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A New P_{II} Protein Structure Identifies the 2-Oxoglutarate Binding Site

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P_{II} proteins of bacteria, archaea, and plants regulate many facets of nitrogen metabolism. They do so by interacting with their target proteins, which can be enzymes, transcription factors, or membrane proteins. A key feature of the ability of P_{II} proteins to sense cellular nitrogen status and to interact accordingly with their targets is their binding of the key metabolic intermediate 2-oxoglutarate (2-OG). However, the binding site of this ligand within P_{II} proteins has been controversial. We have now solved the X-ray structure, at 1.4 Å resolution, of the *Azospirillum brasilense* P_{II} protein GlnZ complexed with MgATP and 2-OG. This structure is in excellent agreement with previous biochemical data on 2-OG binding to a variety of P_{II} proteins and shows that 2-oxglutarate binds within the cleft formed between neighboring subunits of the homotrimer. The 2-oxo acid moiety of bound 2-OG ligates the bound Mg^{2+} together with three phosphate oxygens of ATP and the side chain of the T-loop residue Gln39. Our structure is in stark contrast to an earlier structure of the *Methanococcus jannaschii* GlnK1 protein in which the authors reported 2-OG binding to the T-loop of that P_{II} protein. In the light of our new structure, three families of T-loop conformations, each associated with a distinct effector binding mode and characterized by a different interaction partner of the ammonium group of the conserved residue Lys58, emerge as a common structural basis for effector signal output by P_{II} proteins.

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Introduction

P_{II} proteins are involved in the regulation of many aspects of nitrogen metabolism.[1] They can be regarded as signal integration proteins whose output is their signal-dependent interaction with various target proteins that they may activate or inhibit.[2–4] The universally conserved and probably most ancient signals controlling P_{II} activities are the effector molecules ATP, ADP, and 2-oxoglutarate (2-OG). Additionally, many P_{II} proteins are covalently modified by enzymes whose activities are regulated by another key nitrogen metabolite, glutamine. P_{II} proteins are compact, cylindrically shaped (homo) trimers composed of 12-kDa to 13-kDa subunits from which three long exposed loops, the so-called T-loops, protrude (Fig. 1), as first reported for *Escherichia coli* GlnB.[5] The T-loops are significantly conserved in sequence, but as is apparent from the numerous reported structures of P_{II} proteins, they are structurally very flexible.[3,6] They are vital for P_{II} interactions with many of their targets and are also the sites of reversible covalent modification. In addition to the T-loops, the highly conserved structure of P_{II} proteins is characterized by three lateral intersubunit clefts within which two smaller loops (the B-loops and C-loops) from opposing subunits contribute to an adenyl-nucleotide binding pocket where ADP or ATP can bind competitively (Fig. 1).

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Abbreviations used: 2-OG, 2-oxoglutarate; PDB, Protein Data Bank; NAGK, *N*-acetylglutamate kinase; ACCase, acetyl-CoA carboxylase; Mes, 4-morpholineethanesulfonic acid.

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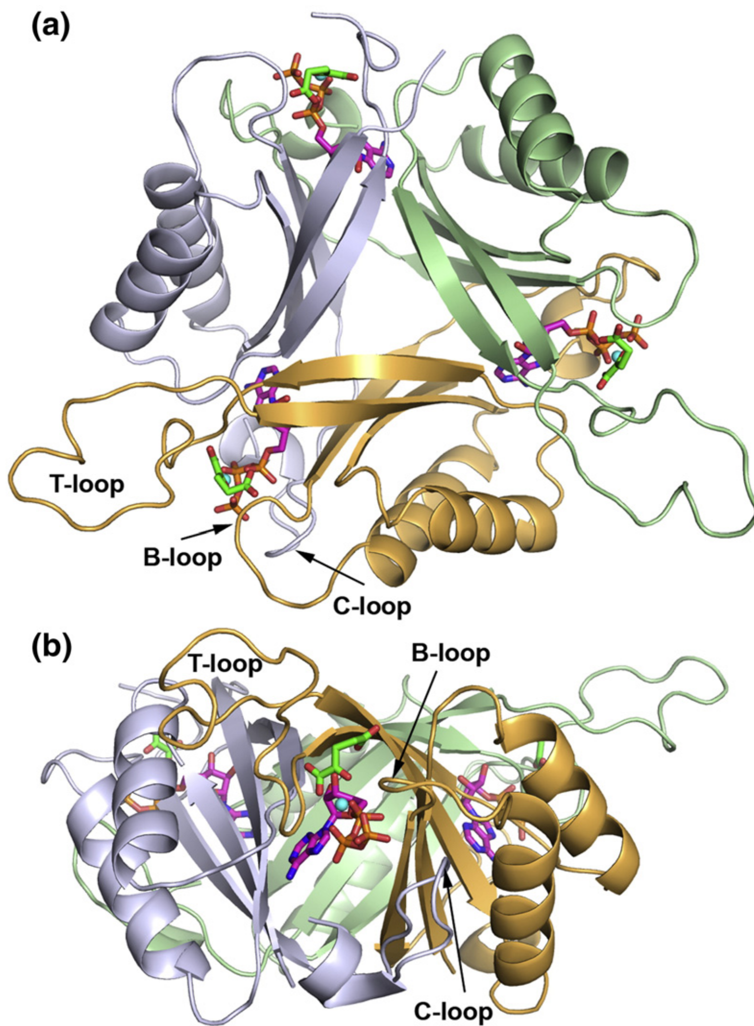

Fig. 1. Cartoon representation of the GlnZ trimer. The trimer with bound ATP (magenta), 2-OG (green), and Mg^{2+} (blue) is shown in top view (a) and side view (b). The T-loop of one protomer (chain A) is partly disordered. The T-loop (residues 37–55), B-loop (residues 82–88), and C-loop (residues 102–105) at one binding site are indicated by arrows.

The binding mode of the other key effector 2-OG, which is assumed to be conserved among all P_{II} proteins, has remained enigmatic and controversial.[3] Here we report a high-resolution structure of a complex of the P_{II} protein GlnZ from *Azospirillum brasilense* complexed with the effectors ATP and 2-OG. GlnZ and its orthologues are specifically involved in the regulation of nitrogenase activity in some nitrogen-fixing bacteria.[7,8] The observed 2-OG binding site is in excellent agreement with biochemical data, in contrast to a previously reported binding mode.[9] This new structure will greatly facilitate an understanding of the link between changes in cellular effector pools and P_{II} signaling.

Results and Discussion

Crystal structure

Purified GlnZ was crystallized in the presence of ATP, 2-OG, and Mg^{2+}, and the structure was solved by molecular replacement using the *E. coli* GlnK trimer as search model [Protein Data Bank (PDB) ID 2GNK].[10] First electron density maps revealed clear density for bound MgATP and 2-OG in all three independent binding sites of the GlnZ trimer (chains A, B, and C) present in the asymmetric unit. Refinement using data up to 1.4 Å resolution, as described in Materials and Methods, led to a final model with an *R*-factor of 0.160 (R_{free} = 0.198) and excellent stereochemistry (Table 1). The final electron density at the effector binding site is shown in Fig. 2. Two of the three T-loops (residues 37–55) of each trimer are completely ordered (Fig. 1) and are observed in the same conformations with similar interactions with neighboring trimers in the crystal lattice. The third, belonging to chain A, appears disordered from residues 43 to 52.

The 2-OG binding site

In our structure, ATP and 2-OG both bind to GlnZ by participating in the coordination of an Mg^{2+} ion (Figs. 2 and 3). Three of the oxygen ligands are provided by the α-phosphate, β-phosphate, and γ-phosphate of ATP, and two of the oxygen ligands are provided by the 2-oxo acid moiety of 2-OG whose 5-carboxy group is involved in a salt bridge with Lys58. The sixth ligand, completing the nearly perfect octahedral Mg^{2+} coordination, is provided

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