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Structure of a membrane-based steric chaperone in complex with its lipase substrate

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Secretion via the type II secretion pathway in Gram-negative bacteria often relies crucially on steric chaperones in the periplasm. Here, we report the crystal structure of the soluble form of a lipase-specific foldase (Lif) from *Burkholderia glumae* in complex with its cognate lipase. The structure reveals how Lif uses a novel α-helical scaffold to embrace lipase, thereby creating an unusually extensive folding platform.

Several proteins do not fold correctly into their native structures without the help of steric chaperones[1]. These chaperones imprint unique structural information onto target proteins while lowering the entropic (un)folding barrier between the native and partially folded states[2]. Kinetic isolation of the native state from folding intermediates seems to be crucial, because such intermediates in dynamic equilibrium with the native protein are susceptible to extracellular proteases[3]. The N-terminal propeptides of subtilisin and α-lytic protease were the first steric chaperones identified. A newly identified class of steric chaperones is associated with the broadly conserved type II secretion system in Gram-negative bacteria, which mediates the transport of many virulence factors across the outer membrane[4]. One such secretion cargo is lipase (LipA), which achieves its active and secretion-competent conformation in the periplasm only upon interaction with its lipase-specific foldase (Lif)[5]. Unlike other steric chaperones, Lif is an inner-membrane protein with a large C-terminal domain protruding into the periplasm. In the absence of Lif, lipase is not secreted and obtains an inactive intermediate folding form, which can be activated *in vitro* by addition of Lif[6].

Despite more than 15 years of active research, the structural biology of Lif has remained elusive. Here, we report the first molecular snapshots of the interaction of this steric chaperone with its cognate lipase. For our structural studies, we used a fully functional truncated Lif from *B. glumae* in which the N-terminal 18 cytoplasmic residues and the membrane anchor are replaced by a histidine-tagged peptide[6,7] (**Supplementary Methods** online). Purified Lif ran at an apparent molecular weight of 65 kDa on a gel-filtration column, which is about twice the expected molecular weight of 35 kDa (**Supplementary Fig. 1** online). Analytical ultracentrifugation, however, showed that this was due to an extended conformation and not to dimerization (**Supplementary Table 1** online).

The crystal structure of the Lif–LipA complex reveals that Lif adopts a previously unobserved fold, featuring an extended α-helical structure consisting of 11 α-helices, which engulfs the lipase (**Fig. 1a** and **Supplementary Table 2** online). Known Lif proteins share low sequence identity but have been predicted to share a common α-helical secondary structure[8]. Our structure corroborates these proposals and can now serve as a prototype for the growing family of Lif proteins (**Supplementary Fig. 2** online). Two distinct, globular minidomains can be identified at the N and C termini of the visible foldase structure (α1–α3 and α9–α11, respectively) on opposite sides of the lipase, separated by an extended helical motif (α4–α8). Notably, all aromatic residues (three tryptophans, five tyrosines and six phenylalanines) are located within these minidomains (**Fig. 1b**), which may explain why Lif has appreciable tertiary structure only in these two segments of the structure. A *Pseudomonas aeruginosa* Lif in which the last 29 residues, containing the C-terminal minidomain, were replaced by the corresponding *B. glumae* residues was unable to activate its

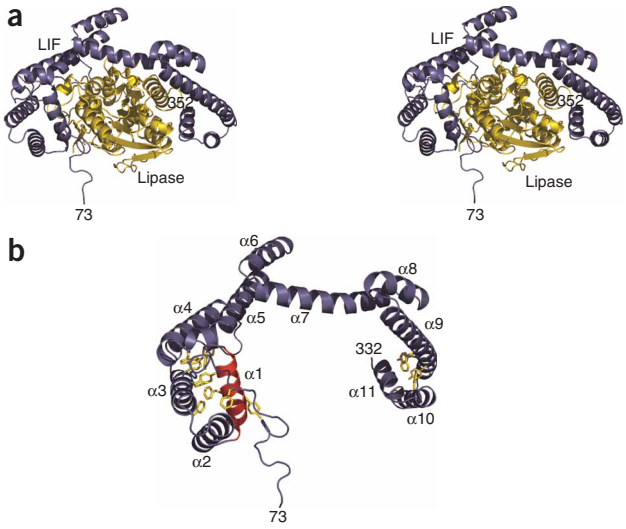

Figure 1 Structure of Lif bound to LipA. (**a**) Stereo view of the Lif–LipA complex. (**b**) The novel α-helical fold of Lif. Aromatic residues in the Lif minidomains are shown in yellow. The highly conserved motif in Lif (RxxFDY(F/C)L(S/T)A) is mapped to the α1 helix (red).

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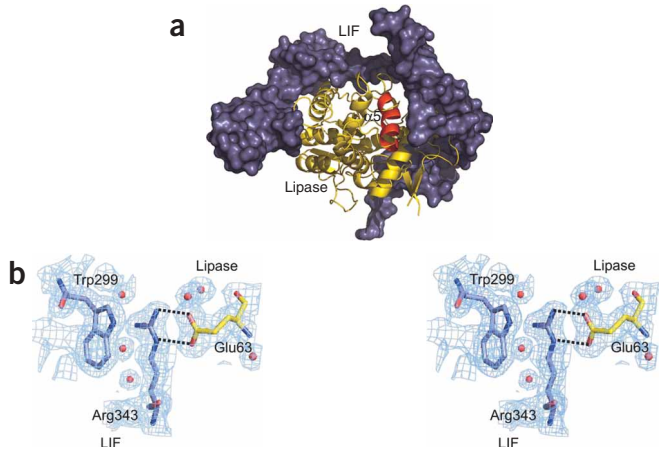

Figure 2 The Lif-LipA interaction. The orientation presented here is rotated 180° around the vertical axis from the view in **Figure 1a**. (**a**) Lif is shown in surface representation; LipA helix α5, covering the active site, is colored red. (**b**) Stereo view of $2F_o - F_c$ electron density (1σ contour) superimposed on the refined model of the Lif–LipA complex, showing the conserved salt bridge between Glu63 of LipA (yellow) and Arg343 of Lif (purple). In the C-terminal domain of Lif, Trp299 interacts with Arg343 in a cation/π-electron stacking mode. Water molecules are shown as red spheres.

cognate lipase, highlighting the significance of this part of the structure[9]. The structure also shows that certain residues that are conserved throughout the bacterial Lifs interact with highly conserved lipase residues[8] (**Supplementary Table 3** online). Furthermore, sequence comparisons have revealed that Lifs have a conserved sequence motif RxxFDY(F/C)L(S/T)A (where x is any residue)[8], which we can now map to the α1 helix in the N-terminal minidomain (**Fig. 1b**). Mutagenesis studies have already shown that these residues are essential for lipase activation[10]. The first 30 residues of the recombinant Lif could not be modeled, suggesting that this part is flexible or unstructured. Indeed, N-terminally truncated Lif from *P. aeruginosa* retained its activity *in vitro*[10]. Possibly this region has a role as a flexible spacer used to ascertain the protrusion of Lif from the inner membrane and perhaps allowing interaction with the type II secretion machinery. Overall, Lif is considerably more flexible than LipA (average *B*-factors of 42 Å^2 and 24 Å^2, respectively), which may reflect the need for a dynamic molecular platform during the folding of lipase.

The structure of LipA in the complex is virtually identical to that of the free native lipase[11] (r.m.s. deviation of 0.5 Å for Cα atoms), and the largest main chain deviations are due to crystal contacts. Like the Lif-free form, LipA in the complex contains a disulfide bridge (Cys190-Cys269), a calcium ion and a conserved *cis*–peptide bond (Gln291-Leu292). Likewise, the structure of the active site is very similar to that in the Lif-free lipase. The α5 helix in Lif-bound LipA (residues 137–147), which forms the lid covering the active site, is in the closed conformation (**Fig. 2a**). Nevertheless, the addition of substrate results in lipase activity without affecting diffraction and thus crystal integrity. This is consistent with the α5 helix of LipA having sufficient space to move in the crystal lattice and, moreover, strongly suggests that the lipase is active in the complex.

The Lif–LipA complex has a notable 5,400 Å^2 of buried solvent-accessible surface area at the interface (**Fig. 2a**) consistent with the high affinity (K_d = 5 nM) between the two partner molecules as measured by surface plasmon resonance. This interaction platform is much larger than the consensus interface of 1,600 ± 400 Å^2 derived from crystallographically observed protein-protein complexes[12], and it is mainly nonpolar, with only 20 intermolecular hydrogen bonds. However, the conserved Glu63 of LipA and Arg343 of Lif form a salt bridge (**Fig. 2b**) and may contribute to the specificity of binding. Given the high affinity and extent of the interaction, the release of LipA from the complex emerges as a real challenge and may only be possible through interaction with the secretion machinery.

Far-UV CD spectroscopy has previously shown that the interaction of Lif with LipA is accompanied by an increase in α-helical structure, while intermediate folding forms of LipA have native-like secondary structure[9]. Furthermore, near-UV CD spectra indicate that Lif lacks a well-defined tertiary structure in the absence of LipA (**Supplementary Fig. 3** online). On the basis of our structural analysis, we conclude that Lif, not lipase, undergoes substantial structural changes in both secondary and tertiary structure upon complex formation. We propose that this occurs predominantly in the spacer region between the minidomains of Lif, where one or more α-helices could form during complex formation, thereby completing the folding platform and helping to compact lipase into its native conformation. The structure presented here provides a platform for further structural, mutational and detailed biophysical studies of the Lif–LipA system.

Accession codes. Protein Data Bank: Coordinates have been deposited with accession code 2ES4.

Note: Supplementary material is available on the Nature Structural and Molecular Biology website.

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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