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Crystal structure analysis of dihydrouridine synthase C from *Escherichia coli*

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1 Introduction

Posttranscriptional modification is an indispensable step for maturation of non-coding RNA. To date, more than 100 modifications have been reported on transfer RNA (tRNA). These modifications play various important cellular roles. Dihydrouridine (D) is one of the most widely conserved tRNA modifications found in bacteria, some archaea, and eukaryotic organisms, and is mostly found in the D-loop of tRNAs for which it is named. Individual tRNAs have varying numbers of D modifications. It was suggested that D modification destabilizes the C3'-endo form ribose conformation associated with base stacking, and consequently increases the flexibility of the tertiary structure of tRNA (Dalluge et al., 1996). D is formed by reduction of the double bond between positions 5 and 6 of the uridine base by dihydrouridine synthase (Dus). Genes encoding four Dus isozymes were found in the genome of Saccharomyces cerevisiae, and their modification sites were identified. Site specificity and nonredundant catalytic functions have been confirmed for three Dus enzymes from Escherichia coli. In a previous study, a unique substrate recognition mechanism in which basic residues located around the active site recognize uridine bases indirectly through a small adapter molecule was proposed based on structure analysis of *Thermus thermophilus* Dus (*Tth*Dus) in complex with tRNA and mutational analyses. However, the adapter molecule has yet to be identified. Therefore, information regarding the adapter molecule is important to gain a further understanding of the reaction mechanism. Furthermore, the relevance of D modification to cancer has been reported, suggesting that elucidation of the mechanism underlying the introduction of D modifications will have important medical implications.

In the present study, to gain insight into the substrate base and/or tRNA recognition mechanism, the crystal structure of DusC from *E. coli* (*Eco*DusC) was determined at a resolution of 2.1 Å [1].

2 Experiment

*Eco*DusC and Se-Met substituted *Eco*DusC were expressed by *E. coli* strain B834(DE3). Crystals of native *Eco*DusC were obtained from reservoir solution consisting of 0.1 M Tris (pH 7.9), 0.2 M sodium acetate, 12% PEG4000, while crystals of SeMet-*Eco*DusC were obtained from reservoir solution consisting of 0.1 M imidazole (pH 8.0), 15% (v/v) isopropanol, and 20% (v/v) glycerol. Data collection was carried out at 100 K after soaking the crystals in crystallization buffer containing 20% glycerol. Single-wavelength anomalous diffraction (SAD) data were collected from SeMet crystals to a resolution of 2.8 Å on beamline BL41XU of SPring-8 (Harima, Japan). The wavelength of 0.9791 Å for data collection was determined based on the fluorescence spectrum of the Se K absorption edge. The data were indexed, integrated, and scaled with HKL2000. The crystal of SeMet-*Eco*DusC belonged to space group $P4_32_12$, with unit cell parameters a = b = 94.5, c = 116.6 Å. A data set was collected from a native crystal to a resolution of 2.1 Å on beamline BL5A of the Photon Factory (Tsukuba, Japan). The data were indexed, integrated, and scaled with HKL2000. The crystal of native *Eco*DusC belonged to space group $P4_32_12$, with unit cell parameters a = b = 93.3 Å, c = 115.5 Å.

The structure of *Eco*DusC was determined by the SAD method with the program SHELX, phenix.autosol, and phenix.autobuild. One molecule of EcoDusC was located in an asymmetric unit. The structure was refined using the native data at 2.1 Å resolution. After several cycles of refinement, the crystallographic R_{work} and R_{free} factors converged to 22.1% and 23.5%, respectively.

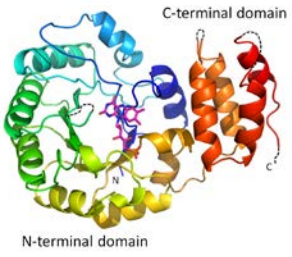
3 Results and Discussion

*Eco*DusC was shown to be composed of two domains: an N-terminal catalytic domain and a C-terminal tRNA binding domain. An L-shaped electron density surrounded by highly conserved residues was found in the active site, as observed for *Tth*Dus. Structure comparison with TthDus indicated that the N-terminal region has a similar structure, whereas the C-terminal domain had marked differences in relative orientation for the N-terminal domain as well as its own structure. These observations suggested that Dus proteins adopt a common substrate recognition mechanism using an adapter molecule, whereas the manner of tRNA binding is diverse.

Fig. 1: Crystal structure of EcoDusC.

References

[1] Chen et al. ***Acta Cryst. F*** in press



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