The Q cycle of cytochrome bc complexes: A structure perspective

William A. Cramer a,⁎, S. Saif Hasan a, Eiki Yamashita b

a Hockmeyer Hall of Structural Biology, Department of Biological Sciences, Purdue University, West Lafayette, IN 47907, USA
b Institute for Protein Research, Osaka University, Suita, Osaka 565-0871, Japan

ABSTRACT

Aspects of the crystal structures of the hetero-oligomeric cytochrome bc1 and bhcf (“bc”) complexes relevant to their electron/proton transfer function and the associated redox reactions of the lipophilic quinones are discussed. Differences between the bhcf and bc1 complexes are emphasized. The cytochrome bc1 and bhcf dimeric complexes diverge in structure from a core of subunits that coordinate redox groups consisting of two histidine-coordinated hemes, a heme bh and bh, on the electrochemically negative (n) and positive (p) sides of the complex, the high potential [2Fe–2S] cluster and c-type heme at the p-side aqueous interface and aqueous phase, respectively, and quinone/quinol binding sites on the n- and p-sides of the complex. The bh, and bhcf complexes diverge in subunit composition and structure away from this core. bhcf Also contains additional prosthetic groups including a c-type heme cp on the n-side, and a chlorophyll and β-carotene. Common structure aspects: functions of the symmetric dimer. (1) Quinone exchange with the bilayer. An inter-monomer protein-free cavity of approximately 30 Å along the membrane normal × 25 Å (central inter-monomer distance) × 15 Å (depth in the center), is common to both bc1 and bhcf complexes, providing a niche in which the lipophilic quinine/quinol (Q/ QH2) can be exchanged with the membrane bilayer. (II) Electron transfer. The dimeric structure and the proximity of the two hemes bh on the electrochemically positive side of the complex in the two monomer units allow the possibility of two alternate routes of electron transfer across the complex from heme bh to bh, intra-monomer and inter-monomer involving electron cross-over between the two hemes bh. A structure-based summary of inter-heme distances in seven bc complexes, representing mitochondrial, chromatophore, cyanobacterial, and algal sources, indicates that, based on the distance parameter, the intra-monomer pathway would be favored kinetically. (III) Separation of quinone binding sites. A consequence of the dimer structure and the position of the Q/QH2 binding sites is that the p-side QH2 oxidation and n-side Q reduction sites are each well separated. Therefore, in the event of an overlap in residence time by QH2 or Q molecules at the two oxidation or reduction sites, their spatial separation would result in minimal steric interference between extended Q or QH2 isoprenoid chains. (IV) Trans-membrane QH2/Q transfer. (i) n/p-side Qh2/Q transfer may be hindered by lipid acyl chains; (ii) the shorter less hindered inter-monomer pathway across the complex would not pass through the center of the cavity, as inferred from the n-side antimycin site on one monomer and the p-side stigmatellin site on the other residing on the same surface of the complex. (V) Narrow p-side portal for QH2/Q passage. The [2Fe–2S] cluster that serves as oxidant, and whose histidine ligand serves as a H+ acceptor in the oxidation of QH2, is connected to the inter-monomer cavity by a narrow extended portal, which is also occupied in the bhcf complex by the 20 carbon phytyl chain of the bound chlorophyll.
Properties that are common to both sets of complexes, or unique to either the bc, or bc/f set, can be defined. These comparisons can be used to describe the evolution of these complexes [31–37]. Recent reviews and discussions have mostly focused either on the bc [38–64] or the bc/f complex [37,65–77], with some on both complexes [78–80]. It has often been assumed in discussions of the bc1 complex that the differences in the structure and prosthetic groups of the two classes of complexes are inconsequential, and that the complexes do not differ significantly in pathways of electron and proton transfer. It will be emphasized in the present discussion that, although the bc1 and bc/f complexes share a common evolutionary origin and many common functions, significant differences exist between the two sets of complexes with respect to details of structure, alternate electron transport pathways, and quinone-mediated redox function.

Structure-function problems discussed recently for the bc1 complex include the role of the monomer and dimer in the electron transport pathway associated with oxidation of ubiquinol [56–58,81], the redox state of the quinone species bound to the n-side of the complex [35,53,80], inter-monomer interactions that may affect the pathway of electron transfer [45,57,82], and consideration of a stochastic approach to a description of the electron transfer reactions in the Q cycle [61].

Recent review topics on the bc/f complex have included unique aspects of structure-function: (i) The photosystem I-linked cyclic electron transport pathway [83–99], absent in mitochondria and purple photosynthetic bacteria that house the bc1 complex. An uncertainty over the participation of the bc/f complex in the cyclic pathway of oxygenic photosynthesis is based on disagreement over whether the quinone-analogue inhibitor, antimycin A, which is a classical inhibitor of the oxidation of mitochondrial cytochrome b [100], and which inhibits cyclic phosphorylation [101,102], does [103] or does not [102] inhibit turnover of the chloroplast heme b2; (ii) the additional heme c1 in the bc1 complex, which was first described by sensitive spectrophotometry [104,105], and subsequently in crystal structures [16,17]; (iii) FNR bound peripherally to the plant bc/f complex may participate along with heme b5, in the cyclic electron transport pathway [88,106].

An understanding of intra-membrane translocation of the large lipophilic Q/QH2 (quinone/quinol) molecules, which is coupled to electron and proton transfer, involves consideration of formidable structure problems. Charge transfer, steric, and kinetic problems associated with quinone translocation across the membrane and the bc complex were recognized [107–111] in the literature that preceded the emergence of crystal structures of the bc complexes.

2. Cytochrome bc1 and bc/f complexes: Common and unique properties

Crystal structures of the hetero-oligomeric cytochrome bc complexes, which have a similar energy transducing function in respiration and photosynthesis, are summarized in Fig. 1a–c, the bc1 complex from (A) yeast mitochondria [15] and (B) the purple photosynthetic bacterium, Rhodobacter sphaeroides, and (C) the bc/f complex from the cyanobacterium, Mastigocladus laminosus [19]. These membrane protein complexes provide the electronic connection between the reducing (dehydrogenase, bacterial photosynthetic reaction center, or photosystem II reaction center) and oxidizing (cytochrome oxidase or photosystem I) electron transport complexes in the respective electron transport chains, while coupling electron transfer within the complex to proton translocation across the membrane. Genomic and hydrophobicity [112] analysis of the cytochrome b subunit allowed prediction of bis-histidine ligation of the two trans-membrane hemes in the N-terminal heme binding domain of the cytochrome b polypeptide in the hydrophobic core of the complex [31,113–115], which was inferred to be identical in the bc/f and bc1 complexes [31], and subsequently corroborated by crystal structures. The Rieske [116] iron–sulfur protein (ISP) subunit of the complex can also be considered part of its basic assembly [33,117], as it is found in cytochrome bc complexes in a wide range of phylla [118], and phylogenetic reconstruction has shown significant congruence of ISP and cytochrome b [119] although the ISP amino acid sequences are less conserved than those of cyt b [23].

2.1. Structures, prosthetic groups

Cytochrome bc complexes contain four common redox prosthetic groups (Table 1) in their redox core: (i) two bis-histidine coordinated b hemes, b5 and b6, whose His ligands bridge two trans-
membrane alpha-helices, the 2nd and 4th of the cytochrome b polypeptide on the p- and n-sides of the complex [31,113,114]. The pattern of heme bridging two trans-membrane helices via two His residues is a frequent structure motif, as subsequently found in the crystal structures of intra-membrane electron transport proteins, such as heme b in fumarate reductase [120] or formate dehydrogenase-N [121] and heme a in cytochrome oxidase [122–125], (ii) a high potential (\(E_{\text{m}} \approx 0.25–0.35\) V) c-type heme that is covalently bound in the p-side aqueous phase domain to the cytochrome f and c1 cluster. The iron–sulfur protein, through this cluster, is the electron acceptor and, through a histidine ligand, a proton acceptor of ubiquinol. (iii) \(PQ^+ + 2e^- + 2H^+ \rightarrow PQH_2 + 2H_2O\). (iv) a flavin in the peripheral FNR subunit of the plant (spinach) complex [96,106,137,138].

Other unique structure features of the bc6 complex include: (v) completely different structures of the extrinsic domain of cytochromes c1 and f [26–28], except for the Cys-X-Y-Cys-His covalent heme binding sequence, and (vi) four small single trans-membrane helical subunits in a “picket-fence” arrangement at the periphery of each monomer of the bc6 complex [16–20].

### 2.2. Polypeptides

Of the 8 and 11 polypeptide subunits that have been defined in the crystal structures of bc6f and respiratory bc1 complexes, respectively, 3–4 that contain the functionally essential redox groups can be considered “core” polypeptides. They contain binding sites for the redox prosthetic groups, the 2 b hemes, the [2Fe–2S] cluster, the high potential c-type heme, and the n- and p-side ubiquinone binding sites. These subunits define the hydrophobic core that corresponds to the 3–4 subunit structure of the bc1 complex of the purple photosynthetic bacterium, Rh. sphaeroides [14].

#### 2.2.1. Additional interacting and/or bound polypeptides

A complete perspective on the atomic structure of the bc complexes should include the less strongly or transiently bound subunits that may be dissociated and lost from the complex during its isolation, purification, or crystallization. For the bc6 complex, these include: (i) the FNR that is found in the plant, but not the cyanobacterial or algal bc6f complex [106,137,138]; (ii) the petP polypeptide seen in cyanobacteria [139]; and (iii) the light-harvesting LHClI chlorophyll protein kinase Stt7-STN7 [140], whose presence on the n-side or stromal side of the complex may respond to quinol oxidation on the p-side.
side [141,142], the correlated (iv) phosphatase [143]; and (v) the petO nuclear-encoded phosphorylatable subunit [144].

2.3. The inter-monomer cavity

A prominent common feature in the structure of bc₁ and bc₆f complexes is the large (30 Å x 25 Å x 15 Å) central cavity which, because of its presumed role in sequestering quinone from the membrane bilayer, has been termed the “quinone exchange cavity” [16]. As discussed below, the term “cavity” may be a misnomer because it is likely that it is mostly filled with lipid acyl chains. Central cavities in dimeric or pseudo-dimeric membrane proteins that sequester substrates and water are found in the structures of transport proteins such as the lac permease [145] and the glycerol-3-phosphate transporter [146].

2.4. Lipids

At least eleven lipid molecules have been defined in the 1.9 Å structure of the yeast bc₁ complex [15,50,59,62,147,148], i.e., 5 1/2 lipids per monomer. Each monomer also contains one peripheral CL, two phosphatidyl-ethanolamines, and two phosphatidic acids (Fig. 2a, b). An additional potential lipid site in each bc₁ monomer is suggested by the presence of one bound undecyl-maltoside detergent molecule. One n-side cardiolipin (CL) is shared between the N-terminal segment of the cytochrome b subunit in each monomer, with a proposed function of a proton antenna for H⁺-coupled reduction of the n-side quinone, Qₙ [59,62,149]. Considering this putative function, the distance between the quinone keto group (seen in the 1NTZ structure) and the nearest CL phosphate oxygen is 10.5 Å, a very large distance for proton transfer [150]. However, the distance for proton hopping is reduced by the presence of His202 (cyt b) between the CL phosphate O and the protonatable nitrogen of the cyt b His202.

A similar number, 7–8 lipid binding sites per monomer, is seen in bc₆f complexes from the cyanobacteria, M. laminosus [19] and Nostoc sp. PCC 7120 [20]. One of these sites in the bc₆f complex is occupied by a natural sulfo-lipid, first seen in the structure of the C. reinhardtii complex [17], whose interaction with n-side segments of the ISP and cytochrome f has been described [151]. The other six lipid binding sites in the monomer of the M. laminosus structure are occupied by four molecules of the detergent UDM and two of the lipid DOPC, whose presence greatly increased the rate of crystal formation of delipidated bc₆f complex [152]. Two additional “natural” lipids, MGDG, for a total of three lipids/monomer, have been assigned in the C. reinhardtii bc₆f complex [17]. Regarding application of the “H⁺ antenna hypothesis” [59,62,149] to the bc₆f complex, there are 2 UDM molecules per dimer near the position of the inter-monomer cardiolipin, CL, in the bc₆f complex. These UDM detergent molecules may replace the natural lipid molecules, e.g., anionic PG, in the detergent-extracted protein complex. Of the four UDM molecules in each monomer, the head groups of three are pointing to, or are in contact with, the n-side aequous phase. Arg207 and Lys208 intervene as possible H⁺ carriers in the path between the O (OAC) of the quinone-analogue, decyl-stigmatellin, which can bind to the Qₙ site in bc₆f [19], and UDM as a putative substitute for CL.

2.5. Inter-monomer interactions: Conformational changes

The ability of one equivalent added per dimer of the p-side quinone-analogue inhibitor, stigmatellin, to completely inhibit electron transfer of the dimeric yeast bc₁ complex, led to the inference of inter-monomer interactions relevant to the electron transfer mechanism of the dimer [45]. A similar “half-sites” inhibition effect in the bc₆f complex has been observed in photosynthetic electron transport, using the p-side inhibitor, DBMIB [153], whose structural basis could be similarly interpreted. The structural basis for inter-monomer interactions and resulting conformational changes, which could be at the root of these “half-site” effects and those observed for the bc₁ complex from yeast [45,57,154] and Paracoccus denitrificans [63], may be contained in the number and nature of the residues involved in close contacts between monomers in bc₁ and bc₆f complexes (Table 2). The number of residues contributed to such interactions by core subunits, (i) cytochrome b (8 trans-membrane helices) and the ISP of the bc₁ complex, and (ii) cytochrome b (4 TMH), subunit IV (3 TMH), and the ISP of the bc₆f complex, are similar in the two complexes. The larger number of interacting residues in the yeast (3CX5) and bovine (1NTZ) bc₁ complexes, compared to the two cytochrome bc₆f complexes, is a consequence of a larger number of bc₁ TMH making inter-monomer contacts: (a) the TMH of cytochrome c₁ makes inter-monomer contact, but that of cytochrome f doesn’t; (b) the small subunits in bc₁ make contacts, but the four small subunits in bc₆f, the petG, L, M, N subunits, which are at the outside periphery of each bc₆f monomer, with one TMH each, do not. One TMH in each monomer with unusual properties is that

<table>
<thead>
<tr>
<th>Structure</th>
<th>3CX5</th>
<th>1NTZ</th>
<th>2QP</th>
<th>2ET4</th>
<th>2ZT9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Close contact, aa pairs</td>
<td>131</td>
<td>120</td>
<td>80</td>
<td>65</td>
<td>66</td>
</tr>
<tr>
<td>Cytochrome b</td>
<td>48</td>
<td>44</td>
<td>55</td>
<td>39</td>
<td>43</td>
</tr>
<tr>
<td>bc₆f Subunit IV</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Rieske [2Fe-2S]</td>
<td>23</td>
<td>16</td>
<td>25</td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td>bc₁-Subunit I</td>
<td>6</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>bc₁-Subunit II</td>
<td>26</td>
<td>24</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>bc₁-Cytochrome c₁</td>
<td>9</td>
<td>11</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>bc₁-14 kDa protein</td>
<td>10</td>
<td>14</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Yeast-Asp-subunit VIII</td>
<td>9</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bovine-bc₁-Subunit XI</td>
<td>-</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
of the Rieske ISP, whose active p-side [2Fe–2S] cluster in one monomer is connected to its TMH by a long glycine-rich disordered flexible loop that spans the trans-membrane domain of the other at a pronounced oblique angle (Fig. 1a–c; described in yellow).

Extensive conformational changes of the [2Fe–2S]-containing subdomain of the Rieske ISP are necessary to accomplish kinetically competent electron transfer from the [2Fe–2S] cluster to the heme of cytochrome c$_1$ or f [3]. For both complexes, the [2Fe–2S] donor–heme c acceptor distance, derived from structures described in PDB 3CX5 and 2E74, 22.5 and 26.1 (seen “edge–edge” in Fig. 5a, b) is too large for competent electron transfer. These distances would result in electron transfer times that are at least 1000 times larger than the –millisecond rate-limiting step of the system [155]. Different crystal forms of bc$_1$ complex show conformations with shorter [2Fe–2S]–heme c$_1$ distances (12.8, 3H1H, [5] and 15.5, 1BE3, [4,156]) that would allow kinetically competent electron transfer [5]. The 15.5 distance described in PDB 1BE3, the cytochrome c$_1$–proximal conformation, is one of three crystallographically determined [2Fe–2S]–heme c$_1$ distances between the [2Fe–2S] cluster and the cytochrome c$_1$ heme that have been defined in the bovine complex. The others are 31.6 in the heme bc$_{1f}$–proximal conformation (PDB ID: 3BBC) and 27.5 in an “intermediate” conformational state (PDB ID: 1BCY [4]). These structure data imply protein conformational changes of the ISP that cause the [2Fe–2S] cluster to alternate between positions that are distal and proximal to the heme of cytochrome c$_1$, the latter allowing competent electron transfer.

The rotation-translation of the cluster-containing peripheral subdomain of the Rieske protein in the bc$_1$ complex is enabled by rotation-translation about the flexible linker region that connects the peripheral domain in one monomer with the trans-membrane α–helix in the other. The necessity of flexibility in this loop was demonstrated through site-directed mutations that are predicted to result in structure changes that decrease the mobility of this linker region [43,157–159]. Substitutions of multiple proline or glycine residues in the loop region of the bc$_{1f}$ complex, or insertions that cause loop elongation had no effect on activity [160], although these mutations are similar to those cited above that markedly decreased activity of the bc$_1$ complex. A crystal structure to demonstrate the cyt f heme-proximal state of the [2Fe–2S] cluster in the bc$_{1f}$ complex, which would be necessary for kinetically competent activity, has not yet been determined.

### 2.6. Inhibitor-induced conformational changes

(a) p-Side quinone analogue inhibitors. For the bc$_1$ complex, the inhibitor stigmatellin, which binds in the p-side entry portal close to an imidazole ligand of the ISP [2Fe–2S] cluster, is present in almost all crystal structures because its presence results in decreased mobility [160] and increased order of the ISP soluble domain, although it does not change the orientation of the cluster itself [161]. For cyt bc$_{1f}$, a structure of the native complex without any bound inhibitor has been obtained [16, 19, 20]. Large conformational changes of the bc$_{1f}$ complex induced by stigmatellin have been reported in a study with 2D crystals [162], although such changes were not seen in a comparison of 3D crystals, native vs. bc$_{1f}$ with stigmatellin [19], for which the RMSD for the PDB ID: 2E74 vs. 2E76 (+ tridecyl-stigmatellin) structures from Mastigocladius laminosus is 1.18 Å.

(b) n-Side inhibitors. RMSD changes of significant amplitude associated with the binding of the known specific inhibitors have not been detected: (i) the RMSD of the native (PDB ID: 1NTM) vs. antimycin A-inhibited (1NTK) bovine bc$_1$ complex is 0.47 Å; (ii) for Nostoc bc$_{1f}$ structures, the RMSD of PDB ID: 2E74 vs. 2E75 (+ NQNO) is 0.43 Å [19]; and (iii) the RMSD for the avian bc$_1$ complex, stigmatellin vs. stigmatellin and antimycin is 0.36 Å (PDB ID: 3H1H) vs. 3H1H. However, antimycin causes a 100–150 mV change in the $E_{m7}$ of a mitochondrial b heme [163], presumably heme bc$_{1}$, and a perturbation of the p-side EPR signal associated with the [2Fe–2S] cluster in the bc$_1$ complex of the photosynthetic bacterium, Rhodobacter capsulatus [64,164,165].

### 3. The Q cycle

The coupling of the oxidation–reduction and deprotonation–protonation of lipophilic quinone/ol (Q/QH$_2$) within the cytochrome bc complex is central to the mechanism of proton translocation in the complex. The proton/electron carriers, ubiquinone (UQ-10; [166,167]) in the respiratory bc$_1$ complex and plastoquinone (PQ-9) in the bc$_{1f}$ complex, contain 10 and 9 isoprenoid groups, respectively. In the extended state, steric problems are anticipated in translocation of these quinones in a rigid extended state across the cytochrome bc or complex, or their reversible insertion into oxidative or reductive niches within the complex. Possible conformational transitions to folded states have been described [168–170]. Based on the observation of oxidant-induced reduction of b-type heme in the respiratory bc$_1$ complex [171], it was proposed that these quinones can cross the cytochrome bc complex and the membrane, as described in the “Q cycle” models proposed by Mitchell [172–174], and in subsequent discussions of this model [46,80,109,111,175,176]. Descriptions of the Q-cycle that illustrate differences between bc$_1$ and bc$_{1f}$ complexes are shown (Fig. 3a, b).

Experimental data that were fundamental to the formulation of the Q cycle models are: (a) oxidant-induced reduction of cytochrome b of the mitochondrial respiratory complex [171,178,179]; (b) a
proton:electron ratio, $H^+/e^- = 2$, for uncoupler-sensitive electronic proton translocation to the p-side aqueous phase by the $b_6$ $b_{10}$ $f_{10}$ $f_{12}$ complexes in the presence of a relatively small $\Delta \psi_{bb}$ $[183,184]$ (for the $b_{10}f_{10}$ complex, there has been debate as to whether the extra $H^+$ translocation, which is electronic, is inhibited in the presence of a large $\Delta \psi_{bb}$ $[185]$, and whether it is $[105,186]$ or isn’t $[187,189]$ specifically associated with the reduction of the hemes b). (c) The model is also strongly supported by the presence of specific Q binding sites of potent quinone analogue inhibitors, e.g., antimycin A and stigmatellin, on both n- and p-sides of the complexes, whose precise locations have been confirmed by: (i) crystal structures; ubiquinone (PDB ID: 3H1H) or antimycin (PDB ID: 3H1I) binding sites have been determined on the n-side of the complex adjacent to heme $b_n$, and the stigmatellin binding site on the p-side (PDB ID: 3H1I) within H-bond distance of the histidine ligand (His181 in yeast) to one of the Fe atoms in the $[2Fe-2S]$ cluster. In the $b_{10}f_{10}$ complex, analogous n- and p-side binding sites of NQNO and tridecyl-stigmatellin have been identified $[19]$; (ii) EPR detection of a ubiquinone free radical intermediate, in the absence, but not in the presence of antimycin $[190]$, and an analogous oxygen-sensitive p-side signal $[191]$. 

Independent data supporting the Q cycle model for redox and $H^+$ transfer reactions in the $b_{10}f_{10}$ complex are less complete because: (i) there is no high affinity n-side inhibitor comparable to antimycin A for the $b_1$ complex, which is partly a consequence of partial occupancy in the $b_{10}f_{10}$ complex of the $b_{1}$-like $Q_p$ site by heme c $[19]$; (ii) in contrast to the $b_1$ complex, the alpha-band absorbance spectra of the two trans-membrane hemes, $b_n$ and $b_{n'}$, cannot readily be distinguished (e.g., Ref. $[105]$; although see Ref. $[192]$). Together with the fact that any $\Delta \Delta \psi_{b}$ between the two hemes is much smaller in the $b_{10}f_{10}$ complex compared to $b_1$, and not resolved in most in situ titrations, a determination of the sequence of reduction of the two hemes in $b_{10}f_{10}$, as accomplished for $b_1$ in chromatophores of the photosynthetic bacteria $[193,194]$, is precluded. (iii) From studies on the $b_1$ complex in Rh. sphaeroides, it is inferred that transfer of the first electron in the two electron quinol oxidation to the ISP, in a proton-coupled electron transfer $[195]$, is the rate-limiting step of the overall Q cycle $[48,196]$ (Fig. 3a, b).

Electron and proton transfer reactions of the $b_{10}$ and $b_{10}f_{10}$ complexes in the context of a Q cycle are summarized (Table 3A, 2). The presence of the unique heme c$_n$ whose covariant attachment to the cytochrome b polypeptide can be detected in SDS-PAGE analysis of the $b_{10}f_{10}$ complex $[197]$, and which is electronically coupled to heme $b_{10}$ $[127,128]$, makes the detailed nature of a “Q cycle” different in the $b_{10}f_{10}$ complex compared to $b_1$: (i) Crystal structures and spectrophotometric analysis showing quinone analogue inhibitors NQNO $[19,128,130]$ and tridecyl-stigmatellin $[19]$ as ligands to heme c$_n$ imply that heme c$_n$ is the n-side PQ binding site. The electronically coupled hemes $b_{n'}c_n$ could provide a 2 electron pathway for reduction of PQ$_n$. The presence of NQNO and stigmatellin as ligands to heme c$_n$ as defined in crystal structures (2E75, 2E76; $[196]$), implies a role in the n-side electron transfer reactions (Table 3B, n-side reactions, ii–iv). The isolation of a plant (spinach) $b_{10}f_{10}$ complex from the green alga C. reinhardtii, containing bound ferredoxin-NADP$^+$ reductase (FRN) $[106]$, and of a supercomplex containing the PSI reaction center and $b_{10}f_{10}$ complexes together with FRN $[96]$, implies that PSI-linked cyclic electron transport provides an alternative source of electrons into the $b_{10}f_{10}$ complex. An FRN-dependent reductive pathway to PQ$_n$, resembles an original formulation of the Q cycle for the $b_1$ complex, in which the one of the two electrons needed for reduction of UQ$_n$, is supplied by a n-side dehydrogenase $[173]$. 

The cyclic pathway may be augmented by an NADH dehydrogenase, implied by studies on chloroplast mutants in Arabidopsis thaliana $[97]$. Regardless of the source of electrons from the cyclic pathway, it is proposed that the Q cycle in the $b_{10}f_{10}$ complex could be completed on the n-side by one electron supplied by the cyclic pathway (Table 3, B3, v), which would complement the electron derived from the p-side reduction of plastoquinol. The possibility can also be considered that two electrons stored on hemes $b_{n'}c_n$ cooperatively reduce PQ (PQ$_n$) bound at the site proximal to heme c$_n$ (Table 3, B3, vi) to PQ$_{2n}$. A consequence of the input of one electron from cyclic electron transport to reduce PQ$_n$ is that only one p-side oxidative turnover of PQ$_{2n}$ would be required to form Q$_n$. 

Another (“activated”) Q cycle model $[53]$ (Table 3, B4) proposes that the quinone species bound at the Q$_n$ site is a semiquinone, which can form a complex and transfer an electron to the higher potential heme $b_{n'}$ (b-150; $[198,199]$) on the other monomer. This most recent and interesting formulation of a modified Q cycle results from consideration of a substantial number of experiments, mainly concerning the flash-induced amplitude and kinetics of the trans-complex electric field and heme b reduction that do not fit the models described in Table 3A, 1B1, 2. The “activated” mechanism employs the dimeric $bc$ complex, in which prompt oxidation of the quinol on the n-side of one monomer reduces heme $b_{n'}$ on the other. The mechanism then requires only one oxidation (turnover) of $Q_nH_2$ to provide the single electron needed to form the quinol $Q_nH_2$ and once primed, minimizes exchange of $QH_2$ and PQ$H_2$ with the membrane $QH_2$ pool. This mechanism was originally proposed for the $b_1$ complex $[53]$ and subsequently for the $b_{10}f_{10}$ complex $[80]$. Two problems with application to the $b_{10}f_{10}$ complex are: (i) the $E_m$ of heme $b_{10}$ in $b_{10}f_{10}$ (Table 1) is approximately 100 mV more negative than that in $b_1$ (Table 1), implying a less favorable equilibration between bound $Q_n$, semiquinone and heme $b_{n'}$ than would occur in the $b_1$ complex, assuming that the $Q_n$ semiquinone has the same $E_m$ in both
4. Pathways for quinone transfer: Consequences of dimer symmetry

Given the above data and logic that are consistent with, and support the Q cycle models shown in Fig. 3a, b with the electron and proton transfer reactions described in Table 3, it is noted that there are no data available on the pathway or trajectories of the lipophilic quinone/ol (Q/QH2) within the bc complex connecting its n- and p-sides. Then, it is important to consider in the context of the atomic structures of the bc complexes (Fig. 1a–c), the possible trans-membrane pathways used by the long chain lipophilic quinones/quinols to transfer electrons and protons within the bc complexes.

4.1. Quinone binding in the dimer: Consequences of symmetry

Although intra-complex transfer of Q, QH2, and semiquinone has been proposed, and is implied in many models of the Q cycle [46,80,109,111,175,176], the presence of lipid acyl chains within the inter-monomer cavity is indicated by at least 11 bound lipids resolved in the yeast complex (PDB: 3CX5), and an Fo–Fc map of the inter-monomer cavity indicating additional lipid acyl chains (Fig. 4a). It is likely that the cavity is occupied by an even higher density of lipid chains than shown, but that most of this lipid is weakly bound and lost during purification and crystallization. The presence of this lipid implies that intra-complex transfer of Q/QH2 through the inter-monomer cavity might be impeded by the lipid chains, although such obstruction would be lessened by the disorder and probable mobility of these chains. Furthermore, a consequence of the C2 symmetry is that the two monomers are arranged so that: (i) the [2Fe–2S] quinol oxidation site and the quinone reduction site (b6 in the bc1 complex and b6c–c6 in bc6) in the two monomers are on opposite faces of the complex (Fig. 4b); (ii) heme b6 in either monomer of the bc1 complex, or hemes b6c–c6 in the bc6 complex, are on the same side of the complex as the 2Fe–2S cluster in the other monomer. Thus, the n-side binding site of antimycin on one monomer is on the same side of the dimeric complex as the p-side binding site of stigmatellin on the other (Fig. 4b), implying that if transfer of Q/QH2 occurs across the bc complex, the transfer trajectory will be on one side of the complex [3,41]. (It is noted that the yeast 3CX5 structure does not have a true C2 symmetry because cytochrome c is bound to one monomer and the partly disordered cardiolipin shared on the n-side between the two monomers is asymmetrically located.)

A side-view (Fig. 4c) of the yeast bc1 complex (PDB ID: 1KB9) shows an apparent cross-over of the ubiquinone isoprenoid tail (UQ-6) bound at the Q6 site in one monomer across the inter-monomer cavity, to the Q6 site portal in the other monomer, located by presence of quinone analogue stigmatellin (Stg). The Stg and UQ-6 pair colored magenta is positioned on one face of the bc1 dimer, while that colored green lies on the other.

**Fig. 4.** (a) Presence of lipid-like molecules in the inter-monomer cavity of yeast cytochrome bc1 complex (PDB ID: 3CX5). The outlined density may correspond to an acyl chain of a lipid or detergent molecule or it may be attributed to the isoprenoid tail of a ubiquinone molecule as found in the Qn site of the yeast bc1 complex (PDB ID: 1KB9). Figure generated in PyMol from PDB 3CX5 and its Fo–Fc map contoured at 3.0 sigma. Negative densities were not included in the analysis. (b) n- and p-side binding sites of quinone analogue inhibitors, antimycin A and stigmatellin (PDB ID: 1PP [216] or 1NTZ [10]), which are on the same side (yellow or blue) of the dimeric complex, implying that if a trans-complex quinone pathway operates for electron and proton transfer, it would be inter-monomer. (c) Yeast bc1 complex (PDB ID: 1KB9) showing (side-view) cross-over of ubiquinone isoprenoid tail (UQ-6, bound at Q6 site) from one monomer across the inter-monomer cavity, to the Q6 site portal in the other monomer, located by presence of quinone analogue stigmatellin (Stg). The Stg and UQ-6 pair colored magenta is positioned on one face of the bc1 dimer, while that colored green lies on the other.
5. Role of the dimer in electron transfer

A dimeric or multimeric structure is a common structure motif in integral membrane proteins, prominent among which are the photo-synthetic reaction centers [200–204]. The electron and proton transfer reactions described in Table 3 do not describe any special function of the dimer. They do not distinguish whether electron transfer across the complex from heme $b_0$ to $b_6$ is (a) intra-monomer [58,61] or (b) inter-monomer with cross-over between the two hemes $b_0$. The latter possibility was suggested after the appearance of the crystal structures of the $b_{c1}$ complex that defined the inter-heme distances [78] and subsequently discussed extensively [56,57,63,82,154,205–207]. A “cross-over model” suggests that a function of the dimer could be to allow a “bypass valve” for a second pathway of trans-membrane electron transfer if the pathway for trans-membrane electron transfer in one monomer is impeded by reduction of the quinone in the Fe binding site in that monomer, or by electron equilibration in that monomer. There are different quantitative descriptions of the electronic connection between the electron donor and acceptor, e.g., whether the distance separation between electron donor and acceptor should be measured “center to center” [208,209] or “edge to edge” [155,210,211]; the electron donor-acceptor distance, in addition to the free energy change, $\Delta G$, and reorganization energy associated with the transfer [212–215] are major determinants of the branching ratio for intra-monomer vs. inter-monomer electron transfer [213]. The crystal structures (Fig. 1a–c) provide donor-acceptor distance to an accuracy of approximately $\pm 0.3–0.5 \text{Å}$ (Table 4A, B) and the identity of the amino acids that bridge the potential electron transfer pathways (Fig. 5a–d). Heme–edge–edge, ring–ring (bypassing side chains), and center–center (Fe:Fe) distances for seven $b_{c1}$ and $b_{c2}$ structures are summarized in Table 4A and B. The seven structures include three $b_{c1}$ respiratory complexes: (i) yeast with bound stigmatellin and cytochrome $c$ bound to one subunit ([15];3CX5), (ii) bovine mitochondria with p-side bound stigmatellin and antimycin A ([216];2A06), (iii) bovine complex with n-side bound ubiquinone-2 (1NTZ), (iv) 3 subunit $b_{c1}$ complex from the photosynthetic bacterium, Rh. sphaeroides, with p-side stigmatellin and n-side antimycin; $b_{c2}$ complexes (2ZT9, 2E74), native structures from two different cytochroma, M. laminosus and Nostoc PCC 7120 (2E74, 2ZT9); and (vii) $b_{c2}$ complex from the green alga, C. reinhardtii (PDB ID: 1Q90) with the p-side bound inhibitor stigmatellin (1Q90).

<table>
<thead>
<tr>
<th>PDB code</th>
<th>3CX5</th>
<th>2A06</th>
<th>1NTZ</th>
<th>2QJP</th>
<th>2E74</th>
<th>2ZT9</th>
<th>1Q90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resolution (Å)</td>
<td>1.9</td>
<td>2.1</td>
<td>2.60</td>
<td>2.60</td>
<td>3.0</td>
<td>3.0</td>
<td>3.1</td>
</tr>
<tr>
<td>R factors</td>
<td>0.245</td>
<td>0.222</td>
<td>0.247</td>
<td>0.244</td>
<td>0.222</td>
<td>0.230</td>
<td>0.222</td>
</tr>
<tr>
<td>Coordinate error (Å)</td>
<td>0.263</td>
<td>0.258</td>
<td>0.283</td>
<td>0.277</td>
<td>0.268</td>
<td>0.259</td>
<td>0.261</td>
</tr>
<tr>
<td>A. Inter-monomer distances (Å)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heme $b_0$–heme $b_6$</td>
<td>10.0</td>
<td>10.5</td>
<td>10.9</td>
<td>10.6</td>
<td>12.9</td>
<td>12.7</td>
<td>12.7</td>
</tr>
<tr>
<td>(edge–edge)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heme $b_0$–heme $b_6$</td>
<td>13.7</td>
<td>14.3</td>
<td>13.8</td>
<td>13.4</td>
<td>15.2</td>
<td>15.1</td>
<td>15.1</td>
</tr>
<tr>
<td>(ring–ring)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heme $b_0$–heme $b_6$ (center–center)</td>
<td>21.2</td>
<td>21.4</td>
<td>20.7</td>
<td>20.7</td>
<td>20.7</td>
<td>22.1</td>
<td>22.0</td>
</tr>
<tr>
<td>B. Intra-monomer distances (Å)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heme $b_0$–heme $b_6$</td>
<td>7.2</td>
<td>7.0</td>
<td>8.6</td>
<td>7.9</td>
<td>7.4</td>
<td>8.2</td>
<td>8.9</td>
</tr>
<tr>
<td>(edge–edge)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heme $b_0$–heme $b_6$</td>
<td>12.2</td>
<td>12.0</td>
<td>12.3</td>
<td>11.9</td>
<td>12.2</td>
<td>12.0</td>
<td>12.2</td>
</tr>
<tr>
<td>(ring–ring)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heme $b_0$–heme $b_6$ (center–center)</td>
<td>20.7</td>
<td>20.4</td>
<td>20.6</td>
<td>20.4</td>
<td>20.6</td>
<td>20.7</td>
<td>20.8</td>
</tr>
</tbody>
</table>

Split Soret band circular dichroism spectra of the mitochondrial $b_{c1}$ [217–219] and $b_{c2}$ complex from C. reinhardtii $b_{c2}$ [220], cyanobacteria and spinach chloroplasts [221], are diagnostic of heme–heme excitonic interactions that arise from the small inter-heme distances required for such interactions. For inter-monomer electron transfer, the only pathway considered is that between the two $b_0$ hemes because of the large distance (–30 Å) in all cytochrome $b_{c2}$ structures between the two hemes $b_0$ and between heme $b_0$ and $b_6$ in different monomers. The pathway between the two $b_0$ hemes, bridged by two Tyr residues and two Phe residues, respectively, in the $b_{c1}$ and $b_{c2}$ complexes, approximately 10 Å in the yeast $b_{c1}$ complex and 13 Å in the M. laminosus $b_{c2}$ complex is shown (Fig. 6c, d). For inter-monomer electron transfer, i.e., “cross-over,” differences in edge–edge and ring–ring distances, distance for closest contact between the two hemes $b_0$, relative to that between $b_0$ and $b_6$, are (10.0–7.2) = 2.8 Å and (13.7–12.2) = 1.5 Å, respectively, for the yeast respiratory $b_{c1}$ complex, which has the best resolution in the set (Fig. 1a, 6a [15]; Table 4A, B). This comparison implies that the intramonomer $b_{0}$–$b_6$ pathway would be favored. The $b_{0}$–$b_6$ edge–edge and ring–ring inter-monomer distances are also greater than the intra-monomer $b_0$–$b_6$ distances for all six of the other representative $b_{c1}$ and $b_{c2}$ complexes considered in Table 4A and B. The difference in the inter- vs. intra-monomer distances (determined edge–edge, ring–ring) is (2.7 Å, 1.5 Å) and (2.3 Å, 1.5 Å) for the $b_{c1}$ complexes described in 2QJP and 1NTZ, and (4.5 Å, 3.1 Å), (5.5 Å, 3.0 Å), and (3.8 Å, 2.9 Å) for the $b_{c2}$ complexes described in 2ZT9, 2E74, and 1Q90. Thus, for all seven cytochrome $b_{c2}$ complexes, the intra-monomer distances, edge–edge and ring–ring, are clearly smaller than the inter-monomer distances. The differences for center–center distances show the same tendency, but are smaller and in some cases do not exceed experimental uncertainty. It is of interest that these inter-heme distances are the same for $bc$ complexes from different sources and are not changed by the presence of n- or p-side quinol analogue inhibitors.

Considering only the distance dependence of the electron transfer rate, the difference in edge–edge and ring–ring distances would predict [155,208–211,222–227] a branching ratio for electron transfer from heme $b_0$ that would significantly favor the intra-monomer pathway. This logic is similar to that used previously to predict a branching ratio that favors the intra-monomer pathway by two orders of magnitude [215]. The latter reference provides an extensive discussion of the intra/inter-monomer electron transfer problem, including the effect of the trans-membrane electric field generated by intramonomer electron transfer that would inhibit the transfer. Recent studies using $b_6$ heme knockout mutants and splicing of the cytochrome $b$ gene have demonstrated that the inter-monomer cross-over branching ratio is >1 [81], and has been estimated to be 2–10 to 1 [228]. Special functions associated with the inter-monomer cross-over pathway, discussed elsewhere for the $b_{c1}$ complex, have been mentioned above. For the $b_{c2}$ complex, a selective pathway for electron transfer is implied from the observation that FNR, in the presence of the “artificial” electron donor NADPH, reduces no more than half of the $b$ heme in the $b_{c2}$ complex [19,229,230]. This half could be the heme $b_0$ in the two monomers [229] or hemes $b_0$ and $b_6$ in one. Selection of the one monomer may result from interaction via n-side docking of an electron donating protein such as FNR [106] (Fig. 3b). In this case, because the chemical reduction is so slow (> seconds) and the half-reduction is an equilibrium level, an explanation solely in terms of differential kinetics of reduction based on differences in inter-heme distances is not adequate.

6. The problem of the p-side portal

Passage of the lipophilic quinol to its oxidation and deprotonation site at the [2Fe–2S] cluster requires that after its entry into the inter-monomer cavity, or transfer from its n-side reduction site, it must pass through a narrow 15 Å long portal that is 10–12 Å and 13–14 Å wide at the cavity-side entrance in $b_{c1}$ and $b_{c2}$ complexes. The nature of this portal for stigmatellin has been described for the yeast $b_{c1}$ complex [7].
The portal is shown with an inserted p-side quinone analogue inhibitor, tri-decyl stigmatellin or stigmatellin (in green) in Fig. 6a, b for the cytochrome $b_6$ and $b_{1}$ complexes, respectively. Such portals are also present in the Qb quinone binding site of the bacterial [231,232] and photosystem II photosynthetic reaction centers [204,233]. The overlap of tridecyl-stigmatellin and the phytyl chain of chlorophyll $a$ also passing through the portal in the $b_6 f$ complex (M. laminosus; PDB ID: 2E76) is shown (Fig. 6c). An expanded view of the Q/QH$_2$ entry/exit portal showing residues within 4 Å of stigmatellin is shown in (Fig. 6d) for the yeast $b_{1}$ complex. Stigmatellin, possessing a chromone ring that forms an H-bond with the His181 ($b_{1}$; PDB ID: 3CX5) or His129 ($b_6 f$; PDB ID: 2E76) ligand of the Rieske $[2Fe–2S]$ cluster, and inhibits electron transfer from the cluster to the heme of cytochrome $c_1$ or f, was defined as a “class Ib” inhibitor [12]. UHDBT is another inhibitor in this class. Myxothiazol and MOAS, which contain a $\beta$-methoxyacrylate ring, were classified as “1a” inhibitors [12]; the binding of myxothiazol in the p-side portal is shown (Fig. 6e).

A better understanding of the insertion and passage of the lipophilic Q/QH$_2$ through the portal could be gained through molecular dynamics analysis, as studied in the passage of ubiquinone through a defect in the ring of light-harvesting (LHI) bacteriochlorophyll molecules surrounding the photosynthetic reaction center [234], and the insertion of a drug molecule into a virus capsid protein [235], which has a formal resemblance to quinol insertion into the p-side entry portal to the [2Fe–2S] cluster. The combination of kinetic and steric constraints of portal entry-extrusion of quinol/quinone in the most frequent description of the Q cycle, described symbolically in Fig. 3a, creates a unique sequence of intra-membrane transfer events that occur twice in the millisecond turnover time of the $b_c$ and $b_{1}f$ complexes: (i) QH$_2$ with its isoprenoid chain of 45–50 carbons must find the narrow portal entry; (ii) traverse its narrow aperture; (iii) transfer 2 electrons and 2 protons; (iv) Q is extruded from the portal after oxidation and deprotonation. As the problem of entry into and from the portal is dynamic, different folded conformations [168,169,234] of the quinone may be relevant to the Q/QH$_2$ passage through the portal. As an indicator of the conformational flexibility of the portal, the average $B$ factors (2) of (i) portal residues and (ii) residues in neighboring trans-membrane helices are 27.2 and 25.8 for the 1.9 yeast $b_{1}$ complex with stigmatellin bound in the portal (3CX5), implying that the portal is relatively ordered in the presence of stigmatellin, an inference previously made for the yeast 2.3 structure (PDB ID: 1EZV), with $B$ factors (measures of disordered regions in the structure) of 37.0 and 35.2$^2$, respectively, for the bound stigmatellin and neighboring portal residues [7]. In contrast, for native $b_6 f$ complex solved in the absence of any quinone analogue inhibitor (2E74), the $B$ factor (60.6$^2$) for the residues lining the p-side portal is substantially larger than that, 44.7$^2$, of residues in the neighboring trans-membrane helices, indicative of greater flexibility or disorder in the structure of the portal, which would facilitate passage of quinol/quinone or the analogue inhibitor. The greater order upon insertion of the quinone analogue inhibitor suggests that entry of the quinone or analogue requires interaction with the walls of the portal, which is reflected in the decreased $B$ values.

In addition to the binding sites of stigmatellin [17,19] and NQNO [19] defined in crystal structures of the $b_{1}f$ complex, the high affinity binding site of the p-side quinone analogue inhibitor DBMIB [236] was found near the p-side aqueous interface, 19 from its site of inhibition.
at the [2Fe–2S] cluster [18]. However, EPR analysis showed one high affinity site for DBMIB to be proximal to the [2Fe–2S] cluster [237,238], and a second low affinity site further away from the cluster. DBMIB inhibition activated by light flashes implies that there is light-activated movement of DBMIB from the distal peripheral site to the inhibitory site proximal to the [2Fe–2S] cluster [239,240]. Thus, from its high affinity binding site determined in the crystal structure, DBMIB traverses a long and labyrinthine pathway to the [2Fe–2S] cluster where it exerts its inhibitory effect.

Acknowledgments

We thank H. Huang and C.B. Post for discussions on molecular dynamics of the quinone isoprenoid chain, and D. Baniulis, D. Beratan, F. Daldal, J. Hempel, G. Kurisu, F. Rappaport, S. Savikhin, S.D. Zakharov, and H. Zhang for helpful discussions. Studies of the authors related to this article were supported by U.S. NIH GM-38323. Diffraction measurements associated with crystal structures of the bc$_f$ complex were carried out with advice from S. Ginell, J. Lanarz, and F. Rotella at Beam-Line19-ID of the Structural Biology Center, Advanced Photon Source, Argonne National Laboratory, operated by the University of Chicago (contract DE-AC02-06CH11357, U.S. Department of Energy, Office of Biological and Environmental Research).

References


R. Covian, B.L. Trumpower, Regulatory interactions between ubiquinol oxidation and ubiquinone reduction sites in the dimeric cytochrome bc1 complex, J. Biol. Chem. 280 (2005) 20905–20912.


P.R. Rich, A.R. Crofts, R.B. Gennis, Assignment of the histidine axial ligands to the cytochrome bc1 and cytochrome bc2 components of the bc1 complex from Rhodobacter sphaeroides by site-directed mutagenesis, Biochemistry 30 (1991) 5747–5754.


C.H. Yen, R. Crofts, R.B. Gennis, Assignment of the histidine axial ligands to the cytochrome bc1 and cytochrome bc2 components of the bc1 complex from Rhodobacter sphaeroides by site-directed mutagenesis, Biochemistry 30 (1991) 5747–5754.


