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Crystal structure of MTA nucleosidase 2 from *Arabidopsis thaliana*

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Introduction

The MTA (Methylthioadenosine) cycle in plants has also been well-characterized because of the synthesis of ethylene and polyamines, which play critical roles in plant physiology. Several genes involved in the plant MTA cycle have recently been identified and characterized. One of these gene products, MTA nucleosidase (MTAN) cleaves the ribosidic bond of MTA to produce the enzymatic products 5'-methylthioribose (MTR) and adenine. Bacteria use MTA/SAH nucleosidase, which cleaves the covalent bond between MTR and adenine in MTA as well as the bond between S-ribosylhomocysteine (SAH) and adenine in SAH. Extensive biochemical and structural studies on MTA/SAH nucleosidase from *E. coli* have provided tremendous information concerning the catalytic mechanism, specific interactions with substrates, and conformational changes during the enzymatic reaction. However, biochemical and structural studies on MTA nucleosidase from plants have been relatively limited in contrast to the bacterial enzyme. Previously, a biochemically and structurally characterization of an MTAN from *Arabidopsis thaliana* (AtMTAN1; AT4G38800) showed AtMTAN1 hydrolyzes MTA and not SAH, in contrast to the bacterial enzyme which does [1,2]. Another MTAN identified in the *Arabidopsis* genome (AtMTAN2; AT4G34840) was examined for its biochemical properties in recent study [2] and as well as this current one [3]. Although sequence alignment between these two plant homologues shows a very high degree of sequence conservation among the residues in the active site, only AtMTAN2 is able to cleave the ribosidic bond in both SAH and MTA, similar to the bacterial enzyme. In order to understand the substrate specificity between AtMTAN1 and AtMTAN2, we have determined the crystal structure of AtMTAN2.

Materials and methods

The purified AtMTAN2 was concentrated to 7 mg/ml in 50 mM Tris-HCl (pH 8.0), 100 mM NaCl, and 1 mM DTT. Crystals were grown in sitting- and hanging-drop formations at 22 ℃ using equal volumes of protein and reservoir solutions. Initial crystallization conditions were obtained using multiple 96-well sitting-drop screens. After extensive optimization, crystals for data collection were grown in hanging-drop over a reservoir solution of 100 mM Bis-Tris (pH 6.5) and 26~32% (w/v) PEG-MME2000 within 3 to 5 days. Crystals were flash frozen and stored in liquid nitrogen with reservoir solution and 25% (v/v) PEG400 as a cryo-protectant. The native diffraction data were collected on a CCD detector at NW12 beamline of the Photon Factory. The diffraction data were processed and scaled using the HKL2000 software package. The MOLREP program was used to obtain phases with the model of AtMTAN1 (PDB ID: 2H8G). The model was rebuilt by the program O and COOT and was refined with CNS including the bulk solvent correction. With the exception of the flexible loop regions, 2-fold non-crystallographic symmetry was tightly maintained during the entire refinement.

Results

Overall superposition of AtMTAN1 and AtMTAN2 performed with the program CNS showed the residues between 216 and 225 in AtMTAN2 contain conformational differences representing an open conformation when compared with AtMTAN1 (Fig. 1A). Interestingly, AtMTAN1 (PDB ID: 2QSU) displayed a more open apo-form in this region compared with our AtMTAN2 structure. AtMTAN1 and AtMTAN2 monomers confirm AtMTAN2 exists in a more open conformation compared with the closed structure observed in adenine-bound AtMTAN1 (Fig. 1B).

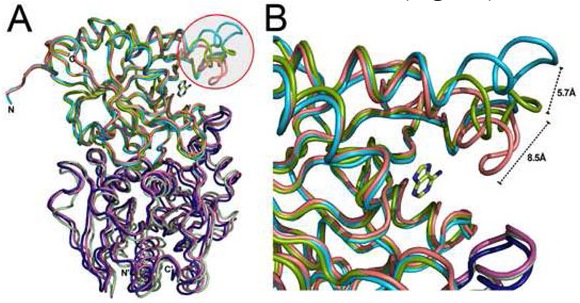

Figure 1. Superposition of AtMTAN1 and AtMTAN2. (A) Backbone superposition of free (cyan), adenine-bound form (salmon) of AtMTAN1, and adenine-bound form (green) of AtMTAN2. The region showing significant structural movement (> 3Å RMSD) is marked with a red circle. (B) Close-up view of the red circle marked in panel (A). The maximal shifts from the adenine bound AtMTAN2 are indicated.

References

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