Electron transfer between yeast cytochrome \( bc_1 \) complex and cytochrome \( c \): a structural analysis

Carola Hunte\textsuperscript{a,*}, Sozanne Solmaz\textsuperscript{a}, Christian Lange\textsuperscript{b}

\textsuperscript{a}Max-Planck-Institute of Biophysics, Dept. Molecular Membrane Biology, Heinrich-Hoffmann-Str. 7, 60528 Frankfurt/M., Germany
\textsuperscript{b}Current address: Leiden Institute of Chemistry, Gorlaeus Laboratories, Leiden University, P.O. Box 9502, 2300 RA Leiden, The Netherlands

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Abstract

The structure of the complex between cytochrome \( c \) (CYC) and the cytochrome \( bc_1 \) complex (QCR) from yeast crystallized with an antibody fragment has been recently determined at 2.97 Å resolution [Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 2800]. CYC binds to subunit cytochrome \( c_1 \) of the enzyme stabilized by hydrophobic interactions surrounding the heme crevices creating a small, compact contact site. A central cation-\( \pi \) interaction is an important and conserved feature of CYC binding. Peripheral patches with highly conserved complementary charges further stabilize the enzyme–substrate complex by long-range electrostatic forces and may affect the orientation of the substrate. Size and characteristics of the contact site are optimal for a transient electron transfer complex. Kinetic data show a bell-shaped ionic strength dependence of the cytochrome \( c \) reduction with a maximum activity near physiological ionic strength. The dependence is less pronounced in yeast compared to horse heart CYC indicating less impact of electrostatic interactions in the yeast system. Interestingly, a local QCR activity minimum is found for both substrates at 120–140 mM ionic strength. The architecture of the complex results in close distance of both \( c \)-type heme groups allowing the rapid reduction of cytochrome \( c \) by QCR via direct heme-to-heme electron transfer. Remarkably, CYC binds only to one of the two possible binding sites of the homodimeric complex and binding appears to be coordinated with the presence of ubiquinone at the Qi site. Regulatory aspects of CYC reduction are discussed.

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1. Introduction

Small diffusible redox proteins facilitate electron transport between large membrane-embedded enzyme complexes in cell respiration and photosynthesis. To promote high turnover and efficiency of the energy converting machinery, binding of the mobile electron carrier proteins has to be both transient and specific.

In mitochondrial respiration cytochrome \( c \) (CYC), a small water-soluble protein that has a covalently attached heme \( c \) group, shuttles electrons between ubiquinol:cytochrome \( c \) oxidoreductase (QCR, cytochrome \( bc_1 \) complex) and cytochrome \( c \) oxidase (COX) in the intermembrane space [2]. QCR (E.C. 1.20.2.2) is a multisubunit, integral membrane protein complex, which passes electrons from ubiquinol to CYC and couples this process to translocation of protons across the inner mitochondrial membrane as described by the Q cycle [3–5].

\[
\text{QH}_2 + 2\text{CYC(Fe}^{3+}\text{)} + 2\text{H}^+_i \rightarrow \text{Q} + 2\text{CYC(Fe}^{2+}\text{)} + 4\text{H}^+_o
\]

Each monomer of the homodimeric complex contains three essential catalytic subunits with prosthetic groups: cytochrome \( b \) (COB) with two \( b \)-type heme groups, cytochrome \( c_1 \) (CYT1) with a \( c \)-type heme, and the Rieske protein (RIP1) containing an \([2\text{Fe–2S}]\) iron–sulfur cluster. Mitochondrial QCRs contain up to eight additional subunits [6].

The Q cycle involves separate catalytic sites for quinol oxidation (Qo site) and quinone reduction (Qi site). Protons are taken up from the matrix side while quinone is reduced to quinol and protons are released to the intermembrane side upon quinol oxidation. Ubiquinol oxidation at the Qo site involves a bifurcated electron transfer. One electron is transferred via the \([2\text{Fe–2S}]\) cluster to heme \( c_1 \), the electron donor for CYC reduction. The second electron is passed via heme \( b_L \) and heme \( b_{11} \) to the Qi site and reduces quinone to...
semiquinone and after a second round of the cycle to quinol.
X-ray structures have been reported for mitochondrial QCRs
from beef and chicken [7–9] and for the complex from the
yeast Saccharomyces cerevisiae [10] and led to major
progress in understanding the enzyme mechanism and
structure–function relationships. Remarkably, the X-ray
structures identified that the extrinsic catalytic domain of
RIP1 is mobile [8]. An alternating movement between the
redox-center domains of COB (b-position) and CYT1 (c-
position) may facilitate the bifurcation of the electron tran-
sfer pathway at the Q_o site [8,11,12].

Despite detailed information about the catalytic mecha-
nism, the binding of CYC to QCR has been debated [13].
The proper encounter complex between CYC and QCR is
critical for ubiquinol oxidation and full turnover of QCR.
Complex formation has to be fast and specific to achieve an
orientation of the reaction partners suitable for fast electron
transfer followed by fast dissociation of the product com-
plex. Structures of transient redox complexes are difficult to
obtain and only few have been determined by X-ray
diffraction or NMR spectroscopy [14–16]. In the past,
binding experiments were carried out using 50 μl affinity
matrix mixed with 0.38 nmol of purified QCR in 100 μl buffer with different ionic strengths [40 mM Tris, pH 7.4,
0.05% undecyl-β-maltopyranoside (UM), NaCl concentra-
tion varied]. After incubation for 20 min, the mixture was
loaded into small column holders, unbound QCR removed
by washing with 100 μl of the same buffer and eluted by addition of 100 μl high salt buffer (500 mM NaCl in the same buffer). Experiments were carried out at 4 °C and room temperature.

For elution experiments, pure QCR samples were bound to 0.5 ml column matrix at an ionic strength of 40 mM.
QCR elution in steps of 1 ml aliquots of buffer with increasing ionic strength was monitored at 415 nm in the
equate fractions.

Enzyme activity was determined by following the reduc-
tion of CYC by QCR with a spectrophotometric assay at
550 nm as previously described [23], but with minor
modifications. Decylubiquinol (80 μM) and CYC (50 μM)
(purified iso-1-CYC or horse heart CYC) were incubated in
assay buffer (40 mM Tris/HCl pH 7.4, 0.05%, variable
concentrations of NaCl) at 25 °C. The reaction was started
by the addition of QCR to a final concentration of 0.625 nM
and followed for 3 min at 550 nm. The reduction rate was
calculated from the initial linear slope after subtracting the
non-enzymatic background.

QCR was quantified by redox absorption spectra using an
extinction coefficient of 28.5 mM−1 cm−1 (563–577 nm,
dithionite-reduced minus ferricyanide-oxidized) [25].

Ionic strength of buffers containing Tris/HCl was calcu-
lated with the simplifying assumption of fully dissociated
buffer molecules.

Amino acid residues of all subunits of yeast QCR, as
well as of yeast CYC, are numbered according to the initial
translation product.

3. Crystallization of the QCR:CYC complex and
structure determination

For crystallization of the yeast QCR:CYC complex, two
major problems had to be overcome. First, protein prop-
erties and conditions of the solution that favor complex
formation had to be found. Second, crystallization was
challenged with one of the components being an integral
membrane protein complex. The latter was addressed by
antibody fragment mediated crystallization, a technique that improves the chances of obtaining well-ordered crystals of a membrane protein by enlarging the polar, solvent exposed surface important for formation of stable crystal contacts [11,26,27]. The antibody fragment FV18E11 derived from the parental antibody mAB18E11 binds to the extrinsic domain of subunit RIP1 and is essential for crystallization of yeast QCR [10,11]. The bound FV fragment does not affect the enzyme activity (unpublished results) and, therefore, should not interfere with enzyme–substrate complex formation. High purity of yeast QCR and FV fragment preparations, as well as the stability of their binding has already been demonstrated by successful crystallization and structure determination of yeast QCR [10]. Furthermore, binding of the antibody fragment provides a spacious crystal packing, in which CYC could, in principle, bind to subunit CYT1 of QCR. Therefore, the yeast QCR:FV18E11 complex was used as binding partner for CYC.

Optimal binding conditions for CYC and QCR were deduced from CYC affinity chromatography experiments (Fig. 1a,b). A series of purified yeast QCR samples with different ionic strengths were prepared and loaded onto CYC affinity columns (Fig. 1a). At room temperature, binding of QCR is high at 80, 100 and 120 mM ionic strength, lower at 140 and 160 mM, and drastically decreased above 160 mM ionic strength. At 4 °C, the amount of bound QCR is lower and binding is only achieved at ionic strengths of 160 mM or below. Also, QCR was bound to the affinity matrix at low ionic strength and eluted stepwise with increasing concentrations of NaCl. At 4 °C, elution of QCR starts at 120 mM and is entirely eluted from the matrix at 130 mM ionic strength (Fig. 1b). The lower ionic strength limit for QCR binding in the elution assay might be caused by a lower QCR to CYC ratio and a higher dilution of the protein during elution.

A bell-shaped ionic strength dependence of yeast QCR activity has been previously shown [28]. This analysis covered a wide range of ionic strengths, whereas we were interested in identifying differences within the physiological limits to optimize complex formation. QCR activity at different ionic strengths were tested using purified yeast QCR and purified iso-1-CYC. From 40 to 120 mM, the turnover number increases from 75 to 102 s⁻¹. Interestingly, a local minimum is observed at 140 mM. The primary structure of CYC is highly conserved in evolution [29] and horse CYC is often used as substrate in yeast QCR activity assays. Remarkably, bell-shaped ionic strength dependence is more pronounced for horse CYC compared to yeast CYC. Turnover numbers decrease below 60 mM and above 160 mM, the highest activity is found at 80 mM. Furthermore, the local activity minimum at medium ionic strength (120–140 mM) is more pronounced.

![Fig. 1. (a) Ionic strength dependent binding of yeast QCR to immobilized yeast iso-1-CYC. Pure QCR samples were bound to the affinity matrix at varying ionic strength. Unbound material was washed off, and bound QCR was quantified at 413 nm in one-step eluates at high salt concentration. In general, binding is higher at 4 °C (black bars) compared to room temperature (gray bars). Best binding occurs below 160 mM ionic strength. (b) Ionic strength dependent elution of yeast QCR bound to immobilized yeast iso-1-CYC. QCR was bound to the affinity matrix at low salt concentration and eluted stepwise with increasing ionic strength at 4 °C. QCR was quantified at 415 nm in the respective eluates. QCR begins to elute at an ionic strength of 120 mM and elutes nearly entirely at 130 mM. (c) Bell-shaped dependence of QCR activity from ionic strength. Higher activities are obtained using yeast iso-1-CYC (black triangles) compared to horse heart CYC (open circles). Remarkably, a local activity minimum at 120–140 mM was found for both substrates.](image-url)
Bell-shaped ionic strength dependence of enzyme activity with an optimum at moderate ionic strength is characteristic for proteins that form stable redox-complexes [30]. This has been described, for instance, for cytochrome f and plastocyanin [31]. At low ionic strength, the low activity could be explained by stronger electrostatic stabilization of the complex, which might not be optimal for electron transfer. Intermediate ionic strength may weaken these interactions and allow rearrangement to a more productive complex. High ionic strength disrupts stabilizing electrostatic interactions. The less pronounced dependence for yeast CYC may indicate, that a smaller portion of the stabilizing interactions are of electrostatic nature and that little rearrangement occurs at low ionic strength. The very reproducible local minima at 120–140 mM ionic strength, i.e. in the physiological range, point to subtle differences in complex formation and will be analyzed in future studies.

Based on these results, QCR:FV complex and CYC were mixed at an ionic strength of 120 mM. Addition of precipitant solution decreases the initial ionic strength in the crystallization drop to approximately 90 mM. Nuclei formation and crystal growth start immediately. The final ionic strength in the mother liquor of the crystal will be slightly higher due to the vapor diffusion set-up. This condition favors CYC binding, allows for high QCR activity and is, in addition, in the range of the proposed physiological ionic strength of the mitochondrial intermembrane space [13].

Although vapor diffusion set-ups are used for crystallization, crystal growth is very fast and often crystals can be seen within 20 min. Nucleation is difficult to control resulting in several hundred small crystals and only few that are suitable for X-ray data collection (Fig. 2). Although these crystals diffract X-rays at synchrotron sources to a resolution better than 2.4 Å, data collection can only be performed at 4 °C up to now. Therefore, large crystals are needed to obtain high resolution data sets. The structure was determined at 2.97 Å resolution using a data set collected at 4 °C from a single crystal at beamline ID14EH3 (ESRF). The structure was solved by molecular replacement [1]. The dimeric QCR:FV fragment complex is present in the asymmetric unit. 2Fo –Fc electron density maps after rigid body refinement and energy minimization of the complex clearly revealed the presence of a single CYC molecule bound to one QCR monomer (monomer B). Distinct electron density allowed the unambiguous placement of the CYC molecule in the structure. The structure of the whole complex was refined to a final Rcryst and Rfree of 22.8% and 26.7%, respectively.

4. Overall description of the structure

The crystallized yeast QCR consists per monomer of nine subunits with a total molecular mass of 225 kDa. The transmembrane region is formed by 24 α-helices. A large polar domain made up mainly by subunits COR1 and QCR2 protrudes into the matrix, whereas the catalytic domains of subunits RIP1 and CYT1 are exposed to the intermembrane space (Fig. 3a). CYC is bound to subunit CYT1 of monomer B of the homodimeric QCR. There is no evidence that the orientation of the substrate molecule is influenced by crystal contacts. Furthermore, no steric hindrance for a CYC molecule bound in equivalent orientation to the recognition site of monomer A could be detected. Therefore, we conclude that the binding mode between the enzyme complex and the substrate molecule is specific. The antibody fragment bound to RIP1 neither interacts with CYC in the β-orientation of the mobile extrinsic domain [1] nor in the c1-orientation, which was modeled by superimposition of the RIP1:FV1BE11 portion of the structure with the c1-orientation of the Rieske-protein in the bovine QCR structure (PDB-entry 1BE3). The crystallographic analysis is in accordance with activity measurements (see above) and clearly shows that the antibody fragment does not interfere with CYC binding.

5. Binding mode of CYC and CYT1

CYC is bound to subunit CYT1 with the two heme clefts facing each other, thereby forming a tightly interacting and complementary contact area (Fig. 3b). Heme c and heme c1 are remarkably close with their pyrrole C rings pointing towards each other. The CBC atoms of the two respective vinyl groups are only 4.5 Å apart. The distance between the two iron centers is 17.4 Å. This is the shortest reported distance between the redox centers of CYC and its redox partners. The interplanar angle of the heme groups is 55°.
Interestingly, this is close to the value found for the CCP:CYC complex structure [14].

Binding of CYC to CYT1 is mainly stabilized by non-polar interactions between amino acid residues that surround the heme crevices and form a contact area of trapezoid shape (Fig. 4a,b). Stabilizing interactions are found between the following residues of CYT1 and CYC, respectively: Ala-103 and Ala-87 (Ala-81), Phe-230 and Arg-19 (Arg-13), Met-233 and Arg-19, Phe-230 and Thr-18 (Thr-12) as well as Ala-168 and Val-34 (Val-28). Residues in brackets refer to the previously used numbering system based on conserved positions of mammalian CYC [1]. At the edge of the heme crevice in CYT1, Ala-103 is as close as 3.4 Å to Ala-

Fig. 3. (a) Overall structure of the complex between the redox partners CYC (yellow) and QCR with antibody fragment Fv[181] (orange) bound to the catalytic domain of RIP1 (green). CYC binds only to one subunit CYT1 (red) of the homodimeric complex. Protein subunits are depicted in ribbon representation. The third catalytic subunit COB is colored in blue, QCR6 is colored in cream. The complex is viewed parallel to the plane of the inner membrane (orientation indicated) with the intermembrane space oriented to the top. (b) The complementary binding interface between CYC and QCR. Transparent surface representation of the catalytic subunits COB (blue), CYT1 (red), and RIP1 (green) of QCR and CYC (yellow) including their cofactors. QCR6 (cream) is shown in ribbon presentation. The first visible residues of the mobile N-terminus of QCR6 are present close to CYC. The figures were generated using the programs Molscript [46] and Bobscript [47].

Fig. 4. Binding interactions at the interface of the yeast QCR:CYC complex. CYT1 (a) and CYC (b) are shown in surface representation viewing the respective contact areas. The associated complex can be visualized by an approximately 180° rotation along the horizontal line. Residues involved in non-polar binding interactions are colored in orange, the cation-π interaction pair is colored in green. Charged and polar residues involved in weak electrostatic interactions are colored in blue and red. In addition, all positively and negatively charged residues of CYC (light green) and CYT1 (pink), respectively, are color-coded.
87 in CYC. This is the shortest distance between the complex partners, which is critical to bring the two heme groups in close proximity for electron transfer.

Most pronounced is the stable planar stacking interaction between Arg-19 of CYC and Phe-230 of CYT1 in a central position of the binding site, with the guanidino group oriented parallel to the aromatic plane. Stacking pairs located at molecular surfaces of proteins are known to be involved in formation of enzyme–substrate complexes [32]. Remarkably, in the complex of yeast CCP and yeast CYC there is also an aromatic residue (Tyr-39 of CCP) at the complex interface near Arg-19 [14]. Interestingly, at position 19 (13) of CYC a positive charge is highly conserved [29] and in position 230 of CYT1 mainly aromatic residues are present [1]. In mammalian CYC, Lys-13 has been shown to be important for binding to QCR [17]. Cation binding to the π face of an aromatic structure provides a relatively strong, non-covalent binding force, the cation–π interaction [33,34]. The stabilization between residues and atoms in van der Waals contact is an electrostatic effect that involves the quadrupole moment of the aromatic ring. We suggest a conserved cation–π interaction pair involving Arg or Lys at position 19 (yeast numbering) of CYC and position 230 of CYT1 as general binding element at the interface of QCR:CYC complexes.

In the past, the binding interaction of CYC and QCR was often thought to be dominated by electrostatic forces as supported by chemical labeling and mutagenesis studies, which assigned conserved negatively and positively charged residues around the heme clefts in CYT1 [20,21] and CYC [19]. However, in the crystal structure, only two weak polar interactions at the periphery of the recognition site are possible between Glu-235 and Lys-92 (Lys-86) as well as between the main chain oxygen atom of Ala-164 and Lys-85 (Fig. 4a,b). The importance of these interactions for binding is supported by the fact that the charge of Glu-235 is conserved in mitochondrial CYT1 [1]. Furthermore, Lys-92 is fully conserved in CYC and its role in CYC binding was shown by chemical labeling studies [19].

In the periphery of the described contact site of CYC and QCR, additional pairs of often conserved residues with opposed charges are found. They would be close enough for indirect polar interactions (e.g. 4.8 Å between Lys-92 of CYC and Asp-232 of CYT1), if water molecules are involved. The conserved charged residues, which surround the hydrophobic patches of CYT1 and CYC, may provide long-range electrostatic interactions, which could direct CYC to its recognition site on QCR as proposed for many transient electron transfer complexes, like the ones involving plastocyanin binding to cytochrome f [16] or binding of cytochrome c2 to the bacterial reaction center [35].

The area of the recognition site is with 880 Å2 [1] the smallest recognition site reported so far for protein–protein recognition sites of low-stability complexes, which in general are below 1600 Å2 [36]. The small interface of the QCR:CYC complex is well suited for a fast turnover. Formation of transient protein complexes is required for the CYC-mediated electron shuttling between QCR and COX. For rapid electron transfer weak protein complexes with short lifetime are expected.

In conclusion, binding interactions of CYC and QCR comprise mainly non-polar interactions, a central cation–π interaction and weak electrostatic interactions. Their conserved and specific nature clearly indicates that the structure resembles the physiological electron transfer complex.

6. Possible role of subunit QCR6 in binding of CYC

Previous studies on QCR from bovine heart mitochondria have shown that in addition to CYT1 a second subunit is involved in the binding of CYC [37]. This so-called hinge protein has a highly acidic N-terminus. The homologous subunit of yeast QCR, namely QCR6, contains 79% negatively charged residues at the N-terminus, which is important for CYC binding [21]. In the high resolution structure of yeast QCR [10], as well as in the structures of vertebrate QCRs [7–9] approximately 40 N-terminal residues of the subunit cannot be located, indicating a high mobility of the N-terminus. Interestingly, in the yeast CYC:QCR complex [1], the visible N-terminus of QCR6 is in close vicinity to the highly conserved lysine residues Lys-92 and Lys-93 of CYC (Fig 3b). However, there is no direct interaction between QCR6 and CYC visible in this structure at the given resolution. Remarkably, the dipole moment of CYC points with the positive side towards the N-terminus of QCR6, which might be an indication for possible ion–dipole interactions between QCR6 and CYC. The difference in ionic strength dependent QCR activity between the use of yeast or horse CYC described above might reflect the pronounced difference in the dipole moment of CYC from these two different sources [38]. It appears that electrostatic components involving QCR6 are important for the orientation of CYC with respect to its binding site.

7. Direct heme-to-heme electron transfer from CYT1 to CYC

Based on the structure of the QCR:CYC complex the kinetics of oxidation of QCR by CYC can be estimated. For calculating the electron transfer rate, the Dutton model for protein electron transfer was chosen using the ET rates package [39]. The architecture of the complex exhibits an edge-to-edge distance of the two c-type heme groups (C3C:C3C) of only 9.4 Å [1] and a packing density ρ of 0.65. This results in a free-energy optimized electron transfer rate of 1.2 × 109 s−1. With a midpoint potential of 270 mV for both CYT1 [40] and CYC [41] the driving force ΔG for electron transfer is zero, assuming no change in redox potentials due to binding. This, together with an estimated reorganization energy of 0.7 eV, results in a calculated
electron transfer rate of $8.3 \times 10^6 \text{s}^{-1}$. The structure of the complex clearly indicates electron transfer from CYT1 to CYC by direct heme-to-heme transfer. As pointed out before, a structural pathway through the intervening protein, via the remarkably close distance between Ala-103 of CYT1 and Ala-87 of CYC appears unlikely [1]. The residues are not conserved and transfer would be considerably slower. However, the hydrophobic patches of both cytochromes do not seem to be tightly sealed, water molecules that are not resolved at the given resolution might be present in the close environment of donor and acceptor and might take part in such a structural pathway. Future studies, including structures of the complex at higher resolution will show, whether stably positioned water molecules are present at the interface.

For the system of bovine heart QCR with a ruthenium-modified horse heart CYC an experimental electron transfer rate of $6 \times 10^4 \text{s}^{-1}$ has been obtained [13]. This discrepancy between the estimated and the experimental rate might either be caused by the bulky ruthenium-label on Lys-72, which is positioned near the complex interface or it might indicate a different binding mode of horse heart CYC. In conclusion, the structural analysis of the reduction of CYC by QCR strongly suggests a fast direct heme-to-heme electron transfer without the contribution of amino acid residues.

8. Half-of-the-sites binding of CYC to the homodimeric QCR

The homodimeric QCR has two identical binding sites for CYC. Remarkably, in the reported crystal structure of the complex, only a single CYC molecule bound to monomer B could be located, in a binding stoichiometry termed half-of-the-sites binding (Fig. 3a,b) [1]. This stoichiometry is unexpected, as the substrate is present in molar surplus in the crystallization set-ups. Furthermore, no major conformational differences between the binding sites of monomer A and B were detected, and the crystal packing does not restrict accessibility to the binding site of monomer A. Thus, the question why only one CYC molecule is bound to the homodimeric enzyme cannot be answered on the basis of this structure. Subtle differences, which are not resolved at the given resolution, may cause different binding properties of the two recognition sites for CYC. It cannot be excluded that the flexible and thus unresolved N-terminus of CYC regulates binding of CYC to QCR, as it was proposed for the homologous hinge protein of bovine QCR [37]. In yeast, deletion of QCR6 led to a 50% decrease in QCR activity, and the authors proposed a half-of-sites mechanism, in which QCR operates with one-half being “silent” upon regulatory events [42].

Each of the two operational units of the homodimeric QCR can, in principal, perform catalysis according to the Q cycle. It is not known whether these units function in a parallel, sequential or independent mode. The specific binding of one CYC molecule to one monomer of QCR indicates that QCR might be able to reduce CYC with the second functional unit not being active, which requires a cross-talk between the two functional units. The latter is supported by a recent study, which demonstrated that yeast QCR shows anti-cooperative activity [43].

Most remarkably, CYC half-of-the sites occupancy coincides with differences in the two ubiquinone reduction sites (Qi sites) of QCR. The Qi site of monomer B with CYC bound, is occupied with a quinone molecule in an orientation known from the high resolution structure of yeast QCR [10]. In contrast, no clear electron density was found at the Qi site of monomer A, indicating a very low occupancy and/or high mobility of the molecule. Furthermore, the bend propionate A of heme $b_1$ that stabilizes the quinone ring plane by non-polar interaction [10,11] is displaced in monomer A supporting a different binding mode in this site.

It appears that the occupancy state of the Qi site affects CYC binding or vice versa. A coordinated binding of both electron acceptors for quinol oxidation, i.e. the presence of both acceptors at the same time [1], might avoid accumulation of perilous intermediates or side products of catalysis. Coordinated binding implies long-range interactions within the molecule. Interestingly, long-range interactions have been observed in cytochrome c oxidase upon binding of CYC by a resonance Raman study [44]. Communication between low- and high-redox potential components has been demonstrated by kinetic studies with yeast QCR and a Qi site-specific inhibitor [45]. Coordinated binding could be controlled by redox-induced subtle conformational changes and long-range electrostatic interactions. Coordinated binding appears to be especially important when one functional unit of QCR is not active, irrespective of whether this occurs in a sequential or independent mode.

Further experiments are required to analyze whether the redox states of different QCR cofactors affect CYC binding and how coordination of the two reduction sites and between the two monomers are achieved. Both coordinated binding and half-of-the sites reactivity suggest that CYC reduction by QCR is regulated in response to the respiratory condition.

9. Conclusions

The structure of the complex between QCR and CYC shows a tight and specific binding interaction between CYC and QCR, which is mediated by a small, compact contact site dominated by non-polar forces. A central cation-$\pi$ interaction appears to be an important and conserved feature of CYC binding. Weak electrostatic interactions involving charged residues in the periphery of the binding site might be important for orientation of the complex. Size and characteristics of the contact site are optimal for a transient electron transfer complex. Distance and orientation of the c-type hemes suggest rapid and direct electron transfer by heme-to-heme contact. The highly specific and conserved
interactions under optimized binding conditions, as well as the fast electron transfer, strongly suggest that the structure resembles the physiological electron transfer complex, although multiple productive conformations in vivo cannot be excluded. Half-of-the sites binding of CYC as well as its coordinated binding with quinone indicate a specific regulatory mechanisms of QCR, which need to be analyzed in future studies.

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