

## Preliminary Crystallographic Data for Cytochromes *c'* of *Rhodopseudomonas capsulata* and *Rhodospirillum molischianum*

Crystallization conditions and unit cell parameters are reported for cytochromes *c'* of *Rhodopseudomonas capsulata* and *Rhodospirillum molischianum*. While both proteins naturally occur as dimers having identical subunits of  $M_r \sim 14,000$ , *R. capsulata* was found to crystallize in the hexagonal space group  $P6_2$  (or its enantiomorph  $P6_4$ ) with one subunit per crystallographic asymmetric unit. This result suggests that the subunits of this molecule are related by exact 2-fold symmetry.

The cytochromes *c'* are a unique class of heme proteins which function as electron carriers of intermediate oxidation-reduction potential (0 to +150 mV) in the electron transport chains of a wide variety of photosynthetic and denitrifying bacteria (Bartsch, 1968; Cusanovich, 1971). With the exception of the monomeric protein isolated from *Rhodopseudomonas palustris*, the cytochromes *c'* thus far examined naturally occur as dimers composed of two identical polypeptide chains of approximately 130 residues. Table 1 compares the physicochemical properties of these molecules with those of two of the most extensively characterized families of heme proteins, the "mitochondrial" *c*-type cytochromes and the oxygen-binding globins. As can be seen, similarities between the cytochromes *c'* and more familiar members of the *c*-type cytochrome class which includes mitochondrial cytochrome *c* and the structurally related bacterial cytochromes  $c_2$ , are limited to the presence of a common Cys-X-X-Cys-His sequence (which furnishes the covalent attachment points for the heme and the fifth co-ordinate ligand of the heme iron) and a generalized oxidoreduction function. In other respects, the cytochromes *c'* appear to share much in common with the oxygen-binding globins. Nevertheless, it is noteworthy that the recent sequence determinations of five species of cytochromes *c'* (Ambler, 1973; Meyer *et al.*, 1975; Ambler, personal communication) give little indication of sequence homology between these molecules and the globins, or the mitochondrial *c*-type cytochromes, aside from the forementioned similarity in the covalent heme binding region common to both the cytochromes *c* and *c'*.

The preceding observations, taken together with the atypical spectroscopic (Imai *et al.*, 1969*a*), ligand-binding (Cusanovich & Gibson, 1973), and magnetic (Maltempo *et al.*, 1974; Rawlings *et al.*, 1977) properties of these molecules, suggest that crystallographic structural studies of the cytochromes *c'* will be significant in a further understanding of both the evolution and structure-function interrelationships of heme proteins.

Preliminary crystallographic data for the monomeric cytochrome *c'* of *R. palustris* have been presented previously (Salemme, 1974). Here we present preliminary data for two dimeric species of cytochrome *c'* which differ substantially in both sequence and various aspects of their physical properties from both the *R. palustris* protein and each other.

*Rhodospirillum molischianum* cytochrome *c'* was crystallized by vapor diffusion

TABLE I

Comparison of physical properties of "mitochondrial" cytochromes c, cytochromes c', and oxygen-binding globins

	Cytochromes c		Cytochromes c'	Oxygen-binding globins
Function	Electron transfer		Electron transfer	Oxygen transfer
Size/monomer	$M_r$ 9000-14,000, 82-134 residues		$M_r$ 14,000, 130 residues	$M_r$ 17,800, 153 residues
Aggregation state	Monomeric		Monomeric, dimeric	Monomeric, tetrameric
Prosthetic group	Protoheme IX, covalently bound by thioether linkages to cysteines; near amino terminus		Protoheme IX, covalently bound by thioether linkages to cysteines; near carboxy terminus	Non-covalently bound protoheme IX
Iron oxidation state	$\text{Fe}^{2+} \rightleftharpoons \text{Fe}^{3+}$		$\text{Fe}^{2+} \rightleftharpoons \text{Fe}^{3+}$	$\text{Fe}^{2+}$ (normally)
Oxidoreduction potential ( $E_m'$ , $\tau$ )	+150 to +350 mV		0 to +150 mV	+60 to +140 mV
Iron co-ordination	Hexaco-ordinate, histidine N and methionine S axial ligands		At least pentaco-ordinate, histidine N probable fifth ligand	Hexaco-ordinate, histidine N fifth ligand, variable non-protein sixth ligand
Spin state	Low		High	High $\rightleftharpoons$ low
Substituent ligands	Normally none		$\text{CO}(\text{Fe}^{2+})$	$\text{O}_2$ , $\text{H}_2\text{O}$ , $\text{CO}$ , $\text{NO}(\text{Fe}^{2+})$ , $\text{NO}$ , $\text{OH}^-$ , $\text{F}^-$ , $\text{N}_3^-$ , $\text{H}_2\text{S}(\text{Fe}^{+3})$
$\alpha$ -helical content† (%)	30		65	63

† Percentage  $\alpha$ -helical content based on known structures of cytochromes c and globins; based on c.d. measurements for cytochromes c' (Imai *et al.*, 1969b).

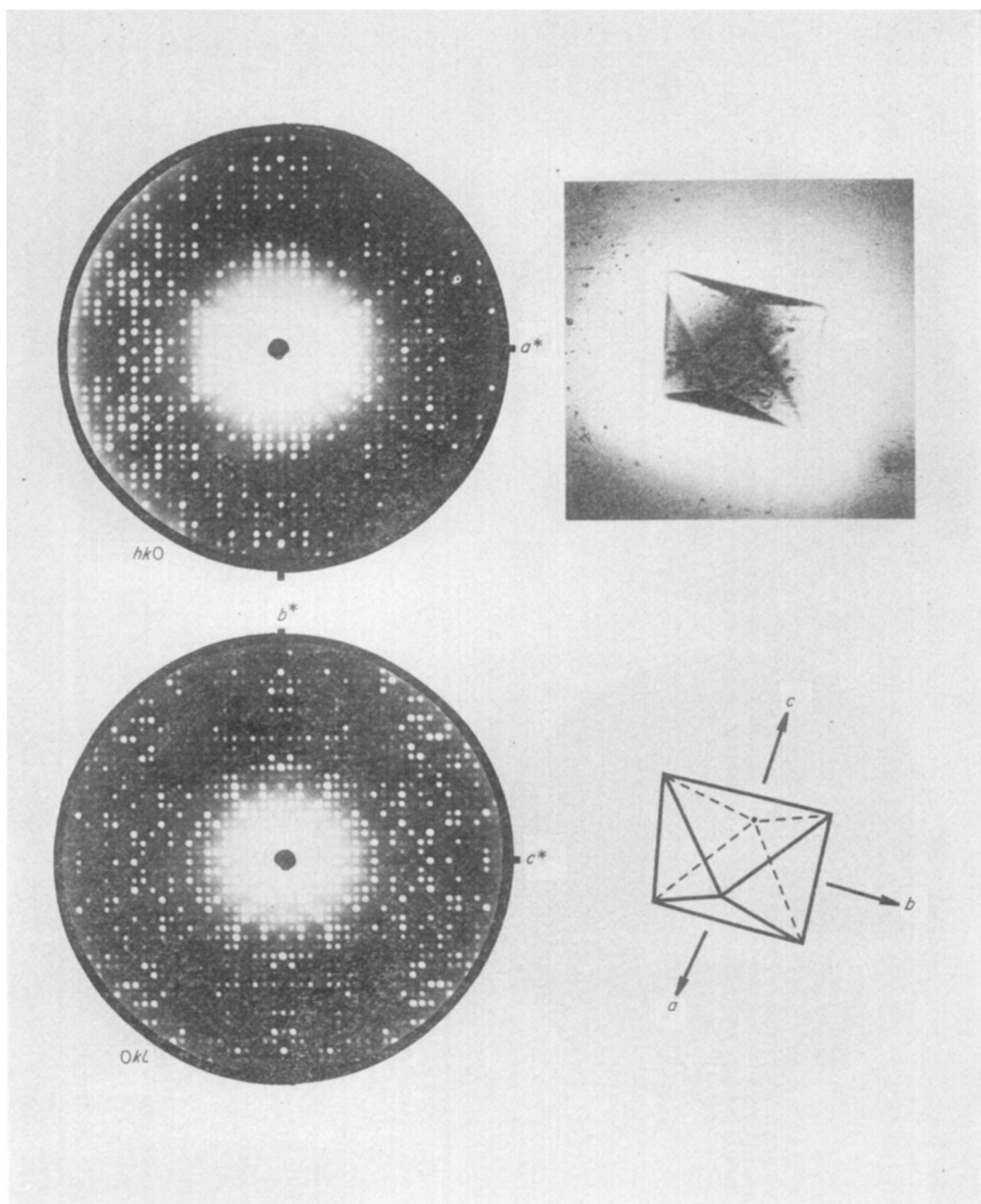


FIG. 1. *R. molischianum* cytochrome *c'*. The space group is  $P2_12_12_1$ . Panels on the left show X-ray precession photographs of the  $hk0$  and  $0kl$  centric projections taken for 14 h at  $f = 10$  cm,  $\mu = 15^\circ$ , using a 760 W Philips fine focus Cu source with a 0.5 mil Ni foil filter. Reflections at the edges of these pictures correspond to a Bragg spacing of about 3 Å. Diffractometer scans of these crystals indicate the presence of strong reflections out to a Bragg spacing of at least 2 Å. Panels on the right show a single crystal photographed through crossed polarizers and the relationship between the crystal habit and unit cell axes. The crystal shown has maximum dimensions of 0.1 mm. Larger crystals are opaque.

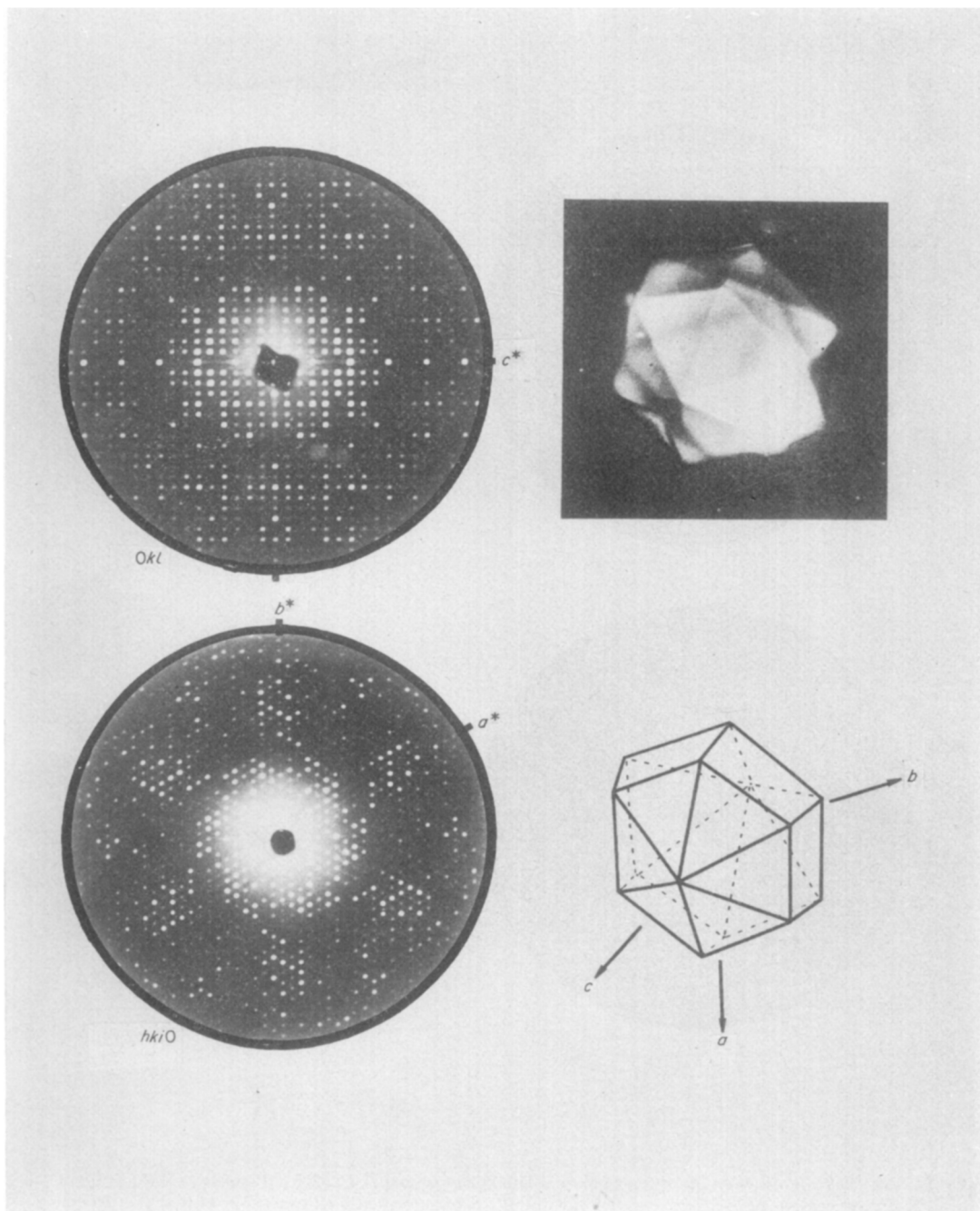


FIG. 2. *R. capsulata* cytochrome *c'*. The space group is  $P6_2$  or  $P6_4$ . Panels on the left show X-ray precession photographs of the  $0kl$  and  $hki0$  zones taken for 20 h at  $f = 10$  cm,  $\mu = 15^\circ$ , using a 760 W Philips fine focus Cu source with a 0.5 mil Ni foil filter. Reflections at the edges of these photographs correspond to a Bragg spacing of about 3 Å. Three-dimensional diffractometer data collected on these crystals typically show 85 to 90% of the total reflections with  $F^2 > \sigma$  to a resolution of 2.5 Å. Panels on the right show a single crystal photographed through crossed polarizers and the relationship between crystal habit and unit cell axes. The crystal shown has maximum dimensions of about 0.1 mm. Larger crystals are opaque.

concentration of a 30 mg/ml protein solution containing 40% saturated ammonium sulfate, 0.05 M-magnesium nitrate, against saturated ammonium sulfate at 4°C. The protein crystallizes as rectangular bipyramids in the orthorhombic space group  $P2_12_12_1$  with  $a = 56.5 \text{ \AA}$ ,  $b = 72.5 \text{ \AA}$ ,  $c = 75.7 \text{ \AA}$ ,  $V = 3.1 \times 10^5 \text{ \AA}^3$  (Fig. 1). Flotation density measurements carried out in mixed ammonium sulfate/potassium phosphate solutions gave a value for  $V_m$  of  $2.37 \text{ \AA}^3/\text{dalton protein}$  (Matthews, 1968). This value corresponds to a molecular weight of 29,600 per crystallographic asymmetric unit suggesting that it contains a single cytochrome  $c'$  dimer. In addition to the excellent diffraction exhibited by these crystals in their non-derivatized state, they appear to survive heavy-atom derivatization and subsequent X-ray exposure without exhibiting the significant diffraction degradation or lack of isomorphism that has been encountered in the study of the *R. palustris* crystals. In the latter case these effects limit the attainable resolution to about 3 Å, whereas centric projections obtained on some four *R. molischianum* derivative crystals show little diffraction degradation or lack of isomorphism.

*Rhodopseudomonas capsulata* cytochrome  $c'$  was crystallized in a free interface diffusion cell (Salemme, 1972) by layering 20  $\mu\text{l}$  of 100 mg/ml protein solution over 20  $\mu\text{l}$  of 65% ammonium sulfate, 0.1 M in sodium citrate buffered with 6 M-HCl to pH 4.2. Initially, the protein crystallizes as hexagonal bipyramids which elongate along the pyramid axes to eventually form hexagonal rods (0.25 mm  $\times$  0.25 mm  $\times$  0.25 mm). Addition of 10% by volume *N,N*-dimethylformamide to the crystallization buffer increased the overall crystal dimensions to 0.5 mm  $\times$  0.5 mm  $\times$  1.0 mm (Fig. 2).

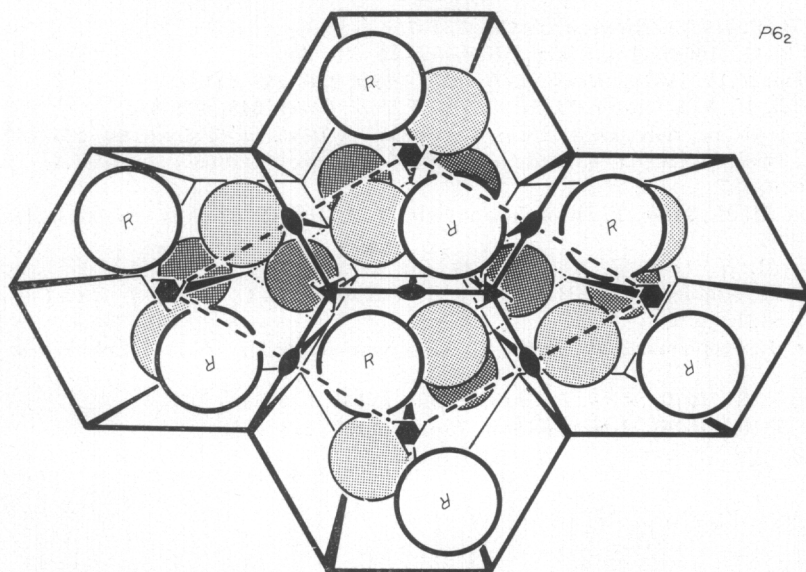


FIG. 3. Subunit packing in the space group  $P6_2$  with unit cell edges shown as broken lines. There are 3 ways in which a dimeric molecule composed of identical subunits, but having a single subunit per crystallographic asymmetric unit, may be accommodated in this space group. The molecular dyad may be coincident with the crystallographic  $6_2$  axis ( $R$ 's base to base), or alternatively, with either of the remaining 2 crystallographic dyads located centrally in the cell ( $R$ 's front to front) or at the cell edge ( $R$ 's back to back).

X-ray investigation of these crystals revealed that *R. capsulata* cytochrome *c'* crystallized in one of the hexagonal enantimorphic space groups,  $P6_2$  or  $P6_4$ , with  $a = b = 72.2 \text{ \AA}$ ,  $c = 52.4 \text{ \AA}$ ,  $\gamma = 120^\circ$ ,  $V = 2.4 \times 10^5 \text{ \AA}^3$ . Flotation density measurements carried out in mixed ammonium sulfate/potassium phosphate gave a value for  $V_m$  of  $2.77 \text{ \AA}^3/\text{dalton}$  protein, corresponding to a molecular weight of 14,000 or one subunit of the cytochrome *c'* dimer in the crystallographic asymmetric unit. Figure 3 shows the packing arrangement in space group  $P6_2$ . As may be seen, there are potentially three different ways in which a dimeric molecule having subunits related by exact 2-fold symmetry may be accommodated in this space group; with the molecular dyad coincident with the crystallographic  $6_2$  axis (*R*'s base to base), or coincident with either of the other two crystallographic dyads (*R*'s front to front or back to back). Since 6 M-guanidine·hydrochloride is required for dissociation of the *R. capsulata* dimer and higher aggregates are not observed, it appears that the chemically identical subunits of this protein are related by an exact 2-fold axis of symmetry.

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