Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization of YloQ, a GTPase of unknown function essential for *Bacillus subtilis* viability

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Received 11 August 2003
Accepted 14 November 2003

YloQ is a putative ATP/GTP-binding protein of unknown function identified from the complete sequence of the *Bacillus subtilis* genome. A gene-knockout programme established that *yloQ* is one of a set of some 270 indispensable genes for the viability of this organism. Crystals of YloQ have been grown from HEPES-buffered solutions at pH 7.5 containing polyethylene glycol and diffraction data have been collected extending to 2.5 Å spacing.

1. Introduction

The genome of *Bacillus subtilis* contains around 4100 genes, of which some 270 have either been shown to be or predicted to be essential (Kunst *et al.*, 1997; Kobayashi *et al.*, 2003). Out of this group of genes, 11 have unknown roles in the physiology of the cell. YloQ, a polypeptide of 298 residues, is one such protein. Although it has not been possible to generate strains harbouring deletions in *yloQ*, low-level expression of this gene (together with the downstream genes *yloR* and *yloS*) is associated with morphological changes in which the cells have a more spherical shape (E. Madec & S. J. Séror, unpublished results). YloQ contains motifs characteristic of a GTP-binding site, including the G1 (Walker A), G3 (Walker B) and G4 signature sequences. The order in which these motifs appear in the sequence of YloQ indicates a circular permutation of a classical GTPase, the G1–G3–G4 motifs appearing in the order G4–G1–G3. Another protein that exhibits this permutation is YjeQ from *Escherichia coli*, the function of which is also unknown (Leipe *et al.*, 2002; Daigle *et al.*, 2002). It has been confirmed that YloQ is a GTP-binding protein with a low intrinsic GTPase activity that is abolished by a mutation in the Walker A motif (Cladière *et al.*, in preparation). YloQ, like YjeQ, also possesses a putative zinc-binding site as deduced from the presence of a Cys-X_4-Cys-*X*-His-X_5-Cys motif towards its C-terminus.

To provide a structural description of this curious GTPase, we have overexpressed, purified and crystallized YloQ from *B. subtilis* and collected preliminary diffraction data from these crystals. It is hoped that combined with biochemical and genetic analyses, the structure of YloQ will define the essential function of this protein and illuminate its contribution to cell physiology.

2. Expression and purification

YloQ was produced as an N-terminally hexahistidine-tagged protein from *E. coli* DH5α harbouring pOMG201, a pBAD plasmid derivative in which expression of the recombinant protein is driven by the arabinose-inducible pBAD promoter. Cells were grown in LB medium supplemented with ampicillin at 310 K to OD_{600} = 1.5. Expression of recombinant His_6-YloQ was induced by the addition of arabinose to a final concentration of 0.05%. Cells were subsequently lysed, the cell extract loaded onto a nickel-chelation column equilibrated with 20 m*M* imidazole and the His-tagged YloQ was eluted with a 20–300 m*M* imidazole gradient. The wild-type protein, which was >90% pure, was identified by its size and its GTPase activity, whereas the matching peak fraction from cells overexpressing a catalytic site mutation in YloQ was completely inactive (Cladière *et al.*, in preparation).

Column fractions were analysed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and those containing YloQ were identified and pooled. The YloQ sample was concentrated by ultrafiltration in an Amicon cell and loaded onto a Superdex S200 (120 ml, Pharmacia) gel-filtration column equilibrated with 30 m*M* HEPES buffer pH 7.5, 100 m*M* NaCl. The gel-filtration profiles suggest that YloQ is present as a mixture of monomers and dimers, a conclusion that was confirmed by analytical ultracentrifugation analysis (Cladière *et al.*, in preparation). This two-step purification procedure yielded 65 mg of pure (~97% as judged by SDS–PAGE) His_6-tagged YloQ suitable for crystallization trials from 2 l of culture grown in a fermentor. YloQ was concentrated to 15–25 mg ml^{-1} in 30 m*M* HEPES pH 7.5, 100 m*M* NaCl and stored as frozen aliquots at 193 K.

3. Crystallization

Crystallization experiments with His_6-tagged YloQ were set up at 291 K using the hanging-drop vapour-diffusion method exploiting a variety of screening kits (Brzozowski & Walton, 2001; Jancarik & Kim, 1991). Initial crystals of YloQ appeared from CSS-I condition No. 6 and CSS-II condition No. 6. The conditions were refined by varying the PEG concentrations and sizes until small single crystals (0.2 × 0.2 × 0.1 mm) were obtained from hanging drops consisting of 1 µl of 10–15 mg ml^{-1} protein and an equal volume of either (*a*) 0.1 *M* HEPES pH 7.5, 0.2 *M* calcium acetate and 10–14% polyethylene glycol 5000 or (*b*) 0.1 *M* HEPES pH 7.5, 0.8 *M* sodium formate and 22% polyethylene glycol 2000 (Fig. 1). These crystals were obtained with pooled protein containing the monomer and dimer fractions following gel-filtration chromatography. Curiously, attempts to grow crystals from the separate monomer and dimer fractions have so far been unsuccessful. Crystals of YloQ have also been grown in the presence of the non-hydrolysable GTP analogue GTPγS. These crystals grew under the same conditions as those of the native protein; however, they have a different needle-like morphology, perhaps indicating that the nucleotide is bound. As yet these crystals are unsuitable for X-ray analysis.

4. Data collection

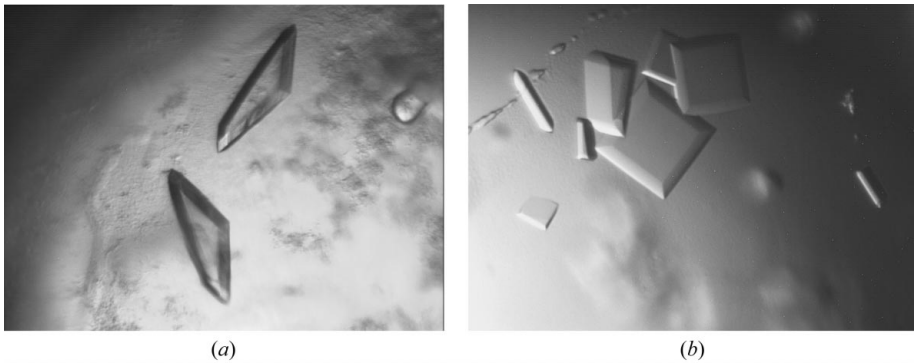
A single crystal grown under condition (*a*) above was transferred into a solution of mother liquor supplemented with 25% glycerol and flash-cooled in liquid N_2. The crystal was exposed to synchrotron radiation on beamline BM14 at the ESRF, Grenoble (λ = 0.9184 Å) and diffraction data were recorded on a MAR CCD detector. A complete three-dimensional data set extending to 2.5 Å spacing was recorded in 360 exposures with a 0.5° oscillation. The exposure time was 25 s and the total data-acquisition time was 2.5 h. The data were processed using *DENZO*, *SCALEPACK* and *TRUNCATE* (Otwinowski & Minor, 1997; Collaborative Computational Project, Number 4, 1994). The crystals belong to space group $P4_{(1/3)}2_12$, with unit-cell parameters $a = b = 47.9$, $c = 274.2$ Å, and have a mosaicity of 0.5°. If there is assumed to be a single YloQ protomer in the asymmetric unit, the solvent content is 46.3% and the Matthews coefficient is 2.3 Å^3 Da^{-1}. 220 313 observations of 11 888 reflections in the resolution range 25–2.5 Å were measured (286 observations of 83 reflections were rejected during scaling). The mean overall redundancy is 6.1 (5.4); figures in parentheses represent the highest resolution shell (2.59–2.50 Å). The data are 99.9% (99.7%) complete with an overall $R_{\rm merge}$ (on intensities) of 0.091 (0.268); the mean $I/\sigma(I)$ is 12.9 (5.5) and the percentage of reflections for which $I/\sigma(I) > 3$ is 85.2 (66.8). A subsequent resolution test suggested that the crystals diffract to 2.2 Å spacing.

5. Discussion

Crystals of YloQ suitable for structure determination have been grown and efforts are currently under way to determine suitable phases for this purpose. Attempts to determine the structure of YloQ by molecular replacement using the coordinates of the Era (Chen *et al.*, 1999) translation factor and GTPase (the two proteins have 37 identical and 63 conserved residues over ∼300 residues) as a search model have proved unsuccessful and *ab initio* phases are sought. We are therefore (i) preparing SeMet-substituted YloQ for crystallization and (ii) screening for conventional derivatives of the existing crystals by soaking in solutions containing heavy-atom compounds, to enable phases to be derived by anomalous dispersion and/or multiple isomorphous replacement methods, respectively.

YloQ is indispensable to *B. subtilis* and it is very likely to be essential in other Gram-positive organisms including pathogens. In *B. subtilis*, the gene encoding YloQ is situated immediately downstream of the genes *prpC* and *prkC* which encode a protein phosphatase (Obuchowski *et al.*, 2000) and a protein kinase (Madec *et al.*, 2002, 2003), respectively. The *prpC-prkC-yloQ* gene arrangement is conserved in several Gram-positive species, including *B. anthracis*, *Listeria monocytogenes* and *Staphylococcus aureus*, and *prkC* has been shown to be essential in the latter (Forsyth *et al.*, 2002). These observations indicate that YloQ is a promising target for the design of drugs targeted against Gram-positive pathogenic bacteria. A three-dimensional structure of the protein will underpin this effort.

This work was supported by grants from the BBSRC (grant 87/C14673) and the Wellcome Trust (grant 057339) to AJW. Lionel Cladière was supported by AVENTIS, France (contract 99040) and SJS also gratefully acknowledges the support of the Association de la Recherche sur le Cancer, Centre National de la Recherche Scientifique and Université Paris-Sud.



(*a*) (*b*)

Figure 1
Crystals of YloQ grown from (*a*) 0.1 *M* HEPES pH 7.5, 0.2 *M* calcium acetate and 10–14% polyethylene glycol 5000 and (*b*) 0.1 *M* HEPES pH 7.5, 0.8 *M* sodium formate and 22% polyethylene glycol 2000. The width of these panels corresponds to ∼1 mm.

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