Acta Crystallographica Section F
Structural Biology and Crystallization Communications
ISSN 1744-3091

Crystallization and preliminary crystallographic studies of UbiG, an *O*-methyltransferase from *Escherichia coli*

Li Xing,[a,b] Yuwei Zhu,[a,b] Pengfei Fang,[a,b] Jing Wang,[a,b] Fuxing Zeng,[a,b] Xuesong Li,[a,b] Maikun Teng[a,b]* and Xu Li[a,b]*

[a]School of Life Sciences, University of Science and Technology of China, 96 Jinzhai Road, Hefei, Anhui 230026, People's Republic of China, and [b]Key Laboratory of Structural Biology, Chinese Academy of Sciences, 96 Jinzhai Road, Hefei, Anhui 230026, People's Republic of China

Correspondence e-mail: mkteng@ustc.edu.cn, sachem@ustc.edu.cn

Received 1 February 2011
Accepted 15 April 2011

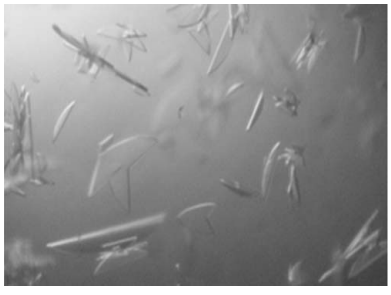
UbiG, an *O*-methyltransferase from the ubiquinone-biosynthesis pathway in *Escherichia coli*, catalyzes two *O*-methyl transfer steps. The primary structures of the *O*-methyltransferase enzyme family used in ubiquinone synthesis are conserved in both prokaryotes and eukaryotes, but their tertiary structures and catalytic mechanisms are not yet known. Here, UbiG with an N-terminal hexahistidine tag was expressed and crystallized. Crystals grown by the hanging-drop vapour-diffusion method diffracted to 2.00 Å resolution and belonged to space group *C*121, with unit-cell parameters a = 119.8, b = 58.6, c = 40.2 Å, β = 105.3°. Both Matthews coefficient analysis and the self-rotation function suggested the presence of one molecule per asymmetric unit in the crystal, with a solvent content of 50.52% (V_M = 2.48 Å^3 Da^{-1}).

1. Introduction

Ubiquinone (coenzyme Q) is a lipophilic substituted benzoquinone that is found in the plasma membranes of prokaryotes and mitochondria and the Golgi vesicles and lysosomes of eukaryotes (Søballe & Poole, 2000). Ubiquinone has various functions in different organisms. In bacteria, ubiquinone is a key component of the respiratory chain that transfers electrons from complex I or II to complex III (Mitchell, 1976). Ubiquinone is also involved in the ArcB/ArcA system, which senses oxygen availability and regulates corresponding reactions (Georgellis *et al.*, 2001). In the *Escherichia coli* periplasm, ubiquinone plays a role as an electron acceptor in the formation of disulfides by DsbA and DsbB (Bader *et al.*, 1999). In higher eukaryotes such as *Homo sapiens*, in addition to participating in the electron-transfer chain to synthesize ATP, ubiquinone is also involved in cell physiology, and deficiencies are closely associated with many diseases such as neurodegenerative disorders (Beal, 2004), diabetes, cancer and muscular and cardiovascular diseases (Søballe & Poole, 2000).

The biosynthesis of ubiquinone first requires the assembly of a quinone head group and a hydrophobic isoprenoid of varying length. Modification of the benzoquinone ring then follows, including C-hydroxylation, decarboxylation, O-methylation and C-methylation, which are carried out by different enzymes (Bentinger *et al.*, 2010). There are two methylation steps during the O-methylation process in ubiquinone synthesis. The first step converts 3,4-dihydroxy-5-hexaprenylbenzoic acid to 3-methoxy-4-hydroxy-5-hexaprenylbenzoic acid and the second step converts demethyl-hydroquinone to hydroquinone (Poon *et al.*, 1999).

In eukaryotes, Coq3 is the enzyme that catalyzes O-methylation reactions in ubiquinone biosynthesis (Poon *et al.*, 1999). A recent report suggested that knockout of *coq3* is related to early developmental arrest in *Caenorhabditis elegans* (Asencio *et al.*, 2009). In *E. coli*, the homologue of Coq3 is UbiG, a 240-residue protein which is required for both O-methylation steps in ubiquinone biosynthesis (Leppik *et al.*, 1976). A single amino-acid mutation of UbiG (L132Q) can result in ubiquinone deficiency in *E. coli* cells, leading to a high



sensitivity to thiols and a failure to grow on succinate (Gulmezian *et al.*, 2006). UbiG can methylate various farnesylated analogues of ubiquinone intermediates and rescue a yeast *coq3*Δ mutant (Poon *et al.*, 1999).

An amino-acid sequence comparison between UbiG and the Coq3 proteins from both *Saccharomyces cerevisiae* and *H. sapiens* indicated that the primary structures of these three proteins are conserved (34% identity), apart from an N-terminal truncation of approximately 80 amino acids in UbiG. The extra 80 N-terminal residues in the Coq3 proteins have been considered to be a mitochondrial targeting sequence that is associated with other polypeptides and a recent report also indicated that the yeast Coq3/Coq4/Coq6 proteins could form an active complex for ubiquinone-biosynthesis activity (Marbois *et al.*, 2005). Compared with the active UbiG monomer (Leppik *et al.*, 1976), it was suggested that the N-terminal ∼80 amino acids of Coq3 proteins play a role in protein–protein interaction but are not necessary for O-methylation catalytic activity. It was also suggested that structure determination of UbiG could reveal the main catalytic mechanisms of the two O-methylation steps in ubiquinone biosynthesis.

2. Materials and methods

2.1. Cloning and expression

Primers of sense strand 5′-GCGCGCATATGAATGCCGAAAA-ATCGCCG-3′ and antisense strand 5′-GACGACTCGAGTTATT-TATTCTGCGTGTGCAGC-3′ (Invitrogen) were used to amplify the target gene by polymerase chain reaction. The PCR fragment was inserted into expression vector pET28b (Novagen, modified) to create recombinant UbiG with a hexahistidine tag (MGHHHHHH) at the N-terminus. The plasmid was then transformed into *E. coli* BL21 (DE3) (Novagen) competent cells. The transformant was grown in 0.4 l Luria–Bertani (LB) medium containing 50 µg ml^{-1} kanamycin at 310 K. When an OD_{600} of 0.8–1.0 was reached, 0.5 m*M* isopropyl β-D-1-thiogalactopyranoside (IPTG) was added to induce expression. After 4 h induction at 310 K, the cells were harvested by centrifugation.

2.2. Purification

Harvested cells were suspended in buffer *A* (consisting of 20 m*M* Tris–HCl pH 7.5, 200 m*M* NaCl) and lysed by sonication on ice. The cell lysate was centrifuged. The clear supernatant was passed through an Ni–NTA column (Qiagen) previously equilibrated with buffer *A*. All unbound proteins were washed out with buffer *A* containing 50 m*M* imidazole. The bound protein was eluted with buffer *A* containing 500 m*M* imidazole. After ultrafiltration to 2 ml using a Millipore 10 kDa centrifugal device, the protein was purified on a Superdex 200 (GE Healthcare) gel-filtration chromatography column using buffer *A*. Fractions containing the desired protein were determined by SDS–PAGE and subsequently pooled, desalted with buffer *B* (20 m*M* Tris–HCl pH 7.5, 40 m*M* NaCl) and ultrafiltrated to 5 ml. DEAE anion-exchange chromatography employing a 0.04–1.0 *M* NaCl gradient was used for further purification. The fractions containing UbiG were then combined and dialyzed using buffer *B*.

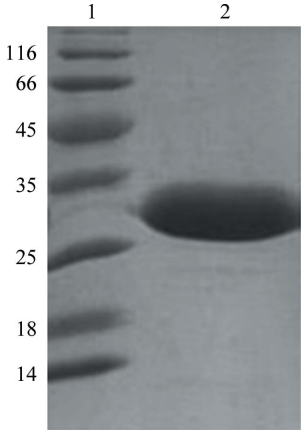


Figure 1
SDS–PAGE analysis of UbiG before crystallization. The protein was analyzed on 15% SDS–PAGE and stained with Coomassie Blue. Lane 1, molecular-weight markers (kDa); lane 2, UbiG after three purification steps.

2.3. Lysine methylation

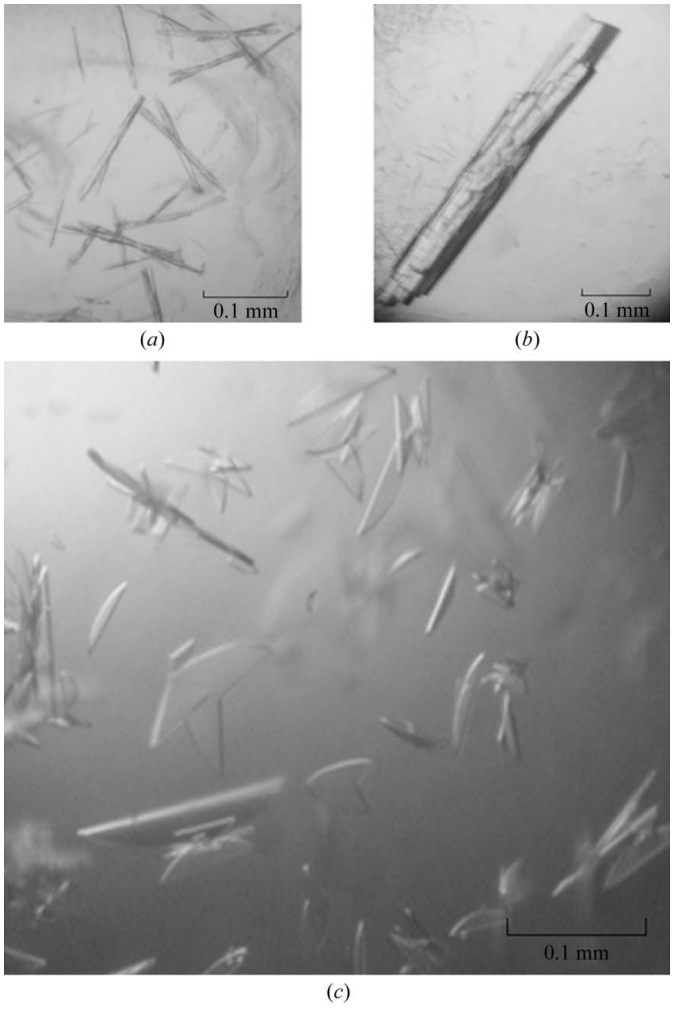
The lysine-methylation reaction was performed basically as described previously (Walter *et al.*, 2006). The UbiG protein was diluted to 1 mg ml^{-1} in 50 m*M* HEPES pH 7.5, 250 m*M* NaCl. 20 µl freshly prepared 1 *M* dimethylamine–borane complex (ABC; Fluka) and 40 µl 1 *M* formaldehyde (Fluka) were then added per millilitre of protein solution. The reaction was carried out at 277 K. After 2 h, a further 20 µl 1 *M* ABC and 40 µl 1 *M* formaldehyde were added per millilitre of solution and the mixture was incubated for a further 2 h. 10 µl 1 *M* ABC per millilitre of solution was then added and the mixture was incubated at 277 K overnight. Finally, the reaction solution was applied onto a Superdex 200 gel-filtration chromatography column pre-equilibrated with buffer *A*.

2.4. Crystallization

The native and reductive lysine-methylated UbiG protein solutions (in buffer *B*) were concentrated to 24 mg ml^{-1} (as calculated from the OD_{280} with an molar absorption coefficient of 31 065 M^{-1} cm^{-1} using an Eppendorf BioPhotometer Plus) by centrifugal ultrafiltration (Millipore, 10 kDa cutoff) prior to crystallization trials. Initial crystallization trials were carried out using the hanging-drop vapour-diffusion method at 287 K with a series of crystallization kits from Hampton Research. Each drop was prepared by mixing 0.25 µl protein solution containing 24 mg ml^{-1} (or 16 mg ml^{-1}) protein with 0.25 µl reservoir solution and was equilibrated against 100 µl reservoir solution. Needle-shaped microcrystals of the native UbiG protein were obtained using a precipitant solution consisting of 0.1 *M* HEPES pH 7.5 and 20% polyethylene glycol 10 000. Another crystal form (thin plate-like microcrystals) of native UbiG was found using a precipitant solution consisting of 10%(*v*/*v*) 2-methyl-2,4-pentanediol (MPD) and 0.1 *M* HEPES pH 7.0. Both native and reductive lysine-methylated UbiG proteins were used for crystallization optimization. After optimization, crystals of high quality were obtained for data collection.

2.5. Data collection and processing

A crystal mounted in a loop was soaked briefly in a cryoprotectant solution consisting of the corresponding reservoir solution supplemented with 25%(*v*/*v*) glycerol and was then flash-cooled in liquid nitrogen. X-ray diffraction data were collected on beamline 17U1 of the Shanghai Synchrotron Radiation Facility (SSRF) using a Jupiter CCD detector. All frames were collected at 100 K using a 1° oscillation angle with an exposure time of 1.2 s per frame. The crystal-to-detector distance was set to 200 mm. The complete diffraction data set was subsequently processed using *HKL*-2000 (Otwinowski & Minor, 1997).



(*a*) (*b*) (*c*)

Figure 2
Crystals of UbiG protein. Crystals were grown (*a*) before (small needle-shaped crystals) and (*b*) after (rod crystals) UbiG reductive-lysine methylation under the same condition consisting of 0.1 *M* HEPES pH 7.5 and 20% polyethylene glycol 10 000. (*c*) Plate-shaped native UbiG crystals obtained using 10%(*v*/*v*) 2-methyl-2,4-pentanediol (MPD) and 0.1 *M* HEPES pH 7.0.

3. Results and discussion

Full-length recombinant UbiG was expressed and purified and migrated as the major band with a molecular weight of 26.6 kDa on SDS–PAGE (Fig. 1). Using reductive lysine-methylated protein instead of native UbiG, large rod crystals grew instead of needle-shaped microcrystals under the same precipitant conditions (Figs. 2*a* and 2*b*).

Because of the poorer diffraction ability of the methylated rod crystals, we finally collected diffraction data from the thin plate-like native UbiG crystals (Fig. 2*c*). A total of 216 diffraction images were recorded from a single crystal. The thin plate-like crystal of native UbiG diffracted to a maximum resolution of 2.00 Å and belonged to the monoclinic space group *C*121, with unit-cell parameters a = 119.8, b = 58.6, c = 40.2 Å, β = 105.3°. The number of molecules in the asymmetric unit is assumed to be one based on the Matthews coefficient (2.48 Å^3 Da^{-1}), with a solvent content of 50.52%. Detailed data-processing statistics are given in Table 1.

Table 1
Data-collection statistics for UbiG.

Values in parentheses are for the highest resolution shell.

Space group	*C*121
Wavelength (Å)	0.9994
Unit-cell parameters (Å, °)	a = 119.8, b = 58.6, c = 40.2, β = 105.3
Resolution limits (Å)	50.00–2.00 (2.07–2.00)
No. of observed reflections	71303
No. of unique reflections	17708
Completeness (%)	97.5 (81.1)
$R_{\rm merge}$† (%)	11.8 (45.1)
Mean $I/\sigma(I)$	19.5 (2.0)
$V_{\rm M}$ (Å^3 Da^{-1})	2.48
No. of subunits per asymmetric unit	1
Solvent content (%)	50.52

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of an individual reflection and $\langle I(hkl)\rangle$ is the average intensity of that reflection.

Structure determination of *E. coli* UbiG will be attempted by the molecular-replacement method using the structure of PH1305 (a putative uncharacterized protein from *Pyrococcus horikoshii* OT3) as a search model (PDB entry 1wzn; H. Mizutani & N. Kunishima, unpublished work; 24% amino-acid sequence identity) and experimental phasing will be carried out if necessary (SeMet-derivative UbiG crystals have already been prepared).

We are grateful to the staff members of SSRF for the collection of diffraction data. Financial support for this project was provided by the Fundamental Research Funds for the Central Universities, the Chinese National Natural Science Foundation (grant Nos. 30900224 and 10979039), the Chinese Ministry of Science and Technology (grant Nos. 2006AA02A318 and 2009CB825500), the Science and Technological Fund of Anhui Province for Outstanding Youth (grant No. 10040606Y11), the Anhui Provincial Natural Science Foundation (grant No. 090413081) and the Education Department of Anhui Province (grant No. 2009SQRZ007ZD).

References

Asencio, C., Navas, P., Cabello, J., Schnabel, R., Cypser, J. R., Johnson, T. E. & Rodríguez-Aguilera, J. C. (2009). *Mech. Ageing Dev.* **130**, 145–153.
Bader, M., Muse, W., Ballou, D. P., Gassner, C. & Bardwell, J. C. (1999). *Cell*, **98**, 217–227.
Beal, M. F. (2004). *J. Bioenerg. Biomembr.* **36**, 381–386.
Bentinger, M., Tekle, M. & Dallner, G. (2010). *Biochem. Biophys. Res. Commun.* **396**, 74–79.
Georgellis, D., Kwon, O. & Lin, E. C. (2001). *Science*, **292**, 2314–2316.
Gulmezian, M., Zhang, H., Javor, G. T. & Clarke, C. F. (2006). *J. Bacteriol.* **188**, 6435–6439.
Leppik, R. A., Stroobant, P., Shineberg, B., Young, I. G. & Gibson, F. (1976). *Biochim. Biophys. Acta*, **428**, 146–156.
Marbois, B., Gin, P., Faull, K. F., Poon, W. W., Lee, P. T., Strahan, J., Shepherd, J. N. & Clarke, C. F. (2005). *J. Biol. Chem.* **280**, 20231–20238.
Mitchell, P. (1976). *J. Theor. Biol.* **62**, 327–367.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Poon, W. W., Barkovich, R. J., Hsu, A. Y., Frankel, A., Lee, P. T., Shepherd, J. N., Myles, D. C. & Clarke, C. F. (1999). *J. Biol. Chem.* **274**, 21665–21672.
Søballe, B. & Poole, R. K. (2000). *Microbiology*, **146**, 787–796.
Walter, T. S., Meier, C., Assenberg, R., Au, K. F., Ren, J. S., Verma, A., Nettleship, J. E., Owens, R. J., Stuart, D. I. & Grimes, J. M. (2006). *Structure*, **14**, 1617–1622.