Biological Science

NW12/2005G048

Crystal structure of 5'-methylthioadenosine nucleosidase from *Arabidopsis thaliana*

Eun Young PARK, Byung-Gil LEE, Byung-Cheon Jeong, and Hyun Kyu SONG*
School of Life Sciences and Biotechnology, Korea University, Anam-Dong
Seongbuk-Gu, Seoul 136-701, South Korea

Introduction

Methylthioadenosine (MTA; 5'-deoxy-5'-methylthioadenosine) is an important metabolite in all living organisms. The MTA cycle has been studied extensively in plant because of the synthesis of ethylene and polyamines, where the maintenance of their levels is important in plant physiology [1]. Many genes involved in the MTA cycle in plant have been isolated and characterized. One of the key enzymes in this cycle is 5'-methylthioadenoside nucleosidase (MTAN).

MTAN (EC 3.2.2.16) is an enzyme that irreversibly cleaves the ribosidic bond of MTA to produce 5'-methylthioribose (MTR) and adenine. Extensive biochemical and structural studies on *E. coli* MTAN have provided a wealth of information regarding the catalytic mechanism, specific interactions with MTA, and conformational changes for the enzymatic reaction [2]. No structural information is available, however, on a plant MTAN, a similar enzyme with different specificity. To gain insight into the structural basis of the differences in substrate specificity, we have determined the high-resolution structure of MTAN from *Arabidopsis thaliana* (AtMTAN).

Materials and methods

The purified MTAN was concentrated to 10 mg/mL in 50 mM Tris-HCl, pH 8.0, 100 mM NaCl, and 1 mM DTT. Crystallization was performed by the hanging-drop vapor diffusion method at 22°C. A monoclinic crystal form of MTAN was obtained with a reservoir solution consisting of 100 mM Tris-HCl, pH = 8.5, 30 % (w/v) polyethylene glycol 3000, and 200 mM sodium acetate. Crystals grew to a maximum size of 0.5 × 0.3 × 0.2 mm^3 within 2 days. The native diffraction data were collected on a charge-coupled device detector at the NW12 beamline of the Photon Factory, Tsukuba, Japan. All diffraction data were processed and scaled using the HKL2000 software package.

Eight selenium and two mercury sites in the asymmetric unit were located with SOLVE. The phases were improved with RESOLVE. The electron density was of sufficient quality to build a nearly complete model of MTAN with the guidance of EcMTAN coordinates (PDB ID: 1Z5P). The protein model was refined with CNS including the bulk solvent correction. A two-fold non-crystallographic symmetry was maintained with tight restraint during the early stages of refinement and was relaxed in the final rounds. Solvent molecules were added using model-phased difference Fourier maps. An unambiguous electron density of adenine was visible, even in the initial MAD-phased map, although the MTAN crystals were obtained in the absence of adenine. The assessment of model geometry and the assignment of secondary structural elements were obtained by using the program PROCHECK.

Results

The crystal structure of AtMTAN has been refined at 1.5 Å resolution. Each monomer of the AtMTAN dimer consisted of seven α-helices, ten β-strands, and two 3_{10}-helices (Figure 1). The first 21 residues (Met1-Glu21) of AtMTAN were not observed in the electron density and, therefore, are likely unstructured. The location of bound adenine is in the deep pocket formed by a monomer and the entrance is partially covered by the adjacent subunit (Figure 1). This flap is important for forming a wide dimeric interface. Structural similarities of AtMTAN with EcMTAN and human MTAP (HsMTAP) are anticipated on the basis of sequence homology, the utilization of the same substrate (MTA) or a combination of the two.

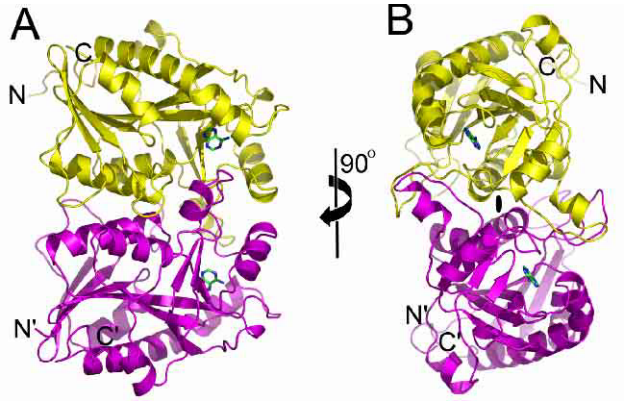

Figure 1. Overall structure of the AtMTAN. (A) Ribbon diagram showing the dimeric AtMTAN structure and (B) a view along the non-crystallographic two-fold molecular symmetry axis. Each monomer in AtMTAN is colored yellow and magenta, respectively. The bound adenine molecules are shown in the stick model. The N- and C-termini of AtMTAN are labeled.

References

[1] J.H. Miyazaki et al., Physiol. Plant 69, 366-70 (1987).
[2] J.E. Lee et al., J. Mol. Biol. 352, 559-74 (2005).

* hksong@korea.ac.kr