Minireview

Bifurcated ubihydroquinone oxidation in the cytochrome bc_1 complex by proton-gated charge transfer

Ulrich Brandt*

Universitätsklinikum Frankfurt, Zentrum der Biologischen Chemie, Theodor-Stern-Kai 7, Haus 25B, D-60590 Frankfurt am Main, Germany

Received 4 April 1996

Abstract The unique bifurcation of electron flow at the ubihydroquinone-oxidation center of the cytochrome bc_1 complex is the energy-conserving reaction of the protonmotive Q-cycle and is prerequisite to vectorial proton translocation. The widely accepted Q-cycle reaction scheme describes the overall electron and proton pathways, but does not address the detailed chemistry of this central step. Based on a model of the ubihydroquinone-oxidation pocket containing two ubiquinone molecules in a stacked configuration, a detailed model for the reactions during steady-state catalysis is proposed. In this proton-gated charge-transfer mechanism the reaction is controlled by the deprotonation of the substrate ubihydroquinone.

Key words: Cytochrome bc_1 complex; Ubiquinone; Q-cycle; Charge-transfer mechanism; Deprotonation

1. Introduction

The cytochrome bc_1 complex transfers electrons from ubiquinol to cytochrome *c* and links this electron transfer to the establishment of a proton gradient across the inner mitochondrial or bacterial plasma membrane. It forms the middle part of the mitochondrial and many bacterial respiratory chains [1-3]. It is also part of the photosynthetic electron-transfer chains of purple bacteria [4] and is a member of a larger family of *bc*-type complexes, which include the cytochrome *bf* complex found in chloroplasts, algae and some Gram-positive bacteria [5].

The subunit composition, topology, structure and mutational analysis of the cytochrome bc_1 complex have been summarized in a number of recent reviews [1,4,6,7]. The redox prosthetic groups of the cytochrome bc_1 complex are contained in three subunits, the diheme cytochrome *b*, cytochrome c_1, and the Rieske iron-sulfur protein. Although the cytochrome bc_1 complexes of mitochondria contain multiple additional subunits which lack prosthetic groups [3,8], these supernumerary subunits are not essential, as demonstrated by the fact that no significant functional differences have been found from the bacterial enzyme in which only the three redox proteins are present [2].

This review reexamines the chemistry of the protonmotive Q-cycle and proposes a mechanism of ubihydroquinone oxidation which ensures tight coupling between electron transfer and vectorial proton transport.

1.1. The protonmotive Q-cycle

The widely accepted reaction scheme known as the protonmotive Q-cycle [9] is depicted in Fig. 1 as numbered electron-transfer reactions. It describes the overall electron and proton pathways within the cytochrome bc_1 complex. For a complete cycle the reaction sequence 1–4 must be completed twice. Essentially two features specific for *bc*-type complexes allow vectorial proton translocation:

(i) At the ubihydroquinone-oxidation center electrons are transferred onto two completely different acceptors, heme b_L ($E_{m7} \approx -20$ mV) and the Rieske Fe_2S_2 cluster ($E_{m7}=+290$ mV).

(ii) Two distinct ubiquinone-reaction centers are located on opposite sides of the membrane. The ubihydroquinone-oxidation center, called center P or center o, on the positive side of the membrane, and the ubiquinone-reduction center, called center N or center i, on the negative side of the membrane.

A bifurcated electron pathway was first proposed by Wikström and Berden [10] to account for the oxidant-induced reduction of cytochrome *b* in mitochondria. Peter Mitchell combined this idea with the Q-loop concept [11] by postulating ubihydroquinone oxidation on the positive side and ubiquinone reduction on the negative side of the membrane and developed a general formulation of a protonmotive Q-cycle [9]. This early version of the Q-cycle did not specify the primary oxidant of ubihydroquinone. This primary oxidant was postulated by Trumpower to be an 'oxidation factor' that transfers electrons onto cytochrome c_1 and not cytochrome *b* [12]. Later he showed that this oxidation factor was the iron-sulfur protein [13] which had already been described by Rieske as a component of the bc_1 complex in 1964 [14]. The first Q-cycle reaction schemes also implied that one of the electrons necessary to reduce ubiquinone on the negative side of the membrane would be donated by a dehydrogenase and not by cytochrome *b*. This is ruled out by the observation that the Q-cycle is operational in isolated bc_1 complex [15]. The observation of a stabilized semiquinone at center N [16] provided the rationale how cytochrome *b* can act as an electron donor for both steps of ubiquinone reduction. This reaction type is also used by other ubiquinone-reduction sites, e.g. the Q_B site of the photosysnthetic reaction center, where a one-electron donor reduces the two-electron acceptor ubiquinone in two consecutive steps.

There is ample evidence for two ubiquinone-reaction centers from the use of specific inhibitors [17] and selective removal of the iron-sulfur center [18]. Their transmembrane arrangement is evident from a wide range of spectroscopic [19,20] and mutational studies [4,6,21].

*Fax: (49) (69) 6301 6970.
E-mail: brandt@zbc.klinik.uni-frankfurt.de

PII S0014-5793(96)00436-X

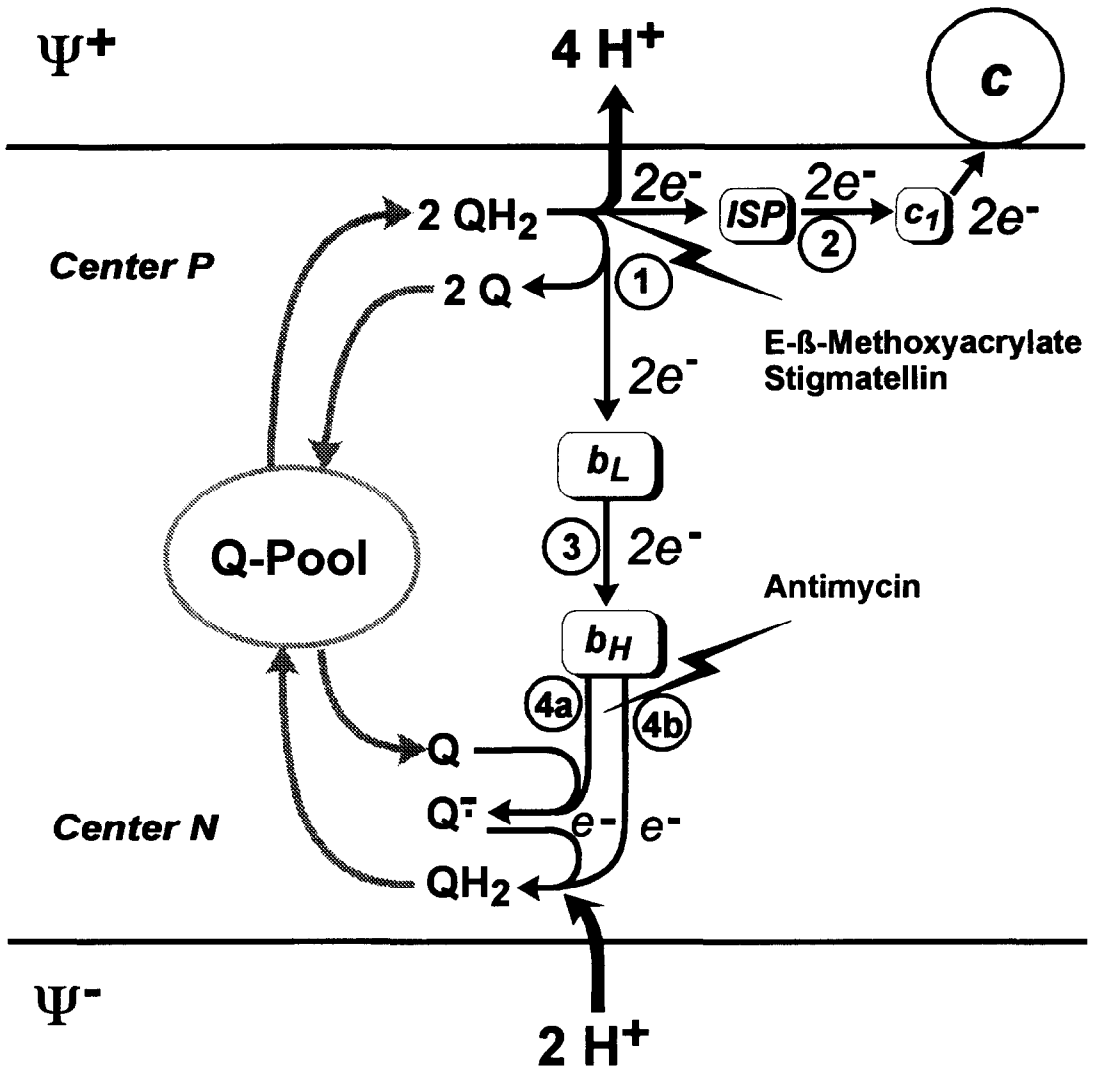

Fig. 1. The protonmotive Q-cycle. The path of electrons from ubihydroquinone to cytochrome *c* through the redox prosthetic groups of the cytochrome bc_1 complex is depicted as a series of numbered reactions shown by solid arrows. Grey arrows indicate exchange of quinones with the Q-pool. Thick arrows indicate proton uptake or release. Jagged arrows point to the sites of inhibiton for the respective inhibitors. ψ^+/ψ^-, positive/negative side of membrane; b_L and b_H, low- and high-potential hemes of cytochrome *b*; c/c_1, hemes c/c_1; FeS, 'Rieske' iron-sulfur cluster; Q, ubiquinone; $Q^{\cdot -}$, ubisemiquinone; QH_2, ubihydroquinone.

2. Chemistry of ubihydroquinone oxidation at center P

The unique bifurcation of electron flow at center P into a high-potential and a low-potential pathway is the energy-conserving mechanism of the bc_1 complex. It is in this reaction where the actual chemistry takes place that drives vectorial proton translocation: One of the two electrons is forced to enter the low-potential pathway according to

$$QH_2 + Fe_2S_2^{ox} + cyt.b_L^{ox} \rightarrow Q + Fe_2S_2^{red} + cyt.b_L^{red} + 2H^+$$

$$\Delta G^{0'} \approx -13 \text{ kJ/mol}^{(1)}$$

thereby preventing the thermodynamically much more favourable, but unproductive reaction

$$QH_2 + Fe_2S_2^{ox} + cyt.c_1^{ox} \rightarrow Q + Fe_2S_2^{red} + cyt.c_1^{red} + 2H^+$$

$$\Delta G^{0'} \approx -39 \text{ kJ/mol}^{(1)}$$

The latter reaction is not even observed in the so-called oxidant-induced reduction experiment, where excess of an oxidant like ferricyanide is added in the presence of substrate and the center N inhibitor antimycin, and both hemes of cytochrome *b* are kept almost fully reduced. In other words, the fact that antimycin is a very efficient inhibitor of the enzyme's steady state demonstrates a tight control of the reaction of some kind, leading to an *obligatory* bifurcation of electron flow, which in turn is prerequisite to vectorial proton translocation. This central question, namely how this control is built into the chemistry of the center P reaction, is not addressed by the reaction scheme of the protonmotive Q-cycle.

(1)Calculated using E_{m7} values of +70, −20, +250 and +290 mV for QH_2/Q, cyt. b_L, cyt. c_1 and Fe_2S_2, respectively.

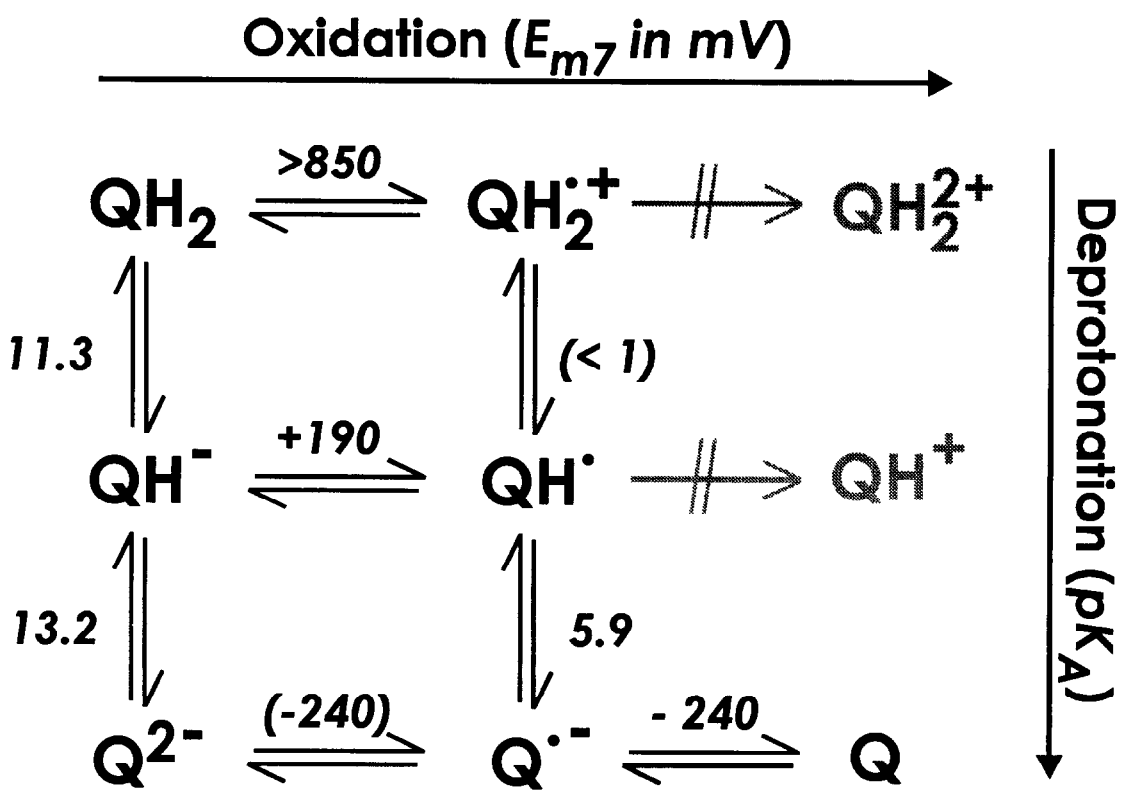

Fig. 2. Ubiquinone intermediates. E_{m7} values and pK_a values were taken from [34]. Values in parentheses are calculated to complete the respective 'square'.

3. Topology and properties of the ubihydroquinone oxidation center

A detailed understanding of any enzymatic mechanism has to consider the structural arrangement of all reactants involved and contributions of the protein moiety to the active site. Good progress is beeing made towards a high-resolution structure of the cytochrome bc_1 complex [22-24]. However, at present indirect evidence has to be used to identify the domains contributing to the center P reaction pocket. Based on the high-resolution structure of a water-soluble fragment of the Rieske iron-sulfur protein from bovine heart [25] and the large body of information available from protein chemistry, enzymology and most notably from mutational studies (see [1,4,6] for recent reviews), we have deduced a structural model of center P [26,27]. According to this model, center P is a fairly large pocket formed by several domains of cytochrome *b*, which is closed by the cluster binding loops of the iron-sulfur protein. Based on changes in the EPR line shape of the iron-sulfur cluster, Ding et al. [28] concluded that in fact two ubiquinone binding sites exist at center P. This is consistent with the earlier observation that E-β-methoxyacrylates are non-competitive inhibitors with respect to ubihydroquinone, indicating that center P accommodates two ligands at a time [29]. More recently, Ding et al. proposed that the more weakly bound species, Q_{OW}, is the substrate and is exchanged during catalysis, while the 10-times more strongly bound species, Q_{OS}, behaves like a prosthetic group and is not exchanged during turnover [30]. An obvious conclusion would be that the E-β-methoxyacrylates are non-competitive inhibitors, because they displace the prosthetic Q_{OS} but not the substrate Q_{OW}.

Thus, it seems very likely that center P accommodates two quinone molecules and the immediate question arises as to how the headgroups bind within the pocket. Several possible arrangements have been discussed by Ding et al. [30]. The authors prefer an edge-to-edge over a stacked configuration as this arrangement could form a bridge between histidines ligating heme b_L and the Fe_2S_2 cluster allowing very fast electron-transfer rates ($>10^{10}$ s^{-1}), needed for the kinetically controlled mechanism discussed in the same work. In fact, the estimated edge-to-edge distance between the two redox centers of 21 Å fits the iron-to-iron distance of 26 Å tentatively determined from X-ray diffraction analysis [22]. However, this could be coincidental, as according to electron-transfer theory [31] rates $\geq 10^6$ s^{-1} could easily be reached across a distance of about 15 Å, which is the worst case for a stacked arrangement of the two quinone headgroups. On the other hand, it has been reported that the affinity for both quinones bound to center P is affected to the same extent by a num ber of quite different point mutations in cytochrome *b* [30,32]. This strongly favours the idea of a rather confined binding domain, as expected from a stacked arrangement, and is difficult to understand assuming an edge-to-edge configuration.

The fact that Q_{OS} induces the stronger EPR line shape change of the iron-sulfur cluster [28] and the picture arising from inhibitor resistant mutants [27] suggest that the 'prosthetic' ubiquinone is bound closer to this redox center and is likely to form a hydrogen bond to one of its histidine ligands

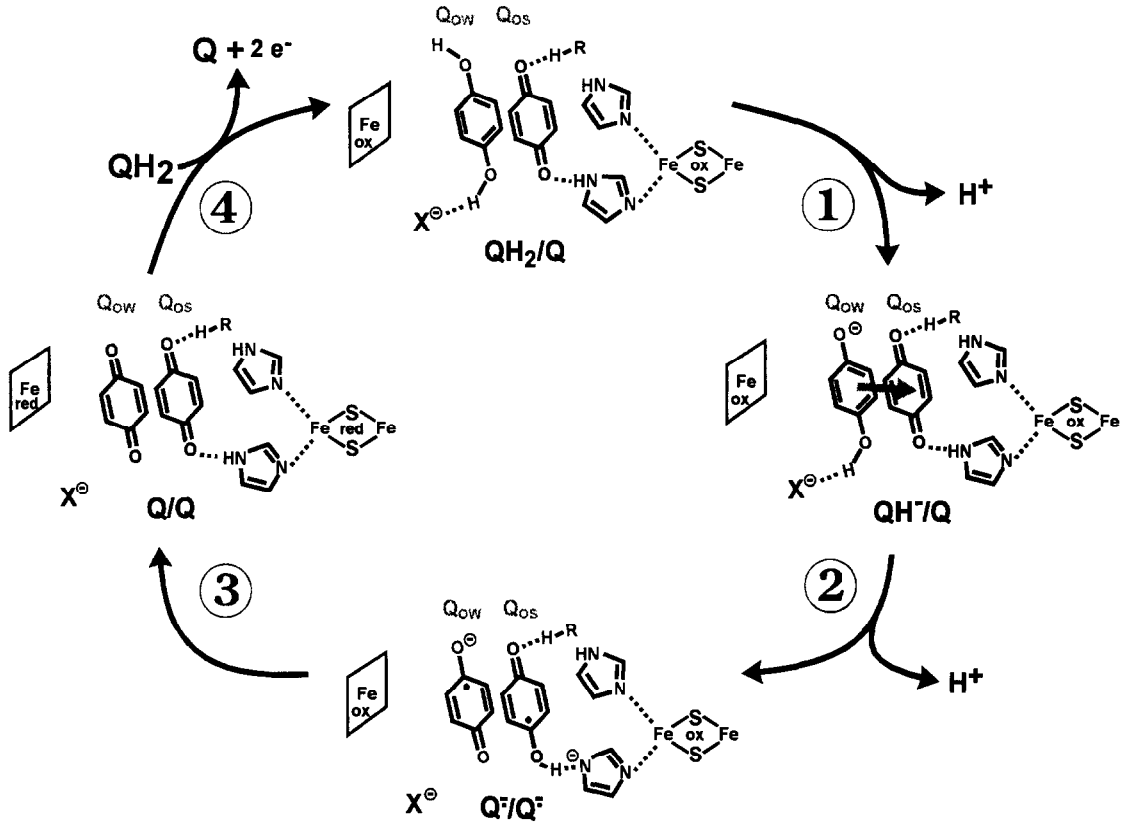

Fig. 3. Proton-gated charge-transfer mechanism of ubihydroquinone oxidation at center P. The four principal steps of ubihydroquinone oxidation are shown. See text for details. Q, ubiquinone; $Q^{\cdot -}$, ubisemiquinone; QH_2, ubihydroquinone; Q_{OS}, 'prosthetic' quinone; Q_{OW}, 'substrate' quinone; X, primary proton acceptor; ox, oxidized; red, reduced.

(see below). Consequently, Q_{OW}, the substrate exchanged during steady state, is expected to bind closer to heme b_L.

4. The activation barrier of ubihydroquinone oxidation

From pre-steady-state measurements a rate constant of 1700 s^{-1} has been determined for the reaction

$$QH_2 + Fe_2S_2^{ox} \rightarrow Q^{\cdot -} + Fe_2S_2^{red} + 2H^+$$

in the bacterial bc_1 complex [33]. As this rate is in the range of the maximal turnover number of the enzyme and all other rate constants are higher by several orders of magnitude, it has been concluded that this step largely controls the overall catalytic rate at saturating substrate concentrations. However, in addition to an electron transfer this step comprises two deprotonation events. For these steps, which cannot be separated experimentally, the following sequence has been proposed [34,35], based on the physical properties of ubiquinone-10 in ethanolic solution (the values given in Fig. 2 were used to calculate standard free energy changes):

$$\begin{aligned} QH_2 &\rightarrow QH^- + H^+ & \Delta G^{0'} &= +25\ kJ/mol \\ QH^- + Fe_2S_2^{ox} &\rightarrow QH^{\cdot} + Fe_2S_2^{red} & \Delta G^{0'} &= -10\ kJ/mol \\ QH^{\cdot} &\rightarrow Q^{\cdot -} + H^+ & \Delta G^{0'} &= -6\ kJ/mol \end{aligned}$$

In fact, this reaction sequence is implicit to all mechanistic models for the oxidation of ubihydroquinone at center P [30,33]. All three reactions are expected to be very fast. Therefore, there is likely to be a thermodynamic limitation of the steady-state rate resulting in a very low steady-state level of an intermediate close to the transition state. From the standard free energy changes it seems straightforward to conclude that it is the first deprotonation step that represents the actual activation barrier. Indeed, the experimental value for the activation energy of around 35 kJ/mol for ubihydroquinone oxidation [33] is quite compatible with this notion. However, the standard free energy changes were calculated based on the physical properties of ubiquinone in 80% ethanol and could be quite different in the bound state. Nevertheless, these values can be used as a starting point and it should be possible to rationalize any deviation by postulating properties of the catalytic site that allow the formulation of a consistent molecular mechanism.

It has been proposed that the activation barrier is linked to the redox event following the first deprotonation, i.e. the formation of a higly unstable semiquinone anion [30,33]. In this case, the deprotonation of the substrate would have to be promoted by the catalytic site to lower or remove the associated thermodynamic barrier. In principle, this could be achieved by two means: (i) a strong base abstracts the proton from QH_2 or (ii) the catalytic site stabilizes the anionic form of the substrate, which is equivalent to lowering the effective pK_a for QH_2. However, the involvement of a strong base can almost certainly be ruled out, as it would have to be regenerated at a later stage of the catalytic cycle. A stabilization of QH^- is also quite unlikely, as this would inevitably lead to a corresponding increase of its midpoint potential. Moreover, the idea of a catalytic site designed to stabilize anions is in-

compatible with the idea of a highly unstable semiquinone anion [30,33]. Finally, center P seems to bind oxidized and reduced ubiquinone with similar affinity [28], which would also be rather unexpected if the reduced form would bind preferentially as QH^-. Thus, it seems necessary to reevaluate the evidence that the formation of a highly unstable semiquinone constitutes the activation barrier. In fact, the only experimental evidence for the formation of a semiquinone at $E_{m7} \approx -300$ to -400 mV at center P reported by De Vries et al. was obtained under conditions where heme b_L was kept fully reduced, i.e. where the semiquinone could not reduce its physiological acceptor [36]. However, as outlined above, this semiquinone is expected to bind in the vicinity of heme b_L, which makes it amenable to electrostatic interaction with this redox center. A consequence of such an interaction would be that the reduced cytochrome significantly lowers the E_{m7} of the semiquinone anion. This means that in the steady-state situation, where heme b_L is oxidized, the semiquinone must have a much more positive midpoint potential than the -350 mV that has been calculated [33] from the stability constants of the species detected in the EPR experiments [36].

Altogether, there is no compelling argument or experimental evidence for the formation of a semiquinone species at center P during steady state with a more negative potential than that observed in solution. On the other hand, when we measured the pH dependence of the activation energy for steady-state turnover of the bovine and yeast cytochrome bc_1 complexes (Brandt and Okun, manuscript in preparation), we found that it decreased linearly from pH 5.5 to 9.0. The slope was 5.7 kJ mol^{-1} pH^{-1} for both organisms, which corresponds nicely to an n=1 deprotonation and can be taken as evidence that in fact a deprotonation event makes up most of the activation barrier.

5. Role of the second quinone

As outlined above, there are good indications that center P accommodates two ubiquinone headgroups with the 'prosthetic' Q_{OS} bound close to or at the iron-sulfur cluster and it appears more likely that they are arranged in a stacked configuration. Such an arrangement calls for a quinhydrone-like charge-transfer complex as an intermediate of ubihydroquinone oxidation at center P. We have formulated a mechanistic concept based on such a quinhydrone-like intermediate [37] and the formation of a quinhydrone at center P has been considered independently by Ding et al. [30]. The most attractive feature of quinhydrone [38] is that it symproportionates into two semiquinone molecules upon deprotonation. This is in line with the idea that ubihydroquinone oxidation is limited by the deprotonation of the substrate and could be used for a preformation of the bifurcated electron flow at center P.

However, the involvement of a symproportionation would require that the second deprotonation takes place prior to the reduction of the redox centers of the bc_1 complex. This deprotonation from QH^- to Q^{2-} is thermodynamically unfavourable (cf. Fig. 2) and it is here where a catalytic mechanism must be formulated.

6. Bifurcation of electron flow by proton-gated charge transfer

The framework of structural, functional and chemical constraints outlined above allows the formulation of a proton-gated charge-transfer mechanism (Fig. 3). This hypothesis proposes a detailed chemistry for the oxidation of ubihydroquinone at center P. The primary difference to other proposed mechanisms [30,33] is that the reaction is controlled by the concentration of QH^- rather than $Q^{\bullet -}$.

A turnover can be divided into four principal steps (see Fig. 3):

6.1. Activation

First one proton has to leave ubihydroquinone (Q_{OW}) and this step forms most of the activation barrier, i.e. QH^- is the intermediate close to or at the transition state. QH^- cannot reduce heme b_L next to it and reduction of the iron-sulfur cluster would have to occur via a transient reduction of Q_{os}.

6.2. Symproportionation

Electron transfer from QH^- onto Q_{os} is equivalent to a symproportionation of the quinone pair, which is catalyzed by the second deprotonation. Correspondingly, the second deprotonation which is even less favourable than the first for the fully reduced ubiquinone (cf. Fig. 2) is facilitated by the symproportionation. This critical step could be promoted significantly by shifting the hydrogen bond between one of the histidine ligands of the iron-sulfur cluster and Q_{OS} towards the quinone. This would stabilize the formed Q_{os}-semiquinone at the expense of a decreasing potential of the cluster. In other words, the effective pK_a for the second deprotonation is lowered by pulling an electron towards the iron-sulfur cluster which could be regarded as acting as a Lewis acid at this point. It also follows that the redox potential of the semiquinone at Q_{OW} is likely to be more negative than that of the semiquinone at Q_{OS}, but that both might be stabilized relative to the free species.

Catalysis of deprotonation and symproportionation could be most easily envisioned by employing properties inherent to a charge-transfer complex, but it should be noted that this is not a crucial point of the model.

6.3. Electron transfer

The double-semiquinone intermediate formed by symproportionation of Q_{OW} and Q_{OS} allows rapid reduction of the two redox centers, thereby completing substrate oxidation. The 'forbidden' reduction of the iron-sulfur cluster by the semiquinone at Q_{OW} is prevented by two mechanisms. (i) Reduced heme b_L is expected to raise the effective pK_a of the ubihydroquinone bound as Q_{OW} by electrostatic interaction. As this means a significant increase of the activation barrier (see step 1), the catalytic cycle will be entered at a much slower rate if heme b_L is reduced. (ii) Direct electron transfer would have to occur via the formation of a Q_{OS} semiquinone and this could be prevented by a significant reorganization barrier.

6.4. Substrate exchange

In the last step ubiquinone is exchanged with ubihydroquinone at the Q_{OW} site and heme b_L and the iron-sulfur cluster are oxidized to complete the cycle.

7. Conclusions

The proton-gated charge-transfer hypothesis provides a detailed mechanism for the chemistry of ubihydroquinone oxi-

dation, which represents the actual energy conserving step within the protonmotive Q-cycle. While each of the proposed steps will have to be scrutinized by careful thermodynamic and kinetic analysis, the fundamental principle that the first deprotonation controls the reaction seems to be inevitable from the experimental evidence available and will have to be included in any mechanistic model.

At this point the model only includes the steady-state mode of the system. Other modes, like the priming reactions occurring if the system is started from a fully reduced quinone pool, are expected to require additional steps, but should be based on the same chemistry.

The model could help one to understand why the steady-state inhibition by center N inhibitors is much less efficient for the *bf* complex than for the bc_1 complex.

References

[1] Brandt, U. and Trumpower, B.L. (1994) CRC Crit. Rev. Biochem. 29, 165–197.
[2] Trumpower, B.L. (1991) J. Bioenerg. Biomembr. 23, 241–255.
[3] Schägger, H., Brandt, U., Gencic, S. and Von Jagow, G. (1995) Methods Enzymol. 260, 82–96.
[4] Gennis, R.B., Barquera, B., Hacker, B., van Doren, S.R., Arnaud, S., Crofts, A.R., Davidson, E., Gray, K.A. and Daldal, F. (1993) J. Bioenerg. Biomembr. 25, 195–210.
[5] Cramer, W.A., Martinez, S.E., Furbacher, P.N., Huang, D. and Smith, J.L. (1994) Curr. Opin. Struct. Biol. 4, 536–544.
[6] Colson, A.-M. (1993) J. Bioenerg. Biomembr. 25, 211–220.
[7] Graham, L.A., Brandt, U., Sargent, J.S. and Trumpower, B.L. (1993) J. Bioenerg. Biomembr. 25, 245–258.
[8] Trumpower, B.L. (1990) Microbiol. Rev. 54, 101–129.
[9] Mitchell, P. (1975) FEBS Lett. 59, 137–139.
[10] Wikström, M.K.F. and Berden, J.A. (1972) Biochim. Biophys. Acta
[11] Mitchell, P. (1961) Nature 191, 144 148.
[12] Trumpower, B.L. (1976) Biochem. Biophys. Res. Commun. 70, 73–80.
[13] Trumpower, B.L. and Edwards, C.A. (1979) J. Biol. Chem. 254, 8697–8706.
[14] Rieske, J.S., MacLennan, D.H. and Coleman, R. (1964) Biochem. Biophys. Res. Commun. 15, 338–344.
[15] Leung, K.H. and Hinkle, P.C. (1975) J. Biol. Chem. 250, 8467–8471.
[16] De Vries, S., Berden, J.A. and Slater, E.C. (1980) FEBS Lett. 122, 143–148.
[17] Von Jagow, G. and Link, T.A. (1986) Methods Enzymol. 126, 253–271.
[18] Trumpower, B.L. (1981) Biochim. Biophys. Acta 639, 129–155.
[19] Semenov, A.Y., Bloch, D.A., Crofts, A.R., Drachev, L.A., Gennis, R.B., Mulkidjanian, A.Y. and Yun, C.-H. (1992) Biochim. Biophys. 1101, 166–167.
[20] Ohnishi, T., Schägger, H., Meinhardt, S.W., LoBrutto, R., Link, T.A. and Von Jagow, G. (1989) J. Biol. Chem. 264, 735–744.
[21] Howell, N. (1993) Mol. Biol. Evol. 10, 488–491.
[22] Xia, D., Yu, C.-A., Deisenhofer, J., Xia, J.-Z. and Yu, L. (1996) Biophys. J. 70, A253.
[23] Yu, C.-A. and Yu, L. (1993) J. Bioenerg. Biomembr. 25, 259–274.
[24] Berry, E.A., Huang, L., Earnest, T.N. and Jap, B.K. (1992) J. Mol. Biol. 224, 1161–1166.
[25] Iwata, S., Saynovits, M., Link, T.A. and Michel, H. (1996) Structure, in press.
[26] Kraiczy, P., Haase, U., Gencic, S., Flindt, S., Anke, T., Brandt, U. and Von Jagow, G. (1996) Eur. J. Biochem. 235, 54–63.
[27] Brandt, U. (1996) Biochim. Biophys. Acta, in press.
[28] Ding, H., Robertson, D.E., Daldal, F. and Dutton, P.L. (1992) Biochemistry 31, 3144–3158.
[29] Brandt, U., Schägger, H. and Von Jagow, G. (1988) Eur. J. Biochem. 173, 499–506.
[30] Ding, H., Moser, C.C., Robertson, D.E., Tokito, M.K., Daldal, F. and Dutton, P.L. (1995) Biochemistry 34, 15979–15996.
[31] Farid, R.S., Moser, C.C. and Dutton, P.L. (1993) Curr. Opin. Struct. Biol. 3, 225–233.
[32] Ding, H., Daldal, F. and Dutton, P.L. (1995) Biochemistry 34, 15997–16003.
[33] Crofts, A.R. and Wang, Z. (1989) Photosynth. Res. 22, 69–87.
[34] Rich, P.R. (1984) Biochim. Biophys. Acta 768, 53–78.
[35] Bendall, D.S. (1982) Biochim. Biophys. Acta 683, 119–151.
[36] de Vries, S., Albracht, S.P.J., Berden, J.A. and Slater, E.C. (1981) J. Biol. Chem. 256, 11996–11998.
[37] Brandt, U. (1994) EBEC Short Rep. 8, 35.
[38] Foster, R. and Foreman, M.I. (1974) in: The Chemistry of the Quinonoid Compounds (Patai, S. ed.) pp. 257–334, Wiley, London.