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# Crystal structure of plant cystathionine β-synthase domain-containing protein in complex with adenosine monophosphate

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## 1 Introduction

The cystathionine *β*-synthase (CBS) domain is a protein domain found in a wide range of proteins in all kingdoms of life, from bacteria to humans. The single CBS pair proteins, CBSXs from *Arabidopsis thaliana*, have recently been identified as redox regulators of the thioredoxin system [1]. Previous crystal structures of CBSX1 and CBSX2 showed unique structural features, including an antiparallel dimer on the central 2-fold axis, a flexible insertion loop followed by an additional helix α5, and an approximately 125° bend in the overall shape on that side of the molecule [1,2]. However, there is no information on how CBSX proteins regulate the activity of Trx and recognize the activator AMP molecule at the atomic level. We determined the crystal structure of CBSX2 in complex with AMP [3], and the structural information clearly explains the specificity of the CBSX protein for only AMP, rather than ADP or ATP.

## 2 Experiment

CBSX2 constructs were overexpressed in *E. coli* BL21(DE3) cells as GST-CBSX2 fusion proteins, and purified using GST affinity chromatography. Then, anion exchange and size exclusion chromatography were used for further purification. Crystallization was performed using the hanging drop vapor diffusion method at 22°C. Initially, co-crystallization of CBSX2 with 0.15 mM AMP was performed using 0.1 M MES pH 6.1, 0.2 M calcium acetate and 21% (w/v) PEG8000. CBSX2-AMP complex crystals belong to a monoclinic space group $P2_1$, with unit cell dimensions of $a$=52.98 Å, $b$=103.64 Å, $c$=69.98 Å and $\beta$=112.22°. X-ray diffraction data of the CBSX2-AMP complex was collected on the beamline NW12A at PF-AR. The structure of the CBSX2-AMP complex was solved using molecular replacement, with CBSX2 from *A. thaliana* as a search model. Subsequently, rigid body and restrained refinement were performed using the PHENIX software program.

## 3 Results and Discussion

The crystal structure of CBSX2 in complex with AMP is determined at 2.6 Å resolution [3]. The structure of dimeric CBSX2 with bound-AMP is shown to be approximately flat, which is in stark difference from the bent form of apo-CBSXs. This conformational change is triggered by a local structural change of the unique α5 helix, and by moving each loop P into an open conformation to accommodate incoming ligands (Fig. 1a). Furthermore, subtle rearrangement of the dimer interface triggers movement of all subunits, and consequently, the bent structure of the CBSX2 dimer becomes a flat structure (Fig. 1b). This reshaping of the structure upon complex formation with adenosine-containing ligand provides evidence that ligand-induced conformational reorganization of antiparallel dimeric CBS domain is an important regulatory mechanism.

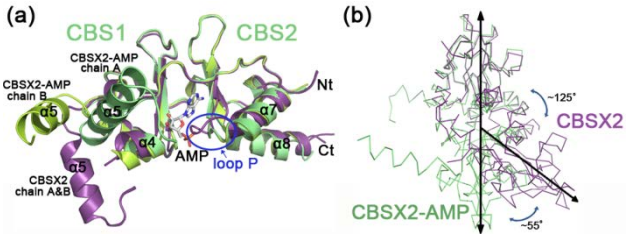


Fig. 1: Structure of CBSX2-AMP complex and comparison with that of apo-CBSXs. (a) Superposition of apo subunit, chain A and B of AMP-bound CBSX2 are shown in a ribbon diagram representation colored purple, green and lemon, respectively.. The loop toward the pocket and AMP molecule are labeled “loop P” and AMP, respectively. (b) Superposition of the Cα structures of CBSX2 and CBSX2-AMP viewed at the side of the molecule.

## References

[1] K.S. Yoo *et al*., *Plant Cell* **23**, 3577 (2011).
[2] B.C. Jeong *et al*., *Biochem. Biophys. Res. Commun.* **430**, 265 (2013).
[3] B.C. Jeong *et al*., *J. Struct. Biol.* **183**, 40 (2013).

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