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Crystal structure of tandem ACT domain-containing protein ACTP from *Galdieria sulphuraria*

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Abstract

The ACT domain is a structurally conserved small molecule binding domain which is mostly involved in amino acid and purine metabolism. Here, we report the crystal structure of a tandem ACT domain-containing protein (ACTP) from *Galdieria sulphuraria*. The two ACTP monomers in the asymmetric unit form a dimer with a non-crystallographic 2-fold axis in a domain-swapped manner, showing a horseshoe-like structure with a central crevice. This structure contributes to expand our knowledge on the structural diversity of ACT domain-containing proteins.

Keywords

ACT domain; ACT domain-containing protein; *Galdieria sulphuraria*

INTRODUCTION

The ACT domain, an amino acid-binding domain named after bacterial aspartate kinase, chorismate mutase, and TyrA (prephenate dehydrogenase), is a structurally conserved regulatory domain which serves as small molecule binding sites in enzymes involved in feedback regulation of amino acid and purine metabolism.¹⁻² The ACT domain is generally incorporated in multidomain proteins in a variety of different arrangements, providing allosteric regulation in response to binding of their respective ligands.^{1,3-4} ACT domain-containing proteins are found in bacteria, archaea, and eukarya, including vertebrates, plants,

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fungi, and single cellular organisms.³ However, most of the ACT domain-containing proteins have not been studied in detail and structural information remains scarce.⁴

In this work, we present the crystal structure of a tandem ACT domain-containing protein ACTP from *Galdieria sulphuraria* (red algae). We show that ACTP forms a domain-swapped dimer with a horseshoe-like structure and a central crevice. The overall structure of ACTP shows structural similarity to a bacterial transcriptional regulator protein GcvR. However, the amino acid sequence of ACTP lacks the conserved motifs of GcvR family members, implying that the molecular function of ACTP may differ from that of GcvR. Our structural characterization of ACTP contributes to expand our knowledge on the structural diversity of ACT domain-containing proteins.

MATERIALS AND METHODS

Native and SeMet-labeled ACTP proteins were cloned and purified following the standard CESG pipeline protocol described in detail elsewhere.⁵⁻⁸ Crystals of ACTP were grown at 293 K by the hanging-drop method from 10 mg ml⁻¹ protein solution in buffer (50 mM NaCl, 3 mM NaN₃, 0.3 mM TCEP, 5 mM MES-NaOH at pH 6.0) mixed with an equal amount of well solution containing 24% (v/v) PEG 8K, 5% (v/v) DMSO, 40 mM MgCl₂ 200 mM NaF, 100 mM PIPES pH at 6.5. Crystals were cryoprotected by soaking in a well solution supplemented with 20% ethylene glycol. X-ray diffraction data for native and SeMet-labeled ACTP crystals were collected at the 23-ID-D beamline at the Argonne National Laboratory Advanced Photon Source. Table I summarizes the data collection statistics. We have solved the crystal structure of ACTP by MAD phasing at 1.80 Å resolution (Table I). Experimental phase information was solved using SHARP⁹ and improved by density modification using DM¹⁰. Phasing statistics are summarized in Table I. The structure was completed using alternate cycles of manual building in Xfit¹¹ and refinement in REFMAC¹². All refinement steps were monitored using an R_{free} value based on 5.1% of the independent reflections. The stereochemical quality of the final model was assessed using PROCHECK¹³ and MolProbity¹⁴. Atomic coordinates and structure factors have been deposited in the RCSB Protein Data Bank (accession code 2NYI).

RESULTS AND DISCUSSION

We have determined the crystal structure of ACTP from *G. sulphuraria*. The final model includes two copies of ACTP monomers and 378 water molecules in the crystallographic asymmetric unit. The R and R_{free} factors were 17.6% and 21.4%, respectively for the 45.6–1.80 Å data (Table I). ACTP monomer consists of 195 amino acids residues and is divided into two separated domains [Fig 1(A)]. The loop region which connects the two domains is missing in the both chains in the asymmetric unit, suggesting that the corresponding loop region is highly flexible. The domains share a ferredoxin-like $\beta\alpha\beta\beta\alpha\beta$ fold comprising a four-stranded anti-parallel β -sheet flanked by two α -helices, resembling the previously reported ACT domain structures [Fig 1(A)].⁴ The C $_{\alpha}$ atoms of domain 1 (2-80) and domain 2 (86-176) can be superimposed with a root mean square (r.m.s.) deviation of 1.2 Å, indicating that the structures of these two ACT domains are highly similar to each other.

It is known that the archetypical ACT domain forms dimers or higher order oligomers, generating a ligand binding site at the interface.^{2,4} The two monomers of ACTP in the asymmetric unit form a dimer with a non-crystallographic 2-fold axis, resulting in a horseshoe-like structure with a central crevice [Fig 1(B)]. ACTP possesses both the face-to-face and the side-by-side domain arrangements between the ACT domains from different chains, exhibiting a domain-swapped dimer structure [Fig 1(B)]. ACTP dimer shows two types of interfaces between the two monomers (interface A and B, indicated by red and blue lines in Fig 1(B), respectively). In the interface A, the β -sheets of domain 1 and 2 from different chains form an extended β -sheet. On the other hand, the β -sheets of domain 1 in the interface B form a β -sandwich through van der Waals packing of a number of hydrophobic residues of Val7, Ala11, Ala39, Leu41, Ala46, Ile48, and Leu50, as well as electrostatic and hydrogen bond interactions formed by side chains of Ser9, Arg37, Asp44, Gln73, and Arg77 [Fig 1(C)].

Program Dali¹⁵ was used to search the Protein Data Bank database for proteins possessing structural similarities to the ACT domains of ACTP. The result shows that the ACT domains of ACTP show high levels of structural similarities ($Z > 12$) to ACT domains of other proteins including glycine cleavage system transcriptional regulator GcvR (PDB id: 1u8s; unpublished data), formyltetrahydrofolate deformylase (PDB id: 3nrb, 3n0v, and 3lou; unpublished data), and phosphoserine phosphatase SerB (PDB id: 3p96)¹⁶. The amino acid sequence identities are in the range of 16 to 28%, and there does not seem to be a strong correlation between the level of structural similarity and sequence identity. Among these ACT domain-containing proteins, GcvR from *Vibrio cholerae* also exhibits a domain-swapped dimer arrangement of two ACT domains resembling the overall structure of ACTP [Fig. 2(A)]. The monomer structure of *V. cholerae* GcvR also shows a high level of structural similarity to that of ACTP ($Z = 15.8$, r.m.s.d. = 4.0 Å over 164 aligned C $_{\alpha}$ residues, and a sequence identity of 20%).

GcvR is a transcriptional regulator protein required for GcvA mediated regulation of *gcvTHP* operon in bacteria, which plays a crucial role in the glycine cleavage system.¹⁷⁻¹⁸ Glycine binding to GcvR disrupts a GcvA/GcvR interaction required for repression of GcvA.^{17,19} However, GcvA/GcvR regulated control of *gcv* genes has not been identified in eukaryotes. Despite the structural similarity between *V. cholerae* GcvR and ACTP, the conserved bacterial GcvR motifs are absent in the amino acid sequence of ACTP [Fig 2(B)]. Considering that the ACT domain is an evolutionarily mobile ligand binding regulatory module that has been fused to different enzymes at various times,^{1,20} it is likely that the molecular function of ACTP differs from that of GcvR and ACTP may play a role in regulation of a different cellular process.

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Abbreviations

ACTP	ACT domain-containing protein
PDB	protein data bank

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Figure 1.

(A) Monomer structure of ACTP. α -helices, β -strands, and loops are colored in yellow, green, and pink, respectively. The missing loop region between domain 1 and 2 is denoted by a dotted line. (B) Dimerization of ACTP. Chain A and B are labeled as A and B and colored in pink and blue, respectively. The interface A and B are indicated by red and blue lines, respectively. (C) Stereo view of the interface B. Residues forming the interface B are shown in sticks and the hydrogen bonds between the residues in dashed lines.

Figure 2.

(A) Dimer structure of ACTP (left) and *V. cholerae* GcvR (right). Chain A and B of ACTP are colored in pink and blue, and Chain A and B of *V. cholerae* GcvR are colored in cyan and yellow, respectively. (B) Multiple sequence alignment of ACTP and bacterial GcvR proteins from *V. cholerae* (SWISS-PROT ID: Q9KQ45), *Escherichia coli* (SWISS-PROT ID: E2QPV4), *Citrobacter rodentium* (SWISS-PROT ID: D2TTB4), and *Yersinia pestis* (SWISS-PROT ID: Q0WCK5). The conserved GcvR motifs are boxed in red. α-helices and β-strands of ACTP are indicated above the sequences as green arrows and yellow lines, respectively.

Table I

Statistics for data collection, phasing, and model refinement

Data collection and phasing ^a	ACTP from <i>Galdieria sulphuraria</i>	
Space group	<i>P</i> 2	
Cell dimensions		
a, b, c (Å)	55.0, 80.0, 55.5	
α, β, γ (°)	90, 111.25, 90	
Data set	Se λ1 (peak)	Se λ2 (remote)
X-ray wavelength (Å)	0.9792	0.9640
Resolution (Å) ^b	80.00b1.80 (1.84–1.80)	80.0–1.85 (1.89–1.85)
Total / unique reflections	292,991 / 39,789	268,412 / 37,307
Completeness (%)	96.3 (83.2)	97.1 (88.8)
<i>R</i> _{merge} (%) ^c	7.1 (36.8)	5.4 (40.6)
Figure of merit ^d for MAD phasing: 0.51		
Refinement	Data set: Se λ1 (peak)	
Resolution (Å)	45.6–1.80	
<i>R</i> _{work} ^e / <i>R</i> _{free} ^f	0.178 / 0.214	
No. of protein atoms / mean B-factor (Å ²)	2,589 ^g / 25.6	
No. of solvent atoms / mean B-factor (Å ²)	370 / 39.8	
Ramachandran plot analysis (for Chain A)		
Most favored regions	144 (97.3%)	
Additional allowed regions	4 (2.7%)	
Disallowed regions	0 (0%)	
R.m.s deviations from ideal geometry		
Bond lengths (Å)	0.017	
Bond angles (°)	1.29	

^aData collected at the Sector 23-ID-D of the Advanced Photon Source.^bNumbers in parentheses indicate the highest resolution shell of 20.^c $R_{\text{merge}} = \sum_h \sum_i |I(h)_i - \langle I(h) \rangle| / \sum_h \sum_i I(h)$, where $I(h)$ is the observed intensity of reflection h , and $\langle I(h) \rangle$ is the average intensity obtained from multiple measurements.^dFigure of merit = $\langle |\sum P(\alpha) e^{i\alpha} / \sum P(\alpha)| \rangle$, where α is the phase angle and $P(\alpha)$ is the phase probability distribution.^e $R_{\text{work}} = \sum ||F_o| - |F_c|| / \sum |F_o|$, where $|F_o|$ is the observed structure factor amplitude and $|F_c|$ is the calculated structure factor amplitude.^f $R_{\text{free}} = R$ -factor based on 5.1% of the data excluded from refinement.^gNumber of non-hydrogen protein atoms included in refinement.