SHORT COMMUNICATIONS

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Cytochrome c_6 from the green alga *Monoraphidium braunii*. Crystallization and preliminary diffraction studies.

By CARLOS FRAZÃO, JOÃO M. DIAS and PEDRO M. MATIAS, *Instituto de Tecnologia Química e Biológica, Rua da Quinta Grande* 6, *Apartado* 127, 2780-*Oeiras, Portugal,* MARIA J. ROMÃO and MARIA A. CARRONDO,* *Instituto de Tecnologia Química e Biológica and Instituto Superior Técnico, Rua da Quinta Grande* 6, *Apartado* 127, 2780-*Oeiras, Portugal,* MANUEL HERVÁS, JOSÉ A. NAVARRO and MIGUEL DE LA ROSA, *Instituto de Bioquímica Vegetal y Fotosíntesis, Universidad de Sevilla y CSIC, Apartado* 1113, 41080-*Sevilla, Spain,* and GEORGE M. SHELDRICK, *Institut für Anorganische Chemie der Universität Göttingen, Tammannstrasse* 4, *D*-37077 *Göttingen, Germany*

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Abstract

Cytochrome c_6, a plastocyanin functionally interchangeable electron carrier between the chlorophyll molecule P700 of photosystem I and cytochrome *f* from cytochrome b_6f complex, has been isolated from the green alga *Monoraphidium braunii* and crystallized by the vapour-diffusion technique in sodium citrate. Crystals belong to space group *R*3, with cell dimensions $a = b = 51.93\,(5)$ and $c = 80.5\,(1)$ Å (hexagonal axes), with one molecule per asymmetric unit. They diffract beyond 1.9 Å under a Cu $K\alpha$ rotating-anode source, with an anomalous signal that allows the positioning of the heme Fe atom in the unit cell.

Introduction

The algal cytochromes c_6 (formerly cytochrome c_{553}) are members of the class I *c*-type cytochromes, which are soluble monoheme and monomeric low-spin heme proteins. This cytochrome is functionally interchangeable with the copper-protein plastocyanin, both as the donor of electrons to the photooxidized chlorophyll molecule P700 in photosystem I (PSI) and as the acceptor of electrons from cytochrome *f* in the cytochrome b_6f complex. In cyanobacteria and some eukaryotic algae, plastocyanin is actually replaced by cytochrome c_6 when cells are grown in low-copper media (Wood, 1978; Sandmann, 1986). It is, thus, of the highest interest to know the similarities and differences, both in structure and function, between these two proteins.

Laser flash photolysis has been used to perform a comparative study of cytochrome c_6 and plastocyanin oxidation by PSI, and has shown that these two proteins exhibit a similar kinetic efficiency when isolated from the same organism (Hervás, De la Rosa & Tollin, 1992; Hervás, Navarro, Ortega, De la Rosa & Bottin, 1994). A detailed comparison of the structural characteristics of these two proteins may, thus, allow us to gain more complete information on how they interact with the reaction partners that they have in common. The conformations in solution and crystal structures of plastocyanin from green algae and higher plants are now well documented (Guss & Freeman, 1983), with two areas in the protein surface being clearly identified as important sites for interaction with PSI and cytochrome *f* (Martinez, Huang, Szczepaniak, Cramer & Smith, 1994; for a review see, Gross, 1993). However, little is known about the structure and physicochemical properties of cytochrome c_6. Sequence alignment of cytochrome c_6 from *M. braunii* against sequences of class I monohemic cytochromes *c*, for which structures have been crystallographically determined and deposited in the Protein Data Bank (Bernstein *et al.*, 1977), did not reveal homologies greater than 24%. This low homology ratio should also take into account that in this cytochrome class there must be four conserved amino acids, the two heme-binding cysteine residues plus the histidine and methionine Fe-atom ligands, within the *ca* 80–90 amino acids of the peptide chain.

The spectroscopic characterization of cytochrome c_6 from the green alga *M. braunii*, which exhibits a molecular mass of 9.3 kDa, an isoelectric point of 3.6, and an unusual methionine–histidine heme axial coordination was recently reported (Campos *et al.*, 1993). In this work, the crystallization and preliminary diffraction analyses of cytochrome c_6 from *M. braunii* are presented.

Materials and methods

Protein purification and crystallization

Cells of *M. braunii* were grown autotrophically at 298 K, in the medium reported by Kessler, Langner, Ludwig & Wiechmann (1963), under the experimental conditions described in Campos *et al.* (1993). Cytochrome c_6 was isolated and purified following the procedure reported by Campos *et al.* (1993).

Crystallization conditions for cytochrome c_6 could be obtained within the pH interval 7.2–7.5 for ammonium sulfate, or at pH 8.0 for sodium citrate as precipitating agents. However, whereas for ammonium sulfate several months are needed to obtain crystalline formations, with sodium citrate single crystals were formed and grown within one month. Therefore, these conditions were used to obtain the diffracting crystals described below. 1 µl of a precipitating solution containing 0.8 *M* sodium citrate and 0.1 *M* glycine at pH 8.0 was added to 3 µl of 2.4 m*M* cytochrome solution in 0.5 m*M* tricine buffer. The sitting drop was deposited on a plastic cylindrical section, 1 mm diameter, located inside the reservoir of a Linbro box, and equilibrated by vapour diffusion against 500 µl of the precipitating solution. Experiments were carried out both at room temperature and in a cold room. The concentration of citrate solution in the reservoir was incremented by 0.2 *M* every 2 to 3 d, according

* Author to whom correspondence should be addressed.

to the temperature of the experiment. Separation of protein from solution became apparent at a concentration of 1.5 *M*.

Crystallographic diffraction data collection and evaluation

Crystallographic analysis was performed with an Enraf–Nonius FR-504 integrating precession camera and an Enraf–Nonius FAST area diffractometer, both coupled to an Enraf–Nonius FR-571 rotating-anode, operating at 40 kV and 75 mA. Zero and first-order precession pictures were obtained for 14° of precession and 60 mm distance. Unit-cell dimensions and intensity-data measurements were obtained using the program *MADNES* (Pflugrath & Messerschmidt, 1989). The crystal-to-detector distance was 40 mm and the detector was tilted by –20°. Graphite-monochromated Cu $K\alpha$ radiation with a collimation of 0.3 mm was used with the FAST area detector. Images were obtained by scanning for 30 s through 0.1° on ω, for three batches of 90° each, interleaved by 60° on ω. Data evaluation and processing from the stored images were carried out on a MicroVAX3100, using the off-line mode of *MADNES*. The reported cell parameters correspond to the mean e.s.d. values calculated from the refined values during data collection. The program *PROCOR* (Kabsch, 1988) was used to obtain profile-fitted intensities, which were further evaluated by the *CCP*4 suite of programs (Collaborative Computational Project, Number 4, 1994). *PRFLCF* split the data into batches of 5°, which were scaled and merged by *ROTAVATA-AGROVATA*, and the individual structure-factor magnitudes were evaluated and statistically improved by *TRUNCATE*. Patterson analysis of the anomalous signal was performed with the *FFT* program from the *CCP*4 suite.

Results and discussion

Crystal data

In the drops where crystals appeared, the protein had completely crystallized, as indicated by the final colourless solution. Crystals of up to 1 mm in the largest dimension (Fig. 1) appeared within 4 weeks, showing a rather irregular morphology. Zero- and first-layer precession photographs (Fig. 2) showed unique threefold rotational symmetry. Further X-ray diffraction data, collected as described above, confirmed the determination of the unit-cell dimensions $a = b = 51.913\,(5)$, $c = 80.5\,(1)$ Å (hexagonal axes). An analysis of the systematic absences showed the crystal to be rhombohedral, space group $R3$ (146). Assuming one molecule per asymmetric unit, the crystal volume per unit molecular weight is 2.24 $Å^3\ Da^{-1}$, which is near the average value found for protein crystals (Matthews, 1968) and indicates a solvent content of 45%.

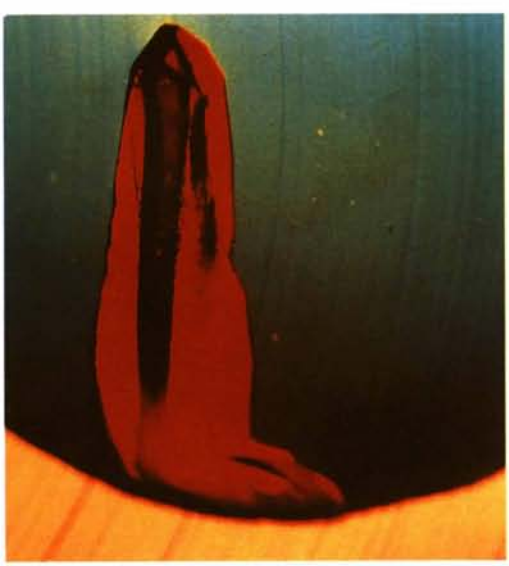
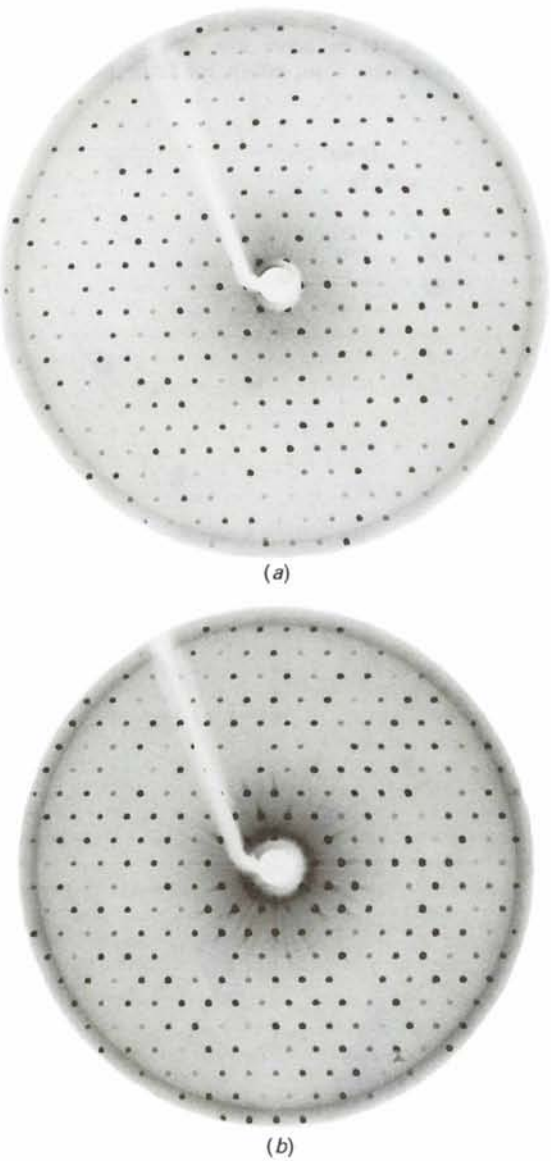


Fig. 1. Crystal of cytochrome c_6 from *M. braunii* (largest dimension *ca* 1 mm)

Diffraction data

26 229 intensities collected within the resolution range 20.0–1.9 Å were merged to give 6287 unique structure-factor



(*a*)

(*b*)

Fig. 2. Precession photographs of cytochrome c_6 from *M. braunii* showing the systematic absences as a result of the rhomboedral Bravais lattice *R* (hexagonal axes), and the point-group symmetry 3 of *hk*0 (*a*) and *hk*1 (*b*).

amplitudes, of which 5119 have their independent Friedel pairs. The data have a completeness of 98.7% (95.0% in the last resolution shell 1.95–1.90 Å), with 96.2% of the data corresponding to $I > 3\sigma$. The calculated reliability factor $R_{merge}(I)$ is 3.6% taking into account the anomalous differences, and 5.6% when Friedel pairs are merged.

A Patterson synthesis was calculated with the anomalous differences as coefficients, $\Delta_{ano} = (||F_o^+| - |F_o^-||)$. The peaks observed in the Harker section $w = 0$, presented in Fig. 3, have a peak height four times above noise level. As iron has its K absorption edge at $\lambda = 1.743$ Å and the data was collected using Cu $K\alpha$ radiation with $\lambda = 1.5418$ Å, these peaks were attributed to the anomalous contribution of that atom, and from them the Fe-atom position in the asymmetric unit can be assigned to one of the positions $(x, y, z) = \pm(0.191, 0.422, 0.0)$.

The presence of one Fe and three S atoms in this protein and the diffraction power of the crystals obtained are a reasonable basis for an attempt to solve this structure directly from higher resolution data. Thus, efforts are being made to obtain synchrotron data with the highest possible resolution, so that the position of the molecule in the asymmetric unit may be directly obtained by automated Patterson interpretation methods.

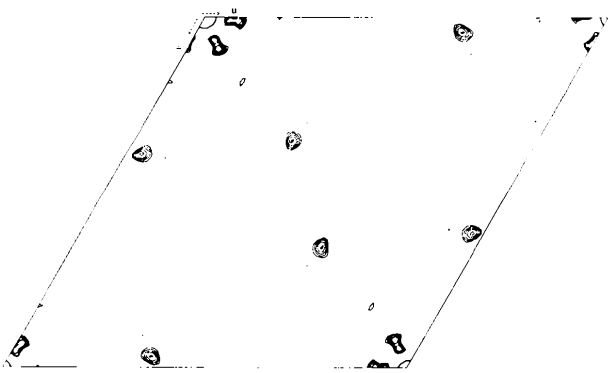


Fig. 3. $w = 0$ section of the 20.0–1.9 Å anomalous Patterson map, with coefficients $>4\sigma$. Plot contoured at 0.5σ levels above 2σ. Coordinates of the peak closest to origin are $u = 0.040$, $v = 0.397$.

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