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On the Evolutionary Relationship of the 4-α-Helical Heme Proteins

THE COMPARISON OF CYTOCHROME b_{562} AND CYTOCHROME *c'**

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The atomic models of the cytochrome b_{562} and cytochrome *c'* monomers have been compared. When the respective heme groups are superimposed, the four α-helices of each nearly coincide. Four aromatic side chains, including the heme ligands, and a methionine occur in spatially equivalent positions in contact with the heme groups. This structural evidence suggests that the two cytochrome families may have diverged from a common molecular ancestor.

Proteins exhibiting similarities in amino acid sequence and function are generally assumed to have arisen through processes of divergent evolution from a common molecular ancestor. X-ray structural studies of members of such protein

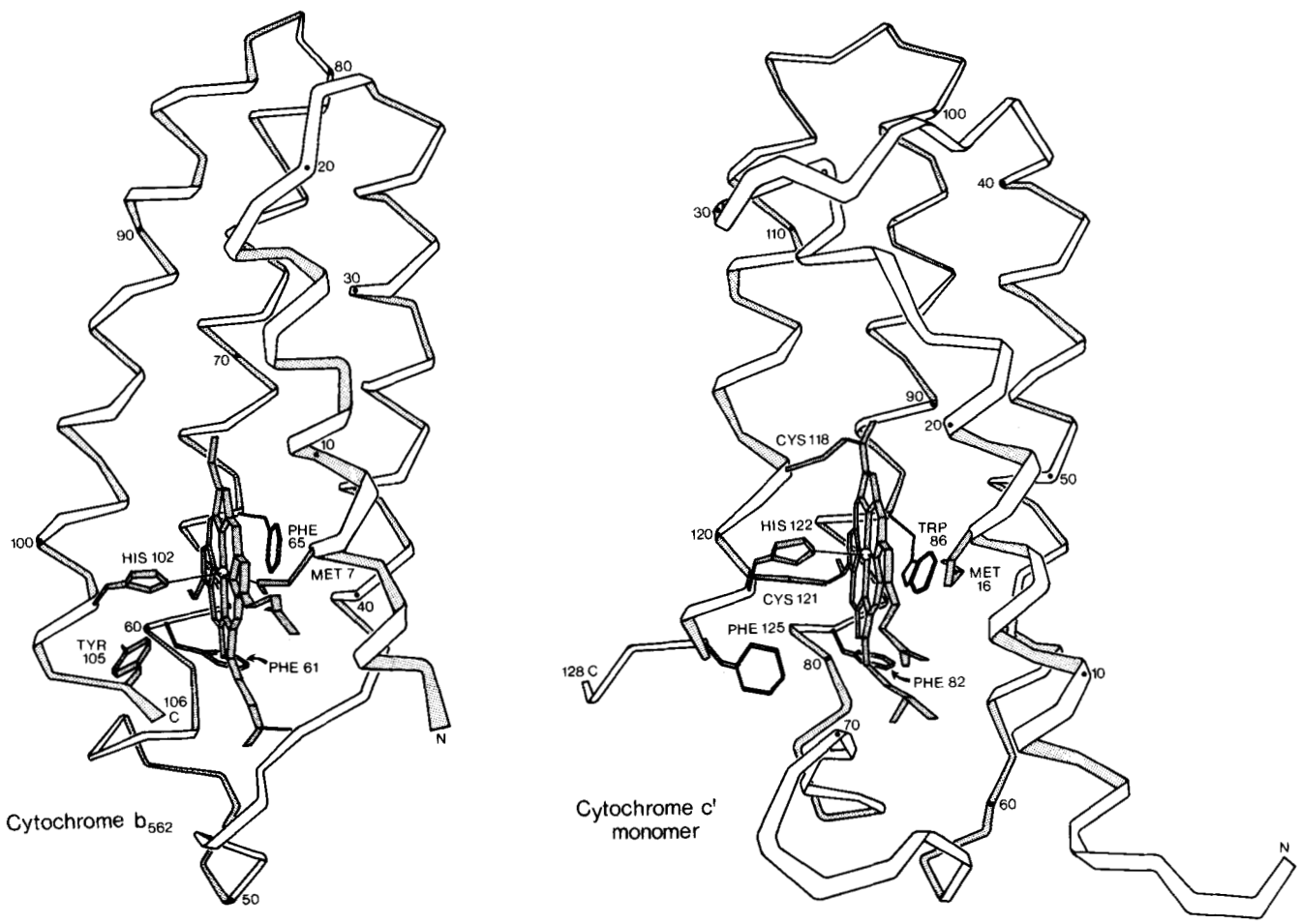

FIG. 1. **Schematic diagrams of *E. coli* cytochrome b_{562} and the *R. molischianum* cytochrome *c'* monomer.** Both molecules are structurally organized as left-twisted bundles of four, sequentially connected, nearly parallel α-helices in which the heme prosthetic groups are situated in the singly connected end of the divergent helical arrays. The low spin heme iron in cytochrome b_{562} is axially ligated by Met 7 and His 105 residues, whereas His 122 provides the single axial ligand in the high spin cytochrome *c'*. Additional thioether linkages formed between cysteine residues, 118 and 121, and the heme vinyl groups, covalently connect the heme and cytochrome *c'* polypeptide. Also shown are aromatic residues adjacent the heme groups in each protein, as well as Met 16 in cytochrome *c'*. In each drawing, every 10th residue is labeled and marked with a *dot*.

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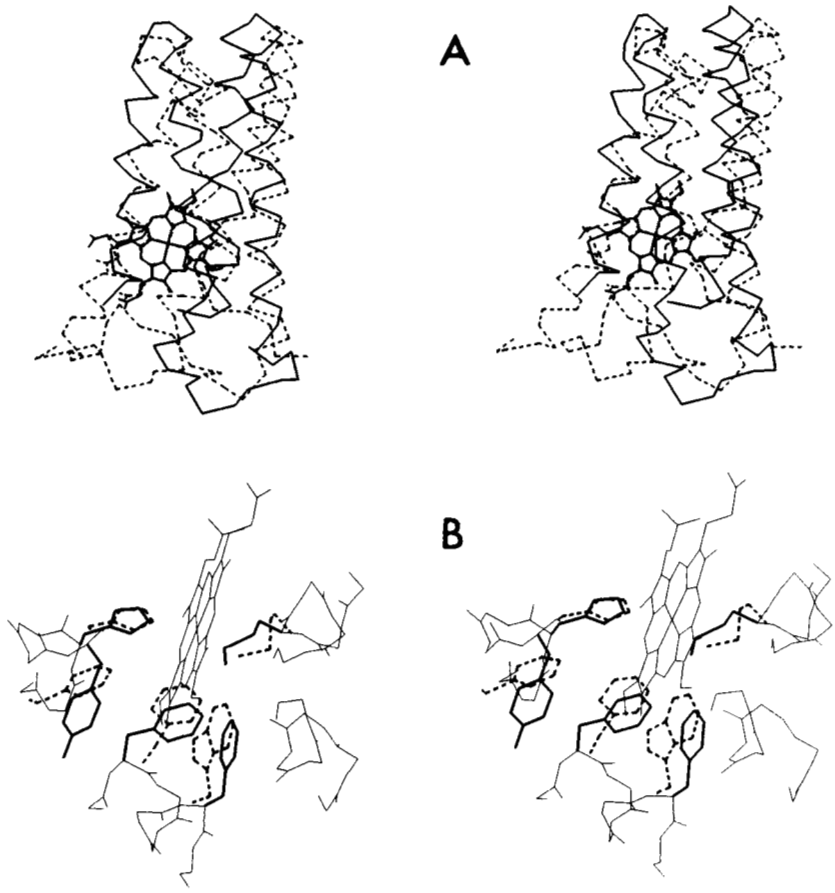

FIG. 2. **Stereoscopic views of cytochrome b_{562} and cytochrome c' superimposed.** *A* shows the resultant correspondence between α-carbons of cytochrome b_{562} and cytochrome *c'* (*dashed*) when the heme groups of the two molecules are superimposed in a graphics system (36). In cytochrome b_{562}, 77 residues, 1–15, 26–43, 58–79, and 84–105, are structurally homologous to residues 10–24, 40–57, 79–100, and 104–125 in cytochrome *c'*. Amino acid identities between molecules occur at 14 of the 77 positions, *i.e.* at Met 7, Thr 9, Leu 10, Met 33, Ala 37, Ala 40, Phe 61, Gly 64, Leu 68, Ala 79, Ala 89, Ala 91, Ala 100, and His 102 (cytochrome b_{562} numbering, see Fig. 1). The α-carbon backbones of the two molecules primarily differ in the lengths of the nonhelical regions. For example, in the cytochrome *c'* molecule which is 22 residues longer than cytochrome b_{562}, extra residues are located at the NH_2 and carboxyl termini, as well as in the loops between the first and second, and second and third helices. Cytochrome b_{562}, in contrast, has an extra residue between the third and fourth helices. *B* shows the arrangement of aromatic residues adjacent to the heme groups of cytochrome b_{562} and cytochrome *c'* (*dashed*) superimposed on portions of the polypeptide backbone of cytochrome b_{562}. The fifth axial heme iron ligands, His 102 in cytochrome b_{562} and His 122 in cytochrome *c'*, and the aromatic residues, Phe 61, Phe 65, and Tyr 105 in cytochrome b_{562} and Phe 82, Trp 86, and Phe 125 in cytochrome *c'*, all share similar spatial orientations relative to their respective heme groups. In addition, the *R. molischianum* cytochrome *c'* molecule contains a methionine residue (Met 16) proximal to the high spin heme iron which, unlike the situation in the low spin cytochrome b_{562} where Met 7 forms the sixth axial ligand to the low spin heme iron, does not ligate the heme iron.

families have further shown that this relationship is manifest in the preservation of the tertiary folding pattern among molecules within a given family (1–6). On the other hand, some tertiary structural arrangements are observed to recur among proteins which lack any apparent similarities in either sequence or function (7–9). In these cases, the recurrence of a particular folding arrangement might be viewed to result from independent evolution of the molecules, so that they eventually converge upon a tertiary arrangement of particular structural stability (10–17). In the present communication, we describe some comparative properties of two structurally similar heme-containing proteins, *Escherichia coli* cytochrome b_{562} and *Rhodospirillum molischianum* cytochrome *c'* (*c*-prime), and assess their possible evolutionary relationship.

Cytochrome b_{562} is a monomeric $M_r \sim 12000$ protein isolated from the soluble extract of *E. coli* (18). The molecule contains a single noncovalently bound protoheme IX prosthetic group whose iron atom is axially ligated by the strong field ligands histidine and methionine, so resulting in the formation of a low spin heme complex in both oxidized and reduced states. The cytochromes *c'* are derived from a variety of photosynthetic and denitrifying bacteria and are generally dimeric molecules composed of identical subunits of $M_r \sim 14000$ (19). In contrast to cytochrome b_{562}, the protoheme IX prosthetic group of cytochrome *c'* is covalently bound to the polypeptide chain through thioether linkages resulting from the condensation of two polypeptide cysteine side chain groups with the heme vinyls. Further, the heme iron in the cytochromes *c'* is axially ligated by a single histidine residue, so giving rise to a high spin heme complex in both molecular oxidation states. However, the oxidation-reduction potential of cytochrome b_{562} (113 mV) lies within the range observed for the cytochromes *c'* (0 to 140 mV).

Crystallographic studies of cytochrome b_{562} (20) and *R. molischianum* cytochrome *c'* (21) have shown these molecules to be structurally organized as sequentially connected, left-twisted bundles of four nearly parallel α-helices. Similarly connected helical arrangements occur in the monomers of hemerythrin (22, 23), tobacco mosiac virus coat protein (24, 25), and apoferritin (26), as well as in the carboxyl-terminal domain of T_4 phage lysozyme (27). The recurrence of this structural motif among these functionally and sequentially disparate proteins has been suggested to arise by convergent evolution as a consequence of sequence-independent factors

governing efficient interhelical packing and polypeptide chain chiral connectivity (17). However, comparison of the cytochrome b_{562} and cytochrome c' monomer structures reveals some similarities which suggest a reassessment of their evolutionary relationship, despite the apparently major differences in their modes of heme attachment and axial ligation. In both molecules, for example, the heme prosthetic groups are situated in the divergent, singly connected ends of their respective 4-α-helical bundles (Fig. 1). The heme groups are also similarly oriented so that their propionic acid side chains point toward the molecular surfaces, where they form hydrogen-bonded interactions or salt links to solvent or surface polar residues.

As shown in Fig. 2*A*, superposition of the heme groups of the two molecules results in a corresponding superposition of the majority of the α-helical regions of the structures. Further similarities exist in the arrangement of specific residues about each heme (Fig. 2*B*) despite the fact that the cytochrome b_{562} and *R. molischianum* cytochrome c' molecules exhibit amino acid identities at only 14 of the 77 structurally homologous positions (Fig. 2). Most notable are the nearly equivalent situations of the histidine residues which form the fifth axial heme iron ligands in both molecules (Figs. 1 and 2). In both cases, this ligand is located near the carboxyl terminus of the molecule and attaches to the same face of the asymmetric heme group. As a result of the similar overall orientation of the heme groups relative to the folded polypeptides, one of the heme faces shows an extensive degree of solvent exposure not observed in other heme protein structures. In addition, the exposure of the histidine heme ligand differs from that seen in most other heme proteins, where the fifth axial coordinate histidine forms a hydrogen-bonded interaction from Nδ1 to an internal backbone carbonyl group (28). The unusual solvent exposure of the axially coordinated histidine residue in both cytochromes b_{562} and c' is apparently manifest in their pH-dependent spectroscopic properties (29–31), *i.e.* both molecules undergo similar changes in their heme spectra at alkaline pH which current model studies (32) suggest may reflect deprotonation of the histidine Nδ1.

Although the heme iron of *R. molischianum* cytochrome c' lacks the sixth axial coordinate methionine ligand of cytochrome b_{562}, the former molecule has a methionine residue located near the heme iron. While this position is not conserved among all species of the high spin cytochromes c' (33, 34), it is to be noted that it is conserved in a sequentially homologous low spin relative, cytochrome c_{556} (35). In addition to actual or potential heme ligands, both cytochrome b_{562} and *R. molischianum* cytochrome c' share three aromatic residues with similar spatial orientations about their heme groups. These are the cytochrome b_{562} residues Phe 61, Phe 65, and Tyr 105 which approximately correspond to residues Phe 82, Trp 86, and Phe 125 in cytochrome c'. Of these residues, positions corresponding to Phe 82 in the *R. molischianum* sequence are occupied by Phe in all but one other cytochrome c' species, while that corresponding to Phe 125 is invariably occupied by Phe or Tyr in other species. In contrast, Trp 86 is not conserved among cytochrome c' species (33, 34).

To summarize, cytochrome b_{562} and cytochrome c' share striking similarities in the position, orientation, and chemical environments of their heme groups, despite the fact that the molecules differ in both their modes of heme attachment and ligation. Although the extent of sequence homology between the proteins is at best described as vestigial (Fig. 2), it is notable that comparisons of various cytochrome c' species also exhibit very limited extents of sequence homology (33). While this situation presumably reflects the relative insensitivity of the 4-α-helical structural arrangement to individual amino acid substitutions (17), the close correspondence in heme orientation, extent of exposure to solvent, and situation of aromatic residues adjacent the hemes would suggest that the cytochromes b_{562} and c' may have diverged from a common molecular ancestor.

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