Crystallographic Study of Bacterial Selenocysteine Formation

Selenium is an essential human micronutrient. It is contained in the 21st amino acid selenocysteine (Sec), which is used in the catalytic centers of some redox enzymes. Sec is synthesized from another amino acid, serine (Ser), on its adapter molecule, $tRNA^{Sec}$. In bacteria, the Sec synthase SelA directly converts Ser to Sec. We determined the crystal structures of SelA with and without $tRNA^{Sec}$. SelA consists of 10 identical subunits that form a star-shaped pentamer of dimers. SelA binds 10 $tRNA^{Sec}$s and forms an 800-kDa protein-RNA complex. Based on the crystal structures, the reaction and substrate recognition mechanisms of SelA are discussed.

The element selenium is an essential micronutrient for human and animal health. In living organisms, it exists in the form of the special amino acid selenocysteine (Sec). Selenium has similar chemical properties to those of sulfur. In Sec, selenium replaces the sulfur atom of cysteine (Cys), which is one of the canonical amino acids. Sec-containing proteins, selenoproteins, function mainly as redox enzymes, such as antioxidant proteins, where Sec is utilized in their catalytic centers. Since the selenol group of Sec is easily deprotonated as compared to the thiol group of Cys (pKa 5.2 *vs* 8.3), Sec has stronger redox activity. Sec is distributed in all three domains of life: bacteria, archaea, and eukaryotes, and there are 25 human selenoproteins [1].

Proteins are macromolecules composed of long chains of amino acid residues. There are 20 types of canonical amino acids, and their sequences are encoded in messenger RNAs (mRNAs), which are transcripts of genes. In addition to the canonical amino acids, Sec is used for a limited number of proteins, and is called the 21st amino acid.

In the translation system, each amino acid is ligated to its cognate adaptor molecule, transfer RNA (tRNA), and is brought to the protein synthesis factory, the ribosome. Aminoacyl-tRNA synthetases (aaRSs) are responsible for the ligation of their corresponding amino acids; e.g., serine (Ser) is ligated to serine tRNA ($tRNA^{Ser}$) by seryl-tRNA synthetase (SerRS) (Fig. 1). However, Sec lacks its own aminoacyl-tRNA synthetase, and is synthesized in a tRNA-dependent manner (Fig. 1). In the first step, SerRS ligates serine to Sec tRNA ($tRNA^{Sec}$), to form seryl-$tRNA^{Sec}$ (Ser-$tRNA^{Sec}$) [2]. In bacteria, the ligated Ser is directly converted to Sec by the Sec synthase SelA [3], while a two-step conversion is performed in eukaryotes and archaea (Fig. 1) [4, 5]. The eukaryotic and archaeal substrate recognition and reaction mechanisms have been well characterized by crystallographic studies [6, 7]. However, the crystal structure of bacterial SelA has not been determined due to its huge molecular mass, which exceeds 500 kDa.

We crystallized the full-length and N-terminally truncated SelA proteins from the bacterium *Aquifex aeolicus*, and determined their crystal structures at 3.2 and 3.9 Å resolutions, respectively [8]. Several mutational and chemical treatments were performed to improve the crystal quality and X-ray diffraction resolution [9].

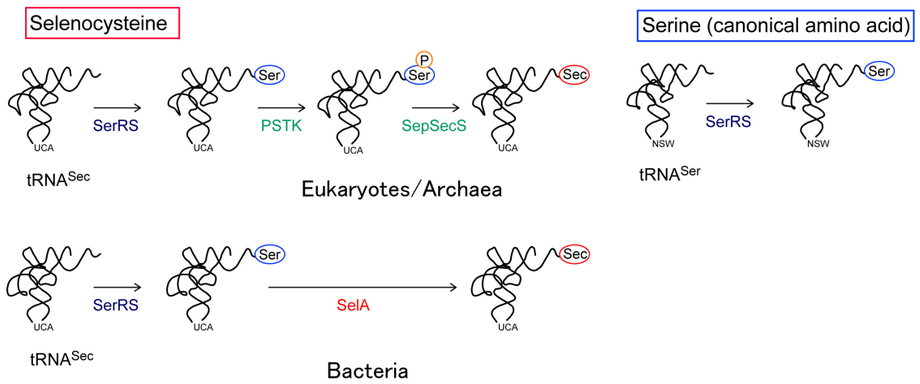

Figure 1: Sec synthesis system. Schematic depiction of $tRNA^{Sec}$-dependent Ser-to-Sec conversion.

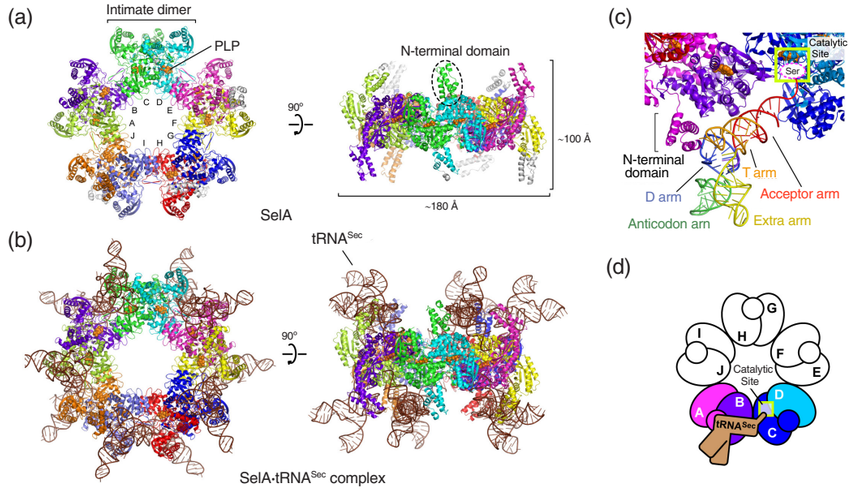

Figure 2: Crystal structures of SelA. (a, b) Overall structures of SelA (without $tRNA^{Sec}$) and the SelA•$tRNA^{Sec}$ complex. (c, d) Close-up view and scheme of the interaction between the SelA subunits and $tRNA^{Sec}$.

SelA is a homodecameric protein composed of 10 subunits, and forms a pentamer of intimate dimers. The overall structure is a star-shaped ring [Fig. 2(a)]. The 10 subunits are identical to each other; therefore, the decamer has regular pentagonal symmetry. Each subunit consists of the N-terminal, core, and C-terminal domains, and the N-terminal domains protrude from the central pentagon [Fig. 2(a)]. There are 10 catalytic sites, where the cofactor pyridoxal phosphates (PLP) are bound. PLP is an active form of vitamin B_6, and is utilized in many enzymes involved in amino-acid metabolism.

The complex of SelA and $tRNA^{Sec}$ was also crystallized [8]. Although the diffraction resolution is 7.5 Å, the positions of the bound tRNAs were identified [Fig. 2(b)]. SelA binds 10 tRNAs, and the total molecular mass is about 800 kDa. Each $tRNA^{Sec}$ interacts with four SelA subunits: subunits A–D [Fig. 2(c), (d)]. The N-terminal domain of subunit A interacts with the D arm region of $tRNA^{Sec}$, subunit B interacts with the acceptor stem, and subunit C interacts with the tip of the acceptor arm. The 3′-terminus of $tRNA^{Sec}$ reaches the catalytic site located on the subunit C-D interface, although $tRNA^{Sec}$ without ligated Ser was used for crystallization.

The Ser-to-Sec conversion requires strict $tRNA^{Sec}$ specificity toward $tRNA^{Ser}$. If SelA reacted with the Ser-ligated $tRNA^{Ser}$, then Sec would be misincorporated into proteins by the Ser codons. The N-terminal domain of SelA interacts with the D arm of $tRNA^{Sec}$ [Fig. 2(c)]. The $tRNA^{Sec}$ D arm has long stem and short loop regions, and forms a unique 3D structure [10, 11]. The specificity is achieved by the distinctive interaction between SelA and the D arm.

In the conversion reaction, subunit A recognizes Ser-$tRNA^{Sec}$, subunits A and B bind Ser-$tRNA^{Sec}$, subunit C interacts with the tip of the acceptor arm, and subunits C and D convert the ligated Ser to Sec [Fig. 2(d)]. Each subunit can perform the four roles. The star-shaped arrangement of the 10 subunits is critical for catalysis and substrate discrimination [8, 12].

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BEAMLINES

BL-5A, BL-17A and AR-NW12A

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