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Crystal structure of *trans*-editing protein AlaX complexed with $_{L}$-serine

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Introduction

Some aaRSs have developed editing mechanism to achieve higher accuracy by attachment of a hydrolytic domain that is specific to mischarged tRNA during evolution. Double sieve discrimination of cognate amino acid *via* activation and editing site have been established through studies of class I aaRSs, however, those of evolutionary distinct class II aaRSs are less known. Class II alanyl-tRNA synthetase (AlaRS) has editing domain harboring the conserved zinc-binding motif, and is known to specifically deacylate mischarged Ser-/ Gly-tRNA^{Ala} [1]. On the other hand, AlaX protein, which are closely related to AlaRS editing domain, are known to present in many organisms, and recently were actually exhibited to have the specific editing activity against mischarged Ser-/ Gly-tRNA^{Ala} *in trans*, which therefore were termed *trans*-editing proteins [2].

To understand the structural basis of amino acid discrimination in an alanyl system, we determined the structure of AlaX from the archaea *Pyrococcus horikoshii* (PhoAlaX), in complex with the non-cognate amino acid, $_{L}$-serine and zinc at 2.8 Å resolution.

Results and Discussion

The crystals of PhoAlaX in complex with serine and zinc were obtained by soaking method using the crystal of apo-PhoAlaX dimer crystals grown by hanging drop vapor diffusion method. The data was collected at 100K using synchrotron radiation sources at BL6A, Photon factory. The diffraction images were analyzed and processed as summarized in Table1.

Table 1. Data collection statistics

Space group	$P2_12_12_1$
a (Å)	34.0
b (Å)	88.5
c (Å)	109.9
Resolution (Å)	50-2.8
No. of Unique reflections	8871
Completeness (%)	100.0
R_{merge} (%)	9.3

The structure was solved by molecular replacement using apo-PhoAlaX structure as a search model, and was refined to an *R*-factor of 22.7 % and an R_{free}-factor of 26.6 % at 2.80 Å resolution, with 154 and 152 residues in two molecules, and one $_{L}$-serine and zinc per molecule, and 107 water molecules.

The overall structure of PhoAlaX closely resembles the editing domain of threonyl-tRNA synthetase, which also harbors the zinc-binding motif. The serine side chain hydroxyl is strongly recognized by hydrogen-bonds with Thr30 hydroxyl, Asp92 carboxyl and one water molecule with distances of 2.8 Å, 3.0 Å and 3.1 Å, respectively, whereas the main chain moiety is only weakly held by the zinc-binding motif, Thr33, and Asn114 with distances of ~3.5Å (Figure1). Remarkably, a side chain hydroxyl of the conserved Thr30 is located near the β-methylene of the bound serine with a distance of 3.7 Å, approximately an atomic radius, and thus, the chemical repulsion of the β-carbon *via* Thr30 hydroxyl is envisaged. In this situation, an aliphatic β-methyl of alanine would not be accommodated with the hydrophilic pocket, whereas a serine hydroxyl would be held strongly by the dense hydrogen bond network with the hydrophilic pocket that compensates the unfavorable interaction between Thr30 hydroxyl and the β-methylene. Such 'chemical discrimination' model was further supported by the analyses of PhoAlaX-T30V and AlaRS-Q584M mutants.

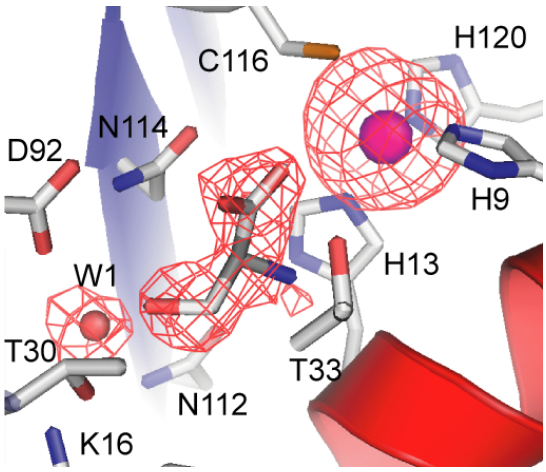

Figure 1. $_{L}$-Serine and Zn^{2+} at PhoAlaX editing site

References

[1] M.A. Swairjo et al., Proc. Natl. Acad. Sci. USA. 102, 988-93 (2005).
[2] I. Ahel et al., Proc. Natl. Acad. Sci. USA. 100, 15422-7 (2003).

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