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Crystal Structure of the Cyanobacterial Signal Transduction Protein P_{II} in Complex with PipX

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P_{II} proteins are highly conserved signal transducers in bacteria, archaea, and plants. They have a large flexible loop (T-loop) that adopts different conformations after covalent modification or binding to different effectors to regulate the functions of diverse protein partners. The P_{II} partner PipX (P_{II} *i*nteraction *p*rotein *X*), first identified from *Synechococcus* sp. PCC 7942, exists uniquely in cyanobacteria. PipX also interacts with the cyanobacterial global nitrogen regulator NtcA. The mutually exclusive binding of P_{II} and NtcA by PipX in a 2-oxoglutarate (2-OG)-dependent manner enables P_{II} to indirectly regulate the transcriptional activity of NtcA. However, the structural basis for these exclusive interactions remains unknown. We solved the crystal structure of the P_{II}–PipX complex from the filamentous cyanobacterium *Anabaena* sp. PCC 7120 at 1.90 Å resolution. A homotrimeric P_{II} captures three subunits of PipX through the T-loops. Similar to P_{II} from *Synechococcus*, the core structure consists of an antiparallel β-sheet with four β-strands and two α-helices at the lateral surface. PipX adopts a novel structure composed of five twisted antiparallel β-strands and two α-helices, which is reminiscent of the P_{II} structure. The T-loop of each P_{II} subunit extends from the core structure as an antenna that is stabilized at the cleft between two PipX monomers via hydrogen bonds. In addition, the interfaces between the β-sheets of PipX and P_{II} core structures partially contribute to complex formation. Comparative structural analysis indicated that PipX and 2-OG share a common binding site that overlaps with the 14 signature residues of cyanobacterial P_{II} proteins. Our structure of PipX and the recently solved NtcA structure enabled us to propose a putative model for the NtcA–PipX complex. Taken together, these findings provide structural insights into how P_{II} regulates the transcriptional activity of NtcA via PipX upon accumulation of the metabolite 2-OG.

Introduction

P_{II} signaling proteins are highly conserved in bacteria, archaea, and plants. Genes coding for P_{II} proteins are divided into three subfamilies: *glnB*, *glnK*, and *nifI*.[1] GlnB and GlnK function as homotrimers to modulate nitrogen assimilation. Heterotrimeric NifI is distinct from the members of the

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Abbreviations used: 2-OG, 2-oxoglutarate; NAGK, *N*-acetyl glutamate kinase; PDB, Protein Data Bank.

other two subfamilies and is found only in nitrogen-fixing archaea and some anaerobic bacteria.[2] P_{II} proteins sense carbon-related, nitrogen-related, and energy-related signals, and interact with diverse target proteins, most of which are involved in the regulation of nitrogen metabolism. A series of structures of P_{II} proteins from bacteria,[3–7] archaea,[8] and plants[9] have been solved. All exist as trimers, with each subunit sharing a highly similar core structure of an antiparallel β-sheet and two α-helices at the lateral surface. The three subunits form a short cylinder, with the functional large flexible loop (T-loop) of each subunit protruding towards the solvent as an antenna to interact with the partner proteins. Most P_{II} proteins sense signals in two modes: through effector-triggered conformational changes and through covalent modifications.[10] Both modes are primarily dependent on the variable T-loop. The binding pattern of the effectors ATP/ADP and 2-oxoglutarate (2-OG) is universal and conserved among P_{II} proteins.[1,11–13] These effectors enable the T-loop to adopt different conformations for interacting with diverse P_{II} partners.[10] ATP is stabilized at the intersubunit cleft of P_{II},[14] and its binding is synergistic with the association of 2-OG.[15] In contrast, the covalent modification patterns are less conserved. Residue Ser49 of P_{II} from the unicellular cyanobacterium *Synechococcus elongatus* PCC 7942 (referred to as *Synechococcus*) or *Synechocystis* PCC 6803 (referred to as *Synechocystis*) is phosphorylated under poor nitrogen conditions,[11] while Tyr51 is nitrificated in the heterocystous cyanobacterium *Anabaena* sp. PCC 7120 (referred to as *Anabaena*).[16] In *Escherichia coli*, P_{II} is regulated by uridylylation at the conserved Tyr51 residue of the T-loop.[17]

To date, only GlnB-type P_{II} has been found in all sequenced cyanobacteria.[18] Because of the absence of 2-OG dehydrogenase in cyanobacteria,[19] the 2-OG produced by the Krebs cycle serves mainly as a carbon skeleton for nitrogen assimilation through the glutamine synthetase–glutamate synthase pathway. The direct link of 2-OG level to nitrogen assimilation makes the metabolite 2-OG an important signal of the carbon/nitrogen balance in cyanobacteria, and this signal is sensed by P_{II} protein.

Three P_{II} partner proteins have been reported in cyanobacteria: *N*-acetyl glutamate kinase (NAGK)[20] and PipX (*P_{II} interaction protein X*) in *Synechococcus*,[20,21] and the membrane protein PamA of unknown function in *Synechocystis*.[22] Of these, only the molecular mechanism of NAGK regulation by P_{II} has been clearly illustrated from the structural point of view.[23] After being first identified in *Synechococcus*,[20,21] PipX has been found exclusively in all cyanobacteria. It specifically interacts only with cyanobacterial P_{II}, and not with *E. coli* GlnB or GlnK, or with *Arabidopsis thaliana* P_{II}.[21] PipX also interacts with the global nitrogen regulator NtcA,[21] which belongs to the Crp/Fnr transcription factor family and regulates a group of cyanobacterial nitrogen assimilation genes. PipX switches between binding P_{II} and binding NtcA, depending on the cellular 2-OG concentration.[21,24] At lower 2-OG concentrations, PipX is bound to P_{II}. Upon 2-OG accumulation, PipX binds to NtcA.[21] 2-OG impairs the interaction between P_{II} and PipX in the presence of ATP, but facilitates the formation of the NtcA–PipX complex. The three proteins P_{II}, PipX, and NtcA are highly conserved in all sequenced cyanobacterial genomes, and NtcA and PipX are exclusively encoded by cyanobacteria, indicating that the 2-OG-dependent swapping of PipX might be a universal mechanism of cyanobacteria.

Here, we present the structure of the P_{II}–PipX complex from *Anabaena*. After the structures of the P_{II}–NAGK complex[23,25] and the AmtB–GlnK[26] complex, our structure represents the third complex of P_{II} with its partner. P_{II} assembles into a trimer as reported and interacts with PipX through the T-loops. The homotrimer of P_{II} resembles an upside-down tripod that holds three PipX molecules, which have only slight interactions with each other. Through comparative structural analysis, we deduced how 2-OG and ATP/ADP affect the interaction between P_{II} and PipX. Further structural simulation provides insight into the swapping of PipX from P_{II} to NtcA.

Results

Overall structure of the P_{II}–PipX complex

The structure of the P_{II}–PipX complex was solved and refined to 1.90 Å resolution. It belongs to space group *P*321, with unit cell dimensions of $a=70.65$ Å, $b=70.65$ Å, $c=88.54$ Å, $\alpha=90.00°$, $\beta=90.00°$, and $\gamma=120.00°$. The asymmetric unit contains one molecule each of P_{II} and PipX, which form a 3-fold symmetric complex with a stoichiometry of 3 P_{II}:3 PipX (Fig. 1a and b). The overall structure of the complex resembles a triangular prism that is 43 Å in height and 64 Å (for the PipX layer) or 40 Å (for the P_{II} layer) in width. Each P_{II} subunit inserts its well-ordered T-loop (Phe36-Leu56) into the lateral cleft of two adjacent PipX molecules, stabilizing the complex via extensive contacts. Three subunits of PipX form a positively charged hole at the center of the P_{II} trimer (Fig. 1c).

Structural comparison of P_{II}

Anabaena P_{II} adopts a ferredoxin-like fold (Fig. 2a), as defined by the Structural Classification of Proteins‡. The overall structure is very similar to the

‡ http://scop.berkeley.edu/data/scop.b.e.bcj.html