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Crystallization and preliminary X-ray analysis of the LOV domain of the blue-light receptor YtvA from *Bacillus amyloliquefaciens* FZB42

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Light–oxygen–voltage (LOV) proteins play an important role in blue-light-dependent physiological processes in many organisms. The LOV domain of the blue-light receptor YtvA from *Bacillus amyloliquefaciens* FZB42 has been purified and crystallized at 277 K using the sitting-drop vapour-diffusion method with 2-ethoxyethanol as a precipitant. A data set was collected to 1.60 Å resolution from a single crystal at 100 K using synchrotron radiation. The LOV domain of YtvA crystallized in space group $C222_1$, with unit-cell parameters a = 64.95, b = 83.76, c = 55.81 Å. The crystal structure of the LOV domain of YtvA was determined by the molecular-replacement method. The crystal contained one molecule per asymmetric unit, with a Matthews coefficient (V_M) of 3.04 Å^3 Da^{-1}; the solvent content was estimated to be 59.5%.

1. Introduction

A large number of light-regulated physiological functions in plants (*e.g.* phototropism and stomata opening) are controlled by the blue-light receptors phototropins (phot; for a review, see Wada *et al.*, 2005). Phot contain two light–oxygen–voltage (LOV) domains (LOV1 and LOV2) in a tandem array, each consisting of ∼110 amino acids and each binding a flavin mononucleotide (FMN) as a chromophore. LOV domains, which belong to the PAS (Per–Arnt–Sim) superfamily (Losi & Gärtner, 2008), have a similar α/β structural motif in various proteins, *e.g.* in blue-light receptors, oxygen-sensor proteins and voltage-gated potassium-channel proteins.

In the dark, LOV domains absorb blue light at around 450 nm (LOV_{447}). Photoexcitation of LOV domains causes a cysteine residue located near to the FMN molecule to form an adduct with the 4a position of FMN. This reaction takes place during the microsecond decay of the FMN triplet state. The photoadduct form (LOV_{390}) is considered to be the signalling state of LOV domains and has been shown to be essential for YtvA to exert its positive regulation on σ^B during environmental stress (Gaidenko *et al.*, 2006; Avila-Pérez *et al.*, 2006). In a thermally driven process, the LOV_{390} state converts back to the LOV_{447} state within minutes.

YtvA from *Bacillus subtilis* was the first prokaryotic counterpart of plant phot to be identified (Losi *et al.*, 2002) and carries a single LOV domain connected to a C-terminal sulfate transporter and anti-σ-factor antagonist (STAS) domain (Aravind & Koonin, 2000). The LOV and STAS domains are connected by a linker region Jα (amino acids 127–146, *B. subtilis* numbering). The STAS domain has recently been shown to bind ATP and GTP (Buttani *et al.*, 2006, 2007), a functionality that is probably linked to the role of YtvA as a positive regulator of the general stress transcription factor σ^B (Akbar *et al.*, 2001). In recent years, LOV-domain proteins have been found in about 13% of all sequenced prokaryotic genomes, making this light-sensing motif the most abundant light-sensing motif among blue-light receptors (Losi, 2006; Losi & Gärtner, 2008).

YtvA from *B. amyloliquefaciens* FZB42 is closely related to YtvA from *B. subtilis*, showing a similar two-domain architecture consisting of a LOV domain and a STAS domain. Isolated LOV domains (amino acids 25–126 in *B. subtilis* YtvA) have a strong tendency to dimerize, which is prevented in the full-length protein but was still observed in a LOV domain carrying the N-terminal extension (amino

acids 1–24; N-terminal cap). Analysis of circular-dichroism measurements showed that both the N-terminal cap and the linker region (amino acids 127–146) between the LOV and the STAS domain from *B. subtilis* are helical and that the central β-scaffold is distorted in the LOV-domain dimers (Buttani *et al.*, 2007). To date, several crystal structures of LOV domains have been determined (Crosson & Moffat, 2001; Fedorov *et al.*, 2003; Nakasako *et al.*, 2008). In order to understand the interaction between the reactive cysteine and FMN during the photocycle, we purified the LOV domain of YtvA from *B. amyloliquefaciens* FZB42 and crystallized it. The protein was overexpressed in *Escherichia coli* with a 6×His tag. In this paper, we report the crystallization and preliminary X-ray analysis of the LOV domain of the photosensor YtvA from *B. amyloliquefaciens* FZB42.

2. Materials and methods

2.1. Protein expression and purification

The genomic DNA of *B. amyloliquefaciens* FZB42 was provided by Professor Rainer Borriss of Humboldt-University, Berlin. The ORF RBAM_027270 was amplified by PCR using the primers 5′-CAG GGA CCC GGT **CAT ATG** GCT GAC TCA AAT GTA TTC GG (forward) and 5′-GGC ACC AGA GCG TT**A AGC TT**A TAC GAC CGG AAG CAC GT (reverse), containing an *Nde*I and a *Hin*dIII restriction site (shown in bold), respectively. Platinum Taq DNA polymerase (Invitrogen, Karsruhe, Germany) was used for PCR. The PCR product was then digested with *Nde*I/*Hin*dIII (NEB, Ipswich, England) and ligated into the digested expression vector pET28a (Novagen–Merck, Darmstadt, Germany). The recombinant proteins carried an N-terminal extension sequence (derived from the cloning vector) including a 6×His tag (MGSSHHHHHHSSGLVPRGSH).

The His-tagged protein was expressed in *E. coli* BL21 (DE3) (Stratagene, Amsterdam, Netherlands) *via* induction with IPTG (final concentration of 0.25 m*M*). The protein was then purified by affinity chromatography and concentrated in 10 m*M* sodium phosphate buffer pH 8.0. The identity of the LOV domain was confirmed by SDS–PAGE and MALDI–TOF. The molecular weight of the purified protein was determined to be 20 480 Da, corresponding to the protein sequence up to position 160 (leucine) and the N-terminal His tag.

2.2. Crystallization

The purified protein was concentrated to 10 mg ml^{-1} in 10 m*M* sodium phosphate buffer pH 8.0 and 10 m*M* NaCl by centrifugation using a Millipore Ultrafree 0.5 centrifugal filter device (10 kDa molecular-weight cutoff, Millipore). Crystallization screening was carried out at 277 K using the sitting-drop vapour-diffusion method. Crystal Screen (Hampton Research, USA) and Cryo 1 (Emerald BioSystems Inc., USA) were used for initial screening. The protein droplets were prepared by mixing 1 µl purified protein solution and 1 µl reservoir buffer solution and were set up in a 96-well plate (Corning, USA) with 100 µl reservoir solution. Crystals suitable for diffraction experiments were obtained using conditions consisting of 35%(*v*/*v*) 2-ethoxyethanol and 0.1 *M* cacodylate pH 6.5. The crystal dimensions were typically 0.1 × 0.1 × 0.1 mm.

2.3. Data collection and analysis

A complete native data set was collected at 100 K on beamline BL14.2 at BESSY II (Berlin, Germany). The detector was a Rayonix 225. 225 frames of 4 s exposure time and 0.8° oscillation were collected. The X-ray wavelength was chosen to be 0.91841 Å. The distance between the crystal and the detector was maintained at 180 mm. In order to collect the data at cryogenic temperature, the crystal was frozen directly in liquid nitrogen and mounted on the goniostat in a nitrogen-gas stream at 100 K. Data collection was carried out in room light. The data set was indexed and integrated using the program *XDS* (Kabsch, 1993). The scaling was carried out with *XSCALE* (Kabsch, 1993). The conditions of the data collection and the results obtained are summarized in Table 1.

Table 1
X-ray data-collection statistics.

Values in parentheses are for the highest resolution shell (1.64–1.60 Å).

Wavelength (Å)	0.91841
Space group	$C222_1$
Unit-cell parameters (Å)	
a	64.95
b	83.76
c	55.81
Resolution range (Å)	33.50–1.60 (1.64–1.60)
Observed reflections	136343
Unique reflections	19550
$R_{\rm merge}$†	0.040 (0.467)
Completeness (%)	95.6 (86.6)
$\langle I/\sigma(I)\rangle$	28.2 (3.7)
$V_{\rm M}$ (Å^3 Da^{-1})	3.04

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of the *i*th observation and $\langle I(hkl)\rangle$ is the mean intensity of the reflections.

3. Results and discussion

The LOV domain of YtvA from *B. amyloliquefaciens* FZB42 was successfully purified, characterized and crystallized. It showed all the salient features of flavin-containing LOV domains such as an absorption maximum at 450 nm, light-induced formation of the photoproduct and a thermally driven dark recovery of the parent state ($\tau_{\rm rec}$ = 25 700 ± 30 s at 293 K). Crystals suitable for diffraction

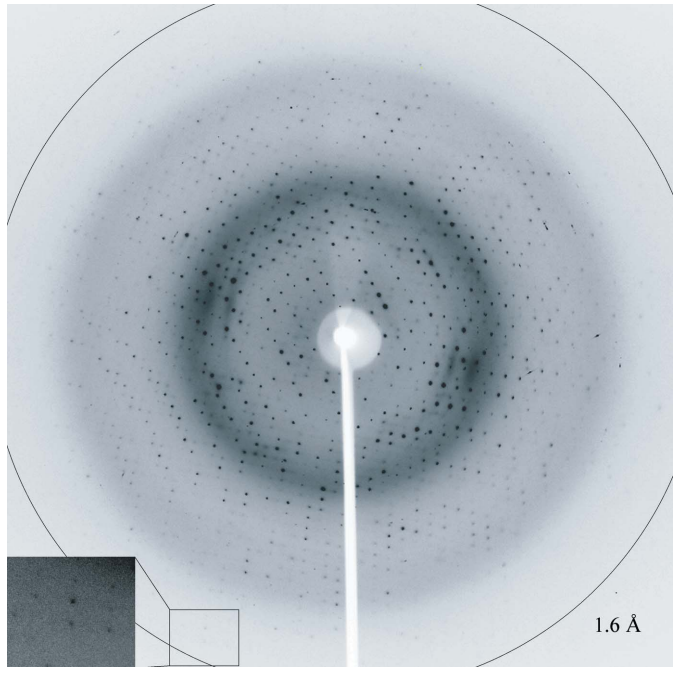

Figure 1
Diffraction pattern of the LOV domain of YtvA from *B. amyloliquefaciens* FZB42.

experiments were obtained using conditions consisting of 35%(*v*/*v*) 2-ethoxyethanol and 0.1 *M* cacodylate pH 6.5. The crystal dimensions were typically 0.1 × 0.1 × 0.1 mm. The crystals diffracted to 1.60 Å resolution (Fig. 1) and belonged to space group $C222_1$, with unit-cell parameters $a = 64.95$, $b = 83.76$, $c = 55.81$ Å. The calculated Matthews coefficient (V_M) of 3.04 Å^3 Da^{-1} with a solvent content of 59.4% indicated the presence of one molecule in the asymmetric unit. The crystal structure of the LOV domain of YtvA from *B. amyloliquefaciens* FZB42 was solved by the molecular-replacement method using the program *MOLREP* (Vagin & Isupov, 2001) from the *CCP*4 package (Collaborative Computational Project, Number 4, 1994). Data for the LOV domain of YtvA from *B. subtilis* (PDB code 2pr6), which has 72% amino-acid sequence identity to the LOV domain of YtvA from *B. amyloliquefaciens* FZB42, were used as coordinates for the search model.

After calculation of the electron-density map using the molecular-replacement solution and initial refinement with the program *REFMAC*5 (Vagin *et al.*, 2004), the electron-density map of FMN was determined. The side chain of the reactive cysteine showed a multiple conformation without binding to FMN, suggesting that the crystal contained the dark state of the LOV domain. Data analysis revealed that two molecules assemble to give a dimer by crystallographic twofold symmetry. The dimer was formed by an interaction between the N-terminal α-helices of each molecule. This is in contrast to the LOV domain of YtvA from *B. subtilis*, in which a dimeric form was observed that was formed by an interaction between the α-helices at the C-terminal end of the LOV domain (Möglich & Moffat, 2007). Dimerization similar to that found in this work for the LOV domain of YtvA from *B. amyloliquefaciens* has also been reported for the redox-sensor domain of NifI (PDB code 2gj3; Key *et al.*, 2007). A total of 115 amino-acid residues (Leu13–Glu128) were traced in the electron-density map. Model building and refinement using the program *SHELX*-97 (Sheldrick, 2008) are now in progress.

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