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Hydrogen bond-linked pathways of peptide units and polar groups of amino acid residues suitable for electron transfer in cytochrome *c* proteins

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Abstract
Electron transfer occurs through heme-Fe across the cytochrome *c* protein. The current models of long range electron transfer pathways in proteins include covalent σ-bonds, van der Waals forces, and through space jump. Hydrogen-bond-linked pathways of delocalized electron units in peptide units and polar side chains of amino acid residues in proteins and internal water molecules are better suited for intramolecular atom-to-atom electron transfer in proteins. Crystal structures of cytochrome *c* proteins from horse (1HRC), tuna (3CYT), rice (1CCR), and yeast (3CX5) were analyzed using pymol software for 'Hydrogen Bonds' marking the polar atoms within the distance of 2.6–3.3 Å and tracing the atom-to-atom pathways linked by hydrogen bonds. Pathways of hydrogen-bond-linked peptide units, polar side chains of the amino acid residues, and buried water molecules connect heme-Fe through axially coordinated Met80-S and His18-N have been traced in cytochrome *c* proteins obtained from horse, tuna, rice and yeast with an identical hydrogen-bonded sequence around the heme-Fe: Asn-N–water-O–Tyr-O–Met-S–heme-Fe–His (HN–C=N)–Pro-Asn-Pro-Gly (peptide unit, HN–C=O)—water-O. More than half of the amino acid residues in these pathways are among the conserved list and delocalized electron units, internal water molecules and hydrogen bonds are conspicuous by their presence.

Keywords Cytochrome *c* · Delocalized electron units · Polar side chains · Hydrogen bonds · Electron transfer · Atom-to-atom pathways

Introduction

Cytochrome *c* is a typical example of electron transfer proteins. Its heme-Fe, held by the four pyrrole nitrogen atoms, undergoes redox changes characterized by differential absorption spectra. It is a water soluble basic protein present ubiquitously on the outer surface of the inner membrane of mitochondria in animal cells and is one of the most conserved proteins [1]. Cytochrome c is in position to receive electrons from cytochrome bc_1 and pass them to cytochrome aa_3 to reduce dioxygen to water [2]. Electrons need to travel across the protein to reach and depart from heme-Fe. The purpose of the study is to find pathways suitable for electron transfer in cytochrome *c* proteins.

Structural elements in intramolecular electron coupling in a protein

Directed electron transfer in a path of "way in, a trap and way out" occurs in proteins, including enzymes, according to Moore and Williams [3]. Proteins in bulk, however, are insulators as the band gap (activation energy) is high 3 eV and above, and this increased further on denaturation [4]. Long-range electron transfer in selective polypeptide structures and the pathway models was revived in 1990 [5–7]. Electron transfer rates were measured by

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using a flash-quench technique from heme-Fe to ruthenium-liganded histidine residues in the surface of cytochrome c protein [7, 8]. Gray et al. [5] included some hydrogen bonds and many covalent bonds for connectivity in the proposed pathways. In contrast to earlier conservative distancing, covalent σ-bonds, van der Waals forces, and through space jump are now included in the suggested electron transfer pathways in cytochrome *c*.

The concept of molecular wire for electron transfer

Lehn and coworkers demonstrated electron transfer across membranes by placing synthetic modified carotenes, called carioviologens, having conjugated double bonds and 'electroactive ends' in the β-ionone rings in vesicles [9]. Resonance shifting of the alternate double bonds in a conjugated chain delocalizes the π-electrons over the entire structure facilitating electron transfer over the distance. This led to the concept of 'molecular wire'. With no delocalization, carbon chains with covalent bonds are likely to be poor for electron transfer.

Application of the molecular wire concept for long range electron transfer in proteins needs several small units of π-electron clouds bridged into a pathway. A protein can be viewed as a polypeptide chain of repeating alternate peptide units (O=C–N–H) and Cα atom of amino acid residues. The delocalized π-electrons of peptide units would have made the polypeptide chain a good molecular wire but for the poorly-conducting σ-carbon bonds of the linking Cα atoms. The ubiquitous α-helix in proteins has an intrinsic helical chain of hydrogen bonds, referred as 'soliton' [10] as a constituent of the helical sequence of alternating peptide group and hydrogen bond [···O=C–N–H···O=C–N–H], named 'suprahelix' [11], indicating its existence over a helix. Such suprahelical sequences can facilitate transfer of electrons provided the π-clouds of the peptide units are bridged by the connecting hydrogen bonds.

Hydrogen bond in electron transfer

By molecular orbital calculations, Chandra et al. [12] found 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", thus supporting electron transfer.

Experimental support for hydrogen bond facilitating electron transfer became available with the early report by Therien et al. [13] including a firm statement: "In contrast to generally accepted theory, electron coupling modulated by hydrogen-bond interface is greater than that provided by an analogous interface composed entirely of carbon–carbon σ-bonds". Nishino et al. [14] recently reiterated that "a H-bond conducts electrons better than a covalent σ bond at short range" based on the experiments with scanning tunneling microscopy measuring the electron transfer "via spontaneous formation of chemical interaction between the tip and sample molecules". These unambiguous new experimental findings support hydrogen bonds, rather than covalent σ bonds, as bridging elements in atom-to-atom electron transfer pathways for intramolecular electron transfer in proteins. We report here that such pathways are present in cytochrome *c* proteins.

Methods used in identifying the pathways

Crystal structures of oxidized forms of cytochrome *c* proteins from different sources (PDB file names in parenthesis), animal-horse (1HRC), fish-tuna (3CYT), plant-rice (1CCR), and microorganism-yeast (*Saccharomyces cerevisiae*) (3CX5), were analyzed using pymol software for 'Hydrogen Bonds' starting from heme by finding the residues less than 4 Å distance and manually marking all the polar atoms including the water molecules which are within the distance of 2.6–3.3 Å except the unconventional one. The unconventional hydrogen bonds described for proline [15] and methionine [16], and buried water molecules are included to make connections in the pathways.

Results

Hydrogen bond-linked pathways in cytochrome c proteins

Heme-Fe, embedded in the surrounding protein with only an edge exposed, is reduced on arrival of an exogenous electron and is reoxidized when the electron departs. Separate paths appear to exist for entry and exit of an electron in horse heart cytochrome c protein as they can be separately blocked by modifying amino acid residues and by specific antibodies [2]. Thus the passing electron travels across the protein. Coordinated to the four pyrrole nitrogen atoms, Fe in the heme is axially connected to His18-N and Met80-S on either side of the plate [17], a conserved structural feature in cytochromes *c*. Together these must be serving as a platform connected to tracks for assisted transfer of electrons using atoms that form hydrogen bonds. We identified, for the first time, pathways consisting of delocalized electron units (peptide and polar groups of side chains of amino acid residues, linked by hydrogen-bonds in cytochromes *c* proteins passing through Met80-S—heme-Fe—His18-N on the two sides of the heme plate to the surface) (Fig. 1). Connectivity by hydrogen bonds, but not by covalent bonds, is the distinctive feature of these pathways.

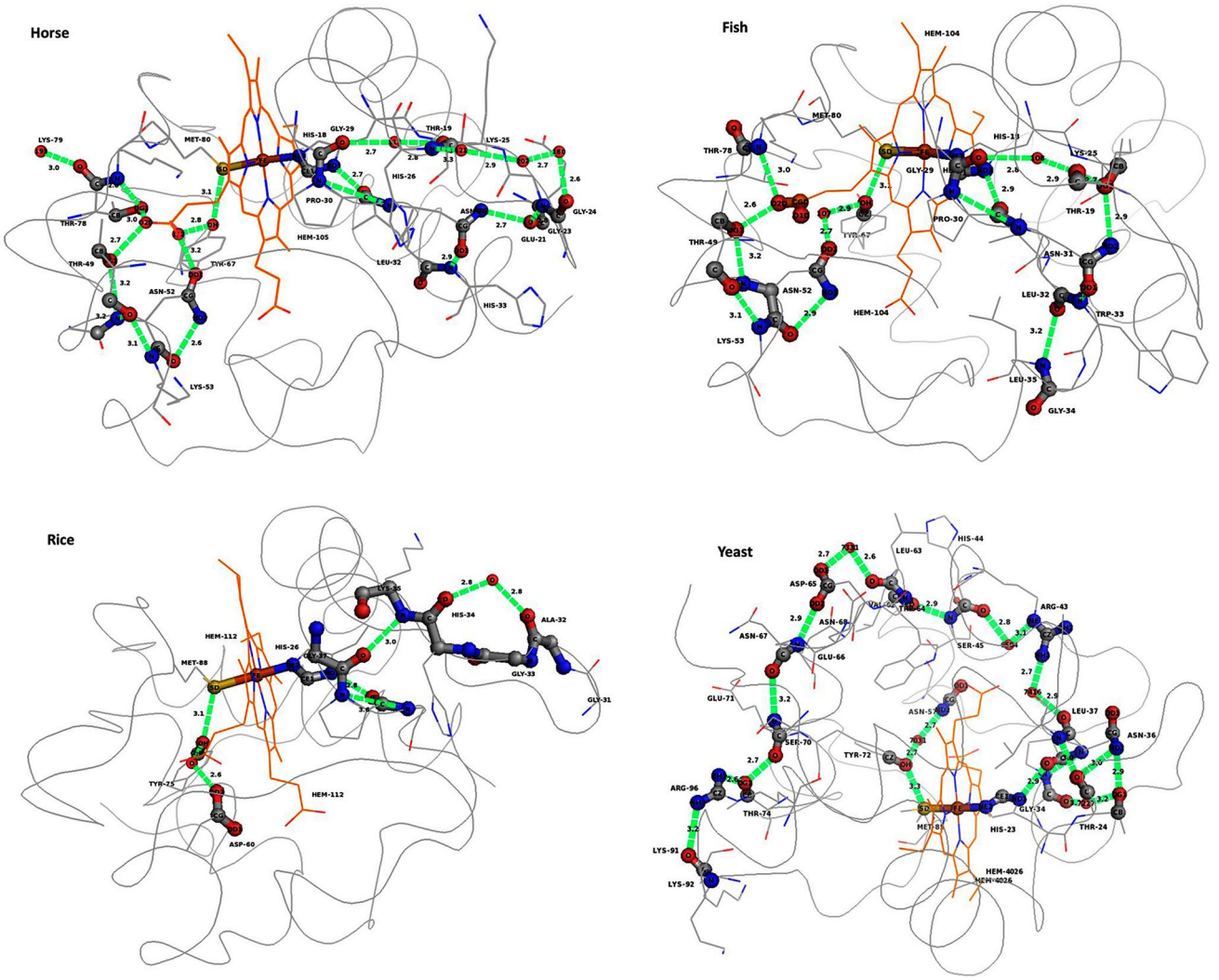


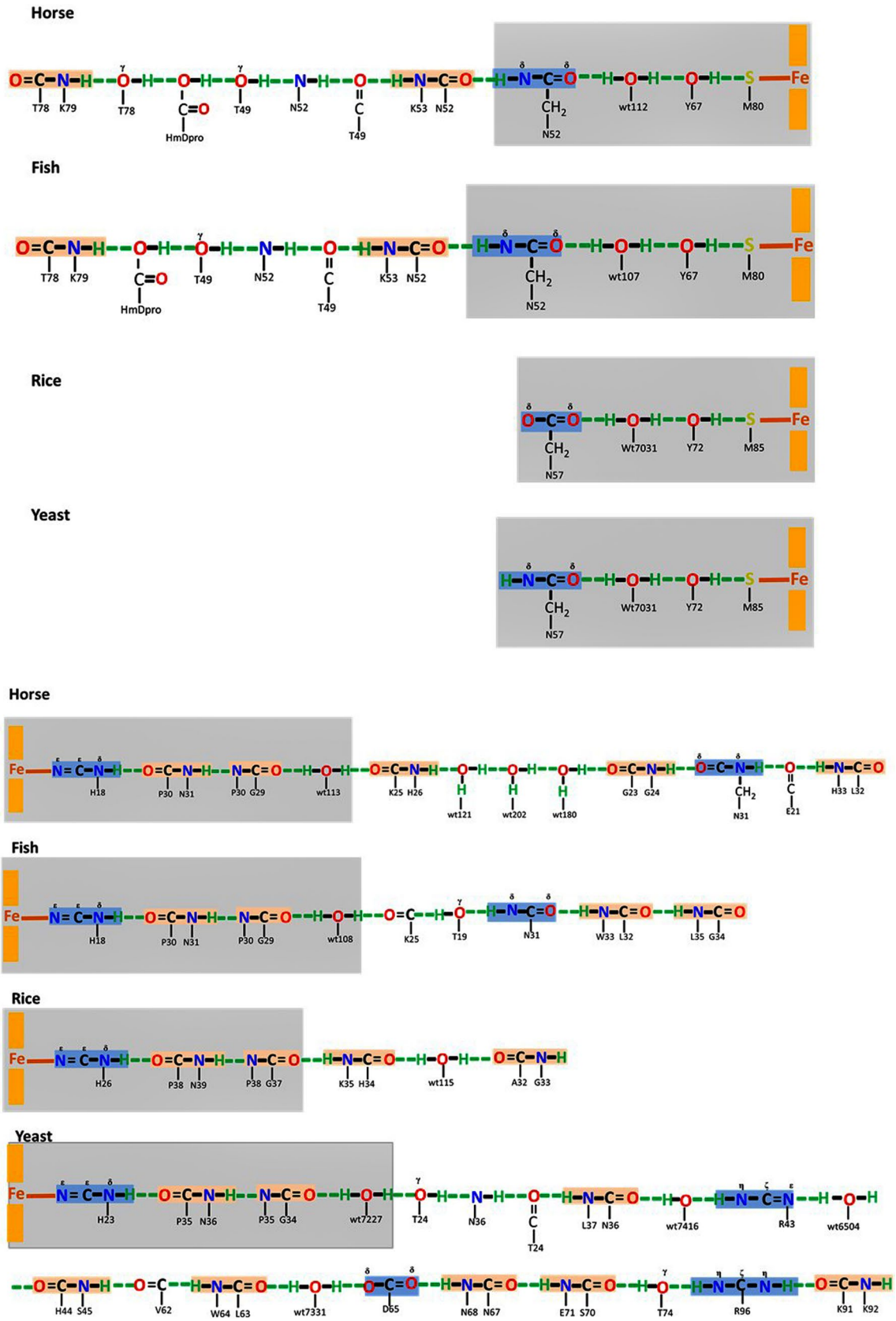
Fig. 1 Pathways linked by hydrogen bonds through heme-Fe in cytochrome c proteins. Hydrogen bonds are shown as green broken lines. The atoms involved in the path are identified as colored spheres [oxygen (red), nitrogen (blue), carbon (light gray), sulfur (yellow), iron (brown)] labeled with the respective name and water (wt)-oxygen with the number. The heme plate is shown in orange line with blue for the pyrrole-nitrogens. The polypeptide backbone is shown as light gray wire in the background. (Color figure online)

Common features of the pathways in cytochrome c from different sources

In the four sources examined the common features are delocalized electron units (peptide units and side chains of histidine, arginine, asparagine, and aspartate), polar side chains (tyrosine-OH, threonine-OH, lysine-NH), buried conserved water molecules (wt)-O, heme-propionate (HmDpro)-OH, all linked by hydrogen bonds (Fig. 2). More than half of the amino acid residues in these pathways are among the conserved list. Delocalized electron units and internal water molecules along with hydrogen bonds are significant by their presence.

Pathways dependent on H-bonds and covalent bonds in linking with His33: a comparison

The pathway emerging from His18 includes His33, one of the ruthenium-modified histidine residues found to be in electronic coupling with heme-Fe [7, 8]. These experiments gave evidence for electron transfer from His18 near the heme-Fe to His33 near the surface. Two pathways are shown together in Fig. 3 for comparison. The pathway described here (A) depends on 10 hydrogen bonds, 4 delocalized electron units. 4 water molecules and no Cα-atom for connectivity. The other pathway (B) used 10 covalent bonds, 1 hydrogen bond, 3 Cα-atoms, but no water molecule, in order to fit with the observed electron transfer to


Horse
T78 K79
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Y67
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Rice
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Wt7031
Y72
M85
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G23 G24
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K35 H34
wt115
A32 G33
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T24
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L37 N36
wt7416
R43
wt6504
Fe
H44 S45
V62
W64 L63
wt7331
D65
N68 N67
E71 S70
T74
R96
K91 K92

◂**Fig. 2** Pathways of atoms O, N, C in delocalized units and side-chains of amino acid residues connected by hydrogen bonds in the cytochrome *c* proteins. The notations are the same as in Fig. 1. Delocalized electron units are shown as follows: peptide units (O=C–N or N–C=O) (orange); His (N^{ε}–C^{ε}–N^{δ}), Asn (N^{δ}–C^{δ}=O) (blue); side chain atoms are identified by their position as superscript; C=O and N–H shown in the pathway correspond to the peptide groups (peptide bond-NH of N52 is hydrogen-bonded to the peptide bond-C=O of T49). The early sections of the pathways on either side of heme-Fe shaded light gray are identical for atleast upto three hydrogen bonds and 10 atoms. (Color figure online)

ruthenium-modified His33 [7, 8]. These flash-quench experiments support both the pathways involving electron transfer between His18 and His33 and the pathway is more efficient in electron transfer when linked with hydrogen bonds compared to covalent σ-bonds [13, 14].

Identical initial segments of the pathways

The initial segments of the pathways (Fig. 4) linking heme-Fe are nearly identical. The internal water molecules near the heme plate, bound by hydrogen bonds with conserved amino acid residues, provide connectivity in the initial segments of the pathways. Without them the pathways remain incomplete. The role of buried water molecules

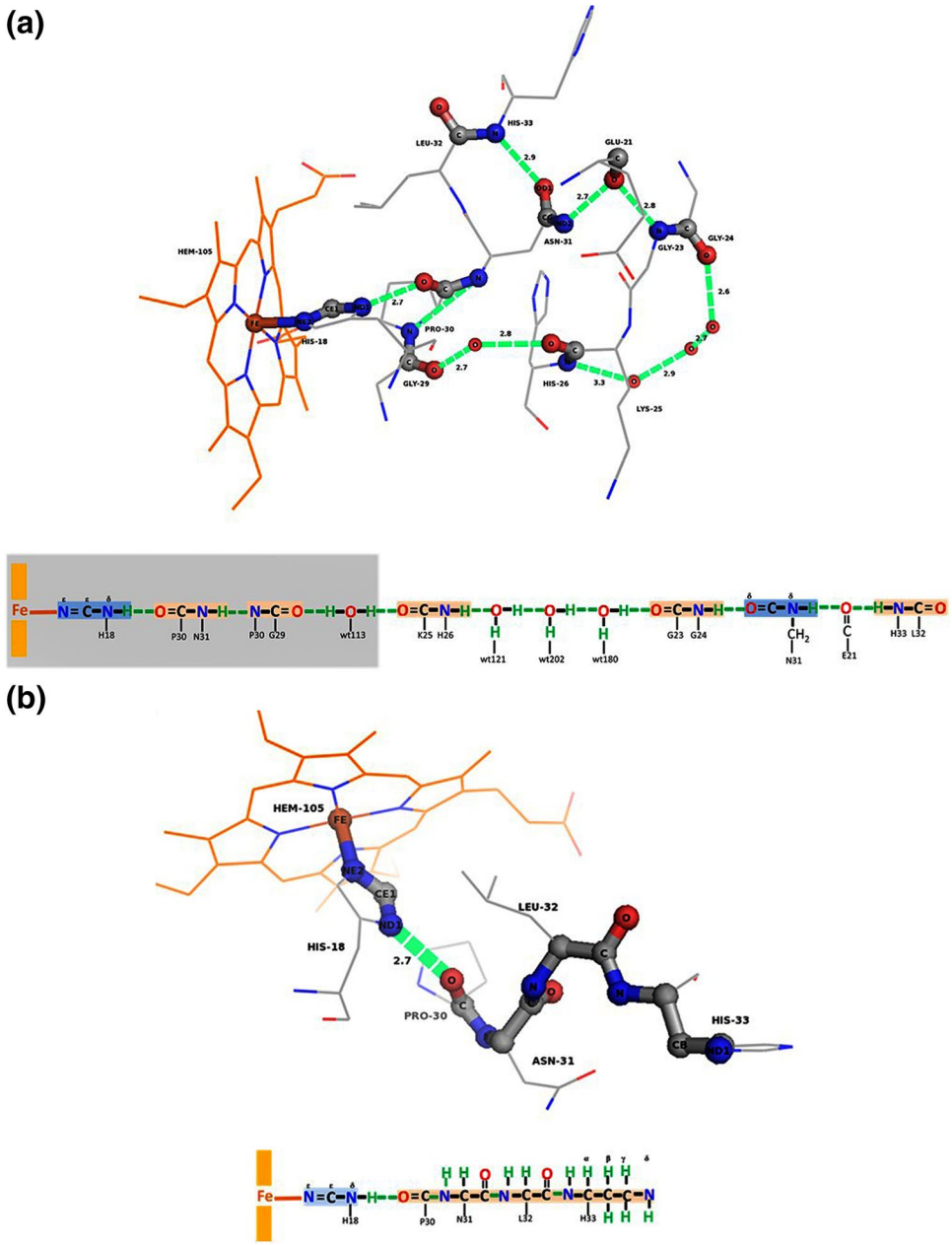

Fig. 3 Pathways dependent on hydrogen bonds and covalent bonds connecting His18 and His33. Notice 11 hydrogen bonds in pathway A and 10 covalent bonds in pathway B for connecting the two histidine residues

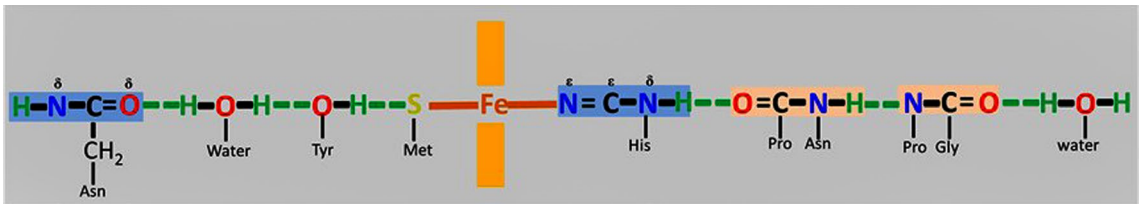

Fig. 4 Identical initial segments of the pathways in cytochrome c in the four sources

Table 1 A comparison of the numbers of the constituents of conserved amino acid residues, water molecules, delocalized electron units (peptide units and polar side chains) and hydrogen bonds in the pathways through heme-Fe of cytochrome c proteins

Source (PDB)	Conserved/total amino acid residues	Bound water molecules	Hydrogen bonds	Peptide units	Polar side chains	Total atoms
Horse heart (1HRC)	10/18	5	21	6	3	64
Tuna fish (3CYT)	12/16	2	17	5	3	55
Rice (1CCR)	7/10	2	8	3	2	31
Yeast (3CX5)	14/24	6	21	6	5	77

in the redox function, suspected for a long time, is thus explained [17].

A comparative distribution of the constituents of the pathways present in these four sources is given in Table 1. Of the total number of amino acids in the range of 104–114 in cytochrome c proteins from over 100 sources analyzed, 38 amino acids are invariant of which 19 are identical in all. The core axial ligand Met80-S–Fe–N-His18 in the heme is fully conserved. The structural elements are similar such that polypeptide backbones fold in cytochrome c proteins with good overlap [1]. In view of these common features it is unsurprising that similar hydrogen-bond-linked pathways connecting delocalized electron units and polar side chains occur in all cytochrome c proteins known for electron transfer.

Discussion

Modification of Tyr67-OH in horse heart protein is known to block reduction of the heme-Fe, but not it oxidation [2], and it is the first to be connected to Met80-S. This can be the pathway for reduction is suggestive. The His18 branch, we surmise, is the oxidation route.

Some branches with multiple ends at the surface occur in the pathways (not shown) probably to accommodate multiple interactions cytochrome *c* is known to have with other mitochondrial cytochromes (bc_1 and aa_3) [18]. In a preliminary study we found similar pathways in these proteins with some links to those in cytochrome *c* (unpublished data) indicating extended electron transport chain.

The buried water molecule, wt112 (horse heart), connects with three conserved residues of Tyr67, Asn52, and Thr78 through hydrogen bonds [19–21], and all these are held close to the heme. This water molecule, along with hydrogen-bond-linked Tyr67 and Asn52, moves consistent with the redox state of cytochrome *c* and is believed to "participate in the mechanism of action of cytochrome *c*" [17, 19]. Interconnection of Met80-S, Tyr67-O, wt112-O, Asn52-N by hydrogen bonds was recorded earlier [19], sans recognition of electron transfer potential.

Delocalized electron systems such as O=C–O–H (Asp, Glu), O=C–N–H (peptide group, Asn, Gln), –N=C–NH (His, Arg) and other reactive groups such as C=O, N–H, O–H, and S–H present in amino acid residues do interconnect by hydrogen bonds into a pathway. Indicating the important constituents of π-electron clouds and H-bonds, these are referred as π-H pathways [22]. Coincidentally π-H pathways utilize the four basic structural features of protein, peptide bond, side chain polar groups, hydrogen bond, and folding. These pathways exist in proteins in general and their functions are not known.

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Compliance with ethical standards

Conflict of interest T. Ramasarma and D. Vaigundan declare that none of them have any conflict of interest.

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