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Decameric Sela•tRNA^{Sec} Ring Structure Reveals Mechanism of Bacterial Selenocysteine Formation

Yuzuru Itoh^{1,2,3}, Markus J. Bröcker⁴, Shun-ichi Sekine^{1,2}, Gifty Hammond⁴, Shiro Suetsugu³, Dieter Söll^{4,5,*}, and Shigeyuki Yokoyama^{1,2,*}

¹RIKEN Systems and Structural Biology Center, Tsurumi, Yokohama 230-0045, Japan

²Department of Biophysics and Biochemistry and Laboratory of Structural Biology, Graduate School of Science, The University of Tokyo, Bunkyo, Tokyo 113-0033, Japan

³Laboratory of Membrane and Cytoskeleton Dynamics, Institute of Molecular and Cellular Biosciences, The University of Tokyo, Bunkyo, Tokyo 113-0032, Japan

⁴Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06520–8114, USA

⁵Department of Chemistry, Yale University, New Haven, CT 06520–8114, USA

Abstract

The 21st amino acid, selenocysteine (Sec), is synthesized on its cognate transfer RNA (tRNA^{Sec}). In bacteria, Sela synthesizes Sec from Ser-tRNA^{Sec}, whereas in archaea and eukaryotes SepSecS forms Sec from phosphoserine (Sep) acylated to tRNA^{Sec}. We determined the crystal structures of *Aquifex aeolicus* Sela complexes, which revealed a ring-shaped homodecamer that binds 10 tRNA^{Sec} molecules, each interacting with four Sela subunits. The Sela N-terminal domain binds the tRNA^{Sec}-specific D-arm structure, thereby discriminating Ser-tRNA^{Sec} from Ser-tRNA^{Ser}. A large cleft is created between two subunits and accommodates the 3'-terminal region of Ser-tRNA^{Sec}. The Sela structures together with in vivo and in vitro enzyme assays show decamerization to be essential for Sela function. Sela catalyzes pyridoxal 5'-phosphate-dependent Sec formation involving Arg residues nonhomologous to those in SepSecS. Different protein architecture and substrate coordination of the bacterial enzyme provide structural evidence for independent evolution of the two Sec synthesis systems present in nature.

The micronutrient selenium is required for human and animal health (1). Selenium is present in proteins in the form of the 21st amino acid, selenocysteine (Sec), in which the thiol moiety of cysteine is replaced by a selenol group (2). Sec is located in the active sites of many redox enzymes and is encoded by a UGA stop codon in all three domains of life (3). Sec lacks its own aminoacyl-tRNA synthetase and is synthesized by the tRNA-dependent conversion of Ser (3). The first step in Sec synthesis is the formation of Ser-tRNA^{Sec} by seryl-tRNA synthetase (SerRS) (3). In bacteria, the selenocysteine synthase Sela then converts the Ser-tRNA^{Sec} to Sec-tRNA^{Sec}. Archaea and eukaryotes use an intermediate step

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*Corresponding author: dieter.soll@yale.edu (D.S.); yokoyama@riken.jp (S.Y.).

Supplementary Materials

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Materials and Methods

Figs. S1 to S9

Tables S1 and S2

References (15–28)

in which the hydroxyl group of Ser-tRNA^{Sec} is phosphorylated by *O*-phosphoseryl-tRNA kinase (PSTK) (4) to give Sep-tRNA^{Sec}, the substrate for the homo-tetrameric enzyme SepSecS, the final synthetic enzyme (5, 6). Both SslA and SepSecS are fold-type-I pyridoxal 5'-phosphate (PLP)-dependent enzymes (7) that use selenophosphate as the selenium donor (3, 8). All Sec-synthesis systems must strictly discriminate Ser-tRNA^{Sec} from Ser-tRNA^{Ser}. tRNA^{Sec} is the longest tRNA (9), and its tertiary structure is quite different from that of a canonical tRNA (8, 10). In archaea and eukaryotes, PSTK discriminates Ser-tRNA^{Sec} from Ser-tRNA^{Ser} for phosphorylation (11), and then SepSecS recognizes Sep-tRNA^{Sec} in a phosphate-dependent manner (8). However, because of the lack of crystallographic studies on SslA, the discrimination mechanism in bacteria has remained elusive. Cryogenic electron microscopy suggested SslA to be a homodecameric enzyme of >500 kD (12, 13), in contrast to the ~220-kD SepSecS homotetramer (8).

Here, we present the crystal structures of full-length decameric *Aquifex aeolicus* SslA (SslA-FL, residues 1 to 452), alone (3.9 Å resolution) and in complex with *Thermoanaerobacter tengcongensis* tRNA^{Sec} (7.5 Å), and of a SslA mutant lacking the N-terminal domain (SslA-ΔN, residues 62 to 452) with and without thiosulfate (3.25 and 3.20 Å, respectively). Biochemical and genetic experiments (table S1) were performed with *Escherichia coli* SslA (*A. aeolicus* numbering is used in this Report, fig. S1).

SslA is a homodecamer in which the 10 subunits form a pentamer of dimers (Fig. 1). Each subunit consists of the N-terminal domain (residues 1 to 66), the N-linker (residues 67 to 89), the core domain (residues 90 to 338), and the C-terminal domain (residues 339 to 452) (fig. S2A). The intimate dimer in SslA contains two catalytic sites formed at the subunit-subunit interface, where the cofactor PLP is covalently linked to a conserved Lys²⁸⁵ (fig. S2, B to E). The N-terminal domain protrudes from the central pentagon, is intrinsically mobile (fig. S3), and only contacts the other core domain of the intimate dimer. The orientation of the C-terminal domain (relative to the core domain) differs substantially from that of the C-terminal domains in other fold-type-I PLP-dependent enzymes (7) (fig. S4). Its orientation creates a large space between the core and C-terminal domains (fig. S2A), which allows the interaction with the neighboring intimate dimer for decamerization (fig. S4B) and the formation of a large cleft between two intimate dimers (Fig. 1).

The structure (Fig. 2A) of *A. aeolicus* SslA complexed to *T. tengcongensis* tRNA^{Sec} at 7.5-Å resolution (cocrystals with the homologous tRNA only diffracted to ~20 Å) revealed that the SslA decamer binds up to 10 tRNA^{Sec} molecules. Despite the low resolution of the ~0.8-MD ribonucleo-protein, the positions of the tRNA^{Sec} molecules were unambiguously detected in the electron density map, except for the CCA terminus (Fig. 2B). Four SslA subunits interact with one Ser-tRNA^{Sec}, with one dimer holding tRNA^{Sec} and the other providing the catalytic site (Fig. 2B). The large cleft (Fig. 1) provides the space for tRNA^{Sec} to approach the catalytic site on the neighboring dimer. Therefore, the proper relative positioning of the two dimers is required to perform the overall reaction and is fixed by the ring closure to form the decameric structure (Fig. 2C). If SslA assumed the quaternary structure of any other fold-type-I PLP enzyme [e.g., the tetrameric SepSecS (8)], the tRNA-binding and catalytic sites could not work together. In fact, a quadruple mutation introduced at the dimer-dimer interface (Thr¹⁹¹-Thr¹⁹²-Asp¹⁹⁹-Tyr²²⁰→Tyr¹⁹¹-Tyr¹⁹²-Arg¹⁹⁹-Pro²²⁰) caused a dimeric quaternary structure (fig. S5A) and abolished SslA activity in vivo (table S1) and in vitro (fig. S5B).

SslA interacts with the D-arm side of the L-shaped tRNA^{Sec} and does not contact either the extra arm or the anticodon arm (Fig. 2B). The N-terminal domain of SslA binds the D arm and the T loop of tRNA^{Sec} (fig. S6, A and B). The SslA-ΔN mutant, lacking the N-terminal domain, did not bind Ser-tRNA^{Sec} or tRNA^{Sec}, whereas the dimeric mutant dSslA, which

retains the N-terminal domain, can still bind tRNA^{Sec} (fig. S5, C and D). The dissociation constant (K_D) for dSela is 624 ± 84 (SD) nM, whereas that for the wild type is 75 ± 13 nM, indicating that more than 90% of the standard change in Gibbs free energy (ΔG°) for tRNA^{Sec} binding is ascribed to the tRNA^{Sec} interactions within one dimer, particularly with the N-terminal domain. The specific interaction between the Sela N-terminal domain and the tRNA^{Sec} D arm accomplishes the discrimination of the substrate Ser-tRNA^{Sec} from the structurally divergent nonsubstrate Ser-tRNA^{Ser} [the maximal six-base-pair D stem and minimal four-nucleotide D loop in tRNA^{Sec} compared with the three-base-pair D stem and 11-nucleotide D loop in tRNA^{Ser} (fig. S7)]. The D stem of *T. tengcongensis* tRNA^{Sec} contains the fifth and sixth pairs G14:U21 [G14:C21 in *A. aeolicus* tRNA^{Sec} (fig. S7)] and C15:G20a, whereas D loop nucleotide U16 forms the unique tertiary base pair U16:U59. The Sela N-terminal domain contacts C15, U16, G20a, and U21 because the shape of the unique D-arm structure of tRNA^{Sec} is complementary to the protein structure (Fig. 2D and fig. S6D). Because of the incompatible shape of tRNA^{Ser}, the corresponding nucleotides in the canonical augmented D helix of tRNA^{Ser} cannot interact with the Sela N-terminal domain (Fig. 2E and fig. S6D). In contrast, the difference in the number of base pairs in the acceptor stem between tRNA^{Sec} and tRNA^{Ser} (eight and seven, respectively) barely contributes to the discrimination, probably because of the inherent flexibility of the N-terminal domain (fig. S6D).

The large cleft described above enables Sela to hold the end of the acceptor stem: G1 from the G1:C72 pair and the G73 discriminator nucleotide interact with the C-terminal domain of the neighboring subunit (Fig. 2B and fig. S6C). The deletion of residues 423 and 424 within the β 18-loop region, which contacts G73 (fig. S6C), yielded completely inactive enzymes (table S1), indicating that the binding of G1 and G73 is required for properly directing the acceptor arm toward the catalytic site. Although the CCA end is not visible, probably because tRNA^{Sec} is not ligated with serine, the length of CCA-Ser is consistent with the distance between G73 in tRNA^{Sec} and PLP in the catalytic site (fig. S6C). Therefore, the large cleft can accommodate the terminal region, from the first base pair of the acceptor stem to the Ser-adenosine moiety of Ser-tRNA^{Sec} (fig. S8A), whereas the N-terminal domain recognizes the tRNA^{Sec}-specific D-arm structure. In contrast, SepSecS binds Sep-tRNA^{Sec} on the surface of the protein globule in a completely different manner from that of Sela and is therefore unable to recognize the D arm (fig. S8, B and C).

We also determined the crystal structure of Sela- Δ N in complex with thiosulfate, which revealed thiosulfate ions (TS1 to TS3) bound to one Sela subunit (Fig. 3A, fig. S9A). TS1 binds in the putative selenophosphate-binding pocket formed by Arg^{86A} (subunit A), Arg^{312B}, and Arg^{315B} (subunit B). Mutations of these residues to Ala resulted in markedly reduced activities. Furthermore, Arg¹¹⁹ and Asp²⁸⁴, which interact with Arg^{312B} and Asp^{86A}, respectively, are required for catalytic activity (table S1). TS2 and TS3 are thought to mimic the phosphate group of the terminal A76 of tRNA^{Sec}. Because the Ala mutations of Asn²¹⁸ and Phe²²⁴ drastically reduced the activity in vivo (table S1), Asn^{218A} and Phe^{224J} might form part of the A76-binding pocket at the interface between the subunits A and J (Fig. 3, B and C). Notably, the interaction of A76 with both Asn^{218A} and Phe^{224J} can only occur in the decameric Sela (fig. S9B). The mechanism of PLP-dependent Ser-to-Sec conversion by Sela (depicted in Fig. 4 and explained in the legend) is similar to that of SepSecS (8) and, unlike other fold-type-I PLP enzymes, involves a series of crucial Arg residues. However, Sela and SepSecS use respective sets of nonhomologous Arg residues.

In summary, the Sela pentamer-of-dimers forms a ring-shaped structure and binds 10 tRNA^{Sec} molecules. Each tRNA^{Sec} interacts with all subunits from the two neighboring dimers, and this interaction critically depends on the pentagonal ring architecture. The Sela N-terminal domain recognizes the tRNA^{Sec}-specific D-arm structure, thereby discriminating

Ser-tRNA^{Sec} from Ser-tRNA^{Ser}. Moreover, the large cleft created between two SslA dimers accommodates the Ser-tRNA^{Sec} 3'-terminal region. Thus, the functional form of SslA differs from that of the tetrameric SepSecS, which recognizes the phosphoserine moiety of Sep-tRNA^{Sec} without tRNA^{Sec}/tRNA^{Ser} discrimination. Moreover, SslA catalyzes Sec formation by using a different set of active-site Arg residues from that of SepSecS. The entirely different structures and divergent catalytic residues of the bacterial and archaeal/eukaryotic Sec synthases are evidence for convergent evolution of two Sec synthesis systems present in nature.

Supplementary Material

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Acknowledgments

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Fig. 1. Structure of SelA

Overall structure of *A. aeolicus* SelA. PLPs are represented as sphere models. The 10 subunits are shown in different colors. The disordered regions in the N-terminal domains are colored gray.

Fig. 2. Structure of the SelA•tRNA^{Sec} complex

(A) Overall structure of *A. aeolicus* SelA complexed with *T. tengcongensis* tRNA^{Sec} (colored brown). (B) Interaction of tRNA^{Sec} with intimate dimers I•J and A•B (close-up view). The omit $F_{\text{obs}}-F_{\text{calc}}$ map (3.0 sigma level) of the tRNA^{Sec} is shown in a blue mesh. The putative position of the CCA region is shown as a dashed line. (C) A schematic diagram of the interaction of tRNA^{Sec} with dimers I•J and A•B. (D and E) Discrimination of tRNA^{Sec} from tRNA^{Ser} by SelA. *T. thermophilus* tRNA^{Ser} [PDB ID 1SER (14)] was docked to the SelA•tRNA^{Sec} complex by superimposing the backbone phosphorus atoms of the T arm. The tRNA^{Sec} surface is complementary to that of the SelA N-terminal domain (D), whereas that of tRNA^{Ser} is not complementary (E).

Fig. 3. Catalytic site of SclA

(A) The catalytic site of SclA-ΔN in complex with thiosulfate. PLP and thiosulfate ions (TS1 to TS3) are shown. TS1 mimics the substrate selenophosphate (Se-P). The subunits of the intimate dimer A•B are colored as in fig. S2A, whereas subunit J is colored gray. (B) Docking model of the Ser-ligated terminal tRNA^{Sec} nucleotide (A76) and Se-P. (C) SclA surface, showing that A76 binds to the boundary of subunits J and A. The docked A76-Ser and G73 of tRNA^{Sec} are connected through C74-C75.

Fig. 4. The mechanism of Sep-to-Sec conversion

In the waiting state, the PLP forms an internal aldimine with Lys²⁸⁵ (A). The amino group of Ser-tRNA^{Sec} replaces Lys²⁸⁵ to form an external aldimine (B), followed by deprotonation of the α position of the Ser moiety (C). Lys²⁸⁵ protonates and eliminates the β hydroxyl group to form a 2-aminoacrylyl moiety (D). The nucleophilic addition of selenophosphate generates a Se-phosphorylated Sec moiety (E), which is hydrolyzed to a Sec moiety and a phosphate (F). Lastly, the α position of the Sec moiety is protonated (G), and Sec-tRNA^{Sec} is released (H).