Finding Novel Antibiotic Targets

Cooks know that getting the water-based and the oil-based components of a dish to agree can take some work. They just don't get along. Cells have exactly the same problem. Getting oily lipids from where they are made to where they need to be in a watery cellular environment can be a daunting task, but not if you are a skilled biochemist, as cells are. One of the ways that cells have developed to solve the oil-water problem is to design a protein that shuttles lipids between biosynthetic enzymes. These proteins, called acyl carrier proteins (ACPs), are found in all domains of life, from bacteria to humans. One particularly interesting version is found in bacteria where ACPs are used in the biosynthesis of membrane lipids that form the bacterial endotoxin called lipid A. Lipid A is an activator of the human immune system and, because it is essential for bacterial survival, represents a possible novel target for antibiotic therapy. Recent work by researchers from the Duke University Medical Center has elucidated the structure of a bacterial ACP interacting with one of the key Lipid A biosynthetic enzymes, LpxD. Their work, conducted at the APS, reveals new information about the catalytic interactions between ACP and LpxD and a surprising new role for ACP in product release that may offer just the target they were seeking.

The researchers crystallized three different versions of the LpxD-ACP complex in order to get structural data on what was going on at each step of the catalytic interaction. The methods for purifying and crystallizing LpxD were already known, their advance was in their ability to make different versions of the ACP and to manipulate the catalytic process to "stall" it at different stages. Central to this was the ability to make ACP with its 4′-phosphopantetheine (4′-PPT) prosthetic arm, which is responsible for binding to the oily cargo, with the 14-carbon acyl chain already loaded (acyl-ACP) and with it unloaded (holo-ACP) [Fig. 1(a) and 1(f)]. Then, they mixed these ACP versions with either catalytically active LpxD or a mutant of LpxD that was not catalytically active. Biochemical tricks to slow catalysis also helped to stall the enzyme in intermediate states.

Three structures were obtained at the SER-CAT 22-BM-D and 22-ID-D beamlines at the APS. Each structure showed that three LpxD molecules combine to form a trimer and that one molecule of ACP binds to each of these through a C-terminal ACP recognition domain (ARD). Aspects of the intact acyl-ACP structure [pre-catalysis, Fig. 1(b)] allowed them to elucidate the basis for the strict 14-carbon acyl chain specificity of LpxD. The structure shows that one end of the acyl chain packs against an amino acid, methionine 290, which acts as a "ruler" that keeps the allowable chain length at 14 carbons.

The second step structure, in which the acyl group had been delivered to the recipient molecule but neither had left the complex, showed that the 4′-PPT prosthetic arm covers the exit site of the enzyme and keeps ACP and the product from leaving [Fig. 1(d)], transparent orange). Interestingly, in the final snapshot of catalysis, the 4′-PPT group moves a significant distance up and out of the way [Fig. 1(d), orange] to trigger release of the new acylated product [Fig. 1(e)] and then finally the holo-ACP [Fig. 1(f)]. These unprecedented structural snapshots provide insight into movements within ACP and uncover key molecular interactions at the protein-protein interface. Molecules that could block acyl-ACP from binding to LpxD would potentially serve as novel antibiotics because the LpxD reaction is essential to bacterial life.

The next step is to determine the structure of LpxD in complex with the acylated lipid product, which will complete the "molecular landscape" of the active site and facilitate the design of novel antibiotics to treat bacterial infections. *— Sandy Field*

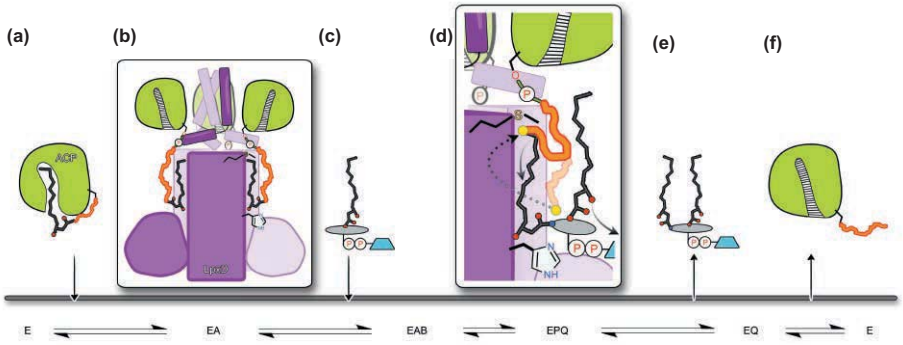

Fig. 1. Molecular model of ordered reaction mechanism between ACP and LpxD. (a) acyl-ACP is shown in green with 4′-phosphopantetheine (4′-PPT) group in orange and lipid "cargo" in black. (b) Three acyl-ACPs bind to LpxD trimer (purple/white). (c) Uridine diphosphate 3-O-(β-OH-C14)-α-D-glucosamine (UDP-acyl-GlcN), which will accept acyl group from ACP, binds next. (d) Acyl group is transferred to UDP-acyl-GlcN, 4′-PPT blocks release (transparent orange), then moves away (orange). (e) UDP-diacyl-GlcN is released. (f) holo-ACP is released.

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