Crystal structure of the N-terminal domain of VqsR from *Pseudomonas aeruginosa* at 2.1 Å resolution

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VqsR is a quorum-sensing (QS) transcriptional regulator which controls QS systems (*las*, *rhl* and *pqs*) by directly downregulating the expression of *qscR* in *Pseudomonas aeruginosa*. As a member of the LuxR family of proteins, VqsR shares the common motif of a helix–turn–helix (HTH)-type DNA-binding domain at the C-terminus, while the function of its N-terminal domain remains obscure. Here, the crystal structure of the N-terminal domain of VqsR (VqsR-N; residues 1–193) was determined at a resolution of 2.1 Å. The structure is folded into a regular α – β – α sandwich topology, which is similar to the ligand-binding domain (LBD) of the LuxR-type QS receptors. Although their sequence similarity is very low, structural comparison reveals that VqsR-N has a conserved enclosed cavity which could recognize acyl-homoserine lactones (AHLs) as in other LuxR-type AHL receptors. The structure suggests that VqsR could be a potential AHL receptor.

1. Introduction

Pseudomonas aeruginosa is a Gram-negative opportunistic human pathogen which causes various types of infections, especially in immunocompromised and cystic fibrosis patients. The pathogenicity of *P. aeruginosa* is mainly owing to the development of biofilms and the expression of extracellular virulence factors (Wu *et al.*, 2000; Hall-Stoodley & Stoodley, 2005), which are controlled by a cell-density-dependent signalling system termed quorum sensing (QS; Davies *et al.*, 1998; de Kievit & Iglewski, 2000). Briefly, *P. aeruginosa* possesses at least three main QS systems: *las*, *rhl* and *pqs*. The two primary QS systems, *las* and *rhl*, generate the autoinducer signals *N*-3-oxododecanoyl-homoserine lactone (3O-C₁₂-HSL) and *N*-butanoyl-homoserine lactone (C₄-HSL), which specifically bind and activate the transcription factors LasR and RhlR, respectively (Bjarnsholt & Givskov, 2007). The third QS system is the *pqs* system, which uses alkyl-quinolone signals as autoinducers to activate the LysR-type transcriptional regulator PqsR (Pesci *et al.*, 1999). Each of these systems is modulated at both the transcriptional level and the post-transcriptional level by a group of key regulators, such as Vfr (Albus *et al.*, 1997), RsaL (de Kievit *et al.*, 1999), QteE (Siehnel *et al.*, 2010) and VqsR (Juhas *et al.*, 2004).

VqsR has been characterized as a major virulence and QS regulator in *P. aeruginosa*. Inactivation of the *vqsR* gene led to decreased acyl-homoserine lactone (AHL) signal production and reduced expression of some QS-controlled virulence factors (Juhas *et al.*, 2004). Gene-chip expression analysis reported that VqsR differentially affected the expression of approximately 200 genes, including numerous genes belonging to the QS network (Juhas *et al.*, 2005). Furthermore, studies revealed that VqsR influences QS systems through directly

Table 1

Macromolecule-production information.

Source organism	<i>P. aeruginosa</i> PAO1
DNA source	Genomic DNA
Forward primer†	5'-GAAATTGGATCCATGGATATCGCATTGCACG-GC-3'
Reverse primer‡	5'-GAAATTCTCGAGTCACTGGACTTCCACCTCC-GC-3'
Cloning vector	pET-15b
Expression vector	pET-15b
Expression host	<i>E. coli</i> BL21 (DE3)
Complete amino-acid sequence of the construct produced§	MGSSHHHHHSGLEVLFGPGSMDIALHGGAWHE-SLGKLEALDRPFFWRILAQLGQFAPVDNWA-ALIFSDSSPLILSFMEEREVEEPDPLISRYI-TGLYLQDPFYQVSRNCRGGFLHLADIVSEDF-ETTEYYNTYFAHYVVTDEVQYNVPLDGERTLC-LSLGSERFSGAEQIALFELLRPWVIALMKKRI-HFEDAVREEAKPIAAAEVEVQ

† The BamHI site is underlined. ‡ The XhoI site is underlined. § The PreScission Protease recognition sequence is underlined.

controlling the expression of the LasR/RhlR homologue QscR (Liang *et al.*, 2012).

VqsR, encoded by PA2591, is a member of the LuxR family of proteins. Sequence analysis showed that VqsR consists of 268 amino acids, with a helix–turn–helix (HTH)-type DNA-binding domain (residues 198–257) at the C-terminus and a region of unknown function (residues 1–193) at the N-terminus. The structure of VqsR has not been solved to date. In this paper, we determined the structure of the N-terminal domain of VqsR (VqsR-N; residues 1–193) at 2.1 Å resolution. Structure comparison and sequence alignment between VqsR-N and homologous structures revealed that VqsR-N forms a similar α – β – α sandwich and has a conserved enclosed cavity, indicating that VqsR could be a potential AHL receptor.

2. Materials and methods

2.1. Macromolecule production

The coding sequence for VqsR-N (residues 1–193) was amplified by PCR from the *P. aeruginosa* strain PAO1 genome and was inserted into a modified pET-15b vector with a PreScission Protease cleavage site to remove the His tag. The constructed vector was then transformed into *Escherichia coli* strain BL21 (DE3) cells. The cells were cultured in Luria–Bertani (LB) medium with 100 $\mu\text{g ml}^{-1}$ ampicillin at 310 K to an OD₆₀₀ of 0.8. Expression of VqsR-N was induced with 0.12 mM isopropyl β -D-1-thiogalactopyranoside (IPTG) at 289 K and the induced cells were harvested at 6000g for 15 min at 277 K after overnight expression.

For protein purification, the cell pellet was resuspended in lysis buffer (25 mM Tris–HCl pH 8.0, 200 mM NaCl) and was lysed by sonication. After ultracentrifugation at 28 370g for 45 min at 277 K, the supernatant was loaded onto a nickel-chelating Sepharose affinity column (GE Healthcare) pre-equilibrated with lysis buffer. To remove contaminants, the column was washed with wash buffer (25 mM Tris–HCl pH 8.0, 200 mM NaCl, 10 mM imidazole), and the Ni–NTA column with His-tagged protein was then incubated with PreScission Protease [100:1(w:w)] at 277 K overnight to

Table 2

Crystallization.

Method	Hanging-drop vapour diffusion
Temperature (K)	293
Protein concentration (mg ml ⁻¹)	20
Buffer composition of protein solution	10 mM Tris–HCl pH 8.0, 100 mM NaCl, 3 mM DTT
Composition of reservoir solution	0.01 M magnesium acetate tetrahydrate, 0.05 M sodium cacodylate trihydrate pH 6.5, 1.3 M lithium sulfate monohydrate
Volume and ratio of drop	2 μl protein solution mixed with 2 μl reservoir solution
Volume of reservoir (μl)	500

remove the His tag. The untagged protein was eluted with elution buffer (25 mM Tris–HCl pH 8.0, 100 mM NaCl). For further purification, the eluted protein was purified using ion-exchange (Source Q, GE Healthcare) and size-exclusion (Superdex 200, GE Healthcare) column chromatography in 10 mM Tris–HCl pH 8.0, 100 mM NaCl, 3 mM DTT. The purified untagged proteins were collected according to the results of SDS–PAGE, and the final concentration of VqsR-N used for crystallization was approximately 20 mg ml⁻¹. Selenomethionine-labelled (SeMet) VqsR-N was produced by expression from *E. coli* strain BL21 (DE3) harbouring the same pET-15b vector. The bacterial cells were cultured in M9 medium supplemented with specific amino acids as well as selenomethionine as a substitute for methionine (Doublié, 1997). The induction conditions and purification procedure were the same as for the native protein. Macromolecule-production information is summarized in Table 1.

2.2. Crystallization

Preliminary crystallization experiments were performed using the sitting-drop vapour-diffusion method at 293 K. For initial screening, a 1:1 ratio of protein solution and commercial screen solutions (1 μl of each) was used. After about a week, crystals were observed in several conditions from Crystal Screen. For further optimization, the hanging-drop vapour-diffusion method was used to optimize the crystallization condition by altering the pH range and salt concentration. Finally, the best crystals were obtained using a reservoir solution consisting of 0.01 M magnesium acetate tetrahydrate, 0.05 M sodium cacodylate trihydrate pH 6.5, 1.3 M lithium sulfate monohydrate at 293 K. SeMet VqsR-N crystals were grown using the same solution. Crystallization information is summarized in Table 2.

2.3. Data collection and processing

To prevent radiation damage, all crystals were soaked in reservoir buffer containing 15%(v/v) glycerol and then flash-cooled in liquid nitrogen. X-ray diffraction data sets consisting of 180 and 360 images were collected from the native and SeMet crystals, respectively, with an oscillation angle of 1°, an exposure time of 0.5 s per frame and a crystal-to-detector distance of 250 mm using a Quantum 315r CCD detector on beamline BL17U1 (wavelength of 0.9791 Å) at Shanghai

Table 3

Data collection and processing.

Values in parentheses are for the outer shell.

	Native VqsR-N	SeMet VqsR-N
Diffraction source	BL17U1, SSRF	BL17U1, SSRF
Wavelength (Å)	0.9791	0.9791
Temperature (K)	100	100
Detector	ADSC Quantum 315r CCD	ADSC Quantum 315r CCD
Crystal-to-detector distance (mm)	250	250
Rotation range per image (°)	1	1
Total rotation range (°)	180	360
Exposure time per image (s)	0.5	0.5
Space group	<i>I</i> 222	<i>I</i> 222
<i>a</i> , <i>b</i> , <i>c</i> (Å)	52.83, 135.20, 150.77	52.98, 134.78, 150.21
α , β , γ (°)	90, 90, 90	90, 90, 90
Mosaicity (°)	0.17–0.25	0.26–0.38
Resolution range (Å)	50.00–2.10 (2.16–2.10)	50.00–2.20 (2.28–2.20)
Total No. of reflections	226958	400208
No. of unique reflections	32086	27810
Completeness (%)	99.9 (100.0)	100.0 (100.0)
Multiplicity	7.1 (7.4)	14.4 (14.9)
$\langle I/\sigma(I) \rangle$	26.04 (7.33)	56.93 (8.46)
$R_{\text{r.i.m.}}$ (%)	7.3 (37.5)	9.7 (49.1)
Overall <i>B</i> factor from Wilson plot (Å ²)	34.69	37.79

Table 4

Structure refinement.

Values in parentheses are for the outer shell.

Resolution range (Å)	36.41–2.10 (2.16–2.10)
Completeness (%)	99.8
No. of reflections, working set	32079
No. of reflections, test set	1626
Final R_{cryst}	0.203 (0.217)
Final R_{free}	0.240 (0.259)
No. of non-H atoms	
Protein	2718
Water	281
Total	2999
R.m.s. deviations	
Bonds (Å)	0.008
Angles (°)	0.888
Average <i>B</i> factors (Å ²)	
Protein	38.7
Water	47.0
Ramachandran plot	
Favoured regions (%)	94.4
Additionally allowed (%)	4.6
Outliers (%)	0.6

Synchrotron Radiation Facility (SSRF), Shanghai, People's Republic of China. Indexing, integration and scaling of the diffraction data were performed with *HKL-2000* (Otwinowski & Minor, 1997). Data-collection and processing statistics are given in Table 3.

2.4. Structure solution and refinement

The selenium single-wavelength anomalous dispersion (Se-SAD) phasing method was used to determine the phase of VqsR-N. Experimental phases were calculated in *PHENIX* with three heavy atoms identified by *AutoSol* (Terwilliger *et al.*, 2009). The quality of the electron-density map obtained after solvent flattening enabled the autobuilding of an initial

model using *AutoBuild* in *PHENIX* (Adams *et al.*, 2010). The initial model was used as a search model for molecular replacement against the native data set using *MOLREP* in *CCP4* (Winn *et al.*, 2011). The resulting model was further refined by alternating rounds of refinement and manual rebuilding using *phenix.refine* (Afonine *et al.*, 2012) and *Coot* (Emsley *et al.*, 2010). The R_{free} value was calculated using 5% of reflections that were randomly excluded from refinement. The resolution limit of the native data was selected as 2.05 Å based on the *R* factors, but the structure refinement yielded a high R_{free} value and a poor Ramachandran plot. The final structure was therefore refined to 2.1 Å resolution. The phasing and final refinement statistics are shown in Table 4. The final model was deposited in the Protein Data Bank (<http://www.rcsb.org>) as PDB entry 5xhx. All molecular structures in the figures were rendered by *PyMOL* (<http://www.pymol.org>).

3. Results and discussion

3.1. Overall and subunit structure

VqsR-N (residues 1–193), which excludes the C-terminal HTH motif, was expressed, purified and crystallized. The crystal structure of VqsR-N belonged to space group *I*222, with unit-cell parameters *a* = 52.8, *b* = 135.2, *c* = 150.8 Å, and was determined at 2.1 Å resolution. In the refined model, each asymmetric unit contains two monomers, and a comparison of the monomers yields an r.m.s.d. value of 0.8 Å, indicating that the two monomers are nearly identical. No density is observed for residues 1–6, 63–66 and 179–193 of chain *A*, as well as residues 1–6, 63–68 and 176–193 of chain *B*. Although mass spectrometry showed that Cys94 was not modified in solution, the electron density for Cys94 indicates that it may be oxidized to a cysteine sulfenic acid (Cys-SOH) in both monomers in the crystal. Similar to other LuxR-type proteins reported in the PDB, VqsR-N forms an α – β – α sandwich, which consists of a

Figure 1

Overall structure of VqsR-N. The structure is depicted as a cartoon diagram. The monomers of this crystallographic dimer are shown in cyan and purple. Secondary-structure elements referred to in the text are labelled.

five-stranded antiparallel β -sheet with strand order 2–1–5–4–3 packed against three α -helices on each side (Fig. 1). Three α -helices, $\alpha 1$ (residues 9–21), $\alpha 2$ (residues 26–37) and $\alpha 6$ (residues 151–174), form one outer layer of the structure and another three α -helices, $\alpha 3$ (residues 70–77), $\alpha 4$ (residues 84–91) and $\alpha 5$ (residues 112–122), form the opposite outer layer. Furthermore, each subunit structure also contains three 3_{10} -helices. Two subunits form a homodimer mainly through the bending part of the long helix $\alpha 6$ in the crystal.

3.2. The homodimer structure of VqsR

VqsR exists as a homodimer in solution, based on gel-filtration results (Liang *et al.*, 2012). As shown in Fig. 1, one asymmetric unit contains two molecules, which dimerize mostly *via* the short bending part of helix $\alpha 6$ (residues 151–161; Fig. 2*a*). According to a review of the structures of

LuxR-type homologues, there are several different dimerization forms for the QS receptors, which have differences in the dimeric interface of the ligand-binding domains (LBDs; Kim *et al.*, 2014). We also noticed that the molecules from two neighbouring asymmetric units simultaneously form another dimeric interaction. In this model, helix $\alpha 1$ and most of $\alpha 6$ (residues 159–170) of one subunit interact with most of helix $\alpha 6'$ (residues 159–170) and helix $\alpha 1'$, respectively, of the other subunit in the dimeric interface (Fig. 2*b*). The crystal-packing interactions of both models were analysed using the PISA server (Krissinel & Henrick, 2007). In the first dimeric model, the area of the interface is $715.0 \text{ \AA}^2$ and the free energy of dissociation is $-7.8 \text{ kcal mol}^{-1}$. For the second model, the values are $938.2 \text{ \AA}^2$ and $-13.6 \text{ kcal mol}^{-1}$, respectively. The larger buried surface area and the smaller free energy of dissociation show that the second model is stable in solution. Furthermore, full-length structures of VqsR were generated

Figure 2
Homodimer structure of VqsR. (*a*, *b*) Cartoon and semi-transparent surface representation of two dimeric models of VqsR-N. The helices involved in the dimeric interface are labelled. (*c*, *d*) Cartoon model of the full-length VqsR structure. The two models of the VqsR-N structure are the same as in (*a*) and (*b*), while the modelled HTH domains are coloured light grey.

based on the two dimeric models of VqsR-N (Figs. 2c and 2d). The HTH domain of VqsR was constructed with the *SWISS-MODEL* web server (Biasini *et al.*, 2014), using the structure of a homologue (PDB code 3qp5; Chen *et al.*, 2011) as a template. The orientations of the two separate HTH domains also indicate that the second dimeric model is more reasonable.

3.3. Structural comparison between VqsR-N and its homologues

Despite a lack of sequence homology to any previously determined structures, a *DALI* search for globally similar

proteins revealed that the overall structure of VqsR-N exhibits an unexpected similar fold to the LBDs of LuxR-type AHL receptors (Holm & Rosenström, 2010), including TraR (PDB entry 2q0o; Chen *et al.*, 2007), LasR (PDB entry 4ng2; Fan *et al.*, 2013), CviR (PDB entry 3qp5; Chen *et al.*, 2011) and SdiA (PDB entry 4lfu; Kim *et al.*, 2014) (Fig. 3a). The highest Z-score for the VqsR-N homologue LasR is 13.6 (r.m.s. deviation of 3.0 Å for 166 equivalent C α positions and 9% sequence identity). AHLs with various acyl-chain lengths are located in the enclosed cavities of these receptors. Similar to these AHL receptors, VqsR-N also has an enclosed cavity between α -helices $\alpha 3$ – $\alpha 5$ and β -strands $\beta 1$ – $\beta 5$ (Fig. 3a).

Figure 3

Structural comparison of VqsR-N with its homologues. (a) VqsR-N (cyan) was superposed with LasR (green), TraR (grey), CviR (salmon) and SdiA (yellow). (b) Residues involved in interaction with the HSL moiety of the AHL are shown as stick models in the same colours as in (a). (c) Close-up view of the AHL-binding cavities of various AHLs in LasR, CviR and TraR. AHLs are shown as stick models in the same colours as in (a). Secondary-structure elements and residues referred to in the text are labelled. (d) Multiple sequence alignment of VqsR-N and its homologues, including LasR, TraR, CviR and SdiA. Conserved residues are shown in blue boxes. The multiple sequence alignment was performed using *ESPrpt* (Robert & Gouet, 2014). Highly conserved residues are shown in white on a red background. Identical and conserved residues stabilizing the HSL moiety of the AHLs are denoted by red triangles and blue dots, respectively.

Structural comparison showed that VqsR-N possesses several strictly conserved residues which stabilize the homoserine lactone (HSL) head groups of the AHLs in other receptors (Zou & Nair, 2009), including Tyr75, Asp83, Pro84 and Ser142 (with Thr replacing Ser in TraR). Although Tyr113, Glu126 and Val127 of VqsR-N are not conserved, their equivalents in AHL receptors are also involved in HSL moiety binding, suggesting that the AHLs could be potential signalling molecules for VqsR (Figs. 3*b* and 3*d*). In the AHL-binding cavities, the orientations of 3O-C₁₂-HSL and AHLs with shorter acyl chains are different (Fig. 3*c*). Fig. 3*c* shows that the end of the antiparallel β -strand β 1 of VqsR-N, especially Phe48, makes a steric clash with the acyl chain of 3O-C₁₂-HSL in the LasR structure. This finding is consistent with a report that VqsR does not bind to 3O-C₁₂-HSL (Liang *et al.*, 2012). In contrast, AHLs with shorter acyl chains from the CviR and TraR structures can extend into the solvent region between α -helix α 3 and β -strand β 2 of VqsR-N. Our structural analyses suggest that VqsR may be a potential receptor for AHLs with shorter acyl chains.

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