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Crystallization and preliminary crystallographic study of the periplasmic domain of the *Escherichia coli* TolR protein

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The TolR protein from *Escherichia coli* is part of the Tol–Pal multiprotein complex used by group A colicins to penetrate and kill cells. All genes of the Tol–Pal system are conserved in Gram-negative bacteria and this system is thought to play a role in the maintenance of the bacterial envelope integrity, although its exact function is not known. The TolR protein comprises 142 amino acids. The periplasmic domain of the TolR protein has been expressed, purified and crystallized. The crystals belong to the tetragonal space group $P4_122$, with unit-cell parameters $a = 46.3$, $c = 178.0$ Å. There are one or two molecules in the asymmetric unit. Frozen crystals diffract to at least 3.2 Å resolution using synchrotron radiation. Selenomethionine-substituted periplasmic TolR protein is currently being produced in order to use multiwavelength anomalous dispersion (MAD) for phasing.

1. Introduction

TolR is a single transmembrane domain protein of 142 residues. It is anchored to the inner membrane of *E. coli* by its N-terminal region. The rest of the protein is localized in the periplasm (residues 44–142) (Kampfenkel & Braun, 1993; Müller *et al.*, 1993). TolR is a component of the Tol–Pal system, which comprises six proteins: TolA, TolQ and TolR are inner membrane proteins, TolB and Orf2 are periplasmic proteins and Pal is a lipoprotein associated with the outer membrane (for reviews, see Lazdunski *et al.*, 1998; Lazzaroni *et al.*, 1999). The Tol–Pal system is organized into two sub-complexes: one in the cytoplasmic membrane between TolA, TolQ and TolR and one associated with the outer membrane between TolB and Pal (Bouveret *et al.*, 1995). These two complexes seem to interact with each other through the interaction of TolA with Pal (Cascales *et al.*, 2000) and with TolB (Walburger, personal communication).

The Tol–Pal system of *E. coli* is involved in the maintenance of outer membrane integrity; since *tol*/*pal* mutants are hypersensitive to drugs and detergents, they release periplasmic proteins in the extracellular medium and form outer membrane vesicles (Lazzaroni *et al.*, 1989; Webster, 1991; Bernadac *et al.*, 1998). However, its physiological role is not precisely defined. It is conserved in many Gram-negative bacteria (Sturgis, 2001) and the *tolA* gene appears to be essential in some pathogenic microorganisms such as *Pseudomonas aeruginosa* or *E. coli* O7 (Dennis *et al.*, 1996; Gaspar *et al.*, 2000).

The inner membrane complex TolA–TolQ–TolR is related to the TonB system, which is composed of TonB, ExbB and ExbD proteins and is involved in the active transport of iron siderophores and vitamin B_{12} across the outer membrane. The TonB system is thought to couple the proton motive force of the inner membrane to these transport processes (for a review, see Braun, 1995). ExbB and ExbD are homologous to TolQ and TolR, respectively, and share some functional reactivity (Braun, 1989; Braun & Hermann, 1993). The organization in the inner membrane of the TolA–TolQ–TolR complex and the TonB system are similar: TolA, TolQ and TolR proteins and TonB, ExbB and ExbD proteins appear to interact with each other *via* their transmembrane segments. The TolA transmembrane domain interacts with the first transmembrane domain of TolQ and with the transmembrane domain of TolR (Derouiche *et al.*, 1995; Germon *et al.*, 1998; Journet *et al.*, 1999). The third transmembrane domain of TolQ interacts with the transmembrane domain of TolR (Lazzaroni *et al.*, 1995). Moreover, the TolR periplasmic region is also involved in several interactions. The TolR periplasmic C-terminal region (residues 117–142), which exhibits a high percentage of conserved residues among the different TolR and ExbD sequenced so far, seems to be involved in the TolQ–TolR and TolA–TolR interactions (Lazzaroni *et al.*, 1995; Journet *et al.*, 1999). TolR has also been shown to dimerize *via* its periplasmic region (Journet *et al.*, 1999).

Bacterial toxins such as group A and group B colicins have been shown to parasite the Tol–Pal and TonB systems, respectively, to cross

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the *E. coli* envelope and reach their cellular target (for a review, see Lazdunski *et al.*, 1998). The N-terminal domain of colicins is involved in this import process. TolA and TolB proteins interact with the N-terminal domain of colicins during translocation (Bénédetti *et al.*, 1991; Bouveret *et al.*, 1997, 1998) and it has recently been shown that TolR also interacts with the N-terminal domain of colicin A *via* its central periplasmic domain (amino acids 44–117) (Journet, personal communication).

A TolR periplasmic domain derivative (residues 44–142) tagged with a six-histidine motif at its C-terminus (TolRII-III-His) has been produced in the periplasm and purified with metal-affinity chromatography. Here, we report crystallization conditions and preliminary X-ray data from the crystals.

2. Results and discussion

2.1. Purification of TolRII-III-His

TolRII-III-His was expressed from BL21(DE3) (Novagen) pETRII-III cells. The previously described pETRII-III plasmid (Journet *et al.*, 1999) encodes the periplasmic domain of TolR (residues 44–142) flanked by three unrelated N-terminus residues (MEF) and a C-terminal seven-residue linker (KLAAALE) preceding the terminus His_6 tag. After initial growth at 310 K of a 1 l culture of BL21(DE3) pETRII-III cells, the temperature was set to 295 K when OD_{600} reached 1 and the TolRII-III-His protein expression was induced by adding 100 m*M* isopropylthio-β-D-galactoside. Cells were harvested after overnight induction ($OD_{600} \simeq 3$) and resuspended in 30 ml 20 m*M* Tris–HCl pH 8 and 30% sucrose in the presence of protease inhibitors (Complete EDTA-free, Boehringer Mannheim). After five minutes of incubation on ice, 30 ml of 10 m*M* Tris–HCl pH 8 was added and the incubation on ice continued for another 15 min. After a 10 min centrifugation at 5500*g*, the supernatant, corresponding to the periplasmic fraction, was filtered through a 0.2 mm Millipore filter. This fraction was applied to a 5 ml column of Cobalt affinity resin (Clontech) equilibrated with 20 m*M* Tris–HCl pH 8 and 100 m*M* NaCl. The column was washed with 20 m*M* Tris–HCl pH 8 and 100 m*M* NaCl and then washed with the same buffer containing 10 m*M* imidazole. TolRII-III-His was eluted from the column with a linear gradient of imidazole (10–500 m*M*). The eluates were collected and the purity of the different fractions was assessed by SDS–PAGE. The fractions corresponding to elution with 100–190 m*M* imidazole were pooled and dialysed against 20 m*M* Tris–HCl buffer pH 8 and 100 m*M* NaCl. The concentration of the resulting preparation was about 4 mg ml^{-1}. The purified TolRII-III-His gave a 12 553.2 Da molecular mass using mass spectroscopy, as expected from the amino-acid composition.

2.2. Crystallization of TolRII-III-His

The TolRII-III-His protein was concentrated to 18 mg ml^{-1} in 10 m*M* Tris pH 7.5 using a centrifugal filter device (Ultrafree Biomax 5K, Millipore, Bedford MA, USA). Precipitation experiments were carried out on the TolRII-III-His protein using various precipitating agents [$(NH_4)_2SO_4$, PEG, NaCl, MPD, ethanol] at various pHs (5, 6, 7, 8, 9). Solutions from Hampton Research Crystal Screens I and II were then used and crystallization trials were performed at 293 K by hanging-drop vapour diffusion using 24-well culture plates. Each hanging drop was prepared by mixing 0.5 µl of the 18 mg ml^{-1} TolRII-III-His with an equal volume of the reservoir solution. The hanging drop on the cover glass was vapour-equilibrated against 500 µl of the reservoir solution in each well of the tissue-culture plate. Two conditions led to crystals. Diamond-shaped crystals were obtained after one month from solution 22 of Crystal Screen II containing 30%(*w*/*v*) PEG 4000, 0.2 *M* sodium acetate, 0.1 *M* Tris buffer pH 8.5 and cubic crystals were obtained from solution 15 of Crystal Screen I containing 30%(*w*/*v*) PEG 8000, 0.2 *M* ammonium sulfate and 0.1 *M* sodium cacodylate buffer pH 6.5. Crystallization conditions were optimized using the *SamBA* software (Audic *et al.*, 1997) and the best crystals were obtained at pH 7.0 using 0.1 *M* imidazole buffer, 35%(*w*/*v*) PEG 8000 and 0.5 *M* $(NH_4)_2SO_4$. Subsequently, 5%(*v*/*v*) glycerol was added to the crystallization medium as a cryoprotectant agent.

2.3. Data collection and processing

A single crystal of dimensions 0.1 × 0.1 × 0.1 mm was collected in a Hampton Research 0.2 mm^3 loop, flash-frozen to 105 K in a cold nitrogen-gas stream and subjected to X-ray diffraction. The data set was collected on a 130 mm MAR CCD at the ESRF synchrotron-radiation facility (ID13, microfocus) at a wavelength of 0.96 Å. Data collection was carried out with oscillation angles of 1.0° and a crystal-to-detector distance of 180 mm. The total oscillation range collected was 96°. Space-group determination was performed using the autoindexing option in *DENZO* (Otwinowski, 1993). The crystals belong to the tetragonal space group $P4_122$ (or enantiomorph $P4_322$), with unit-cell parameters a = 46.33, c = 177.97 Å. The packing density for one monomer of TolRII-III-His (12.5 kDa) in the asymmetric unit of these crystals (volume 381 857 Å^3) is 3.8 Å^3 Da^{-1}, corresponding to an approximate solvent content of 67.7%, while for two monomers the packing density is 1.9 Å^3 Da^{-1}, corresponding to an approximate solvent content of 35.3%; both values are reasonable for globular proteins (Matthews, 1968), but the higher solvent content would explain the extreme fragility of the crystals as well as their temperature sensitivity.

The data set was processed using the *MOSFLM* package (Leslie, 1990; Kabsch, 1993; Campbell, 1995; Steller *et al.*, 1998); the *SCALA* program from the *CCP*4 package (Collaborative Computational Project, Number 4, 1994) was used for the scaling and data reduction of the native data set. The crystal diffracted to 3.2 Å and 58 329 reflections were measured in the resolution range 36.5–3.2 Å. This was reduced to a data set of 3615 unique reflections with an R_{sym} value of 8.7. This represents a completeness of 99.3% with a multiplicity of 6.0 and an average $I/\sigma(I)$ of 4.1. For the highest resolution shell, 2089 reflections were measured in the resolution range 3.31–3.2 Å, corresponding to 352 unique *hkl*, an R_{sym} value of 46.6, an average $I/\sigma(I)$ of 1.6, a completeness of 99.3% and a multiplicity of 5.9. We are currently producing crystals of selenomethionine-substituted TolRII-III-His in order to solve the TolR structure using the MAD method (Hendrickson *et al.*, 1990).

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References

Audic, S., Lopez, F., Claverie, J.-M., Poirot, O. & Abergel, C. (1997). *Proteins*, **29**, 252–257.

Bénédetti, H., Lazdunski, C. & Lloubès, R. (1991). *EMBO J.* **10**, 1989–1995.

Bernadac, A., Gavioli, M., Lazzaroni, J. C., Raina, S. & Lloubès, R. (1998). *J. Bacteriol.* **180**, 4872–4878.

Bouveret, E., Derouiche, R., Rigal, A., Lloubes, R., Lazdunski, C. & Bénédetti, H. (1995). *J. Biol. Chem.* **270**, 11071–11077.

Bouveret, E., Rigal, A., Lazdunski, C. & Bénédetti, H. (1997). *Mol. Microbiol.* **23**, 909–920.

Bouveret, E., Rigal, A., Lazdunski, C. & Bénédetti, H. (1998). *Mol. Microbiol.* **27**, 143–157.
Braun, V. (1989). *J. Bacteriol.* **171**, 6387–6390.
Braun, V. (1995). *FEMS Microbiol. Rev.* **16**, 295–307.
Braun, V. & Hermann, C. (1993). *Mol. Microbiol.* **8**, 261–268.
Campbell, J. W. (1995). *J. Appl. Cryst.* **28**, 236–242.
Cascales, E., Gavioli, M., Sturgis, J. & Lloubès, R. (2000). *Mol. Microbiol.* **38**, 904–915.
Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.
Dennis, J. J., Lafontaine, E. R. & Sokol, P. A. (1996). *J. Bacteriol.* **178**, 7059–7068.
Derouiche, R., Bénédetti, H., Lazzaroni, J. C., Lazdunski, C. & Lloubès, R. (1995). *J. Biol. Chem.* **270**, 11078–11084.
Gaspar, J. A., Thomas, J. A., Marolda, C. L. & Valvano, M. A. (2000). *Mol. Microbiol.* **38**, 262–275.
Germon, P., Clavel, T., Vianney, A., Portalier, R. & Lazzaroni, J. C. (1998). *J. Bacteriol.* **180**, 6433–6439.
Hendrickson, W. A., Horton, J. R. & LeMaster, D. M. (1990). *EMBO J.* **9**, 1665–1672.
Journet, L., Rigal, A., Lazdunski, C. & Bénédetti, H. (1999). *J. Bacteriol.* **181**, 4476–4484.
Kabsch, W. (1993). *J. Appl. Cryst.* **26**, 795–800.
Kampfenkel, K. & Braun, V. (1993). *J. Bacteriol.* **175**, 4485–4491.
Leslie, A. G. W. (1990). *Crystallographic Computing.* Oxford University Press.
Lazdunski, C. J., Bouveret, E., Rigal, A., Journet, L., Lloubès, R. & Bénédetti, H. (1998). *J. Bacteriol.* **180**, 4993–5002.
Lazzaroni, J. C., Fognini-Lefebvre, N. & Portalier, R. (1989). *Mol. Gen. Genet.* **218**, 460–464.
Lazzaroni, J. C., Germon, P., Ray, M. C. & Vianney, A. (1999). *FEMS Microbiol. Lett.* **177**, 191–197.
Lazzaroni, J. C., Vianney, A., Popot, J. L., Bénédetti, H., Samatey, F., Lazdunski, C., Portalier, R. & Géli, V. (1995). *J. Mol. Biol.* **246**, 1–7.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Müller, M. M., Vianney, A., Lazzaroni, J. C., Webster, R. E. & Portalier, R. (1993). *J. Bacteriol.* **175**, 6059–6061.
Otwinowski, Z. (1993). *Proceedings of the CCP4 Study Weekend. Data Collection and Processing*, edited by L. Sawyer, N. Isaacs & S. Bailey, pp. 56–62. Warrington: Daresbury Laboratory.
Steller, I., Bolotovsky, R. & Rossmann, M. (1998). *J. Appl. Cryst.* **30**, 1036–1040.
Sturgis, J. N. (2001). In the press.
Webster, R. E. (1991). *Mol. Microbiol.* **5**, 1005–1011.