Materials and methods

Protein Expression and Purification

The *lida* gene was amplified by PCR and cloned into pET-28a expression vector to generate a recombinant protein containing a 6×His-tag at the N-terminus. The His-tagged LidA protein was overexpressed in *E. coli* strain BL21 (DE3). An overnight culture in 200 ml of LB medium was transferred into 4 L of LB medium with 50 μg/ml kanamycin and continuously grown at 37 °C until its OD_{600} reached 0.6. The expression of LidA was induced by adding isopropyl-1-thio-b-D-galactopyranoside (IPTG) to a final concentration of 0.5 mM for 6 hours at 30 °C.

The selenomethionine(SeMet)-substituted LidA protein was overexpressed in *E. coli* strain B834 (DE3). The single colony was first cultured in 20 ml of LB medium with 50 μg/ml kanamycin overnight, which was then transferred into 200 ml of medium comprising 20% LB medium and 80% M9 medium and continuously grown at 37 °C and 210 rpm. When its OD_{600} reached 0.6, the cells were harvested by centrifugation at 2000 rpm at 4 °C. The cells were resuspended in M9 medium and transferred into 4 L M9 medium containing 50 μg/ml kanamycin and 50 μg/ml selenomethionine, and continuously grown at 37 °C and 210 rpm. When its OD_{600} reached 0.6, IPTG was added to a final concentration of 0.5 mM to induce the expression of selenium-labeled LidA for 20 hours at 18 °C. The cells were collected and resuspended as mentioned above.

Both the native and selenium-labeled cells were sonicated, and the lysate was centrifuged at 38,900*g* for 30 min twice at 4 °C. The supernatant was collected and clarified further through a 0.22-μm filter. The native and selenium-labeled LidA proteins were purified by nickel affinity chromatography followed by a gel-filtration chromatography (Superdex-200 size exclusion column, GE Healthcare, USA). At last, the pure native and selenium-labeled proteins were desalted to the buffer containing 10 mM HEPES (pH 7.5) and 200 mM NaCl and concentrated to 20 mg/ml for further

studies. The truncation of $LidA_{60\text{-}594}$ was expressed and purified as the full-length LidA.

Crystallization and Data Collection

Native proteins were used for initial crystal screening with commercial kits (Hampton Research, USA) at 20°C by sitting-drop vapor-diffusion method. Each drop included 1 μl of protein solution and 1 μl of reservoir solution and was equilibrated against 150 μl of reservoir solution. Both native and selenium-labeled LidA were used for crystal optimization and data collection. After optimization, the best crystal was achieved from 200 mM Trimethylame N-oxide, pH 8.0, 20% (w/v) PEG 2000 MME and 1% glycerol.

Both native and selenium-labeled crystals were soaked in 20%-glycerol-contained cryo-protectant and were subsequently flash-frozen in liquid nitrogen for further crystallographic experiments. X-ray diffraction data were collected on MAR 225 image plate detector at a wavelength of 0.9795 Å for selenium-labeled crystal and 0.9792 Å for native crystal at beamline BL17U of Shanghai Synchrotron Radiation Facility (SSRF). Both datasets were indexed, scaled, and merged by using HKL2000.

Structural Determination and Refinement

Single-wavelength anomalous dispersion (SAD) data of selenium-labeled LidA crystal at a 2.9-Åresolution were employed to solve phasing problem. With Shelx, 8 of 18 selenium sites in one asymmetric unit were located. Phase calculation, phase refinement, and initial model building were performed using PHENIX. AutoSol . The 60%-covered initial model was further built manually in Coot. Data of native LidA crystal to a 2.1-Å resolution were used for alternate cycles of manual adjustment in Coot and refinement with PHENIX.refine. The statistics of diffraction data and refinement are summarized in Table 1.

Surface Plasmon Resonance (SPR) measurements

Phosphatidylinositol 3-phosphate diC4 [PtdIns(3)P, P-3004] were obtained from Echelon. The SPR binding experiments were performed with a four-channel BIAcore 100 biosensor system (BIAcore, Inc.). Purified $LidA_{60-594}$ was diluted to 50 μg/ml with running buffer [20 mM HEPES (pH7.5), 150 mM NaCl] and immobilized onto CM5 sensor chips (GE Healthcare) according to standard procedures. PtdIns(3)P diluted with the same buffer to concentrations of 0, 15.625, 31.25, 62.5, and 125 μM was injected into the flow cells at a rate of 50 μl/min at 25˚C. For affinity assay, binding and dissociation were monitored with a BIAcore X. The data were corrected by subtracting the signal of the reference cell and fitted by the BIAevaluation software version 4.1 (GE Healthcare).

Table 1

Table 1. LidA Diffraction Data and Refinement Statistics

	$LidA_{60-594}$	Se-Met LidA
Crystallographic data		
d_{min} (Å)	2.1 (2.15 ~	2.90 (2.97 ~
Wavelength (Å)	0.9792	0.9795
Measured reflections	35,304	25,537
Average redundancy	10.6	3.6
Mean I/σ(I)	8.0	13.0
Completeness (%)	99.9 (99.7)	97.4 (80.2)
R_{merge} [a]	0.147	0.072 (0.317)
Refinement statistics		
Bragg spacing (Å)	46.0 – 2.10	42.4 – 2.9
Space group	$P2_12_12_1$	$P2_12_12_1$
Cell parameters		
a (Å)	64.5	64.4
b (Å)	164.4	169.1
c (Å)	55.6	58.3
Reflections in working set	33,550	
Reflections in test set	1,754	
R_{cryst} [b]	0.203	
R_{free} [c]	0.249	
r.m.s.d. bonds (Å)	0.004	
r.m.s.d. angles (°)	0.78	
Average *B*-factor (Å^2)	42.0	
No. of waters	422	

Values in parentheses indicate the corresponding statistics in the highest resolution shell.

[a] $R_{merge} = (\Sigma|I_i - \langle I_i \rangle|)/\Sigma|I_i|$, where I_i is the integrated intensity of a given reflection.

[b] $R_{cryst} = (\Sigma||F_o| - |F_c||)/\Sigma|F_o|$, where F_o and F_c denote observed and calculated structure factors, respectively.

[c] R_{free} is equivalent to R_{cryst} but calculated using randomly chosen 10% reflections as the test set, which were excluded from refinement process.

Figure 1.

Structure-based sequence alignment of two TBR motifs and their adjacent α-helical hairpin αh1 and αh2. The suare-knot β-hairpins βh1 and βh3 are highly homologous, especially for the β-turn and 3_{10} helix between two β-strands. The canonical β-hairpins βh2, βh4 and βh5 are also homologous.

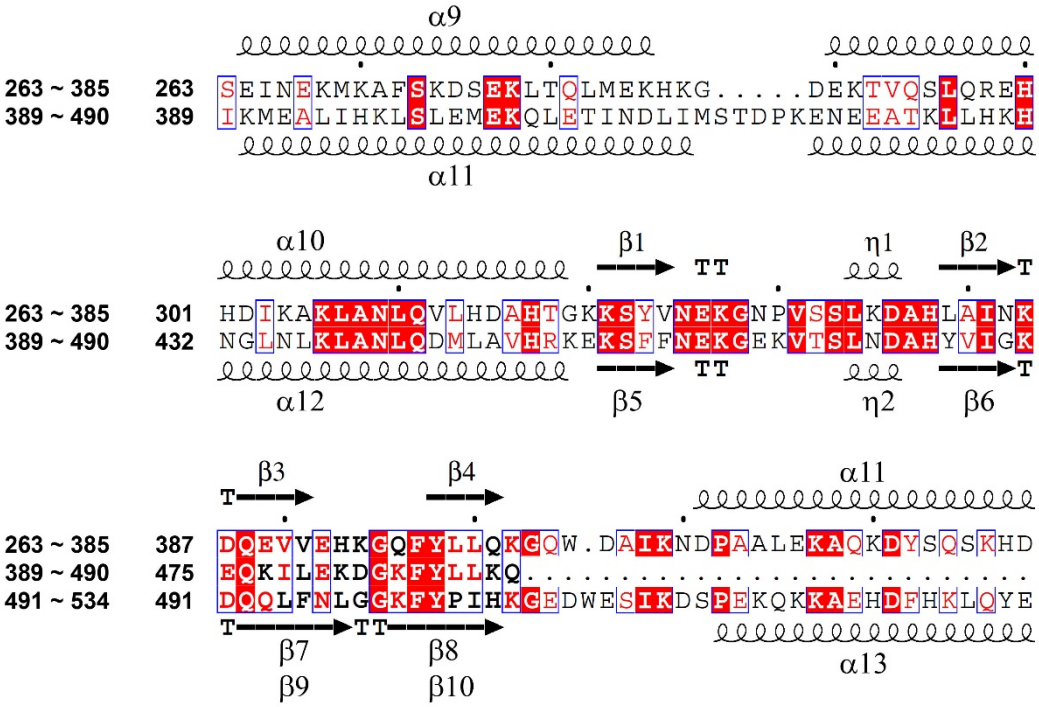

Figure 2.

SPR sensorgrams for binding of PtdIns(3)P and $LidA_{60-594}$. The concentration range of PtdIns(3)P is from 0μM for the bottm curve to 125 μM for the top curve. The reaction is around 300 seconds. The K_d was 3 mM.