# Cytochrome *c'*: A Dimeric, High-Spin Heme Protein

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The cytochromes *c'* constitute an unique class of bacterial heme proteins (Bartsch, 1978). With one exception (Dus et al., 1967), the cytochromes *c'* are isolated at ~28,000-MW dimers (Cusanovich, 1971). The heme iron of the covalently bound protoporphyrin IX prosthetic group is high spin in both oxidation states (Ehrenberg and Kamen, 1965; Rawlings et al., 1977), although the molecules' magnetic properties manifest several unusual features that differentiate them from other high-spin heme proteins (Maltempo et al., 1974; Maltmepo, 1976). Additionally, the cytochromes *c'* bind only the uncharged ligands CO and NO at the sixth iron coordination site (Taniguchi and Kamen, 1963; Gibson and Kamen, 1966), in contrast to most other high-spin heme proteins, which also bind charged axial ligands (Perutz, 1979). The cytochrome *c'* heme spectra is sensitive to solvent pertubations (Emptage, et al. 1977) and undergoes a reversible high- to low-spin conversion at alkaline pH or in the presence of organic solvents (Imai et al., 1969a; Strekas and Sprio, 1974; Kitagawa et al., 1977) without apparent loss of protein secondary structure (Imai et al., 1969b). Extensive amino acid sequence studies of various species of cytochrome *c'* indicate an unusual interspecific variability (Ambler et al., 1979). This variability is reflected in many of the spectroscopic and physiochemical properties of the molecule (e.g., Cusanovich and Gibson, 1973). Despite its relative abundance in bacterial cells (Bartsch and Kamen, 1960), cytochrome *c'* is difficult to detect in intact cells or membrane vesicles (Kakuno et al, 1971; Dutton and Leigh, 1973; Corker and Sharpe, 1975), which suggests that the spectroscopic properties of cytochrome *c'* as isolated may differ from those in vivo (Kakuno et al., 1971).

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## The Tertiary and Quaternary Structure of Cytochrome *c'*

The 2.5-Å resolution x-ray crystallographic analysis of *Rhodospirillum molischianum* cytochrome *c'* (Weber et al., 1980) has shown the subunits of this dimeric molecule to be structurally organized as a left-twisted array of four, nearly parallel $\alpha$ helices. The interconnected helices form a divergent bundle with the heme group packaged in its more open end (Weber and Salemme, 1980). Similar 4-$\alpha$-helical arrangements have been observed in the crystallographic structures of tobacco mosaic virus coat protein (Stubbs et al., 1977; Bloomer et al., 1978), the hemerythrin subunit (Hendrickson et al., 1975; Stenkamp et al., 1978), horse spleen apoferritin (Banyard et al., 1978) and the monomeric, low-spin cytochrome $b_{562}$ from *Escherichia coli* (Mathews et al., 1979). As we show elsewhere (Weber and Salemme, 1980), the common folding pattern exhibited by these molecules principally reflects the requirements of reg-

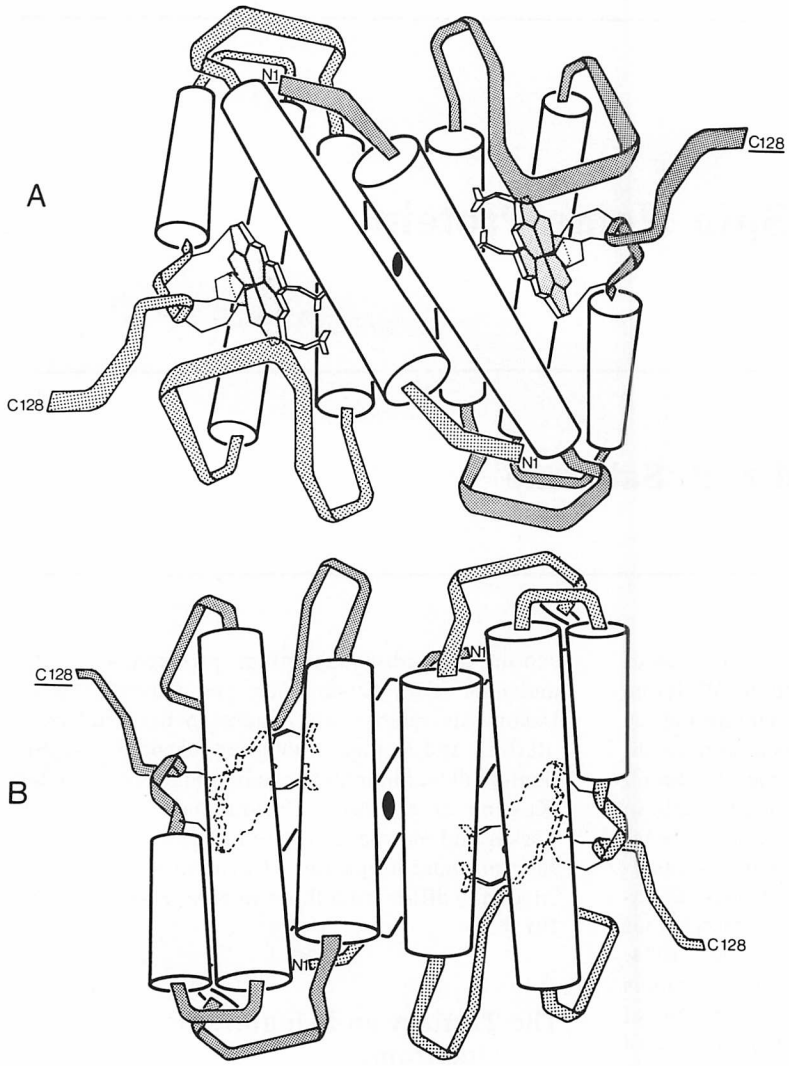


**Figure 1.** Schematic representations of the *R. molischianum* cytochrome *c'* dimer that illustrate its structural characteristics when viewed along the molecular diad. Each monomer is organized as a left-twisted bundle of four, roughly parallel $\alpha$ helices, here represented as tubes. The heme groups, situated at one end of the helical bundle, are oriented roughly parallel to each other and to the subunit interface. In the dimer, the heme groups and regions of extended polypeptide between helices (shaded) face the same side of the molecule (**A**), whereas the opposite face is primarily composed of $\alpha$ helices (**B**).

ular interhelical packing, which are relatively independent of amino acid sequence. The appearance of this functionally versatile structural arrangement in cytochrome *c'* consequently suggests that the unusual interspecific sequential variation (Ambler et al., 1979) can nevertheless be accommodated within a common structural framework. In contrast, the variation in the observed physicochemical properties among the various species appear to more directly reflect differences in their amino acid sequences.

As shown in Figures 1A and 1B, the cytochrome *c'* dimer, when viewed along its diad symmetry axis, possesses two distinct faces. On the "heme" side of the dimer (Figure 1A), the approximately parallel heme groups have maximal exposure to solvent and are surrounded by extended loops of polypeptide chain. In contrast, the surface of the opposite side is composed primarily of $\alpha$ helices (Figure 1B). The situation of both hemes on the same surface of the molecular dimer suggests that the protein may be involved in physiological oxidoreduction processes, which require the transfer of two electrons. Although the heme irons of the dimer are separated by approximately 20 Å, it is of interest to note that the dimer interface incorporates an intimate helix-packing interaction between diad related helices whose interior residues are proximal to the sixth axial coordination site of each heme group. This suggests that structural effects accompanying heme ligation or oxidoreduction may be mechanically

transmitted between the hemes in the dimeric molecule.

In summary, the molecular configuration of the dimeric cytochrome c' shows that the heme groups face the same side of the molecule and are potentially mechanically linked by an intimate interhelical interaction at the dimer interface. This suggests both that the hemes may interact with each other and/or simultaneously interact with a physiological oxidoreductase.

## Summary

The cytochromes c' are a class of high-spin heme proteins found in a variety of photosynthetic and denitrifying bacteria. The tertiary structure of *Rhodospirillum molischianum* cytochrome c' has been determined at 2.5-Å resolution by x-ray crystallographic techniques. The dimeric molecule is composed of identical ~14,000-MW subunits, each of which incorporates a covalently bound protoheme IX prosthetic group. Each monomer is structurally organized as a left-twisted bundle of four, nearly parallel $\alpha$ helices, sequentially connected by interventin regions of extended polypeptide. In the dimer, the 4-$\alpha$-helical arrangement of the cytochrome c' subunit is qualitatively repeated at the dimer interface, as a result of the pairwise interaction of two helices from each monomer. The relative orientation of the two heme groups in the resulting molecular configuration suggests both that they may interact with each other and/or simultaneously interact with a physiological oxidoreductase.

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