Biological Science

NW12/2009G062

X-ray crystallographic analysis of SecDF periplasmic domain

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Introduction

The transport of proteins across cell membranes is one of the most fundamental and essential cellular activities across all organisms. In bacteria, protein translocation across the cytoplasmic membrane is driven by dynamic interplays between the protein-conducting SecYEG channel (Sec translocon) and the SecA ATPase [1-3]. We have determined the structure of a membrane component SecDF, which associates with SecYEG and enhances the protein translocaiton, and elucidated its molecular mechanism by a series of biochemical and biophysical approaches [4]. The flexible first periplasmic (P1) domain of SecDF plays an important role in protein translocation. Here, we report the crystallization and X-ray analysis of the P1 domain from *Thermus thermophilus*. The high-resolution structure of P1 domain determined by a multi-wavelength anomalous dispersion method enabled us to correctly build the model of the periplasmic domains in full-length *T. thermophilus* SecDF [4].

Results and Discussion

The selenomethionine labeled P1 domain ($SecDF_{35\text{-}263}$) was crystallized using the sitting-drop vapour-diffusion method (Fig. 1). The crystals were transferred into a cryoprotectant solution and then flash-cooled in a nitrogen-gas stream at 100 K. The X-ray diffraction data sets of selenomethionine labeled P1 domain were collected at 100 K using an ADSC Quantum 210 detector on beamline NW12. The crystals diffracted X-ray to 2.7 Å resolution and belonged to space group *C*2. We collected a four-wavelength multi wavelength anomalous diffraction data set at the absorption peak (λ = 0.97923 Å), the inflection point (λ = 0.97931 Å), a high-energy remote point (λ = 0.98319 Å) and low-energy remote point (λ = 0.96408 Å). The diffraction data sets were processed using DENZO/SCALEPACK. The data collection statistics of the dataset at the absorption peak are summarized in Table 1. We identified the Se sites using the programs SHELXC and SHELXD, and obtained an initial electron density map, calculated using the program SHARP. The model of P1 domain was manually built with the program Coot and then refined using the program Refmac with R_{work} = 23.0% and R_{free} = 27.2 % at 2.7 Å resolution, until the R_{free} factor converged (Fig. 2).

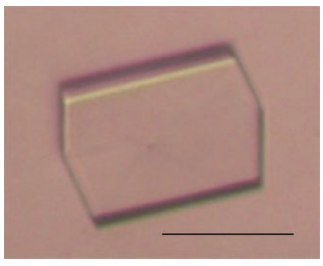

Fig. 1. Selenomethionine labeled crystal of SecDF P1 domain. The crystal was obtained by sitting-drop vapour diffusion with reservoir solution containing 20% PEG 3350,0.2 M sodium thiocyanate and 3% trehalose. The scale bars represent 100 μm

Table 1 : Data collection statistics

	Se-Met labeled P1
Space group	*C*2
Cell dimensions	a = 160.6 Å, b = 35.8 Å c = 180.9 Å, β ••••••••Å
Wavelength	0.97923 Å
Resolution	50.0-2.70 Å (2.75-2.70 Å)
R_{sym}	0.066 (0.106)
$I/\sigma I$	50.5 (17.1)
Completeness	97.2 (96.4)
Redundancy	9.5 (7.9)

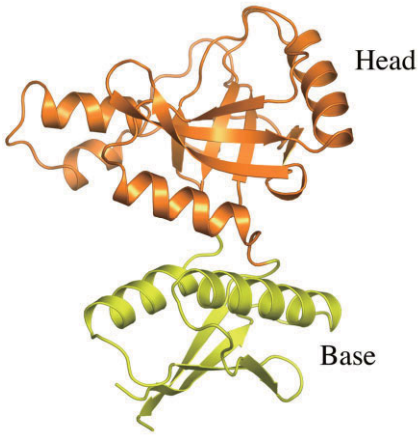

Fig. 2. Structure of P1 domain. P1 domain consists of Head and Base subdomains.

References

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