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Functional flexibility of electron flow between quinol oxidation Q_o site of cytochrome bc_1 and cytochrome *c* revealed by combinatory effects of mutations in cytochrome *b*, iron-sulfur protein and cytochrome c_1 ☆

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ABSTRACT

Transfer of electron from quinol to cytochrome *c* is an integral part of catalytic cycle of cytochrome bc_1. It is a multi-step reaction involving: i) electron transfer from quinol bound at the catalytic Q_o site to the Rieske iron-sulfur ([2Fe-2S]) cluster, ii) large-scale movement of a domain containing [2Fe-2S] cluster (ISP-HD) towards cytochrome c_1, iii) reduction of cytochrome c_1 by reduced [2Fe-2S] cluster, iv) reduction of cytochrome *c* by cytochrome c_1.

In this work, to examine this multi-step reaction we introduced various types of barriers for electron transfer within the chain of [2Fe-2S] cluster, cytochrome c_1 and cytochrome *c*. The barriers included: impediment in the motion of ISP-HD, uphill electron transfer from [2Fe-2S] cluster to heme c_1 of cytochrome c_1, and impediment in the catalytic quinol oxidation. The barriers were introduced separately or in various combinations and their effects on enzymatic activity of cytochrome bc_1 were compared. This analysis revealed significant degree of functional flexibility allowing the cofactor chains to accommodate certain structural and/or redox potential changes without losing overall electron and proton transfers capabilities. In some cases inhibitory effects compensated one another to improve/restore the function. The results support an equilibrium model in which a random oscillation of ISP-HD between the Q_o site and cytochrome c_1 helps maintaining redox equilibrium between all cofactors of the chain. We propose a new concept in which independence of the dynamics of the Q_o site substrate and the motion of ISP-HD is one of the elements supporting this equilibrium and also is a potential factor limiting the overall catalytic rate.

1. Introduction

Cytochrome bc_1 is one of the enzymes engaged in oxidative phosphorylation. As a part of electron transport chain it catalyzes net oxidation of quinol (QH_2) and reduction of cytochrome *c* in reactions that contribute to building up the proton motive force which is further utilized to produce ATP [1,2].

Cytochrome bc_1 is a homodimeric protein complex. Each monomer consists of three to eleven subunits, depending on a species [3–5]. The three subunits, cytochrome *b*, cytochrome c_1, and iron-sulfur protein (ISP), form a catalytic core, within which all steps of catalytic reaction take place. The subunits of the catalytic core contain the redox cofactors assembled into two chains: high potential c-chain (including [2Fe-2S] cluster and heme c_1 embedded in iron-sulfur protein and cytochrome c_1, respectively) and low potential b-chain (including heme b_L and heme b_H embedded in cytochrome *b*). These chains of cofactors connect electronically the catalytic sites of the enzyme forming a characteristic H-shaped electron transfer system (Fig. 1) [6]. In each monomer the c-chain connects the quinol oxidation site (Q_o site) with cytochrome *c* reduction site, while the b-chain connects the Q_o site with the quinone reduction site (Q_i site) on the opposite side of the membrane. In addition, the two monomers are linked functionally by a two-hemes b_L bridge in the center of the dimer [6,7].

Cytochrome bc_1 operates according to the Q cycle [8,9]. Upon oxidation of QH_2 at the Q_o site two protons are released into the intermembrane space and two electrons are directed into the different chains: one electron is transferred via c-chain to reduce one molecule of cytochrome *c* while the second electron is transferred via b-chain to form a semiquinone at the Q_i site. Oxidation of the second QH_2 molecule at the Q_o site results in reduction of the second cytochrome *c* and

Abbreviations: ISP, iron-sulfur protein; ISP-HD, head domain of ISP; WT, wild type; QH_2, quinol; E_m, redox midpoint potential; *R.*, *Rhodobacter*

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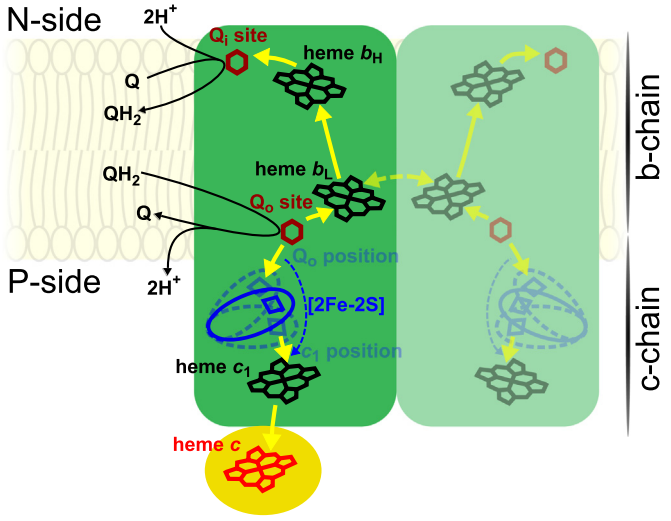

Fig. 1. Schematic representation of the catalytic core of dimeric cytochrome bc_1. Each monomer of the core (shown as green round rectangle) consists of cytochrome *b*, ISP and cytochrome c_1. The arrangement of cofactors in the c- and b- chains is depicted for both monomers (green and transparent green). The c-chain connects quinol oxidation site (Q_o site) with cytochrome *c* reduction site by [2Fe-2S] cluster and heme c_1 embedded in iron-sulfur protein and cytochrome c_1, respectively. Within the c-chain, ISP-HD, a domain containing [2Fe-2S] cluster (blue diamond) moves (blue dotted arrow) between the Q_o position and the c_1 position. The b-chain connects the Q_o site with quinone reduction site (Q_i site) by heme b_L and heme b_H embedded in cytochrome *b*. Yellow arrows represent the paths of electron transfer within the complex, including the inter-monomer electron transfer between hemes b_L (yellow dotted arrow). The connections of all four catalytic sites in the dimer form an H-shaped electron transfer system. For simplicity, the details of Q cycle reactions are highlighted only for one of the monomers and the scheme does not consider stoichiometry of the Q cycle.

the completion of reaction at the Q_i site (reduction of semiquinone to QH_2) with a two-proton uptake taking place at this site [9]. The coupling of reactions of the Q_o and Q_i sites allows translocation of protons across the membrane. In this way cytochrome bc_1 contributes directly to the generation of the proton motive force.

The focus of the present study is electron transfer between the Q_o site and cytochrome *c* along the c-chain. The operation of this chain involves an intriguing mechanistic event: large-scale movement of the head domain of iron-sulfur protein (ISP-HD) between the Q_o site (Q_o position) and cytochrome c_1 (c_1 position) [5,10–14]. This movement separates two interaction domains: in the Q_o position ISP-HD interacts exclusively with the occupant of the Q_o site while in the c_1 position – it interacts exclusively with heme c_1. Although it is generally agreed that the movement itself is not rate-limiting for catalysis [13,15], its exact mechanism remains elusive. Nevertheless, several important insights into this mechanism emerge from biochemical and structural analyses [11,13,15–23]. For example, the fraction of ISP-HD in each of the positions can be estimated from the crystal structures of cytochrome bc_1 from different species. It was estimated to be < 0.1 for the Q_o position occupancy, approximately 0.5 for the c_1 position occupancy and the unaccounted occupancy was around 0.4 [23]. This may suggest a rather broad distribution of positions of ISP-HD in solution. Indeed, spectroscopic study revealed that an average equilibrium position of ISP-HD in solution is between the Q_o- and c_1- positions [19]. Furthermore, the native distribution of positions of ISP-HD can be altered by various mutations such as the well-described alanine insertions in the neck region connecting ISP-HD with hydrophobic anchor [13]. $1Ala^{ISP}$ and $2Ala^{ISP}$ introduce steric constrains to the motion of ISP-HD, resulting in ISP-HD captured at the Q_o site for milliseconds ($1Ala^{ISP}$) or seconds ($2Ala^{ISP}$) [13]. It causes an increase in population of ISP-HD at the Q_o position [19,24]. The opposite effect on the Q_o position occupancy was observed in recent studies with $G167P^b$ mutant in bacterial cytochrome *b* (a mimic of mitochondrial disease mutation $S151P^b$) which displayed a decrease in occupancy of the Q_o position by ISP-HD [25]. Mutations affecting ISP-HD motion may introduce rate-limiting step to the Q-cycle.

Another effective way of introducing barrier for electron transfer between the Q_o site and cytochrome *c* is to affect the redox potential difference between individual cofactors. Among various factors determining the redox potential (E_m) of [2Fe-2S] cluster, the formation of hydrogen bond between hydroxyl group of serine and one of the sulfur atoms of [2Fe-2S] cluster appears crucial [26–29]. Removing this specific hydrogen bond by substitution of serine with other amino acid, such as alanine (mutation $S158A^{ISP}$ in *Rhodobacter capsulatus*) causes a decrease in E_m of [2Fe-2S] cluster by > 100 mV in comparison to the wild type (WT) [26,29]. Such low E_m of [2Fe-2S] cluster on one hand renders oxidation of QH_2 at the Q_o site energetically less favorable, but on the other hand it favors the electron transfer from ISP to cytochrome c_1. The former effect appears dominant as the turnover rate of $S158A^{ISP}$ is much lower than in WT. A large change in E_m of heme c_1 is observed in mutants that change the ligation pattern for the heme iron [30–33]. For example, substitution of heme-ligating methionine with lysine (mutation of $M183K^{c1}$ in *R. capsulatus*) decreases E_m of heme c_1 for > 200 mV in comparison to the WT [30,32]. This makes electron transfer from ISP to cytochrome c_1 energetically uphill. The barrier is large enough that it decreases the enzymatic activity to such a low level that the enzyme is non-functional in vivo [32].

Yet another way to introduce barrier for electron transfer between the Q_o site and cytochrome *c* is to affect the catalytic reaction of the Q_o site by mutating one of the important amino acids of the substrate-binding pocket. For example, a mutation $E295Q^b$ in a highly conserved PEWY motif of cytochrome *b* significantly slows down the reaction of the Q_o site influencing thereby the observed rate of reduction of cytochrome *c* via c-chain [34–37].

In this work we compared the effects of introducing all three types of barriers (i.e. barriers created by impediment in the motion of ISP-HD, change in E_m of ISP and/or heme c_1, and impediment in the catalytic quinol oxidation) separately and in various combinations. The combinations corresponded to the double mutants where the two out of three barriers were introduced simultaneously. The results offer interesting new insights into the mechanism of electron flow from the Q_o site to cytochrome *c*.

2. Materials and methods

2.1. Preparation of *R. capsulatus* mutants

R. capsulatus strains bearing single mutations: $1Ala^{ISP}$, $2Ala^{ISP}$, $G167P^b$, $S158A^{ISP}$, $M183K^{c1}$ and $E295Q^b$ were constructed previously [13,25,29,32,34]. The strains bearing double mutations $E295Q^b$/$1Ala^{ISP}$, $E295Q^b$/$G167P^b$, $M183K^{c1}$/$1Ala^{ISP}$, $M183K^{c1}$/$2Ala^{ISP}$ and $M183K^{c1}$/$S158A^{ISP}$ were constructed by ligating appropriate restriction fragments derived from plasmids carrying single mutations, and introducing them into a suitable genetic background as described [38].

2.2. Bacterial cultures and tests of photoheterotrophic growth

R. capsulatus strains were grown under semi-aerobic/dark conditions at 30 °C, on plates or in liquid cultures (shaking at 110 rpm) in mineral-peptone-yeast-extract (MPYE) medium supplemented with 10 μg/ml kanamycin as described in [39]. The tests of bc_1-dependent growth under photosynthetic conditions were performed as follows. One colony of each mutant was cultured semi-aerobically in 2 ml of MPYE overnight. Subsequently, the cells were diluted to OD_{600} = 0.1 and 100 μl of such suspension was seeded on the MPYE-agar plates supplemented with 10 μg/ml kanamycin. The cells were grown in