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Crystallization and preliminary X-ray study of a new crystal form of cytochrome *c′* from *Rhodobacter capsulatus*

YOSHIKI HIGUCHI,[a] YOJI OKAMA,[a] TOSHIYUKI KANDA,[a] SHINTARO MISAKI,[a] NORITAKE YASUOKA,[a] TERRY E. MEYER[b] AND MICHAEL A. CUSANOVICH,[b] *at* [a]*Department of Life Science, Himeji Institute of Technology, 1479-1 Kanaji, Kamigori, Ako-gun, Hyogo 678-12, Japan, and* [b]*Department of Biochemistry, University of Arizona, Tucson, Arizona 85721, USA*

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Abstract

A new crystal form of diheme cytochrome *c′* from *Rhodobacter capsulatus* has been obtained and preliminary crystallographic experiments have been performed. The crystals belong to the space group $P2_12_12$ with unit-cell dimensions of $a = 47.82$, $b = 72.59$, $c = 34.32$ Å. The assumption that an asymmetric unit of the crystal contains one half of the homodimer molecule indicates that the monomers in the dimeric molecule may be related by a crystallographic twofold axis. Crystals diffract up to 1.7 Å resolution using the X-ray beam from synchrotron radiation, and 11 127 unique structure factors were obtained with an $R_{\rm merge}$ of 7.1% from 52 922 indexed reflections. Structure analysis by means of molecular-replacement methods is now underway.

1. Introduction

The cytochromes *c′* are typically dimeric proteins with a molecular weight approximately 28 kDa. They are classified as class II cytochromes *c* in the cytochrome *c* superfamily (Pettigrew & Moore, 1987), and are found in photosynthetic and denitrifying bacteria (Bartsch, 1978; Meyer & Kamen, 1982). They are considered to be electron carriers for some enzymes, but substantial biological function *in vivo* is still uncertain. Cloning and site-directed mutagenesis of the *Chromatium vinosum* cytochrome *c′* have made it possible to establish the relationship between structure and physicochemical properties of the cytochrome *c′* (Even, Kassner, Dolata, Meyer & Cusanovich, 1996). Since the heme Fe atom of cytochromes *c′* is in the high-spin state (Taniguchi & Kamen, 1963), they have unique spectroscopic (Horio & Kamen, 1961; Imai, Imai, Sato & Horio, 1969) and ligand-binding properties (Cusanovich & Gibson, 1973) compared with class I cytochromes *c*.

The cytochrome *c′* from *Rhodobacter capsulatus* was reported by Bartsch (1971) and has been studied intensively. This is a dimeric protein, with one heme and 129 amino-acid residues in monomer, and the redox potential is +51 mV (Bartsch, 1971). *Rb. capsulatus* cytochrome *c′* was crystallized in space group $P6_2$ or $P6_4$ with $a = b = 72.2$, and $c = 52.4$ Å by Weber & Salemme (1977), but the structure was not reported. Structures for three cytochromes *c′* have been determined at atomic resolution to date. They are from *Rhodospirillum molischianum* (Weber, Howard, Xuong & Salemme, 1981; Finzel, Weber, Hardman & Salemme, 1985), *Rhodospirillum rubrum* (Yasui *et al.*, 1992), and *Chromatium vinosum* (Ren, Meyer & McRee, 1993). Though all of them are dimeric in solution, the sequence homology between cytochromes *c′* from *Rb. capsulatus* and the above strains is at most 30%. Therefore, we have crystallized the cytochrome *c′* from *Rb. capsulatus* in order to determine the three-dimensional structure by the X-ray method. In addition, the ^{1}H, ^{15}N and ^{13}C NMR spectra of *Rb. capsulatus* cytochrome *c′* have been assigned (Caffrey, Simorre, Brutschet, Cusanovich & Marion, 1995) and a three-dimensional structure in solution is imminent. Thus, it is timely that the crystal structure be determined for comparison.

2. Crystallization

A new crystal form of cytochrome *c′* from *Rhodobacter capsulatus* was obtained using the sitting-drop vapor-diffusion method. The crystallization droplets contained 20 µl of protein solution at a protein concentration of 10 mg ml^{-1} with 100 m*M* ammonium sulfate and 15%(*w*/*v*) polyethylene glycol 8000 as precipitating agents. These droplets were vapor equilibrated against a 0.5 ml of reservoir solution of 200 m*M* ammonium sulfate and 30%(*w*/*v*) polyethylene glycol 8000 at room temperature. The red plate-like crystals were grown to the largest size of 0.7 × 0.7 × 0.1 mm in three weeks (Fig. 1).

3. Diffraction experiments

A crystal was sealed into a thin-glass capillary tube with a small amount of the mother liquor. Diffraction study was performed at the Photon Factory (BL-6A) of the National Laboratory for High Energy Physics in Tsukuba using the X-ray beam from the synchrotron radiation source with a Weissenberg camera for protein crystallography (Sakabe, 1983). Two diffraction data sets from two different axis settings were collected in order to

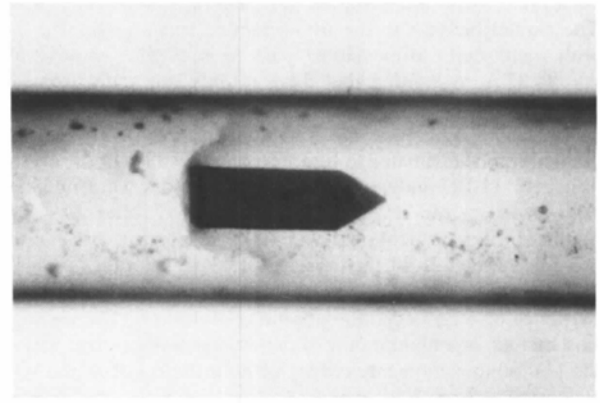

Fig. 1. A single crystal (0.7 × 0.2 × 0.05 mm) of cytochrome *c′* from *Rhodobacter capsulatus* mounted in a thin capillary tube.

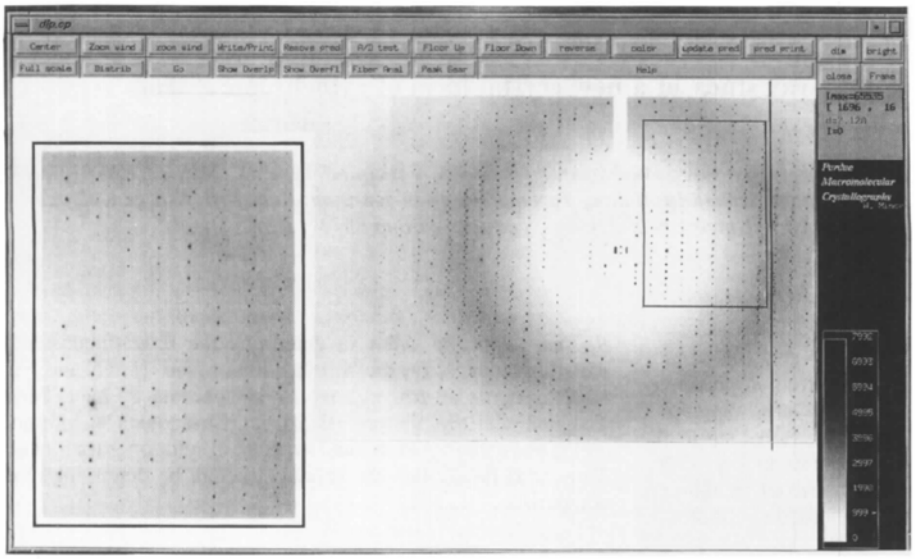

Fig. 2. A typical diffraction pattern of a crystal of cytochrome c' from *Rhodobacter capsulatus*. The images were recorded using the X-ray beam from the synchrotron radiation on a Weissenberg camera for macromolecular crystallography with a Fuji imaging plate as a detector system. The enlarged image of the boxed area is shown in the left of the figure. The figure shows the diffraction image of the crystal rotated along the b axis in a rotation range of 7°. The X-ray was approximately parallel to the crystallographic a axis.

obtain the complete data set in Weissenberg geometry at 288 K. One data set was collected over 90° in steps of 7.0° with rotation along the b axis. The second data set was obtained over 90° in steps of 11° with rotation along the c axis. The coupling constant (ratio of rotation step and cassette movement along rotation axis) was set to 2.0° mm^{-1}, and the overlap of rotation angles between consecutive images was 1° for both data sets. The radius of a Weissenberg camera cassette was 286.5 mm, and helium gas was filled in the beam path from the beam collimator to the camera cassette to reduce the background of diffraction images during the data collection. Data sets were collected from only one crystal in order to reduce various errors which are caused by the difference of absorption and scattering power between crystals. The wavelength of the X-ray beam was tuned to 1.0 Å.

4. Results and discussion

The crystal diffracted up to 1.7 Å using Fuji imaging plates as a detector system (Fig. 2), and was stable in the X-ray beam for the duration of the data collection. Data processing was performed with the program *DENZO* (Otwinowski, 1986). The crystal belongs to the orthorhombic space group $P2_12_12$ with unit-cell dimensions of $a = 47.82$, $b = 72.59$, $c = 34.32$ Å. Assuming that there is only one cytochrome c' monomer molecule in the asymmetric unit, the V_M value is calculated to be 2.14 Å^3 Da^{-1} (Matthews, 1968). The solvent content can be estimated to be approximately 42% of the crystal unit cell. 11 127 independent structure factors were obtained with an $R_{\rm merge}$ of 7.1% from 52 922 indexed reflections. The number of independent observations corresponds to 80% of the possible reflections of the crystal at 1.7 Å resolution.

The self-rotation function shows no clear evidence of the existence of a non-crystallographic twofold axis. The assumption that an asymmetric unit of the crystal contains one half of the homodimer molecule coincides with the result of the self-rotation function. This means that monomers in the dimeric molecule may be related by a crystallographic twofold axis. The structure determination by the molecular-replacement method using atomic coordinates of other cytochromes c' deposited in the Protein Data Bank is now in progress.

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