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Crystallization and preliminary X-ray analysis of the *Rhodobacter capsulatus* magnesium chelatase BchI subunit

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The *Rhodobacter capsulatus* BchI protein is one of three subunits of Mg chelatase, the enzyme which catalyzes the first committed step of chlorophyll and bacteriochlorophyll biosynthesis. The BchI protein was produced with an inducible T7 RNA polymerase expression system in *Escherichia coli*. The protein was purified from the soluble cell-extract fraction and crystallized from polyethylene glycol solution. The crystals diffract to a minimum Bragg spacing of 2.1 Å. The space group is $P6_3$ with unit-cell dimensions $a = b = 90.6$, $c = 84.1$ Å.

1. Introduction

Photosynthetic organisms commit a large part of their resources to the synthesis of chlorophyll (Chl) or bacteriochlorophyll (Bchl). However, Bchl/Chl synthesis shares a common biosynthetic pathway with a second end-product, haem. The branch point of haem and Chl/Bchl synthesis is at the level of protoporphyrin IX utilization for the metal-ion chelation. Ferrochelatase, a monomeric enzyme, inserts a Fe^{2+} ion to make protohaem and Mg chelatase inserts a Mg^{2+} ion to make Mg-protoporphyrin IX on the chlorophyll branch (Beale & Weinstein, 1990).

Mg chelatase is a complex enzyme consisting of three separate subunit types (Willows *et al.*, 1996; Walker & Willows, 1997) and requires ATP for activity (reviewed in Walker & Willows, 1997). In the photosynthetic bacterium *Rhodobacter capsulatus*, the three subunits are named BchI, BchD and BchH and have subunit molecular masses of 38 kDa, 59 kDa and 129 kDa, respectively. Homologues of these subunits have been identified in other photosynthetic bacteria (Petersen *et al.*, 1996) as well as in cyanobacteria, algae, and plants (Luo & Weinstein, 1997; Jensen *et al.*, 1996). Of the three subunits, BchI is the most highly conserved among species. It has an ATPase consensus sequence and has demonstrated ATPase activity.

Because of the high similarity of the amino-acid sequences of BchI homologues from a diverse range of organisms from photosynthetic bacteria to higher plants (Walker & Willows, 1997), it is likely that this subunit has a similar role in the Mg chelatase reaction in all of these organisms. The three-dimensional structure of the different subunits of Mg chelatase will be very important in helping to define the functions and interactions of the three subunits. In this work we present a rapid high-yield purification procedure, crystallization and preliminary X-ray analysis of the *R. capsulatus* Mg chelatase BchI subunit.

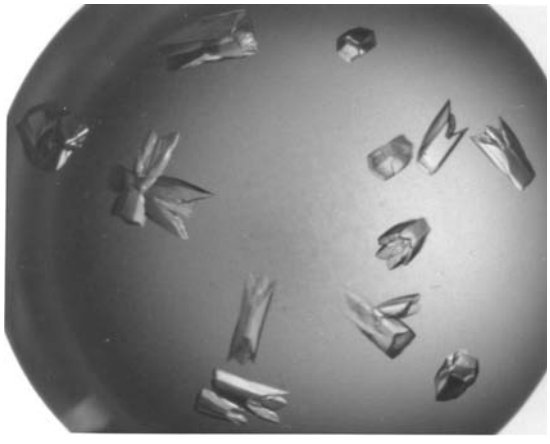

Figure 1
Crystals of BchI. Crystals formed in 3 d at room temperature using the hanging-drop vapour-diffusion method by mixing 5 µl of 18 mg ml^{-1} BchI with 5 µl of 16%(*w*/*v*) PEG 2000, 0.1 *M* Tris–HCl pH 7.4.

2. Materials and methods

2.1. Gene cloning and expression

The plasmid pRPS404 (Marrs, 1981), which contains a 44 kbp region of the *R. capsulatus* photosynthetic gene cluster (Bollivar *et al.*, 1990), was used as a polymerase chain reaction (PCR) template throughout. A modified T-vector was constructed between the *Eco*RI and *Bam*HI sites of pBluescript (KS) as described (Kwak & Kim, 1995) and this was used to subclone the *bch*ID and *bch*I genes as described below. The oligonucleotides 5′-GGATCATCTTGCGGAAACTGT-3′ and 5′-ATGACTACCGCCGTCGCTCGACTT-CAACCCTCTGCT-3′ were used to amplify the *bchID* region of the photosynthetic gene cluster by PCR. The 3 kbp PCR product was then cloned directly into the T-vector to yield plasmid pKSBchID. This cloning construct created an *Nde*I site at the *bch*I translation start site. A portion of the insert containing the *bch*I gene and the N-terminal portion of the *bch*D gene was then subcloned between the *Nde*I and *Bam*HI sites of pET3a to create pETBchI. *Escherichia coli* strain BL21 (DE3)

pLysS containing pETBchI was grown at 310 K in 1 l of LB medium containing 100 mg ml^{-1} ampicillin and 34 mg ml^{-1} chloramphenicol until the A_{600} of the culture was 0.8. Protein expression was induced by the addition of isopropyl β-D-thiogalactopyranoside to 1 m*M*. After 4 h, the cells were harvested by centrifugation at 4000*g* for 10 min.

2.2. Protein purification

The cells from 1 l of culture were resuspended in 20 ml of 50 m*M* tricine–NaOH pH 8.0, 15 m*M* $MgCl_2$, 4 m*M* dithiothreitol. The suspension was then frozen at 253 K, thawed once and lysed by passage through a French pressure cell at 1.38×10^8 Pa. The lysate was centrifuged at 30000*g* for 30 min and polyethylene glycol of average molecular mass 8000 (PEG 8000) was added to the supernatant to a concentration of 10%(*w*/*v*). The solution was placed on ice for 30 min and then centrifuged for 30 min at 10000*g*. The pellet was discarded and PEG 8000 was added to 25%(*w*/*v*). After 2 h on ice, the solution was centrifuged at 30000*g*. The supernatant was discarded and the precipitate was washed once with 30%(*w*/*v*) PEG 8000 and then redissolved in 50 m*M* tricine–NaOH pH 8.0, 15 m*M* $MgCl_2$, 4 m*M* dithiothreitol. The solution was loaded onto a ResourceQ (Pharmacia) column pre-equilibrated in the same buffer and then eluted with a linear gradient of 0–1 *M* NaCl in this buffer, in 10 column volumes. The BchI protein eluted at 0.3 *M* NaCl. This BchI was then desalted into 10 m*M* Tris–HCl pH 8.0 by Sephadex G-25 column chromatography and lyophilized. The yield of protein from 1 l of culture during a typical purification is shown in Table 1.

2.3. Crystallization

Crystal screen I (Hampton Research, Riverside, CA) was used for finding suitable preliminary crystallization conditions. Screening was performed at 277 K with the hanging-drop vapour-diffusion technique.

2.4. Data collection

Prior to freezing, 2 µl of PEG 400 was added to the drop with the crystals. The drop was allowed to equilibrate for a few minutes, after which the crystals were transferred in a loop (Hampton Research) to a vapour stream from liquid nitrogen at 100 K (Oxford Cryosystems). Although the crystals in Fig. 1 look rather imperfect, the 'wings' could easily be detached from them in the drop prior to data collection, yielding single crystals with a clear hexagonal shape. Data were collected at beamline BL711 at the MAX II synchrotron, Lund, Sweden using a MAR Research imaging plate. The use of cryo-freezing and synchrotron radiation allowed an improvement of the resolution from 2.9 Å at the laboratory source (Rigaku RU200BEH) to 2.1 Å. Data were integrated and scaled using *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997).

Table 1
Purification of BchI.

The BchI content as a proportion of the total protein was estimated at each stage on a Coomassie Brilliant Blue G-250-stained SDS–PAGE gel using a Bio-Rad 690 Densitometer. Total protein content was estimated using the protein assay kit from Bio-Rad USA. BchI activity was monitored at each stage by its ability to reconstitute Mg chelatase activity with BchD and BchH proteins (Willows *et al.*, 1996).

Purification stage	Total protein (mg)	BchI (mg)	Yield (%)
Lysate supernatant	420	84	100
10–20% PEG precipitate	204	56	67
ResourceQ column eluate	35	35	42

Table 2
Details of data collection and processing.

X-ray source	MAX II, BL711
Wavelength (Å)	0.958
Cell dimensions (Å)	$a = b = 90.12$, $c = 83.67$
Resolution (Å)	20.0–2.1
Number of unique reflections	22070
Completeness (%)	99.0
Multiplicity	$\geq$3 for 90% of reflections
$I/\sigma(I)$	>3 for 95% of reflections
R_{sym}	0.066
In the highest resolution shell	
Resolution (Å)	2.17–2.10
Completeness (%)	99.6
Multiplicity	$\geq$3 for 91% of reflections
$I/\sigma(I)$	>3 for 89% of reflections
R_{sym}	0.22

3. Results and discussion

A protein concentration of 15 mg ml^{-1} was used in the initial screen. In initial experiments, needle-like crystals up to 1 mm long were obtained in 8%(*w*/*v*) PEG 8000, 0.1 *M* Tris–HCl pH 8.5. Microcrystals were also obtained with 0.4 *M* potassium/sodium tartrate after approximately four weeks at 277 K. The crystals grown in PEG 8000 were extremely fragile and appeared to be tubular. To obtain thicker crystals, the following were evaluated systematically: pH, PEG average molecular mass, PEG concentration, buffer composition and buffer concentration.

The largest crystals ($0.1 \times 0.1 \times 0.5$ mm) were obtained using hanging drops. The reservoir solution was 16%(*w*/*v*) PEG 2000, 0.1 *M* Tris–HCl pH 7.4. The drops consisted of 5 µl of 18 mg ml^{-1} BchI mixed with 5 µl of reservoir solution at room temperature. Under these conditions, crystals suitable for diffraction formed within a few days (Fig. 1).

The crystals were analyzed as described in §2 and diffracted to at least 2.1 Å resolution. The space group is $P6_3$ with one molecule in the asymmetric unit. The solvent content of the crystals was 53% and the Matthews coefficient V_m was 2.62 Å^3 Da^{-1}, which is in the range usually observed for protein crystals (Matthews, 1968). The mosaicity of the crystals was about 0.5° as refined by *SCALEPACK*. Details of data collection and processing for the synchrotron cryo-data are presented in Table 2. The unit-cell dimensions for the room-temperature data are greater ($a = b = 92.44$, $c = 85.38$ Å) than those for the cryo-data, which is an effect of crystal freezing. The search for heavy-atom derivatives is in progress.

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