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Alternative pathway linked by hydrogen bonds connects heme-Fe of cytochrome *c* with subunit II-CuA of cytochrome *a*

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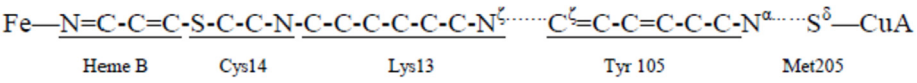
ABSTRACT

The bridging element for electron transfer in proteins is the hydrogen bond according to the new experimental perspective in preference to carbon-carbon σ-bond presently used. The purpose of this study is to identify an alternative pathway linked by hydrogen bonds suitable for electron transfer from heme-Fe of cytochrome *c* to subunit II-CuA of cytochrome *a*. A pathway consisting of 15 delocalized electron systems including peptide bonds, 5 polar groups of side chains of amino acid residues and 8 water molecules, linked by 27 hydrogen bonds, exists between the two metal electron centers of heme-Fe of cytochrome *c*, cytochrome *c* and of subunit II-CuA of cytochrome *a*. Pathways built of delocalized π-electron systems, polar groups and water molecules linked by hydrogen bonds may be considered for intramolecular and intermolecular electron transfer in proteins.

1. Introduction

The mitochondrial electron transport chain consists of multiple proteins (flavoproteins, iron-sulfur proteins, cytochromes) that carry the electrons of the oxidizable substrates down the energy gradient from negative to positive redox potential. Finally, four electrons are transferred from cytochrome *c* to cytochrome oxidase for reduction of dioxygen to two molecules of water [1–3]. The initial receiving center of electrons from heme-Fe (reduced form, 550 nm) of cytochrome *c* was identified as the subunit II-CuA (oxidized form, 830 nm broad [4]) of cytochrome *a* [5,6]. The two metal electron centers, are embedded 23 Å apart in the respective proteins. The path connecting them, by implication, traverses the intervening polypeptide structure.

The program of pathways plugin for visual molecular dynamics through proteins was developed to identify and tunneling pathways and calculate relative electronic couplings for electron transfer [7,8]. This was employed to trace "the most probable electron transfer pathway" between heme-Fe (coordinated to pyrrole-N atom of cytochrome *c*) and CuA (coordinated to Met207-S atom of subunit II of cytochrome *a*) [9]. This 41.9 Å long, atom-to-atom pathway shown below consists of 23 atoms, linked by 4 double bonds, 18 σ bonds of side chains of the amino acid residues and 2 through-space (…) jumps. It passes through hemeB-pyrrole ring, thioether-linked cys14, a peptide bond-linked Lys13 of cytochrome *c*. It then continues through space jumps, between Lys13-N^{ζ} to subunit II-Tyr105-C^{ζ}, and another between Tyr105-N^{α} and Met205-S^{δ} to reach CuA of subunit II of cytochrome *a*. Hydrogen bonds and internal water molecules, conspicuous in the interior of several proteins including cytochromes, are absent in the proposed pathway.



Evidence is now available supporting electron transfer in pathways of delocalized electron systems linked by hydrogen bonds. Lehn and coworkers [10] described that significant electron transfer occurred in a carbon chain of carioviolagen, because its conjugated

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double bonds are capable of resonance shifting to provide delocalized π-electrons over the length of the structure. By theoretical calculations, Chandra et al. [11] found that "delocalization of an extraneous electron is pronounced when it enters low-lying virtual orbital of the π-electronic structures of peptide units linked by hydrogen bonds." Electron coupling mediated by hydrogen bonds, in preference to carbon-carbon σ-bond, now received support from experiments on photo-induced transfer [12] and scanning tunneling microscopy [13]. According to this new perspective, hydrogen bond is the most likely bridging element in electron transfer pathways in proteins.

Water molecules present buried in cytochrome *c* [14] and also at its interface with subunit II [9] have been proposed to have functional significance. In the context of supporting their redox function, water molecules linked by hydrogen bonds were found to bridge the gaps in probable electron transfer pathways in cytochrome *c* [15]. This new perspective prompts searching for pathways involving the delocalized electron systems, hydrogen bonds and water molecules suitable for intramolecular and intermolecular electron transfer in proteins. Such an alternative pathway between heme-Fe of cytochrome *c* and CuA of cytochrome *a* linked by hydrogen bonds is reported here.

2. Methods used in identifying the pathways

Crystal structures of cytochrome *c* (1CRC) and of the complex of cytochromes *c and a* (5YI5) were analyzed using pymol software for 'Hydrogen Bonds' starting from heme by finding the residues less than 4 Å distance and marking all the polar atoms including the water molecules which are within the distance of 2.6–3.3 Å except for the unconventional hydrogen bond described for proline [16] with multiple conformations [17].

Hydrogen bonds provide connectivity to several delocalized electron systems with π-electron clouds [O═C–O–H (Asp, Glu), O═C–N–H (Asn, Gln and peptide bonds), –N═C–NH (His, Arg)], to polar groups of –NH (Lys) and of –OH (Ser, Thr, Tyr and to water molecules). These occur randomly in proteins. The pathways are identified by their sequence linked by hydrogen bonds occurring at appropriate distance. They are referred as π-H pathways [18], indicating the importance of their π-electron clouds and H-bonds.

The π-H pathway on the His18 side of the heme plate of cytochrome *c* is considered the oxidation route as this extends to the peptide bond (Lys86-Lys87) docked with the oxidant, ferricyanide, through a water molecule at the interface (PDB: 5C0Z).

3. Results

The purpose of this investigation is to identify a π-H pathway between the two metal electron centers of heme-Fe of cytochrome *c* and of subunit II-CuA of cytochrome *a*. The pathway follows the oxidation route that facilitates transfer of electron from the reduced form of cytochrome *c* protein [15]. The pathway is traced by the polar side chains and peptide bonds of amino acid residues with water molecules bridging the gaps that occur in the two proteins at appropriate distances and linked by hydrogen bonds (Fig. 1).

The pathway starts from heme-Fe of cytochrome *c* and passes through the axially coordinated His18 followed by two peptide bonds Pro30-Asn31 and Pro30-Gly29. Hydrogen bonds link these three delocalized electron units and a water molecule to initiate the pathway. The connectivity of the path between Asn31-NH and Pro30-N is made possible by the intervention of an unconventional N–H––N hydrogen bond of proline. This unique segment of the atom-to atom pathway shown below is understandably conserved in cytochrome *c* proteins obtained from a variety of sources [15].

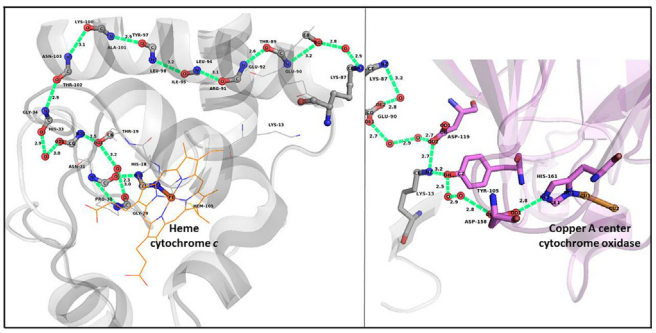
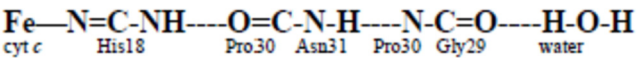


Fig. 1. Pathway between cytochrome c-heme-Fe and cytochrome oxidase subunit II-CuA linked by hydrogen bonds. The sections of the pathway in cytochrome *c* (1CRC) (left panel) and in the complex of cytochromes *c and a* (5YI5) (right panel) with the common point at Lys87-N at the interface (thin vertical line). The atoms involved in the path are identified as colored spheres [oxygen (red), nitrogen (blue), carbon (light grey), sulfur (yellow) and iron (brown)] with the respective names and water. The heme plate (orange line), the polypeptide backbone and the secondary structure (light grey cartoon) are shown in the background. Hydrogen bonds (green broken lines) connect the whole pathway.



One structural feature observed was the punctuating appearance of water molecules which indeed provide bridges between polar groups and delocalized electron systems at certain points, all linked by hydrogen bonds for continuity of the pathway. The pathway is extended by the water molecule with Thr19 and Asn31 and another water molecule which then extends the pathway by hydrogen-bonded string of 7 peptide bonds, 6 of which form part of suprahelix [19], on the C-terminal α-heix, reaching the interface through a water bridge, Thr89 and Lys87. The atom-to-atom connectivity in the pathway between the two metal centers is shown in Fig. 2.

Interface water molecules bridge the pathway between the two proteins at Lys87 and Glu90 (both of cytochrome *c*) and Asp119 (subunit II-cytochrome *a*). Here, Lys13 of cytochrome *c* comes into position to bridge Asp119 and Tyr105 from where it proceeds via two water molecules, Asp158 and His161, and finally reaches by coordination the CuA center.

The full pathway thus formed between Fe and Cu atoms is 120.2 Å long and consists of 27 hydrogen bonds, 15 delocalized electron systems including 9 peptide units, 2 amino groups (lysines), 11 hydroxy groups (2 threonines, 1 tyrosine, 8 water molecules), with a total of 85 atoms including H-atoms (Figs. 1 and 2).

4. Discussion

The pathway of delocalized electron systems, polar groups and

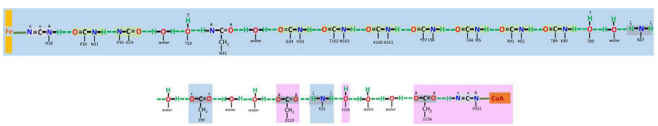


Fig. 2. Atom to atom π-H pathway between heme-Fe of cytochrome *c* and CuA of subunit II-cytochrome oxidase. The pathway is built based on the structure analysis of horse cytochrome *c* (1CRC) and cytochrome oxidase (5IY5). Cytochrome *c* (blue background); cytochrome *c* oxidase subunit II (pink background); interface (no background). Identification of the atoms is the same as in Fig. 1. The unconventional hydrogen bond of pro30 and the hydrogen bond between His18 and Pro30 are included to arrive at this pathway.

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water molecules assembled by hydrogen bonds (120.2 Å) ensures electron transfer between the two metal centers (23.0 Å), albeit longer than the pathway of carbon-carbon σ-bonds and through space jumps (41.9 Å).

The invariant and irreplaceable amino acid residues, His18, Pro30, Lys87 and Lys13 of cytochrome *c* [14] and Tyr105 and His161 of subunit II of cytochrome *a* [9] are now found to play essential roles in pathway connectivity between the two proteins.

Internal and interface water molecules of cytochrome *c and a* are known to 'construct hydrogen bond network' between the two proteins and purported to have redox function [9,14]. Bridging gaps in the hydrogen-bonded pathway by these water molecules explains their essential role in redox function.

The four structural features of a protein, peptide bonds, side chain polar groups, hydrogen bonds and folding, covering a large number of amino acid residues and spanning a large volume of the protein are implicated in this pathway formation.

Declaration

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Conflicts of interest

None.

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References

[1] S.H. Speck, D. Dye, E. Margoliash, Single catalytic site model for the oxidation of ferrocytochrome *c* by mitochondrial cytochrome *c* oxidase, Proc. Natl. Acad. Sci. U.S.A. 81 (1984) 347–351.

[2] K. Sakamoto, M. Kamiya, M. Imai, K. Shinzawa-Itoh, T. Uchida, K. Kawano, S. Yoshikawa, K. Ishimori, NMR basis for interprotein electron transfer gating between cytochrome c and cytochrome c oxidase, Proc. Natl. Acad. Sci. U.S.A. 108 (2011) 12271–12276.

[3] S. Ferguson-Miller, D.L. Brautigan, E. Margoliash, Correlation of the kinetics of electron transfer activity of various eukaryotic cytochromes c with binding to mitochondrial cytochrome c oxidase, J. Biol. Chem. 251 (1976) 1104–1115.

[4] I. Szundi, G.L. Liao, O. Einarsdóttir, Near-infrared time-resolved optical absorption studies of the reaction of fully reduced cytochrome oxidase with dioxygen, Biochemistry 40 (2001) 2332–2339.

[5] P. Rich, Mitochondrial cytochrome c oxidase. Catalysis, coupling and controversies, Biochem. Soc. Trans. 45 (2017) 813–829.

[6] S. Yoshikawa, A. Shimada, Reaction mechanism of cytochrome c oxidase, Chem. Rev. 115 (2015) 1936–1989.

[7] W. Humphrey, A. Dalke, K. Schulten, VMD: visual molecular dynamics, J. Mol. Graph. 14 (1996) 27–38.

[8] I.A. Balabin, X. Hu, D.N. Beratan, Exploring biological electron transfer pathway dynamics with the pathways plugin for VMD, J. Comput. Chem. 33 (2012) 906–910.

[9] S. Shimada, K. Shinzawa-Itoh, J. Baba, S. Aoe, A. Shimada, E. Yamashita, J. Kang, M. Tateno, S. Yoshikawa, T. Tsukihara, Complex structure of cytochrome c–cytochrome c oxidase reveals a novel protein–protein interaction mode, EMBO J. 36 (2017) 291–300.

[10] T.S. Arrhenius, M. Blanchard-Desce, M. Dvolaitzky, J.-M. Lehn, Molecular devices: carioviologens as an approach to molecular wires-synthesis and incorporation into vesicle membranes, Proc. Natl. Acad. Sci. Unit. States Am. 83 (1986) 5355–5359.

[11] A.K. Chandra, B.S. Sudhindra, M. Vijayan, T. Ramasarma, A theory on the migration of an extraneous electron across hydrogen bonds in polypeptides, J. Theor. Biol. 74 (1978) 1–9.

[12] P.J.F. De Rege, S.A. Williams, M.J. Therien, Direct evaluation of electronic coupling mediated by hydrogen bonds, implications for biological electron transfer, Science 269 (1995) 1409–1413.

[13] T. Nishino, N. Hayashi, P.T. Bui, Direct measurement of electron transfer through a hydrogen bond between single molecules, J. Am. Chem. Soc. 135 (2013) 4592–4595.

[14] G.W. Bushnell, G.V. Louie, G.D. Brayer, High-resolution three-dimensional structure of horse heart cytochrome *c*, J. Mol. Biol. 214 (1990) 585–595.

[15] T. Ramasarma, D. Vaigundan, Hydrogen bond-linked pathways of peptide units and polar groups of amino acid residues suitable for electron transfer in cytochrome *c* proteins, Mol. Cell. Biochem. (2018), https://doi.org/10.1007/s11010-018-3445-2 in press.

[16] R.N.V.K. Deepak, R. Sivaramakrishnan, Unconventional N-H…N hydrogen bonds involving proline backbone nitrogen in protein structures, Biophys. J. 110 (1967) 1967–1979.

[17] G.J. Bartlett, A. Choudhary, R.T. Raines, D.N. Woolfson, $n \rightarrow \pi^*$ interactions in proteins, Nat. Chem. Biol. 6 (2010) 615–620.

[18] T. Ramasarma, A perspective of biological supramolecular electron transfer, Indian J. Biochem. Biophys. 36 (1999) 379–397.

[19] T. Ramasarma, M. Vijayan, Suprahelical arrangements of hydrogen bonds in peptide helices, FEBS Lett. 41 (1974) 307–309.

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