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Proton processing at the Q_o-site of the bc_1 complex of *Rhodobacter sphaeroides*

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Introduction

In seven different crystals of the bc_1 complex in the native state (reviewed in Berry et al., 2000), the position of the extrinsic domain of the iron sulfur protein (ISP) has been found in eight different configurations, in none of which it would be competent in catalysis. The different positions map a trajectory between binding sites on cyt c_1 and cyt b subunits, and we have suggested that movement between these sites is essential for catalysis (Zhang et al., 1998, Crofts et al. 1999a, b). The domain acts as a tethered substrate, and participates in formation of an ES-complex at both catalytic interfaces. We have discussed at length the nature of the ES-complex formed at the Q_o-site, and have proposed a proton coupled reaction for the first electron transfer, between QH_2 and the oxidized ISP. We have speculated on the nature of the second proton and electron transfer reactions in which heme b_L is reduced, and favored a mechanism involving a single quinone occupant (Crofts et al. 1999a, b). In this paper, we review the nature of these two processes in the light of recent developments.

Characteristics of the Q_o-site reaction. The partial reactions leading to quinol oxidation, as assayed by flash activation of the complex in *Rb. sphaeroides*, include the reactions by which an oxidizing equivalent reaches the Q_o-site, formation of the ES-complex, and two electron transfers from quinol, one each to high and low potential chains, accompanied by release of two protons. Detailed kinetic studies as a function of temperature, redox poise and pH have allowed us to dissect these reactions, and to establish that the rate limiting process is the oxidation of QH_2 by ISP_{ox} (~1.5 10^3 mol.mol-bc_1^{-1} s^{-1}), with an activation barrier of ~65 kJ.mol^{-1} (Hong et al., 1999). This proceeds from an ES-complex involving an interaction between QH_2 and the dissociated ISP_{ox} that is favored by a binding constant, K~14. This value can be detected in kinetic experiments, from the differential binding of quinol when the ISP is oxidized (observed as a displacement of the apparent midpoint of the quinone pool), and in the displacement of the pK of ISP_{ox} (observed from the pH dependence of the reaction when [QH_2] is maintained constant) (Crofts et al., 2000). The activation barrier is affected neither by pH, nor by varying substrate concentration, indicating that neither dissociation of QH_2 nor formation of the ES-complex contributes the high activation energy. Both substrates show normal saturation behavior. The properties of the reaction are not affected by the presence of antimycin at the Q_i-site. The energy landscape for the quinol oxidation is summarized in Fig. 1.

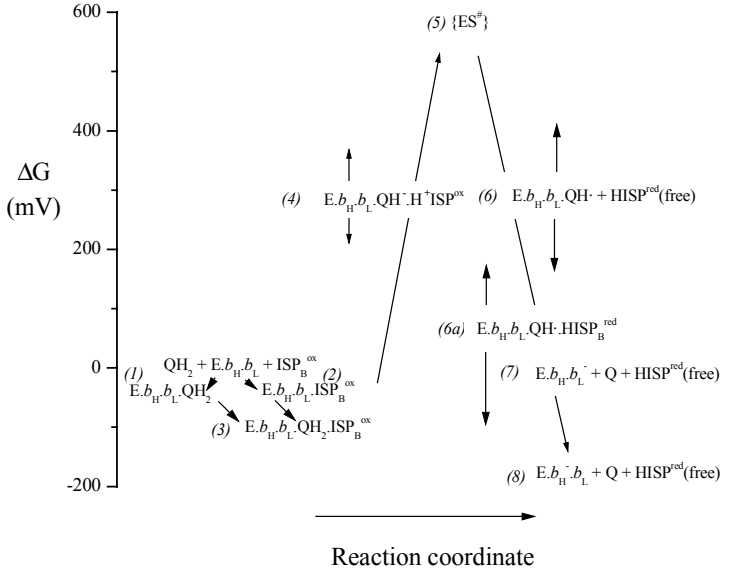

Fig. 1. The energy profile for quinol oxidation. Reactions 1-3, formation of ES-complex; 4, proton transfer; 5, activation barrier for electron transfer; 6, 6a, alternative models for product state; 7, second electron transfer; 8, e-transfer from b_L to b_H. Vertical arrows show range of realistic values.

Nature of the ES-complex. None of the native structures currently available has an occupant at the Q_o-site. In the absence of direct structural information, we may speculate about the nature of the ES-complex on the basis of indirect evidence, and the structural information on inhibitor binding (Crofts et al. 1999a, b). Structures containing inhibitors of both classes acting at the site are available from Berry's work (Berry et al., 2000, Crofts et al., 1999a, b). They reinforce conclusions from a large body of previous work that the inhibitors bind in a common volume, with the different classes occupying different domains. We have suggested that the ES-complex has the same liganding groups as stigmatellin, - His-161 of the ISP and Glu-272 of cyt b. However, whereas stigmatellin H-bonds with the reduced ISP, with His-161 acting as H-bond donor, quinol forms a H-bond with the dissociated His-161 of ISP_{ox}, and contributes as H-bond donor. This configuration accounts for the pH dependence of electron transfer, since only the dissociated ISP_{ox} can act as substrate, and its concentration varies with pH above the pK at ~7.6. The difference between this value and the apparent pK revealed by the pH dependence (~6.4) is attributable to the binding constant involved in formation of the ES-complex, which removes the dissociated form (Hong et al., 1999; Crofts et al., 2000).

The first electron transfer. As a consequence of the above configuration, reduction of ISP_{ox} by quinol involves transfer of both an electron and a H^+. Several lines of evidence suggest that this is the partial process responsible for the high activation barrier. The strongest argument comes form the dependence of the rate on driving force (Hong et al., 1999; Guergova-Kuras et al., 1999). Many mutant strains have been reported in which the E_m values of either ISP or heme b_L are modified (Brasseur et al. 1996; Denke et al., 1998; Schroter et al., 1998), and there are experimental conditions under which the E_m of b_L might be expected to vary. In summary, the data show changes in rate of the overall reaction with changes in E_m of ISP, consistent with electron transfer to ISP controlling the rate. None of the data indicate that the potential of b_L has a controlling effect. Perhaps most compelling is the fact that the rate of QH_2 oxidation is the same whether heme b_H is reduced or oxidized before starting the reaction (Hong et al., 1999). The coulombic effect induces a change in E_m of b_L from -15 mV (seen in mutant strains in which the b_H ligands are changed to prevent binding of heme b_H) to -90 mV seen in standard redox titrations. Also, rates of quinol oxidation are similar in mitochondrial, bacterial and chloroplast complexes, with $E_{m,\,bL}$ values of -40, -90, and -150 mV, respectively, but similar overall driving force. These results suggest that electron transfer to b_L is not rate-determining (Hong et al., 1999).

Paradoxical features of the first electron transfer. If the reaction involves an ES-complex with the characteristics above, the electron transfer occurs through the distance of the H-bond and the histidine ring, - ~6-7 Å (Fig. 2). This short distance should favor a rapid electron transfer. Using the parameters suggested by Moser et al. (1995), an intrinsic rate constant of $\sim 10^{10}$ s^{-1} would be expected. In order to account for the observed rate ($\sim 1.5\ 10^{3}$ s^{-1}), a high value for the reorganization energy, $\lambda > \sim 2.0$ V, must be invoked (Hong et al., 1999). While this value is consistent with the high activation energy, it is anomalously high compared to expected values ($\lambda < \sim 1.25$ V). In order to resolve this paradox, we have suggested, following similar work in model systems (Cukier and Nocera, 1998) and the two-electron gate of reaction centers (Grainge et al., 1999), that the electron transfer is constrained by the proton transfer. We note that the intrinsic rates for H^+-transfer through H-bonds are very rapid (Kresge and Silverman, 1999), and suggest that a simplified approach in which the constraining effect of the proton is represented by the probability of attaining an appropriate configuration might provide an adequate description:

$$\underset{}{} \quad \overset{\Delta G_{proton}}{} \qquad \overset{\Delta G^{\#}_{electron}}{}$$

$$E.b_L.QH_2.ISP_{ox} \leftrightarrows E.b_L.QH^-.H^+ISP_{ox} \leftrightarrows ES^{\#} \rightarrow E.b_L.QH\cdot.ISP_{red}$$

The energy of activation is given by the sum of positive contributions from two improbable processes, but the quantum mechanical constraints of Marcus theory apply only to the electron transfer part. We may adapt the equation of Moser et al. (1995) to include an additional term:

$$\log_{10}k = 13 - 0.6\,(R - 3.6) - 3.1\,(\Delta G^{o} + \lambda)^2 / \lambda - (pK_{donor} - pK_{acceptor}).$$

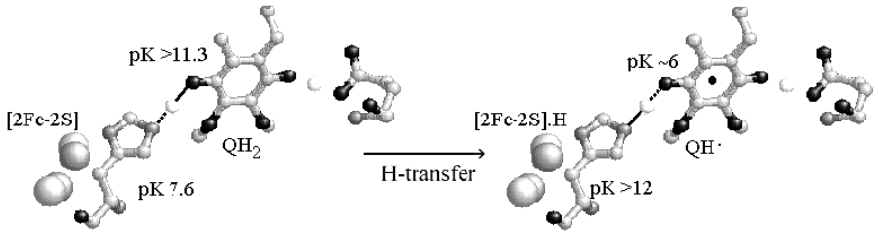

Fig. 2. Suggested configuration of the ES-complex, and the first electron transfer reaction.

Using literature values for the pK of quinol as donor (>11.3) and ISP_{ox} as acceptor (7.6 for the free, or 6.5 for the bound form), the probability of attaining the proton transferred state will modify the activation barrier by ~4 orders of magnitude (~0.24 V), leaving a smaller component to be accounted for by the Marcus term, with reasonable values for λ . Values for λ of 0.75 – 1.25 V, depending on choice of pK for bound or free ISP_{ox}, provide a good fit, and these are in the range expected for intra- or inter-molecular electron transfer (Moser et at., 1995).

A mechanism requiring transfer of the proton through the H-bond is strongly supported by the anhydrous nature of the interface between ISP and cyt b at the docking site (Hunte et al., 2000, and Fig. 3). There are no waters (or space for water) closer than 6.8 Å to the histidine ring, and so no obvious possibility of H-bond exchange, if the ES-complex has a similar configuration.

The second electron transfer. Because the first electron transfer is rate determining, no direct kinetic information on the critical parameters determining the second electron transfer reaction is available. Controversy in this area is dominated by the question of whether one or two quinone species occupy the site during catalysis. This question is not critical for the first electron transfer, but determines all discussion of subsequent processes. We have argued a preference for a single occupancy model at length elsewhere (Crofts et al., 1999a, b). The

double-occupancy model (Ding et al., 1992, 1995) was an ingenious explanation for a line in the ISP EPR spectrum found at g_x=1.783 in chromatophores extracted so as to contain ~1 free quinone/bc_1 complex, that differed from the lines at g_x=1.80 in quinone replete or at g_x=1.77 in fully extracted samples. However, the data showed some features difficult to reconcile with the hypothesis (see Crofts et al., 1999 for references).

1. Differential effects of mutation in conferring resistance to inhibitors have been reported at several positions around the Q_o-site (reviewed by Brasseur et al., 1996), and the failure to find any such differential effect on the phenomena associated with the Q_{os} and Q_{ow} species (strong and weak binding quinones) was a serious discrepancy. The structures showed a fairly clear-cut correlation between the binding sites of myxothiazol and stigmatellin, the resistance conferred by mutation, and the distribution of residues that impinged on their binding domains, bringing this discrepancy into a sharper focus.

2. The second discrepancy brought out by the structures was the finding of a vacant Q_o-site but occupied Q_i-site (now a feature common to all native mitochondrial structures so far reported). There was a clear prediction from the relative binding affinities estimated by Ding et al. (1992, 1995) in chromatophores, that the Q_o-site should contain a strongly bound quinone (Q_{os}), binding there in preference to the Q_i-site, but the opposite was observed. However, the differential affinity might be less in mitochondrial complexes, and not therefore applicable to the structures.

3. Ding et al. (1992, 1995) showed that either myxothiazol or stigmatellin could eliminate both EPR signals, which was interpreted as displacement of both quinones. The structures were quite consistent with this observation, since inhibitors of both classes (exemplified by stigmatellin and myxothiazol), overlapped in a common of volume, which would likely be shared by any other occupant(s). However, this feature of the structures then made it difficult to see how the same constraints would not apply to binding of two quinones (Crofts et al., 1999a, b). Nevertheless, Brandt and colleagues have shown evidence that the Q_o-site inhibitors displace 2 quinones from the bc_1 complex (Bartoschek, S., Brandt, U. et al., personal communication), bringing a renewed interest to this controversy.

Now that structures are available, proponents of double occupancy models are faced with a philosophical dilemma. In order to explore the double-occupancy mechanism, it is necessary to introduce two quinones by modeling, but this leads to substantial displacements of structure, and in our own studies, to a considerable increase in the energy of the system, showing that the resultant configuration is less stable. Unfortunately, this distortion undoes the value of the structures in understanding mechanism, because they are only useful to the extent that they do not require improbable distortion. For the single-occupancy case, the structures provided for the first time sufficient detail to allow some speculation about mechanism, but because they show no native occupant, they contain no direct information about the binding of the quinone substrate(s) or participation in turnover. The liganding of the inhibitors, the changes in structure with different occupants, and the attempt to meld this information into the expanding volume of physico-chemical data, have allowed us to speculate indirectly on the nature of the ES-complex, and possible pathways for further processing of the electrons and protons, as outlined above for the first electron transfer (Crofts et al., 199a, b). Quinones can be modeled at the site in either the stigmatellin- or myxothiazol-binding domains with minimal distortion or energy penalty, and, on the basis of such structures, we have speculated that a single occupant (the semiquinone formed after the first electron transfer) might move between these two positions during catalysis.

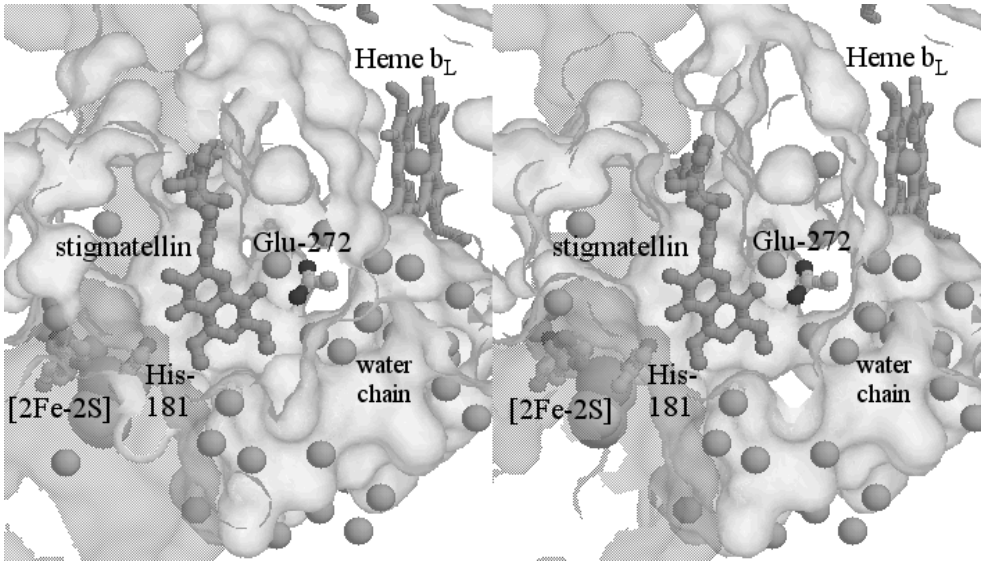

Fig. 3. The waters at the Q_o-site. ISP His-181 (equivalent to His-161 in beef), and Glu-272, are shown liganding stigmatellin (all as ball and stick models). Cyt b (white) and ISP (gray, transparent) are shown by their surfaces. Water molecules are shown by their O-atoms, represented by 1 Å spheres. Note the anhydrous, tightly packed interface between ISP and cyt b around His-181 (left), and the water channel, leading from Glu-272 past the heme b_L propionates, to the exterior (right). Within the pocket, the carboxylate O-atom of Glu-272 that is not involved in H-bond formation is H-bonded by two H_2O. Coordinates are from 1ezv. Stereo image is for crossed-eye viewing.

Although none of our data disprove the double-occupancy model, consideration of the role of Glu-272 requires additional comment. Participation of Glu-272 in the liganding of stigmatellin, and its change in orientation on binding of myxothiazol, suggest a plausible pathway for release of the second proton. In the mechanism we have proposed, release of a proton from QH·, and transfer through the H-bond to the Glu-272 carboxylate, followed by rotation of the acid to the position in the myxothiazol structure, would ferry the proton to an aqueous channel leading to the external phase (Crofts et al., 1999a, b). This speculation is strongly supported by the structure of Hunte et al. (2000) (see Fig. 3), in which crystallographic waters have been identified, which reveals the water chain previously modeled by computational methods (Izrailev et al., 1998). The structures show that the volume occupied by Glu-272 in the stigmatellin structure overlaps the volume that would be occupied either by myxothiazol, or by a second quinone if it sat where myxothiazol binds, so occupancy by a second quinone would not be possible without distortion. However, occupancy of this domain proximal to heme b_L, for instance by movement of the semiquinone, would certainly be facilitated by movement of the side chain to the position in the myxothiazol structure.

Acknowledgments

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