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Connecting CuA with metal centers of heme *a*, heme a_3, CuB and Zn by pathways with hydrogen bond as the bridging element in cytochrome *c* oxidase

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ABSTRACT

Pathways formed of delocalized π-electron systems and polar groups of polypeptide chains bridged by hydrogen bonds are referred as π-H pathways. Suitable for electron transfer, these pathways in cytochrome *c* oxidase connect CuA, the source of electrons distributed in cytochrome *c* oxidase, with the metal centers, heme *a*, heme a_3, CuB, the constituents of the catalytic binuclear center. The unusually rapid electron transfer between heme *a* and heme a_3 would have been facilitated by the link pathway of a long sequence of alternate peptide unit and hydrogen bond spanning Pro336-Val374, referred as suprahelix, between these hemes. Two pathways between CuA center and zinc center, share some portions with purported proton-translocating channels, designated "K" and "D".

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1. Introduction

The redox active metal center of cytochrome oxidase-subunit II, CuA, is known to receive electrons from cytochrome c [1,2] and transfer four of them to dioxygen bound between heme a_3-Fe and CuB, the catalytic site referred as binuclear center (BNC), yielding two molecules of water [3,4]. There is a consensus that CuA → heme *a* → heme a_3 sequence and direct connections between CuA to heme a_3 and CuB [5–8] are present. Calculations by quantum-mechanical and molecular-mechanical (QM-MM) e-pathway scheme and plotting unpaired electron spin-density on removing one residue at a time indicated potential electron paths between CuA and CuB, and also heme *a* and heme a_3 [8]. This approach is limited to identifying amino acid residues between the donor and the acceptor which are conjectured to "simply act as bridge mediators between a longer through space jump" sans any clue on their connectivity. "The most probable electron transfer pathway" between heme-Fe of cytochrome *c* and CuA of cytochrome oxidase, obtained by calculating relative electron couplings by the pathways plugin program [9], depended on covalent σ-bonds of the amino acid residues and through space jumps [10]. The paths of electron transfer from CuA to other metal centers in cytochrome oxidase are less defined.

The concept of "tunneling" dominates studies on electron transfer in cytochrome oxidase [5–10] and in proteins in general [11,12]. A new perspective emerged that the bridging element is the hydrogen bond between delocalized electron systems, known for electron transfer [13], and polar groups to form pathways in proteins [14–16]. We described pathways, linked by hydrogen bonds, passing through heme-Fe in cytochrome *c* protein [17] and from there reaching CuA center in subunit II protein of cytochrome oxidase [18]. Occurrence of such pathways connecting CuA, the source of electrons distributed in cytochrome oxidase, with the metal centers, heme *a*, heme a_3, CuB and Zn is shown in this sequel report.

2. Methodology

Crystal structure of cytochrome c in complex of cytochromes c oxidase (PDB ID:5IY5) was analyzed using pymol software for 'Hydrogen Bonds' starting from CuA center, Zn center, heme *a*, and heme a_3-CuB binuclear center by locating residues below 4 Å distance and marking the polar atoms, including the water molecules, which are within the distance of 2.6–3.3 Å. Whenever more than one hydrogen bond is encountered the short and/or the strong (in

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terms of distance and the most favored angle between the donor and acceptor) one is selected. The hydrogen bonds of side chain N-atoms of residues of His61, His376 and His378 with atoms within 3.3 Å are identified. A helical chain of alternate hydrogen bonds and peptide units, present on and owing its existence on α-helix is referred as 'suprahelix' [19]. This offers a pathway of delocalized electrons connected by hydrogen bonds. Three such independent helical pathways span the length of α-helix. Examples are found where a part of a connecting pathway uses two suprahelices in the same α-helix for making a U-turn. The pathways between two centers are built of delocalized π-electron systems (peptide bonds, side chains of some amino acid residues), polar groups and buried water molecules linked by hydrogen bonds and are referred as the π-H pathways [20].

3. Results

Pathways bridged by hydrogen bonds are ubiquitous in the interior of membrane proteins of cytochrome oxidase. These are tracked starting from CuA center wherefrom electrons are distributed. Invariably the metal centers are coordinated to one or more of histidine residues (Fe and Cu), or to cysteine residues (Zn) to which the pathways reach.

The binuclear center, the active site of cytochrome oxidase, comprises of heme a_3-Fe coordinated to His376 and CuB metal center coordinated to His 290, His 291 and His 240. There are four pathways from CuA leading to the binuclear center, each led by a separate histidine residue. The first two pathways are connected to heme a_3-Fe, one directly and another via heme a-Fe (Fig. 1). The other two paths lead to copper B via His290 and His291 coordinately bonded to CuA (Fig. 2).

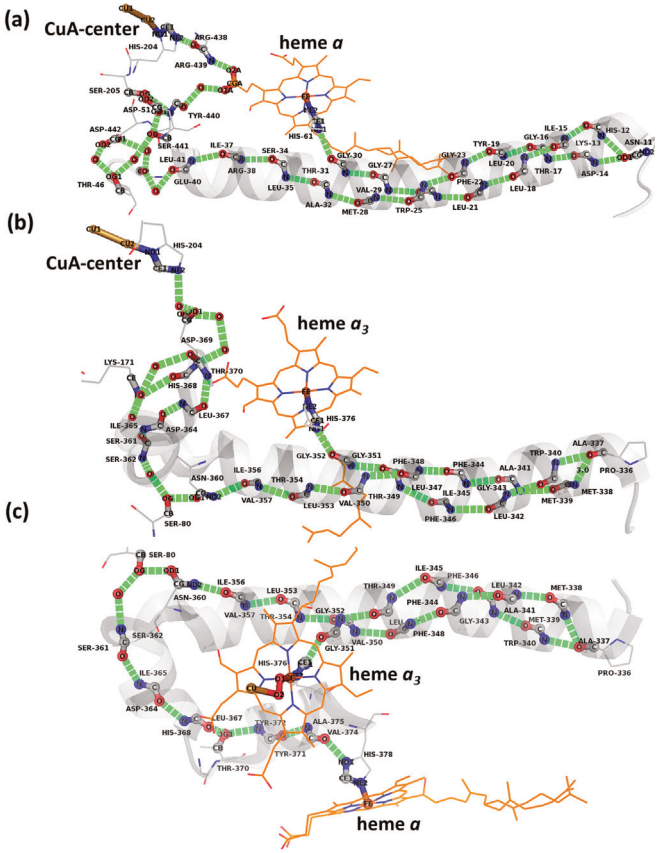

Fig. 1. π-H pathways connecting CuA and hemes a and a_3. (a) Connecting CuA to heme a; (b) CuA to heme a_3; (c) heme a to heme a_3. Each of the above three pathways makes a U-turn (right edge) using two suprahelices in the respective helix to reach the heme.

3.1. Pathways connecting CuA to heme a and heme a_3

The CuA center is held by C-terminus β-sheet domain of subunit II that also interacts with cytochrome c. CuA is connected to heme a by a pathway consisting of peptide units (Arg438-Arg439, Tyr440-Ser441), hydroxyl groups (Ser205, Ser441, Thr46), carboxy groups (Asp51, Asp442), and water molecules. In the last phase it enters the 'suprahelix' spanning Asp14-Glu40, makes a U-turn at Asp14 and finally reaches His61 coordinated to heme a-Fe (Fig. 1**a**).

The pathway between CuA and heme a_3 starts from His204 and passes through carboxy group (Asp369), an amino group (Lys171), water molecules, and several peptide units (Asp369-Thr370, His368-Asp369, Leu367-His368) among others. After reaching peptide unit Ser361-Ser362, a water molecule, hydroxyl group of Ser80 and amide group of Asn360 forms a π-H pathway. At Asn360 side chain the hydrogen bond network reenters the helix, goes on to the peptide unit Ile356-Val357, extends as a suprahelix upto Pro336, moves over to another strand on the same α-helix and reverses direction. At Gly351 it leaves the suprahelix and is connected by a hydrogen bond to His376 coordinated to heme a_3-Fe (Fig. 1**b**).

Another π-H pathway interconnects heme a and heme a_3 (Fig. 1c). It starts from His378, follows up to Thr370, a short independent path covering a bent suprahelix, and then joins the path described for CuA to heme a_3 (Fig. 1**b**). It then passes through Val357 to Pro336, reverses direction by entering another strand towards Gly352, all the while moving in an effective path of a long suprahelix spanning Pro336-Val374.

3.2. Pathways from the CuA center to CuB center

There are two independent, interacting routes starting from CuA center to CuB center through His290 and His291. One pathway involves side chains of Asp369 and Arg438, His368-Asp369 peptide unit, side chain of Lys171, peptide units (Leu367-Val366, Asp364-Leu363, Asp360-Ser361, Thr306-Phe305), Arg303 (peptide-C=O), hydroxyl groups (Thr306, Thr309) and water molecules that surround a magnesium atom without coordination to any amino acid residue unlike other metal centers (Fig. 2a).

Another pathway passes through the peptide bond of Arg438-Arg439, propinyl-carboxy groups of ring A and ring D of heme a, side chain of Arg439, heme a propinyl-carboxy group of ring D, water molecules in between and is finally connected to CuB via His291 (Fig. 2b).

3.3. Pathways between CuA-center and Zn-center

The zinc center is formed by four coordinate bonds of the metal with Cys60, Cys62, Cys82 and Cys85. It has a possible role, together with CuA center, in the exchange of electrons and protons (like in a voltaic cell) and is surmised to be the proximate source of protons.

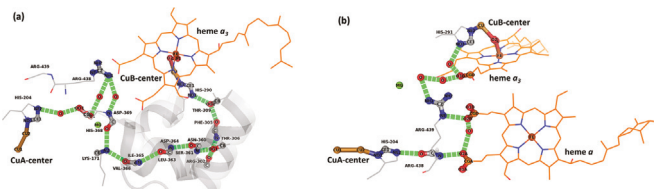

Fig. 2. π-H pathways from CuA to CuB.

There are multiple pathways between CuA center and zinc center. Two of them described here utilize suprahelices of varying length: the helix span Pro182-Leu215 in the first (Fig. 3a) and the helix span Pro444-Arg480 in the second (Fig. 3b). These two share a common path of two water molecules and a peptide unit near the Zn atom. Apart from, these paths, the N-terminus helices Pro13-Leu46, Gln59-Asp86 of cytochrome oxidase subunit-II also connects path between CuA center and zinc center. Interesting characteristic of this pathway is that it is built of two large suprahelices of 38 and 36 residues, a relatively large fraction among the pathways.

3.4. Pathways leading away from the Zn-center

A pathway starting from Cys60 of Zn center links the suprahelix (Asp300-Ser322), water molecules and peptide units (Ile70-Val69 and Tyr270-Gly269) and hydrogen bonds. The pathway further extends from the helix to the binuclear center via Thr316, a water molecule, farnesyl keto group, and the unusual covalently linked rings of Tyr244-His240 [21] and this histidine is coordinately associated with CuB (Fig. 4**a**). Coincidentally, this pathway also shares part of the K path that includes the amino acid residues His240, Tyr244, Lys319, shown in Fig. 4a [22].

The second pathway uses both the paths and extends up to His290 of binuclear center through a series of peptide units that span two helices 270-289 and 260-251, connected by the hydrogen bond network with the help of Lys319 (Fig. 4b). The side chain of Lys319 has been reported to move close to the binuclear center, within 3.5 Å with the helix connecting to His290.

The third pathway includes Cys60 and Cys62, peptide unit (Thr68-Val69), water molecules, His395, Tyr261, Ser335, the suprahelix spanning Ser335-Gly351, and reaches His376 coordinated to heme a_3-Fe (Fig. 4**c**).

The zinc center is also connected by a fourth π-H pathway (Fig. 4**d**) to Asp91 residue, prompting its name “D path”, proposed to generate proton gradient in the inter-membrane space [4,21].

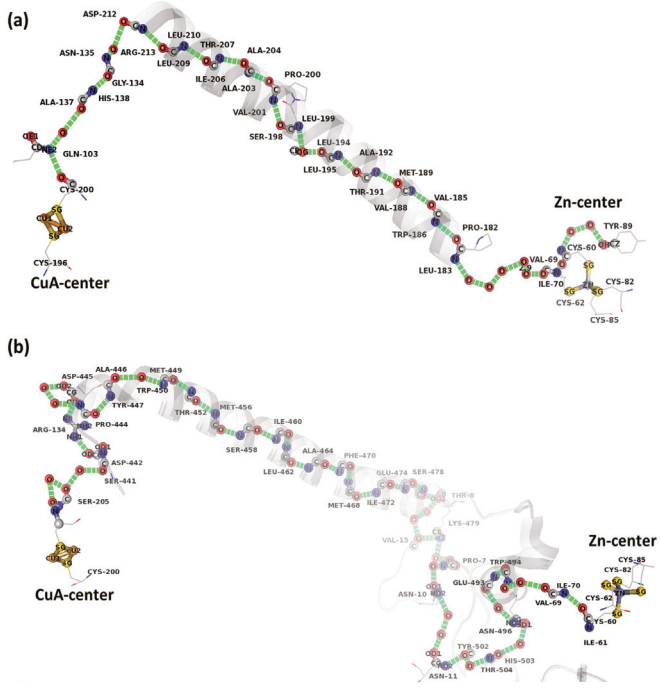

Fig. 3. π-H pathways from CuA to zinc center through different helices (subunit I).

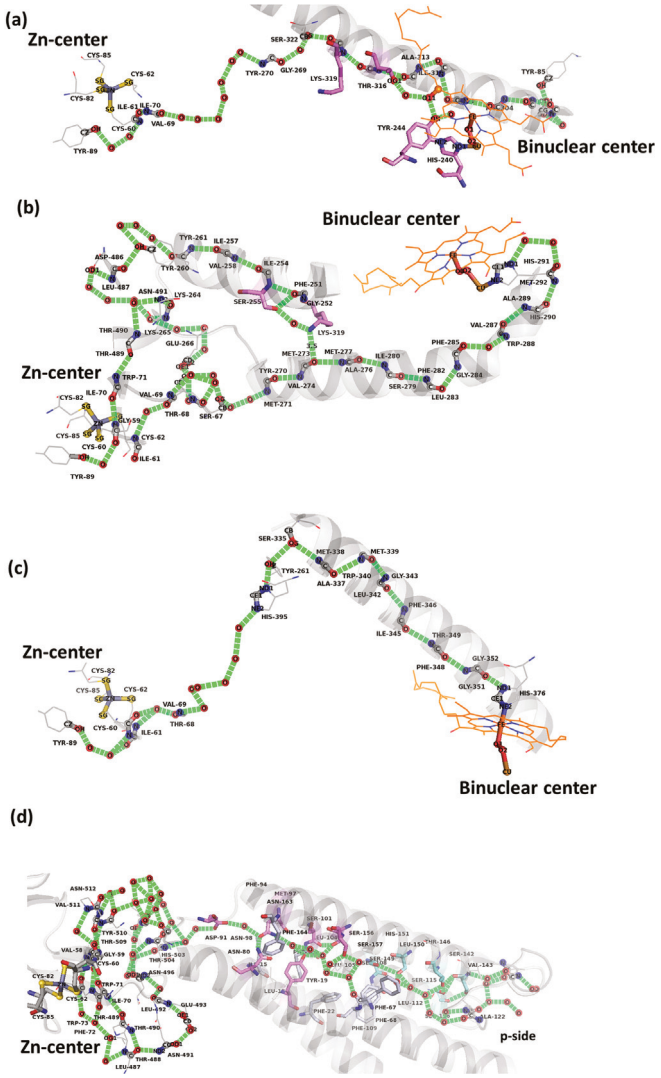

Fig. 4. π-H pathways from Zn-center to binuclear-center. (a) zinc center to CuB via His240 (K path: His240, Tyr244, Lys319); (b) zinc center to heme a_3 via His376; (c) zinc center to copper B via His291; (d) zinc center to binuclear center (D path: Asp91, Asn80, Asn98, Asn163).

The D path is formed by the lining of residues Asn80, Asn98, Asn163, Asp91 and Ser101, Ser156, Ser157, Thr146, Tyr19 are hydrogen bonded with the water molecules (colored pink). After Ser157 there is a break in the hydrogen bond network because of protrusion of aromatic rings of residues Phe22, Phe67, Phe68, Phe102, and Phe109 possibly involved in the regulation of D path. Apart from these residues Phe164, Leu105, Leu112, Leu113, Leu150, Val83, Val128, Ile75, Ile87 and Ile168 also line the path. In the path described here apart from the residues identified earlier, hydrogen bonding of residues Ser108, Ser115, Ser142, Ser149 and Thr146 (cyan, Fig. 4D) with water molecules line the path, visualized to enable translocation of protons to the inter-membrane space.

4. Discussion

Hydrogen bonds are now recognized to mediate electron transfer [14,15]. Chandra et al. [12] stated that “delocalization of an extraneous electron is pronounced when it enters low-lying virtual orbital of the π-electronic structures of peptide-linked by hydrogen bonds” based on theoretical calculations. Found over α-helices and

named 'suprahelices', sequences of alternative peptide and hydrogen bonds, are abundant in membrane proteins and can act as molecular wires for electron transfer in mitochondrial cytochrome chain. Together with other delocalized electron units and polar side chains of amino acid residues, hydrogen bonds can bridge atom-to-atom π-H pathways between two centers spread in the protein interior. Once an exogenous travelling electron enters a pathway it is committed to reach the other end. Such a defined travel is possible in these pathway, unlike the 'through space jump', as the electron travels in the antibonding orbital, always moving on an atom-to-atom basis.

These pathways are now tracked passing through cytochrome *c* across heme-Fe [17], between cytochrome *c* and CuA centre [18] and, in this report, from CuA centre to the other metal centers and between other centers in cytochrome *c* oxidase as detailed below: CuA to CuB (21.7 Å → 42.7 Å, 68.0 Å), CuA to heme *a* (19.4 Å → 158 Å), CuA to heme a_3 (22.1 Å → 144.1 Å), heme *a* to heme a_3 (13.3 Å → 121.9 Å), CuA to Zn (63.5 Å → 124.6 Å, 229.5 Å), Zn to CuB (43.4 Å → 150.9 Å, 163.3 Å), Zn to heme a_3 (42.4 Å → 96.0 Å) [distances shown in parenthesis: point-to-point → π-H pathways]. The π-H pathways are 2–9 fold longer compared to the point-to-point distances of the metal centers. These hydrogen-bonded pathways, notwithstanding their winding, are likely to be effective in electron transfer rather than the alternatives of covalent σ-bonds and through space jumps [10].

The bridging element of electron transfer in polypeptide structures is the hydrogen bond indicating that its quality (distance and angle) or its break [23] can modulate the rates. The most stable and standard distance of the hydrogen bond link is found in suprahelix appearing over an α-helix, and this, short or long, invariably appears in the π-H pathways. Electron transfer between heme *a* and heme a_3 in cytochrome oxidase is known to be unusually fast [24,25] but the suggestion of "exceptionally low reorganizing energy" as the cause does not have consensus. A possible explanation emerges on comparing the pathways. Suprahelix, expected to ensure rapid electron transfer, occurs to a relatively large extent in the pathway between heme *a*-Fe and heme a_3-Fe (Fig. 1c).

Dioxygen binding between heme a_3-Fe and CuB initiates the catalytic cycle. It is followed by its reduction by four electrons, concomitantly consuming four protons. Cytochrome c is the apparent source of electrons to CuA center. The source of protons is inconclusive. The Zn- center is a possibility. We surmise on arrival of an electron at the metal center, the coordinated cysteine becomes a Cys-$S^{\cdot}$ radical releasing the proton. When the electron leaves the metal center the reduced cysteine is reformed picking a proton from the external source. The two molecules of water formed finally are displaced into the water pool near the 'Magnesium center' [26]. Diffusion channels for the entry of dioxygen into the active site have been proposed [27,28]. The helices spanning residues Ile229-Leu358 forms hydrophobic core and the chain of polar residues Lys264, Lys319, Thr326, Thr259, Thr316, Ser255, Ser322, Ser262, Trp323, and Tyr244 may facilitate entry of dioxygen.

Transfer of electrons for reducing the substrate, dioxygen, held between heme a_3-Fe and CuB in the binuclear center is the apparent role of the π-H pathways. These two metal centers are connected to CuA, the source of electrons, with two direct pathways to CuB, and two to heme a_3-Fe (one direct and one via heme *a*-Fe), coincidentally providing two electrons on either side of the dioxygen.

A binuclear center holding dioxygen offers a model for adding twin electrons on each oxygen atom to form water. Hemin molecule catalyzing oxygen reduction by dithiothreitol (DTT) exhibited cooperativity [29]. Based on this, a complex represented as dithiol-heme-O=O-heme-dithiol was proposed as the active species of the reaction that yields water, the fully reduced product with no sign of partially reduced oxygen. This is the only chemical model known to us typical of the terminal electron sink, the binuclear center, heme a_3-Fe-O=O-CuB, of cytochrome oxidase.

The π-H pathways utilize all the four levels of protein structure for their formation. Peptide bonds and the polar groups of side chains of amino acid residues (primary structure) are linked by hydrogen bonds (secondary structure), with the chain spanning the folded polypeptide in the interior (tertiary structure) as well as across subunits (quaternary structure) employing large area of polypeptide covering a large volume explaining the need for large proteins. They are ubiquitous in interior of globular proteins and more so in the hydrophobic core of membrane proteins wherein electron transfer and accompanying energy transfer occurs. Being linked by hydrogen bonds that facilitate electron transfer these unique pathways in proteins expectedly can act as molecular wires, as it were, over long distances and thus in multiple protein functions.

Authors declare

"No conflict of interest".

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