Structural Insights into tRNA-Dependent Cysteine Synthesis by Transsulfursome

In many methanogenic archaea, Cys-tRNA^{Cys}, which is used to synthesize protein and produces cysteine, is synthesized through an indirect pathway by a large protein complex, transsulfursome. This complex consists of two enzymes, SepRS and SepCysS, and a scaffold protein, SepCysE. Here, we report two crystal structures of transsulfursome: the structure of SepCysE-SepCysS-tRNA^{Cys} showing the interaction among SepCysE, SepCysS and tRNA^{Cys}, and the structure of SepRS-SepCysE(N-helix) characterizing the binding manner of SepRS and SepCysE. Combining SEC-SAXS results, we established the architecture of transsulfursome, which indicates the dynamic conformation of transsulfursome mediated by SepCysE for Cys-tRNA^{Cys} synthesis in the indirect pathway.

Aminoacylation of tRNA is the key step in translation, where aminoacyl-tRNA synthetases (aaRSs) ligate the cognate amino acid to the specific tRNA (direct pathway). Generally, there are 20 kinds of amino acids in cells, and accordingly 20 kinds of aaRSs each responsible for its specific amino acid. Therefore, aaRSs are crucial for living creatures and so are almost invariable or highly conserved throughout evolution. Surprisingly, whole genome sequencing of methanogens showed the absence of CysRS and enzymes responsible for cysteine biosynthesis in ancient life [1]. Further, it was discovered that Cys-tRNA^{Cys} is synthesized by enzymes in two-step reactions (indirect pathway). The first enzyme, SepRS, attaches a phosphoserine (Sep) to tRNA^{Cys} (Sep-tRNA^{Cys}), and then the second enzyme, SepCysS, converts mischarged Sep-tRNA^{Cys} to Cys-tRNA^{Cys} [2]. This indirect pathway not only provides the Cys-tRNA^{Cys} for protein synthesis, but also paves the way for *ab initio* biosynthesis of cysteine to satisfy the cellular requirement in methanogens. However, even a decade after the discovery, the mechanism of how the two enzymes work together was still unclear.

In our previous research, we found that a scaffold protein, SepCysE, is required to bridge SepRS and SepCysS to form a stable ternary complex, named transsulfursome [3]. However, how the three proteins interact with each other to assemble into transsulfursome, and how tRNA^{Cys} is transferred between each active site of SepRS and SepCysS, remain unclear. Here, we report the structure of transsulfursome and its working mechanism by using crystallography analysis and small-angle X-ray scattering (SAXS) [4].

To understand the recognition of tRNA^{Cys} by SepCysS in the second step of the indirect pathway, firstly, the complex of SepCysE-SepCysS was overexpressed and purified, followed by crystallization together with tRNA^{Cys}. Crystals ($P6_522$) were obtained and the structure was solved by the molecular replacement method using the structure of SepCysE-SepCysS as a search model. In the asymmetric unit, there is a SepCysS dimer and a SepCysE dimer binding only one tRNA^{Cys}. The structure revealed the interaction between SepCysE-SepCysS and tRNA^{Cys}, where SepCysS recognizes the U73 discriminant of tRNA^{Cys} and the C-terminal domain of SepCysE binds the elbow of tRNA^{Cys} to stabilize the tRNA^{Cys} in the second-step reaction of the indirect pathway.

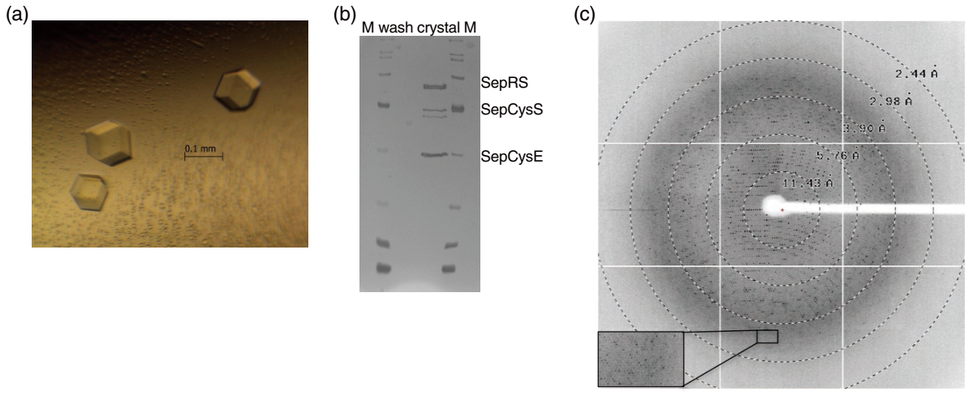

Figure 1: Crystals of transsulfursome. **(a)** Photo of the crystal, **(b)** SDS-PAGE analysis of the crystal, **(c)** X-ray diffraction of the crystal.

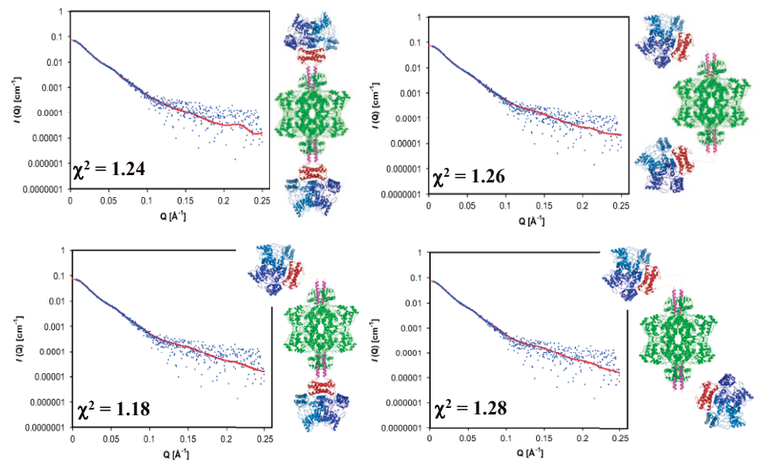

Figure 2: SAXS analyses showing the domain flexibility of the transsulfursome. The theoretical curves (red) calculated from different models of transsulfursome (green: SepRS; red: SepCysE; blue: SepCysS) fit well to the SAXS scattering data (blue) with similar χ^2 value.

Thereafter, transsulfursome (SepRS, SepCysS and SepCysE) complexed with tRNA^{Cys} was reconstituted *in vitro*, and then crystallized [**Fig. 1(a)**]. SDS-PAGE and Urea-PAGE analysis of crystals confirmed the presence of all three proteins [**Fig. 1(b)**], but no tRNA^{Cys}. X-ray diffraction data were collected [**Fig. 1(c)**] at 3.1 Å resolution on BL-5A beamline and crystallographic analysis showed the space group as $I2_13$. The structure was solved by the molecular replacement method, but only SepRS tetramer with two additional helix dimers was identified, while SepCysE and SepCysS were disordered in the crystal structure. Further model building and sequence analysis indicated that the two helix dimers are the N-terminus of SepCysE. This structure disclosed the interaction between SepRS and SepCysE, as the N-terminal helix dimer of SepCysE inserts into the cleft of SepRS formed by two anticodon-binding domains at each side of SepRS tetramer.

Based on these structures, a model of the whole transsulfursome was built (**Fig. 2**), and SAXS with size exclusion chromatography (SEC-SAXS) was used to validate the model in solution. A sample was loaded onto a Superdex 200 Increase 10/300 GL column (GE Healthcare) and the collected fractions were directly exposed to X-rays at beamline BL-10C. Simulation using crystal structure to fit to scattering intensity data was carried out using CRYSOL programs. Various models were generated by changing the distance and orientation of two rigid structures, SepRS-SepCysE(N-helix) and SepCysE(NTD)-SepCysS, connected by a flexible loop of SepCysE (**Fig. 2**). Simulation results suggested that different structures of transsulfursome with various tilt angles between SepRS-SepCysE(N-helix) and SepCysE(NTD)-SepCysS existed in solution. This flexibility of transsulfursome is essential for Cys-tRNA^{Cys} synthesis, which was confirmed by *in vivo* assay experiments using loop-deletion mutants of SepCysE. Such dynamic structure of transsulfursome may be required for tRNA transfer between each active site on SepRS and SepCysS.

REFERENCES

[1] C. J. Bult, O. White, G. J. Olsen, L. Zhou, R. D. Fleischmann, G. G. Sutton, J. A. Blake, L. M. FitzGerald, R. A. Clayton, J. D. Gocayne, A. R. Kerlavage, B. A. Dougherty, J. –F. Tomb, M. D. Adams, C. I. Reich, R. Overbeek, E. F. Kirkness, K. G. Weinstock, J. M. Merrick, A. Glodek, J. L. Scott, N. S. M. Geoghagen, J. F. Weidman, J. L. Fuhrmann, D. Nguyen, T. R. Utterback, J. M. Kelley, J. D. Peterson, P. W. Sadow, M. C. Hanna, M. D. Cotton, K. M. Roberts, M. A. Hurst, B. P. Kaine, M. Borodovsky, H. –P. Klenk, C. M. Fraser, H. O. Smith, C. R. Woese and J. C. Venter, *Science* **273**, 1058 (1996).

[2] A. Sauerwald, W. Zhu, T. A. Major, H. Roy, S. Palioura, D. Jahn, W. B. Whitman, J. R. Yates 3rd, M. Ibba and D. Söll, *Science* **307**, 1969 (2005).

[3] Y. Liu, A. Nakamura, Y. Nakazawa, N. Asano, K. A. Ford, M. J. Hohn, I. Tanaka, M. Yao and D. Söll, *Proc. Natl. Acad. Sci. U.S.A.* **111**, 10520 (2014).

[4] M. Chen, K. Kato, Y. Kubo, Y. Tanaka, Y. Liu, F. Long, W. B. Whitman, P. Lill, C. Gatsogiannis, S. Raunser, N. Shimizu, A. Shinoda, A. Nakamura, I. Tanaka and M. Yao, *Nat. commun.* **8**, 1521(2017).

BEAMLINES

BL-5A and BL-10C

M. Yao[1] and M. Chen[1,2] ([1] Hokkaido Univ., [2]TSRI)