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Structure

Structures of the Carbon-Phosphorus Lyase Complex Reveal the Binding Mode of the NBD-like PhnK

Highlights

- Cryo-EM structures of a small, asymmetric C-P lyase complex at 7.8 Å resolution
- Only one PhnK binds to the dimeric core complex due to steric hindrance
- The NBD-like PhnK binds to a cytoplasmic protein, distinct from NBD-TMD interaction
- Binding of PhnK exposes the active site residue, Gly32 of PhnJ

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In Brief

Yang et al. examine the binding of the NBD-like PhnK to the C-P lyase core complex using single-particle cryo-electron microscopy. PhnK binds to the core complex through its α helices 3 and 4, and exposes the active site residue, Gly32 of PhnJ.

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Structures of the Carbon-Phosphorus Lyase Complex Reveal the Binding Mode of the NBD-like PhnK

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SUMMARY

The carbon-phosphorus (C-P) lyase complex is essential for the metabolism of unactivated phosphonates to phosphate in bacteria. Using single-particle cryo-electron microscopy, we determined two structures of the C-P lyase core complex $PhnG_2H_2I_2J_2$, with or without PhnK. $PhnG_2H_2I_2J_2$ is a two-fold symmetric hetero-octamer. Its two PhnJ subunits provide two identical binding sites for PhnK. Only one PhnK binds to $PhnG_2H_2I_2J_2$ due to steric hindrance. PhnK is homologous to the nucleotide-binding domain (NBD) of ATP-binding cassette transporters. The α helices 3 and 4 of PhnK bind to α helix 6 and a loop (residues 227–230) of PhnJ, in a different mode from the binding of NBDs to their transmembrane partners. Moreover, binding of PhnK exposes the active site residue, Gly32 of PhnJ, located near the interface between PhnJ and PhnH. This structural information provides a basis for further deciphering of the reaction mechanism of the C-P lyase.

INTRODUCTION

Phosphorus is essential for life. Currently, this element is most abundant as phosphoric acid and phosphate esters (Wackett et al., 1987). However, certain organisms can acquire needed phosphorus under conditions of low phosphate directly from organophosphonate compounds, a class of compounds that contain a carbon-phosphorus (C-P) bond that must be enzymatically cleaved to phosphate (McGrath et al., 2013). Phosphonates have been proposed as a major source of phosphorus on prebiotic earth and large quantities of phosphonates are discharged annually into the environment as agricultural herbicides and industrial detergents (Ternan et al., 1998). In bacteria such as *Escherichia coli*, the C-P bond in unactivated organophosphonates can be enzymatically cleaved to form phosphate and an alkane by the multi-subunit C-P lyase complex (Chen et al., 1990). In *E. coli*, the C-P lyase complex is encoded by the 14 genes (*phnCDEFGHIJKLMNOP*) contained within the *phn* operon (Chen et al., 1990; Metcalf and Wanner, 1993a, 1993b). Previous genetic and biochemical studies have demonstrated that seven of the genes in this operon (*phnGHIJKLM*) are critical for expression of the proteins that are required for the enzymatic cleavage of the C-P bond during the transformation of phosphonates to phosphate (Metcalf and Wanner, 1993b). The individual subunits of the *E. coli* C-P lyase complex that are essential for the enzymatic cleavage of the C-P bond in methylphosphonate have been reconstituted and characterized in vitro (Kamat et al., 2011, 2013). The series of enzymatic steps catalyzed by the C-P lyase complex is illustrated in Figure 1A. The initial reaction of the C-P lyase pathway is catalyzed by a nucleotide phosphorylase, PhnI, in the presence of PhnG, PhnH, and PhnL in which ATP and methylphosphonate are converted to adenine and α-D-ribose-1-methylphosphonate-5-triphosphate (RPnTP). The RPnTP is hydrolyzed by the phosphohydrolase PhnM to produce pyrophosphate and 5-phosphoribosyl-1-phosphonate (PRPn). PRPn serves as a substrate for the cleavage of the C-P bond by PhnJ through an S-adenosyl methionine-dependent radical-based reaction, transforming PRPn to produce 5-phosphoribosyl-1, 2-cyclic phosphate (PRcP) and methane (or corresponding alkane).

Fragments of the C-P lyase complex containing $PhnG_2I_2$, $PhnG_2H_2I_2J_2$, and $PhnG_2H_2I_2J_2K$ can be expressed and purified in high yield (Jochimsen et al., 2011). We have used mass spectrometry and H/D exchange methods to construct a low-resolution interaction map of the $PhnG_2H_2I_2J_2K$ complex that illustrates how the individual subunits associate with one another to form larger multi-subunit complexes (Ren et al., 2015). Interestingly, the demonstrated stoichiometry indicates that one copy of PhnK binds to the dimeric core complex ($PhnG_2H_2I_2J_2$). The X-ray crystal structure of the core complex $PhnG_2H_2I_2J_2$ has been recently reported (Seweryn et al., 2015). It revealed an intertwined network of subunits PhnG, PhnH, PhnI, and PhnJ with self-homologies. However, how PhnK binds to the core complex remains unclear.

From sequence analysis, PhnK is homologous to the nucleotide-binding domain (NBD) of ATP-binding cassette (ABC) transporters. PhnK has all the NBD motifs: Walker A, Walker B, ABC signature, A-loop, D loop, Q-loop, and switch H-loop. The role of PhnK in the C-P lyase pathway is unclear: PhnK is apparently not required for any of the essential reactions of the C-P lyase pathway in vitro (Kamat et al., 2011). However, the *E. coli ΔphnK* strain is phosphonate growth deficient (Metcalf and Wanner, 1993b). PhnK was previously mapped in a groove close to the two-fold symmetry axis of the core complex $PhnG_2H_2I_2J_2$ based on a low-resolution density map from negative stain electron microscopy (negative stain EM) (Seweryn et al., 2015). The ABC