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Crystallization and preliminary X-ray diffraction studies of cytochrome c_6 from *Porphyra yezoensis*

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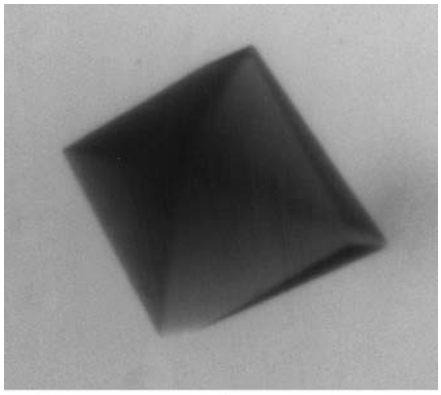
Cytochrome c_6 from the red alga *Porphyra yezoensis* has been purified and crystallized by the sitting-drop vapour-diffusion method. Two different crystal forms, tetragonal and orthorhombic, were obtained. The tetragonal crystals belong to space group $P4_12_12$ or $P4_32_12$, with unit-cell dimensions a = 49.33 (2), c = 83.70 (10) Å. The orthorhombic crystals belong to space group $P2_12_12_1$, with unit-cell dimensions a = 46.74 (2), b = 49.42 (1), c = 37.11 (1) Å. Absorption spectra of the crystals showed that the tetragonal form was oxidized and the orthorhombic form was reduced.

1. Introduction

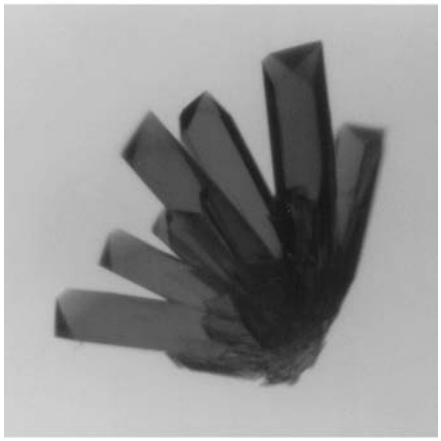
The physiological function of cytochrome c_6 (cyt c_6) is electron transfer from the cytochrome b_6f complex to P700 in photosystem I. Cyt c_6 is a member of the class I *c*-type cytochromes which are water-soluble proteins with a haem *c*. Cyt c_6 has a low-spin haem iron and has a high redox potential. Recently, the structures of cyt c_6 from the green algae *Chlamydomonas reinhardtii* (Kerfeld *et al.*, 1995) and *Monoraphidium braunii* (Frazão *et al.*, 1995; Banci *et al.*, 1996; Banci *et al.*, 1998) and the cyanobacterium *Synechococcus elongatus* (Beissinger *et al.*, 1998) have been reported. The overall structures of these cyt c_6s are similar to each other. The sequence identities between *P. yezoensis* cyt c_6 (Sasaki *et al.*, 1999) and *C. reinhardtii* cyt c_6 (Kerfeld *et al.*, 1995), *M. braunii* cyt c_6 (Campos *et al.*, 1993) and *S. elongatus* cyt c_6 (Beissinger *et al.*, 1998) are 48.9, 50.6 and 67.1%, respectively. On the other hand, plastocyanin (Pc), containing copper as the central ion, is also known to have the same function and nearly the same size and redox potential as cyt c_6. However, no structural similarities can be recognized between them. Cyt c_6 is mainly composed of α-helices, while Pc contains β-sheets. The higher plants use Pc alone as the electron carrier between the cytochrome b_6f complex and P700. Some algae can switch between using the two proteins in response to changes in the environmental concentration of copper ions. The red alga *P. yezoensis* presented here possesses only cyt c_6, which is composed of 85 amino-acid residues with a redox potential of 342 mV (Sugimura *et al.*, 1968).

2. Protein purification

Frozen *P. yezoensis* was homogenized with 20 m*M* phosphate buffer (pH 7.0) and 5 m*M* sodium ascorbate. The homogenate was filtered through four layers of gauze. 2% acrinol solution was added to the crude extract. The solution was then fractionated with 50–100% saturation of ammonium sulfate and desalted by passage through a Sephadex G-25 column equilibrated with 10 m*M* phosphate



(*a*)



(*b*)

Figure 1
Crystals of cytochrome c_6 from *P. yezoensis*. (*a*) Tetragonal form, (*b*) orthorhombic form.

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Table 1
Crystal data and data-collection statistics.

	Tetragonal	Orthorhombic
Crystal data		
Space group	$P4_12_12$ or $P4_32_12$	$P2_12_12_1$
Unit-cell parameters (Å)	a = 49.33 (2)	a = 46.74 (2)
	c = 83.70 (10)	b = 49.42 (1)
		c = 37.11 (1)
Data collection		
X-ray source	PF BL-6A	Cu $K\alpha$
Wavelength (Å)	1.00	1.54
Resolution (Å)	1.79	1.60
Last resolution shell (Å)	1.85–1.79	1.67–1.60
Number of observations	27841	95723
Number of unique reflections	9484	13688
$R_{\rm merge}$ (%)	4.0	6.5
Last resolution shell (%)	20.1	22.2
Completeness (%)	93.4	98.5
Last resolution shell (%)	92.5	95.9

buffer (pH 7.0). The desalted solution was applied to a DEAE–cellulose column equilibrated with 20 m*M* phosphate buffer (pH 7.0). The bound proteins were eluted with 100 m*M* phosphate buffer (pH 7.0). Fractions with $A_{553}/A_{280} \geq 1$ were pooled and concentrated by ultrafiltration.

3. Crystallization and data collection

Crystallization was carried out using the sitting-drop vapour-diffusion method at 287 K. Two different crystal forms, tetragonal and orthorhombic, were obtained.

3.1. Tetragonal form

A 5 µl droplet of 35 mg ml^{-1} cyt c_6 solution in 10 m*M* phosphate buffer (pH 7.0) was mixed with an equal volume of reservoir solution (200 m*M* citrate buffer at pH 4.0 containing 1.5 *M* ammonium sulfate). This droplet was equilibrated against 1 ml reservoir solution. Dark red tetragonal bipyramidal crystals grew to a typical size of 0.4–0.5 mm along one edge in a week (Fig. 1*a*). Diffraction data were collected at room temperature on synchrotron-radiation beamline 6A at the Photon Factory (Tsukuba, Japan) using a wavelength of 1.00 Å on a 286.5 mm screenless Weissenberg camera with 200 × 400 image plates (Sakabe, 1991). Data were processed and scaled using *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997). The crystals belong to space group $P4_12_12$ or $P4_32_12$, with unit-cell dimensions a = 49.33 (2), c = 83.70 (10) Å. For one molecule of molecular mass 9.7 kDa per asymmetric unit (Z = 8), the Matthews coefficient is 2.5 Å^3 Da^{-1} and the solvent content is 51% (Matthews, 1968). The crystals diffract to 1.8 Å resolution.

3.2. Orthorhombic form

A 5 µl droplet of 15 mg ml^{-1} cyt c_6 solution in 10 m*M* phosphate buffer (pH 7.0) was mixed with an equal volume of reservoir solution (200 m*M* citrate buffer pH 3.2 containing 2.2 *M* ammonium sulfate and 200 m*M* sodium ascorbate). Orange tetragonal prismatic crystals grew to typical dimensions of 0.5 × 0.2 × 0.2 mm in 10 d. The crystals formed clusters which had to be cut in order to obtain single crystals (Fig. 1*b*). Diffraction data were collected on a Rigaku R-AXIS IIC image-plate detector using graphite-monochromated Cu $K\alpha$ radiation from a Rigaku RU-200 rotating-anode X-ray generator operating at 40 kV and 100 mA with a 0.3 × 0.3 mm focal spot. The crystal-to-detector distance was set at 60 mm. Diffraction measurements were performed at room temperature. Data were processed using the *PROCESS* package (Rigaku, Japan). The crystals belong to space group $P2_12_12_1$, with unit-cell dimensions a = 46.74 (2), b = 49.42 (1), c = 37.11 (1) Å. For one molecule per asymmetric unit (Z = 4), the Matthews coefficient is 2.2 Å^3 Da^{-1} and the solvent content is 44%. The crystals diffract to 1.6 Å resolution.

Absorption spectra of the crystals showed that the tetragonal form was oxidized and the orthorhombic form was reduced. Crystal data and data-collection statistics are listed in Table 1.

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