrome bc_1 complexes of mitochondria, are not essential for catalysis. The subunit composition, topology, structure, and mutational analysis of the cytochrome bc_1 complex have been summarized in other contributions to this issue and a number of recently published reviews (Brandt and Trumpower, 1994; Gennis *et al.*, 1993; Colson, 1993; Graham *et al.*, 1993; Brasseur *et al.*, 1996; Crofts and Berry, 1998). The unique bifurcation of electron flow at the

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ubiquinol–oxidation center (center P or Q_O) into a high-potential and a low-potential pathway is the energy-conserving reaction within the Q-cycle reaction scheme. This step is prerequisite for vectorial proton translocation as it results in the "recycling" of every second electron by transferring it across the membrane to the ubiquinone–reduction center N (Q_i).

The recently published X-ray structures of the cytochrome bc_1 complexes from bovine (Xia *et al.*, 1997; Iwata *et al.*, 1998) and chicken heart mitochondria (Zhang *et al.*, 1998) support the overall picture that had emerged from previous extensive studies on this respiratory chain complex. Most importantly, the protonmotive Q cycle (Mitchell, 1975) as the general reaction scheme of the cytochrome bc_1 complex is strongly supported by the molecular structure. However, the structural data also revealed some unexpected features of which the mobility of the Rieske iron–sulfur protein is most notable. It became clear that several important issues still have to be resolved to understand the mechanism of the cytochrome bc_1 complex. Structural information has revived two ideas that have been put forward earlier, namely that ubiquinone can move directly from center P to center N without entering the ubiquinone pool and that there is significant electron transfer between the two monomers (Xia *et al.*, 1997; Crofts and Berry, 1998). However, the current focus of interest is on three closely linked questions that relate directly to the mechanism of ubiquinol oxidation at center P: (i) Is there a second "prosthetic" ubiquinone at center P? (ii) What are the individual steps of ubiquinol oxidation and how are they controlled? (iii) What is the function of the moving domain of the Rieske iron–sulfur protein.

The current views on these issues have been discussed in detail recently (Kim *et al.*, 1998; Crofts and Berry, 1998; Brandt, 1998) and will, therefore, only be briefly summarized here. This review will focus on some controversial aspects of ubiquinol oxidation in the cytochrome bc_1 complex.

GENERAL ASPECTS OF UBIQUINOL AT CENTER P

The ubiquinol oxidation at center P by two entirely different acceptors is a unique reaction only found in the cytochrome bc_1 complex. It is this reaction that drives vectorial proton translocation: One of the two electrons enters the low-potential pathway according to

$$QH_2 + Fe_2S_2^{ox} + \text{cyt. } b_L^{ox} \rightarrow Q + Fe_2S_2^{red} + \text{cyt. } b_L^{red} + 2H^+ \quad \Delta G^{o'} \approx -13 \text{ kJ/mol} \quad (2)$$

thereby preventing the unproductive, but thermodynamically more favorable reaction

$$QH_2 + 2\ Fe_2S_2^{ox} \rightarrow Q + 2\ Fe_2S_2^{red} + 2\ H^+ \quad \Delta G^{o'} \approx -43 \text{ kJ/mol} \quad (3)$$

($\Delta G^{o'}$ values were calculated for the bovine enzyme using E_{m7} of +70, +290 and −20 mV for QH_2/Q, Fe_2S_2, and cyt b_L, respectively.) The latter reaction is observed not even in the so called oxidant-induced reduction experiment, indicating tight control of the reaction that leads to an *obligatory* bifurcation of electron flow.

It is evident from Eq. (2) that the driving force for ubiquinol oxidation at center P results from the highly positive midpoint potential of the Rieske iron–sulfur cluster (Trumpower and Edwards, 1979; Wikstrom and Berden, 1972) and that the first electron transfer must be reduction of this redox center by ubiquinol. However, this thermodynamic relationship only provides information on the overall reaction. To understand the mechanism and how it is controlled, it will be necessary to identify and analyze the individual intermediates of ubiquinol oxidation.

Ubiquinone-induced changes of the EPR line shape of the Rieske iron–sulfur cluster suggest that ubiquinol oxidation at center P may involve two ubiquinone molecules (Ding *et al.*, 1992, 1995). However, independent and more direct evidence and/or detection of two ubiquinone molecules at center P in a high-resolution structure are clearly needed to decide with certainty whether these two ubiquinones really exist. Obviously, answering this question is prerequisite to defining the exact chemical nature of the ubiquinone intermediates. Possible mechanistic implications of two ubiquinones at center P, like a shortening of distances for electron transfer (Ding *et al.*, 1992) and a charge-transfer chemistry of ubiquinol oxidation have been proposed (Brandt, 1996).

Some questions related to the intermediates of ubiquinol oxidation are not strictly related to the issue whether one or two ubiquinones can bind to center P. These questions are: (i) What are the energetics of the intermediates at center P, and, particular, how is ubiquinol deprotonated and how stable is the semiquinone? (ii) What is the mechanistic function of the Rieske iron–sulfur cluster, apart from providing the driving force for the reaction?

The observation that the water-soluble domain of the Rieske iron–sulfur protein can turn by as much as about 60° around a hinge region connecting it to its transmembrane helix (Zhang *et al.*, 1998; Kim *et al.*, 1998; Iwata *et al.*, 1998) adds another unique, mechanical aspect to the mechanism at center P. Again several questions arise from this observation: (i) Is the movement of the Rieske domain passive or is it triggered by the electron transfer reactions? (ii) What is the timing of the movement and how is it controlled? (iii) Is the Rieske movement an accidental feature of the cytochrome bc_1 complex or is it required for the obligatory bifurcation of electron flow at center P?

Overall, the bifurcated ubiquinol oxidation at center P turns out to be rather complex. To limit the number of possible intermediates and reaction sequences it is necessary to integrate all restraints imposed by experimental evidence. The following sections are an attempt to use these restraints to define a thermodynamic and kinetic framework of the reaction and to integrate a function for the Rieske movement and a possible role of a second ubiquinone.

KINETICS AND THERMODYNAMICS OF UBIQUINOL OXIDATION AT CENTER P

The rate constants for individual steps of the protonmotive Q cycle have been determined by rapid kinetic techniques and were built into kinetic models (Crofts and Wang, 1989; Orii and Miki, 1997). Simulations based on these kinetic models did not take into account an activation barrier that was found experimentally to be in the range of 30 to 40 kJ/mol at neutral pH for the steady state (Brandt and Okun, 1997) and the rate of heme b_H reduction (Crofts and Wang, 1989). It has been proposed that a highly unstable semiquinone (E_m for $Q^{\bullet -}/Q = -300$–400 mV) is the transition state responsible for this barrier (Crofts and Wang, 1989). However, when Orii and Miki tried to incorporate this assumption into their comprehensive kinetic model, the resulting simulation was incompatible with the experimental data (Orii and Miki, 1997). Moreover, it was recently shown that the EPR detectable semiquinone radical originally assigned to center P by de Vries *et al.* (1981) is not abolished by the addition of inhibitors like myxothiazol, MOA-stilbene, or stigmatellin that are known to block this ubiquinone binding site.

On the other hand, the presteady-state measurements used for the kinetic models clearly identify the oxidation of ubiquinol at center P as the slowest step within the protonmotive Q cycle that is, therefore, likely to control the steady-state rate. To account for this problem, Orii and Miki suggested that the bifurcated reaction at center P is under "delicate kinetic control" and that the timing of the reaction is governed by the redox state of the Rieske protein (Orii and Miki, 1997).

To address this problem we have analyzed the pH dependence of the steady-state reaction of mitochondrial cytochrome bc_1 complex (Brandt and Okun, 1997). The central conclusion from our results was that the observed pH dependent activation barrier reflects the deprotonation of ubiquinol:

$$QH_2 \rightleftharpoons QH^- + H^+ \quad \Delta G^{\neq}\,(pH = 7) \approx +40\ \text{kJ/mol} \tag{4}$$

On the other hand, it has been reported that changes in catalytic rate caused by point mutations of the Rieske protein in *Saccharomyces cerevisiae* (Deṇke *et al.*, 1998) and *Paracoccus denitrificans* (Schroter *et al.*, 1998) correlated very well with a corresponding shift of the redox midpoint potential of the iron–sulfur cluster, as predicted by the fundamental principle of the Q cycle that the high potential of the Rieske iron–sulfur cluster drives reduction of the low potential heme b_L, sometimes called the "redox seesaw." This seems to be in stark contrast to Eq. (4), if one equates the step responsible for the thermodynamic activation barrier with the so called rate-limiting step (Snyder and Trumpower, 1998; Crofts and Berry, 1998). However, rate limitation can be a rather complex phenomenon and can rarely be explained by looking at a single step in a reaction sequence. This is true, in particular, if the reaction under study is bifurcated as is the ubiquinol oxidation in the cytochrome bc_1 complex and if all individual rate constants involved are large compared to the catalytic rate. In fact, the observed pH dependence of the activation barrier was not reflected in the pH dependence of the steady-state rate of bovine and yeast cytochrome bc_1 complex (Brandt and Okun, 1997), which was instead governed by the protonation state of two groups essential for catalysis.

This somewhat puzzling situation can be understood by analyzing the reaction profile for the initial steps of the Q cycle shown in Fig. 1 that has been reduced to the essential steps. The scheme describes ubiquinol oxidation at center P as a sequence of interconversions according to:

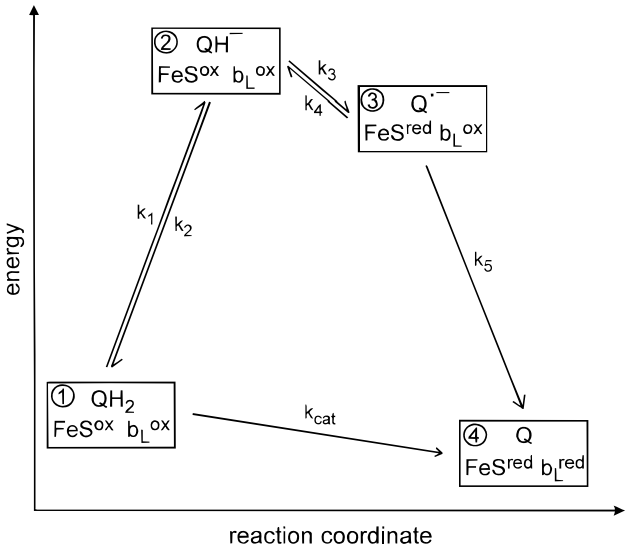

Fig. 1. Reaction profile of the first steps of the protonmotive Q cycle. Qualitative illustration of the proposed energy profile for the oxidation of ubiquinol. The boxes indicate the approximate energy levels of the reaction intermediates. For simplicity, binding and release of the substrate quinone (left) were omitted. See text for further details. b_L^{ox} and b_L^{red}, oxidized and reduced low-potential heme of cytochrome *b*; FeS^{ox} and FeS^{red}, oxidized and reduced Rieske iron–sulfur cluster; Q, quinone; Q^-, semiquinone; QH_2 QH^-, protonation states of ubiquinol.

$$① \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ② \underset{k_4}{\overset{k_3}{\rightleftharpoons}} ③ \overset{k_5}{\rightarrow} ④ \qquad (5)$$

The transition from intermediate 3 to 4 is treated as irreversible, as the *net* rate constants for the two preceding steps are expected to be much slower. If one assumes that all other rate constants of the protonmotive Q cycle are much greater, it follows that the net catalytic rate constant k_{cat} is the major determinant of the overall steady-state rate (Crofts and Wang, 1989; Orii and Miki, 1997). This also implies that intermediate 4 rapidly decays and is converted back to intermediate 1. Using the concept of net rate constants (Cleland, 1975) it can then be deduced that:

$$k_{cat} = \frac{1}{\frac{1}{k_5} + \frac{k_4 + k_5}{k_3 k_5} + \frac{k_2 + [k_3 k_5/(k_4 + k_5)]}{k_1 k_3 k_5/(k_4 + k_5)}} \qquad (6)$$

The denominator is the sum of the net rate constants for the transitions intermediates 1 to 2, intermediates 2 to 3, and intermediates 3 to 4 (from right to left). It becomes immediately obvious from Eq. (6) that all rate constants contribute to k_{cat} and that the net rate constants can be small even if all individual forward and back rate constants are very large. In effect, the overall rate is governed by kinetic control of the occupancy of intermediates 2 and 3 that, in turn, depends on the relative energy level of all four intermediates.

This simple kinetic model offers straightforward explanations for both the pH dependence of the activation barrier E_{act} (Brandt and Okun, 1997) and the changes in steady-state activity caused by shifting the midpoint potential of the Rieske iron–sulfur cluster by point mutation (Denke *et al.*, 1998; Schroter *et al.*, 1998):

According to Fig. 1, the first deprotonation of ubiquinol is solely responsible for the activation barrier and E_{act} is expected to be proportional to pH, as was observed for the steady-state reaction of yeast and bovine cytochrome bc_1 complex (Brandt and Okun, 1997). At first sight, it seems a paradox, however, that a decrease in E_{act} had no significant effect on the steady-state rate. To understand this, one has to consider that the pH dependence of the midpoint potential of the Rieske iron–sulfur cluster can be described by two pK_a values, namely, 7.7 and 9.1, that were assigned to the two histidines liganding the cluster (Link, 1994). The second pK_a was found to be responsible for the decrease of the steady-state rate above pH 8, as protonation of this group is required for catalytic activity (Brandt and Okun, 1997), but does not affect the activation barrier, i.e., only those enzymes in which this group is protonated will be active. The other group with a pK_a of 7.7 leads to a significant drop of the Rieske midpoint potential above pH 7 (Link, 1994), which, if taken by itself, would increase the energy level of intermediate 3 (cf. Fig. 1). However, the expected effect on k_{cat} was not observed (Brandt and Okun, 1997). This is likely to be due to the fact that the semiquinone should exhibit a pH dependence of its midpoint potential that goes parallel to that of the iron–sulfur cluster as it is estimated to also have a redox-dependent pK_a value in the neutral range. Thus, the ΔG between intermediates 2 and 3 and their relative steady-state occupancy should not change very much with pH. However, this also means that pH-dependent changes of the ΔG between intermediates 3 and 4 and of k_5 are expected to go parallel with the changes in E_{act} (Fig. 2A), which has opposite and seemingly compensatory effects on k_{cat} and the steady-state rate.

If, in contrast, the midpoint potential of the Rieske iron–sulfur cluster is lowered by point mutation (Denke *et al.*, 1998; Schroter *et al.*, 1998), the energy level of intermediate 3 is increased, changing k_3, k_4 and k_5 and, therefore, k_{cat} (Fig. 2B; see Eq. 6). In

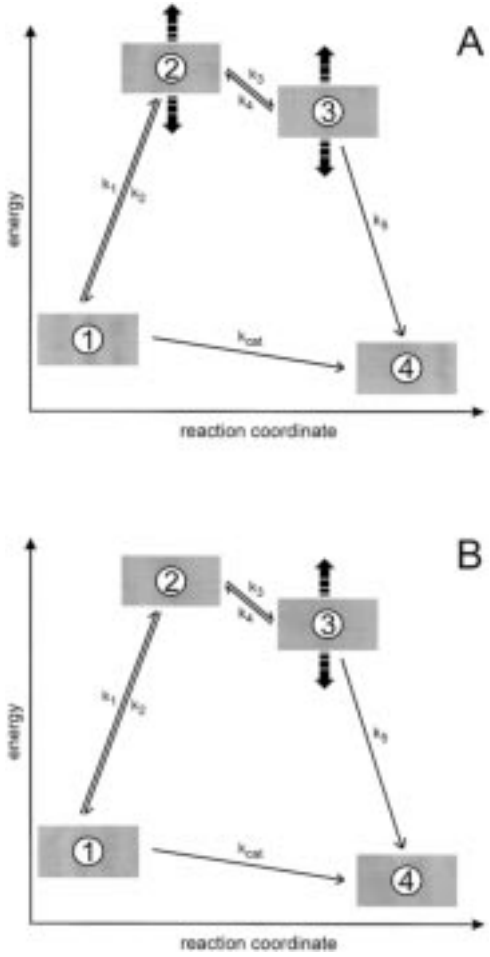

Fig. 2. Influence on the energy levels of intermediates in ubiquinol oxidation. Effects of changes in pH (A) and midpoint potential of the Rieske iron–sulfur cluster by point muations (B) on the energy level of the intermediates, defined in Fig. 1, are indicated by hatched arrows. See text and Fig. 1 for further details.

particular, the steady-state occupancy of intermediate 2 is increased, which will critically affect the net rate for the interconversion of intermediates 1 to 2. Note that, in this case, the steady-state rate, but not E_{act} are changed, because the relative energy levels of intermediates 2 and 3 directly affect the trapping of the ubiquinol anion. Thus, the midpoint potential of the Rieske iron–sulfur remains a major determinant of the steady-state rate as it determines the trapping efficiency for the ubiquinol anion. Therefore, control of ubiquinol oxidation is not thermodynamic but kinetic as predicted by Orii and Miki (1997). Therefore, the model presented here works without a strong base for the deprotonation of ubiquinol and without stabilization of the ubiquinol anion. Rate limitation is explained by the delicate kinetic balance between endergonic deprotonation of ubiquinol and a first electron transfer occuring at very low ΔG. Remarkably, the pH dependence of the activation barrier has not been observed to the same extent in presteady-state kinetics with bacterial chromatophores by measuring the rates of heme b_L or heme b_H reduction (Crofts and Wang, 1989; Crofts and Berry, 1998). The likely reason is that Eq. (6) does not apply because these experiments were not performed under conditions of substrate saturation and without removing the product rapidly (Cleland, 1975). Under these conditions, intermediates 2 and 3 reach no steady state and kinetic control is expected to be quite different. This may have obscured the effect of pH on the thermodynamic barrier of ubiquinol deprotonation in these experiments.

MIDPOINT POTENTIAL OF THE SEMIQUINONE ANION AT CENTER P

The general kinetic model for ubiquinol oxidation presented in the previous section provides some clues of what to expect for the midpoint potential of the semiquinone anion transiently formed at center P: Clearly, there seems to be no reason to postulate a highly unstable semiquinone with a midpoint potential of −300 to −400 mV to account for the activation barrier of ubiquinol oxidation (Crofts and Wang, 1989). Therefore, the question is to what extent the semiquinone is thermodynamically stabilized by center P.

A pronounced stabilization of the semiquinone intermediate because of tight binding to one of the histidines liganding the reduced iron–sulfur cluster is the critical element of the proton-gated affinity change mechanism proposed by Link (Link, 1997). According to this model, reoxidation of the iron–sulfur cluster requires electron transfer to heme b_L and the semiquinone state should build up under the conditions of oxidant-induced reduction in the presence of antimycin. The fact that this species cannot be observed by EPR spectroscopy could be explained by tight magnetic coupling between the semiquinone radical and the reduced iron–sulfur cluster, but this has not been confirmed by experimental evidence, so far. In the reaction scheme of Fig. 1, stabilization of the semiquinone would result in a correspondingly lower energy level of intermediate 3 (cf. Fig 2B). This would make trapping of QH^- more efficient, but make it less dependent on the midpoint potential of the Rieske iron–sulfur

cluster. In quantitative terms, the observed 2.5-fold increase in steady-state rate per 60 mV change in potential of the iron–sulfur cluster is consistent with the Marcus theory, if electron transfer occurs between a donor and acceptor of similar midpoint potential (Snyder and Trumpower, 1998), i.e., the ΔG for the interconversion of intermediates 2 to 3 is small. However, the donor midpoint potential to consider is not that of the QH^-/Q or QH_2/Q couple as proposed by Snyder and Trumpower, but that for the one electron oxidation couple $QH^-/QH^{\bullet}$. In 80% ethanol the midpoint potential for this one electron oxidation of QH^- is +190 mV (Rich, 1984; Brandt, 1996), which compares well to +290 mV for the iron–sulfur cluster. Thus, it is not necessary to postulate stabilization of the semiquinone at center P. An advantage for a very low occupancy of the semiquinone state under all conditions would be that formation of superoxide is minimized.

If a second, prosthetic quinone (Ding *et al.*, 1995) would be a component of center P, this could be easily incorporated into the model discussed here and would offer some advantages to the mechanism: It would shorten distances for electron transfer making it more efficient and may preform the bifurcation of electron transfer by symproportionation of a charge transfer complex (Brandt, 1996). However, this issue remains controversial (Crofts and Berry, 1998) and no experimental evidence has been presented so far to resolve it. Therefore, the reader is referred to detailed discussions on this topic that have been published earlier (Ding *et al.*, 1995; Brandt, 1998; Crofts and Berry, 1998).

MOVEMENT OF THE "RIESKE" PROTEIN

As early as 1967, Baum and co-workers noted that the conformational stability of the cytochrome bc_1 complex increases when the iron–sulfur protein becomes reduced and that, at the same time, this protein becomes more susceptible to proteolytic digestion (Baum *et al.*, 1967). These authors also speculated that these observations might indicate involvement of different conformational states of the protein during catalysis. Thus, the idea of a redox-linked conformational change of the cytochrome bc_1 complex, which seems very attractive in the light of the emerging molecular structures, is rather old.

Based on specific redox-dependent affinity changes observed with different inhibitors of center P, we later proposed the catalytic-switch model that employs two conformational states of center P to explain the obligatory bifurcation of electron flow at center P (Brandt *et al.*, 1991; Brandt and von Jagow, 1991).

The molecular basis of these observations was revealed by the high-resolution structures of the bovine (Kim *et al.*, 1998; Iwata *et al.*, 1998) and chicken (Zhang *et al.*, 1998) cytochrome bc_1 complexes: The water-soluble part of the Rieske protein was found to turn by about 60° around a hinge region connecting it to its transmembrane helix and several substates induced by the addition of inhibitors or crystal packing have been reported. Considering that the iron–sulfur cluster is only capable to accept an electron from the proposed ubiquinol-binding site in the so called *b* position, but can donate an electron to heme c_1 only in the so called c_1 position at high enough rates, it is almost inevitable to conclude that the water-soluble domain of the Rieske protein has to switch between the two conformations during turnover. This notion is supported by the observation that reducing the flexibility of the hinge region of the Rieske protein by introducing prolines or cysteine-disulfide bridges through site-directed mutagenesis dramatically reduced catalytic activity (Tian *et al.*, 1998).

The important question arises whether the movement is largely a passive diffusion of the iron–sulfur protein between two docking sites on cytochrome *b* and c_1, or whether redox changes trigger a switching between these two states in a fashion similar to that proposed in the catalytic-switch model (Brandt *et al.*, 1991; Brandt and von Jagow, 1991). The latter alternative seems more attractive, as conformational changes linked in particular to the redox state of the iron-sulfur cluster were demonstrated by a significant body of experimental evidence (Brandt *et al.*, 1991; Brandt and von Jagow, 1991; Baum *et al.*, 1967; Rieske *et al.*, 1967; Palmer and Degli Esposti, 1994).

The catalytic-switch model was based on extensive inhibitor binding studies (Brandt *et al.*, 1991; Brandt and von Jagow, 1991) that turned out to be fully compatible with inhibitor-induced changes of the position of the hydrophilic domain of the Rieske domain observed in the high-resolution structures. In a modified version of the catalytic-switch model (Brandt, 1998), it was possible to accommodate the moving Rieske with very little changes to the original proposal.

The modified catalytic-switch mechanism (Fig. 3) not only incorporates the movement of the Rieske

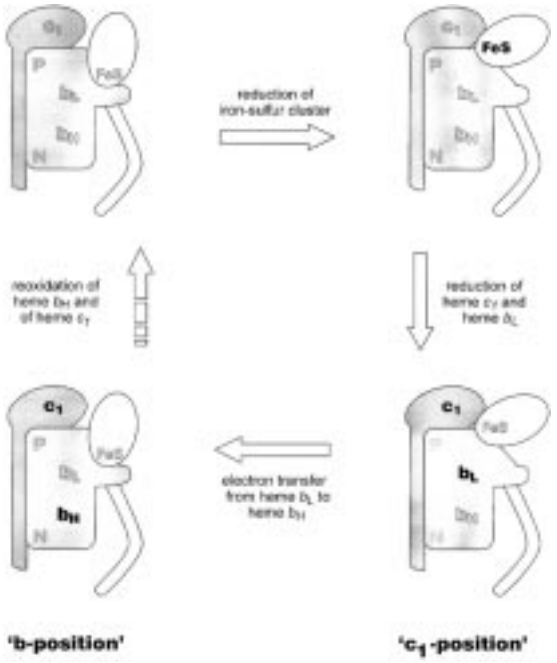

Fig. 3. Schematic representation of the modified catalytic-switch mechanism at the ubiquinol oxidation center of cytochrome bc_1 complex (Brandt, 1998). See text for further details. The three catalytic subunits and the orientation of the Rieske protein are based on the structure of the chicken enzyme (Zhang *et al.*, 1998). b_L, b_H, c_1, heme b_L, b_H, c_1; FeS, Rieske iron–sulfur cluster; *p*, positive side; *n*, negative side. Oxidized and reduced redox centers are shown in outline and bold letters, respectively.

protein into the center P reaction, but assigns a specific function to this unusual phenomenon. In the *b* position, both the iron–sulfur center and heme b_L are oxidized (cf. left side of Fig. 3), ensuring that ubiquinol is only oxidized at center P when a bifurcation of electron flow is possible. As electron transfer from heme b_L to heme b_H triggers return to the *b* position, the complex stays in the c_1 position even when center N is blocked by antimycin and the iron–sulfur cluster is oxidized by heme c_1 because heme b_L stays reduced. This may help to prevent further electron transfers at center P.

The conformational changes required to trigger the transitions between the different positions of the Rieske protein may be rather subtle and, therefore, improvement of the crystallographic data of the oxidized and partially reduced complex will be necessary to show whether such structural changes indeed occur.

Like the proposed prosthetic quinone, a redox-triggered movement of the Rieske protein could add another functional element that could help to optimize the kinetic control of the general mechanism outlined in Fig. 1 and ensure obligatory bifurcation of electron flow at center P.

CONCLUSIONS

According to the kinetic model presented here, ubiquinol oxidation at center P is kinetically controlled by trapping unstable ubiquinol anion and semiquinone species that are present at very low occupancy during steady state. The model accommodates both, the pH dependence of the steady-state activation barrier and the influence of the Rieske midpoint potential on the catalytic activity of the cytochrome bc_1 complex.

It will require further experimental evidence to decide with certainty whether a second, prosthetic ubiquinone and a redox-triggered catalytic switch of the Rieske iron–sulfur protein are components of the mechanism of ubiquinol oxidation at center P of the cytochrome bc_1 complex. However, both features could help to optimize the delicate kinetic control of the basic mechanism.

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