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COMMUNICATIONS

Preliminary Crystallographic Data for Cytochrome c′ of *Rhodopseudomonas palustris*

The cytochromes c' constitute a unique class of heme proteins of intermediate oxidation-reduction potential (0 to +150 mV) which occur in a variety of photosynthetic and denitrifying bacteria (1). Their physiological role is best defined in the photosynthetic organisms, where they function as electron carriers in the cyclic photophosphorylating electron transport chain (2). In general, cytochromes c' are isolated as dimers consisting of identical chains of $M_r \simeq 14000$ daltons, each chain having a protoheme-IX prosthetic group covalently bound through thioether linkages to cysteine residues located near its carboxy terminus. The cytochromes c' are distinguished from the nearly ubiquitous "c-type" cytochrome class (which includes mitochondrial cytochrome c, the bacterial cytochromes c_2, as well as a host of smaller related molecules of bacterial and photosynthetic origin) by a variety of unusual magnetic (3), spectroscopic (4), and ligand-binding (5) properties. Further, the recent amino acid sequence determination (T. Meyer and R. P. Ambler, personal communication) of several species of cytochrome c' has shown that they exhibit virtually no sequence homology with the c-type cytochromes. The novel characteristics of the cytochromes c', therefore, render them interesting candidates for crystallographic studies relating to the structure–function properties of heme proteins.

Crystallization attempts were undertaken using a free interface diffusion technique (6) with cytochromes c' derived from the denitrifying bacterium *Pseudomonas denitrificans*, and two species of purple nonsulfur photosynthetic bacteria, *Rhodospirillum rubrum* and *Rhodopseudomonas palustris*.

Ps. denitrificans cytochrome c' was observed to crystallize as hexagonal bipyrimids under a wide range of conditions. None of the crystals obtained, however, were large enough for X-ray work.

R. rubrum cytochrome c' was crystallized in a free diffusion cell by layering 50 μl of 30 mg/ml protein solution over 50 μl of 65% saturated unbuffered ammonium sulfate at 37°C. These crystals grew as long rods (0.2 × 0.2 × 0.6 mm) in 1–3 wk (see Ref. 6). X-ray investigation of these crystals revealed that this protein crystallized in an orthorhombic space group (probably $P222_1$) with a = 98.4, b = 122.4, c = 108.3 Å. From a calculation based on the assumption that these crystals have a crystal volume per unit of molecular weight V_M = 2.37 Å³/dalton (the mean value for 116 different crystal forms surveyed by Matthews (7)), it is inferred that the crystallographic assymetric unit of this crystal form contains a tetramer of dimers, although Langridge (8) has reported a crystalline form of this protein in space group $P2_12_12_1$ having a single dimer in the crystallographic asymmetric unit. Since the former crystal form was considered to have a prohibitively large molecular weight per asymmetric unit and the protein could not be induced to crystallize in the form studied by Langridge, crystallization attempts were subsequently focused on cytochrome c' derived from *Rps. palustris*.

Rps. palustris cytochrome c' was crystallized in two forms. One form, Type A, was initially crystallized by layering 50 μl of 30 mg/ml protein solution over 100 μl of unbuffered 65% saturated ammonium sulfate in a free diffusion cell at 37°C. These crystals grew in 1 mo as flat rectangular prisms about 0.3 × 0.3 × 0.2 mm in size (see Ref. 6). X-ray examination of Type A crystals revealed that they invariably crystallized as twins in the monoclinic space group $P2$ or $P2_1$, with a = 40.7, b = 108.3, c = 19.9 Å, β = 104.5°, $V = 8.49 \times 10^4$ Å³. A multitude of attempts to reduce the twinning of the Type A crystals by varying crystallization conditions proved ineffectual.

In the course of these experiments, however, a second untwinned crystalline form, designated Type B, was obtained by layering 50 μl of 30 mg/ml protein solution over 50 μl 65% saturated ammonium sulfate 0.1 M in $Mg(NO_3)_2$ at 37°C. Type B crystals grew as flat rectangular beveled prisms (0.2 × 0.2 × 0.1 mm) in about 1 mo, and are in the orthorhombic space group $P2_12_12_1$, with a = 39.5, b = 77.6, c = 107.8 Å, $V = 3.30 \times 10^5$ Å³.

The density of both the A and B forms of the *Rps. palustris* cytochrome c' crystals were measured by flotation in mixed ammonium sulfate–sodium phosphate solutions. The observed densities for Type A crystals equilibrated against 75% saturated ammonium sulfate, and Type B crystals

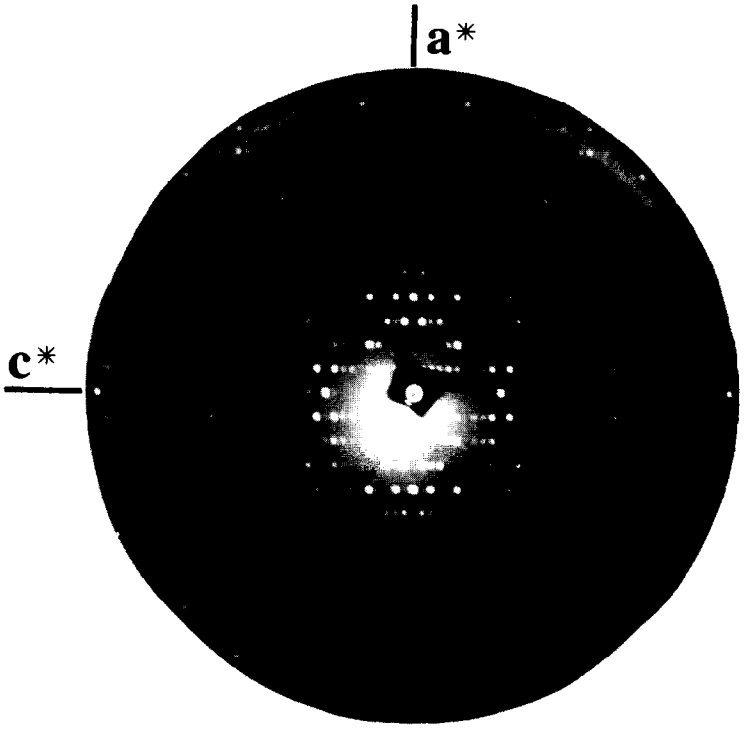

FIG. 1. X-ray precession photograph of the b^* projection of *Rps. palustris* Type B crystal, taken for 20 hr, f = 10 cm, μ = 15°, using a Philips fine focus Cu X-ray source with a 0.5 mil Ni foil filter. The edges of the photograph correspond to a Bragg spacing of 3 Å.

equilibrated against 65% saturated ammonium sulfate 0.1 M $Mg(NO_3)_2$ were 1.27 g/cc and 1.26 g/cc, respectively. Assuming the partial specific volume of protein in the crystalline lattice to approximate the dilute solution value of 0.74 g/cc (7) the calculated values of V_M for the A and B crystal forms are 2.92 and 3.01 Å^3/dalton. The resultant estimated molecular weights per crystallographic asymmetric unit are 14,500 daltons for the monoclinic Type A crystals (two unique positions in either the $P2$ or $P2_1$ cell) and 27,400 daltons for the orthorhombic Type B crystals (four unique positions in the $P2_12_12_1$ cell). Since the known molecular weight of a monomer of *Rps. palustris c'* is approximately 13,700 daltons, these results suggest that a single monomer constitutes the asymmetric unit in the Type A crystals, whereas a dimer constitutes the asymmetric unit of the Type B crystals.

Although it is usually desirable to undertake structural studies upon a crystal form having a minimum molecular weight per crystallographic asymmetric unit, structural studies are currently being extended on the *Rps. palustris* cytochrome *c'* orthorhombic Type B crystals containing two molecules in the crystallographic asymmetric unit, since they diffract to a Bragg spacing of at least 2.5 Å and it has not been possible to overcome the persistent twinning characteristic of the monoclinic Type A crystals.

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