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Structural basis for recognition of the type VI spike protein VgrG3 by a cognate immunity protein

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ABSTRACT

The bacterial type VI secretion system (T6SS) is used by donor cells to inject toxic effectors into receptor cells. The donor cells produce the corresponding immunity proteins to protect themselves against the effector proteins, thereby preventing their self-intoxication. Recently, the C-terminal domain of VgrG3 was identified as a T6SS effector. Information on the molecular mechanism of VgrG3 and its immunity protein TsaB has been lacking. Here, we determined the crystal structures of native TsaB and the VgrG3C–TsaB complex. VgrG3C adopts a canonical phage-T4-lysozyme-like fold. TsaB interacts with VgrG3C through molecular mimicry, and inserts into the VgrG3C pocket.

Structured summary of protein interactions:
VgrG3 and **TsaB** bind by x-ray crystallography (View interaction)
TsaB and **TsaB** bind by x-ray crystallography (View interaction)
VgrG3 and **TsaB** bind by cosedimentation in solution (View interaction)
TsaB and **TsaB** bind by cosedimentation in solution (1, 2)
TsaB binds to **VgrG3** by surface plasmon resonance (1, 2, 3, 4, 5, 6, 7)
VgrG3 and **TsaB** bind by molecular sieving (View interaction)
TsaB and **TsaB** bind by molecular sieving (View interaction)
VgrG3 and **TsaB** bind by x ray scattering (View interaction)

1. Introduction

The type VI secretion system (T6SS), a macromolecular nanomachine, is involved in the interaction with prokaryotic and eukaryotic cells [1]. The T6SS organelle is structurally and functionally analogous to contractile phage tails, including the spike, tube, sheath, and baseplate components [2–6]. Valine–glycine repeat protein G (VgrG), together with hemolysin coregulated protein (HCP), is secreted and required for the function of the T6SS machine, and represents the hallmark of T6SS [7]. Gram-negative bacteria expressing T6SS secrete and deliver toxic effector proteins into neighboring cells. The T6SS effectors destined for the periplasm degrade the cell wall and cell membrane of the receptor cells to kill the competitor cells, and the cognate immunity proteins expressed by the bacterium bind the effectors to prevent self-intoxication [8–13].

At present, several crystal structures of T6SS effector–immunity pairs have been determined [11,14–21], but these effectors are single-domain proteins and considered to transit via the HCP tube [22]. VgrG3 from *Vibrio cholera* consists of a bacteriophage-spike-like N-terminal domain (VgrG3N) acting as an important

Abbreviations: T6SS, the type VI secretion system; VgrG, valine–glycine repeat protein G; TsaB, the type VI secretion antitoxin B protein; AUC, analytical ultracentrifugation; SPR, surface plasmon resonance; SAXS, small-angle X-ray scattering

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component of T6SS apparatus and a C-terminal domain (VgrG3C) which functions as a peptidoglycan-targeting glycoside hydrolase [10]. Recent studies have shown that VgrG3C plays a pivotal role in the fight against competitor cells as a unique T6SS effector and the type VI secretion antitoxin B protein (TsaB) inhibits VgrG3C to prevent self-killing [10,12]. However, the structure of the VgrG-related effector has not yet been reported, which restricts our understanding of the VgrG and T6SS mechanisms.

Here, we first report the crystal structure of the VgrG-related T6SS effector (VgrG3C). VgrG3C displays a phage-T4-lysozyme-type fold. Compared with its structural homologs, VgrG3C displays several unique features, which are associated with its substrate specificity. The catalytic residues of VgrG3C are identified in this study. The immunity protein TsaB interacts extensively with VgrG3C, destroying the enzymatic activity of VgrG3C. Unlike other T6SS immunity proteins, TsaB tends to form oligomer. Importantly, the oligomerization of TsaB is involved in the inhibition of VgrG3C. Structural clarification of the VgrG3C–TsaB complex has revealed the inhibition mechanism. Our work also extends our understanding of the role of T6SS in interspecies competition.

2. Materials and methods

2.1. Protein expression and purification

The DNA encoding the C-terminal domain of VgrG3 (amino acids 731–1009) was amplified by PCR from the genomic DNA of *Vibrio cholerae*, and ligated into a BamHI/XhoI-digested pET21a(+) (Novagen) vector. The plasmid of VgrG3C was transformed into Escherichia coli BL21 (DE3) competent cells for induced expression with 0.2 mM isopropyl-β-D-1-thiogalactopyranoside (IPTG) at 289 K overnight. The cells were harvested by centrifugation and resuspended in buffer containing 20 mM Tris pH 8.0, 150 mM NaCl, 2 mM β-Mercaptoethanol, 0.1 mM Phenylmethylsulfonyl Fluoride (PMSF), 10 μg/ml DNaseI and 1 mM $MgCl_2$. Cells were lysed by French press and cell debris was removed by centrifugation at 30000×g for 50 min. The supernatant was purified by nickel affinity chromatography. The expression and purification of TsaB (amino acids 27–122) were performed as described previously [23]. The elution of VgrG3C was concentrated and directly applied to gel filtration (Superdex™ 200 10/300 GL, GE Healthcare). The fractions of VgrG3C and TsaB were mixed, and then applied to gel filtration chromatography. The buffer for gel filtration contained 20 mM Tris pH 8.0, 150 mM NaCl and 1 mM DTT. Pooled the complex peak and concentrated to ~12 mg/ml prior to the crystallization trial.

2.2. Crystallization and data collection

VgrG3C-TsaB complex was crystallized at 277 K using sitting-drop vapour-diffusion technique. The crystals of the native complex were grown by mixing 1 μl of protein solution with 1 μl reservoir solution consisting of 0.1 mM HEPES pH 7.6 and 16% (*w*/*v*) PEG3350. The crystals were transferred into cryoprotectant solution (reservoir solution supplemented with ethylene glycol up to 10% (*v*/*v*). The selenomethionine (Se-Met) complex was purified and crystallized essentially in the same conditions as native complex. The crystallization of TsaB was performed as described previously [23]. The X-ray diffraction data were collected at beamline 3W1A of Beijing Synchrotron Radiation Facility (BSRF), China. All datasets were processed with HKL2000 [24].

2.3. Structure determination and refinement

The structure of complex was solved by single-wavelength anomalous diffraction (SAD) method, the initial phases were determined using AutoSol in the PHENIX [25–27] software package, and density modification and automatic model building were subsequently performed using AutoBuild routine in PHENIX [28]. Further model was manually built by the program COOT [29]. All the structure refinements were performed by phenix.refine [30] of the PHENIX package. The final model quality was checked with MolProbity [31] from the GUI of PHENIX. The structure of TsaB was determined by molecular replacement method with AutoMR [26] from the GUI of PHENIX using TsaB of Se-Met complex structure as a search model. Detailed data collection and refinement are presented in Table 1. All the structure figures were generated by PyMol [32].

2.4. Analytical ultracentrifugation (AUC)

For the AUC experiment, proteins were purified as described above. AUC was performed using Beckman XL-I analytical ultracentrifuge equipped with an An60Ti rotor speed of 60000 rpm at 293 K. All the data were processed by SEDFIT program [33].

2.5. Surface plasmon resonance (SPR)

Binding affinity of effector and immunity was measured by the technique of surface plasmon resonance (SPR) using a BIAcore T100 (GE Healthcare) with single-cycle kinetics method. TsaB (1 μg/ml in 10 mM sodium acetate, pH 4.5) was immobilized on a CM5 sensor chip (GE Healthcare) up to level of 100RU. VgrG3C and its mutants were injected over the sensor chip surface in running buffer (10 mM HEPES pH 8.0, 150 mM NaCl and 0.005 % (*v*/*v*) Tween-20) for 60 s at a flow rate of 30 μl/min. The dissociation occurred in running buffer for 720 s at the same flow rate and the sensor chip was regenerated with 10 mM NaOH for 20 s. Data were analyzed by 1:1 binding model using BIAcore T100 Evaluation software (GE Healthcare).

2.6. Small-angle X-ray scattering (SAXS)

SAXS experiments were performed at beamline 1W2A station in BSRF using a MARCCD165 detector. The proteins were diluted to 2–4 mg/ml in SAXS buffer (20 mM Tris pH8.0, 150 mM NaCl and 0.2 mM DTT). The experimental one-dimensional data were generated by PRIMUS [34] software from ATSAS suite. Subsequently, the

Table 1
Data collection and refinement statistics.

	SeMet-VgrG3C-TsaB	Native-TsaB
Data collection		
Space group	*C2*	$P2_12_12_1$
Resolution (Å)	50.00–2.40(2.49–2.40)*	50.00–2.79(2.85–2.79)
R_{sym} or R_{merge}	7.3(54.8)	7.7(40.1)
$I/\sigma I$	27.6(2.9)	25.0(3.0)
Completeness (%)	99.7(98.1)	98.4(99.4)
Redundancy	7.5(6.1)	5.9(5.7)
Refinement		
Resolution (Å)	38.16–2.40	44.12–2.79
No. reflections	19076	15700
R_{work}/R_{free}	21.09/25.60	22.26/27.96
No. atoms		
Protein	2343	4361
Water	107	38
B-factors		
Protein	49.66	56.77
Water	48.34	34.58
R.m.s. deviations		
Bond lengths (Å)	0.009	0.0134
Bond angles (°)	1.048	1.545

* Values in parentheses are for highest-resolution shell.