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Crystallization and preliminary crystallographic studies of *Escherichia coli* CcmG/DsbE protein

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CcmG/DsbE is a typical thiol/disulfide oxidoreductase, exhibiting a specific reducing activity in a highly oxidizing environment, and is involved in electron transfer during the maturation of *c*-type cytochromes. *Escherichia coli* CcmG/DsbE (residues 19–185) has been crystallized using the hanging-drop vapour-diffusion technique. The crystals belong to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters $a = 35.48$, $b = 48.52$, $c = 84.78$ Å. X-ray data have been collected to 1.9 Å resolution.

1. Introduction

CcmG is one of the periplasmic protein thiol/disulfide oxidoreductases (Fabianek *et al.*, 1998) and is encoded by the *ccmG* gene in *Escherichia coli* (Thöny-Meyer *et al.*, 1995). CcmG exhibits a specific reducing activity in a highly oxidizing environment and is involved in electron transfer during the maturation of *c*-type cytochromes (Fabianek *et al.*, 1998).

CcmG is also called DsbE and was considered to be a disulfide-bond (Dsb) family member (Bardwell, 1994; Debarbieux & Beckwith, 1999); it is a substrate of DsbD that keeps CcmG in a reduced state (Fabianek *et al.*, 1998; Katzen & Beckwith, 2000).

CcmG is a member of the thioredoxin superfamily. The proteins in this superfamily share the thioredoxin (Trx) fold, which is characterized by a common active-site motif with general sequence Cys-*X*-*X*-Cys and two structural motifs, $\beta\alpha\beta$ and $\beta\beta\alpha$ (Bardwell, 1994; Martin, 1995). The highly hydrophobic N-terminal segment of *E. coli* CcmG anchors the protein onto the cytoplasmic membrane and the soluble C-terminal Trx domain faces toward the periplasm (Fabianek *et al.*, 1998).

The crystal structure of CcmG from *Bradyrhizobium japonicum* (residues 39–194) has recently been determined (Edeling *et al.*, 2002) from crystals grown by the microseeding technique (Edeling *et al.*, 2001) and is the only three-dimensional structure of CcmG so far reported. This structure revealed a modified Trx fold with two unique structural features, an unusually acidic active site and an adjacent groove formed from two inserts in the Trx fold (the N-terminal insert and the central insert), which were proposed to be the structural features necessary for the redox activity of CcmG (Edeling *et al.*, 2002). However, direct biochemical evidence of how CcmG functions in mediating electron transfer is still unavailable and the detailed redox mechanism is unknown.

CcmG from *E. coli* contains 185 amino-acid residues and shares 31% sequence identity with CcmG from *B. japonicum*. Asp97 is one of the three acidic active-site residues in *B. japonicum* CcmG and corresponds to Ala85, a hydrophobic residue, in *E. coli* CcmG. The N-terminal insert forming the groove in the *B. japonicum* CcmG structure corresponds to a segment containing a deletion of seven amino acids and an insertion of two amino acids in *E. coli* CcmG. These differences suggest that the three-dimensional structure of CcmG from *E. coli* might provide a further structural basis for understanding the redox mechanism of CcmG. *E. coli* CcmG (residues 19–185) and the N-terminal 57-residue truncated Trx domain have previously been overexpressed and purified in our laboratory and their properties and functions have been reported (Li *et al.*, 2001*a*,*b*). Here, we present the crystallization and preliminary crystallographic studies of *E. coli* CcmG (residues 19–185).

2. Method

The crystallization conditions of *E. coli* CcmG (residues 19–185) were first screened using the sparse-matrix sampling method (Jancarik & Kim, 1991). Three of the 50 conditions gave aggregates of tiny needle-shaped or thin plate-shaped crystals, all using PEG 4K as precipitant with or without 2-propanol. Various concentrations of the precipitants and different buffers with various pH values were further tested using the hanging-drop vapour-diffusion method and approximately 150 drops were set up, but little improvement was achieved. Seeding was also tried but failed. The conditions reported for the crystallization of *B. japonicum* CcmG were also tested, but no crystals were grown; however, crystallization succeeded when $(NH_4)_2SO_4$ (1.8–2.0 *M*) was used as the major precipitant and a small amount (2–4%) of PEG 4K was substituted for

Table 1
Crystal data and data-collection statistics of *E. coli* CcmG.

Values in parentheses correspond to the data in the highest resolution shell (1.97–1.90 Å).

Space group	$P2_12_12_1$
Unit-cell parameters (Å)	
a	35.483
b	48.515
c	84.775
No. molecules per AU	1
V_M† (Å^3 Da^{-1})	1.94
Resolution (Å)	1.9
No. unique reflections	12145
R_{merge}‡ (%)	6.2 (45.6)
Data completeness (%)	99.7 (97.0)
Data with $I > 3\sigma(I)$ (%)	72.5 (34.6)

† The volume per unit protein molecular weight (Matthews, 1968). ‡ $R_{merge} = \sum(|I_i - \langle I_h\rangle|)/\sum(I_i)$, where I_i is the ith observation of the intensity of the reflection h and I_h is the mean intensity of the reflection h from multiple measurements.

the PEG 400 in the previously used conditions. Three droplets gave good single crystals, one in each drop, without seeding. The three single crystals all have prismatic morphology. The crystal of CcmG used for X-ray data collection was grown at 293 K in a droplet composed of 2 µl of protein solution and 2 µl of reservoir solution. The protein solution contained 20 mg ml^{-1} CcmG, 25 m*M* Tris–HCl pH 8.0, 250 m*M* NaCl and 1 m*M* EDTA. The reservoir solution consisted of 0.1 *M* HEPES buffer pH 7.8, 2.0 *M* $(NH_4)_2SO_4$ and 2%(*w*/*v*) PEG 4K.

A crystal of CcmG was sealed in an X-ray capillary and X-ray data were collected at 293 K using a diffractometer equipped with a CCD detector. The data were processed using *HKL*2000 (Otwinowski & Minor, 1997).

3. Results

Crystals of CcmG with dimensions of 0.3 × 0.17 × 0.1 mm grew within a month. The X-ray data were 99.7% complete at 1.9 Å resolution, giving an R_{merge} of 6.2%. The crystal data and data-collection statistics are shown in Table 1. Structure determination is in progress.

4. Discussion

It was reported that crystals of *B. japonicum* CcmG were grown from microseeded hanging drops containing 1 µl of 40 mg ml^{-1} protein and 1 µl of a reservoir solution containing 0.1 *M* HEPES pH 6.5, 2%(*v*/*v*) PEG 400 and 2.0 *M* $(NH_4)_2SO_4$ (Edeling *et al.*, 2001). As a comparison, *E. coli* CcmG was crystallized without seeding, with PEG 4K instead of PEG 400 and at a higher pH value and a lower protein concentration. Both proteins were in an oxidized state (Li *et al.*, 2001*b*; Edeling *et al.*, 2001), albeit under different crystallization conditions. The CcmG proteins from the two species share only 31% identity in amino-acid sequence and the *B. japonicum* CcmG samples for crystallization were prepared in Milli-Q water without any additives such as buffers or salts (Edeling *et al.*, 2001), whereas the *E. coli* CcmG samples used for crystallization contained 25 m*M* Tris–HCl pH 8.0, 250 m*M* NaCl and 1 m*M* EDTA.

The CcmG proteins from the two species crystallize in the same space group and the unit-cell parameters *a* and *b* of the two species are similar to each other; however, the *c* parameter of the *E. coli* crystals is 5.4 Å shorter than that of *B. japonicum* CcmG. This is probably related to the difference in the length and sequence of the N-terminal segment between the two species, which may lead to different conformation of this segment and may affect the crystal packing.

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