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Authors' Review

Seryl-tRNA Synthetases: Enzymes with Multiple Personalities*

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Abstract. Seryl-tRNA synthetases (SerRS) are essential enzymes responsible for aminoacylation of cognate transfer-RNAs ($tRNA^{Ser}$) with serine, which is then incorporated into the proteins being synthesized on the ribosome. Although tRNA synthetases are usually a very conserved class of enzymes, phylogenic analyses revealed the existence of two distinct types of serine-charging enzymes; a standard or bacterial-type SerRS is found in the majority of organisms (prokaryotes, eukaryotes and archaea), while a highly diverged methanogenic-type SerRS is confined to the methanogenic archaea. We give a short overview on our recent biochemical and structural contributions to the understanding of different molecular mechanisms of tRNA serylation.

Keywords: seryl-tRNA synthetase, transfer RNA, synthetase crystal structure, synthetase: tRNA model

STRUCTURAL AND FUNCTIONAL FEATURES OF AMINOACYL-tRNA SYNTHETASES

The genetic code is based on the correct translation of each codon in mRNA to its cognate amino acid. One key step in ensuring accuracy during the translation of genetic information is the correct attachment of an amino acid to its cognate tRNA by the aminoacyl-tRNA synthetase (aaRS) family of enzymes. This process requires that each aaRS selectively binds its respective amino acid, ATP, and the cognate tRNA. Aminoacylation of tRNA is a two-step reaction: the first is the activation reaction, in which the aminoacyl-adenylate is formed, followed by the second step where transfer of the amino acid to the tRNA occurs and the ester bond is formed at the 3'-adenosine of the macromolecule.[1,2]

The aaRS family is divided into two structurally unrelated classes, I and II, with 10 members in each,[3] which have evolved from different ancestors[4] (besides rare exceptions[5]). Each class is characterized by a class-defining catalytic domain containing short conserved sequence motifs.[6,7] Class I aaRSs comprise a catalytic center built around a Rossmann fold where the two signature sequences HIGH and KMSKS are systematically found, while class II enzymes possess a central antiparallel β-sheet with three conserved motifs; motif 1, 2 and 3. Divergent structures of the representatives of the two classes result in functional differences with respect to ATP and tRNA binding in each class.[8,9]

Numerous biochemical and structural studies have led to idea that aaRSs are modular enzymes, containing several domains appended to the catalytic core and having special functions. These motifs provide tRNA-binding determinants, and in some cases function extraneous to aminoacylation.[10–12]

Within each class, subclasses can be identified containing more closely homologous synthetases that often share a common tRNA anticodon-binding module.[7] In class II, the largest subclass is class IIa, which contains the Ser-, Thr-, Pro-, Gly- and His-tRNA synthetases.[7]

TWO TYPES OF SERINE-SPECIFIC tRNA SYNTHETASES

During many years our group has significantly contributed to elucidating the structure, function and evolution of seryl-tRNA synthetases (SerRS), which have the essential role of aminoacylating cognate $tRNA^{Ser}$ isoacceptors with serine.[13] Serine code is highly degenerated and requires the employment of multiple tRNA isoacceptors for decoding of six codons. Besides, SerRSs also serylate selenocysteine-specific tRNAs ($tRNA^{Sec}$) and

* Dedicated to Professor Emeritus Drago Grdenić, Fellow of the Croatian Academy of Sciences and Arts, on the occasion of his 90th birthday.

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thus participate in the incorporation of seleno-cysteine into proteins.[14] Interestingly, two distinct types of serine-charging enzymes have been found in nature: bacterial-type SerRS, that is wide-spread and functions in

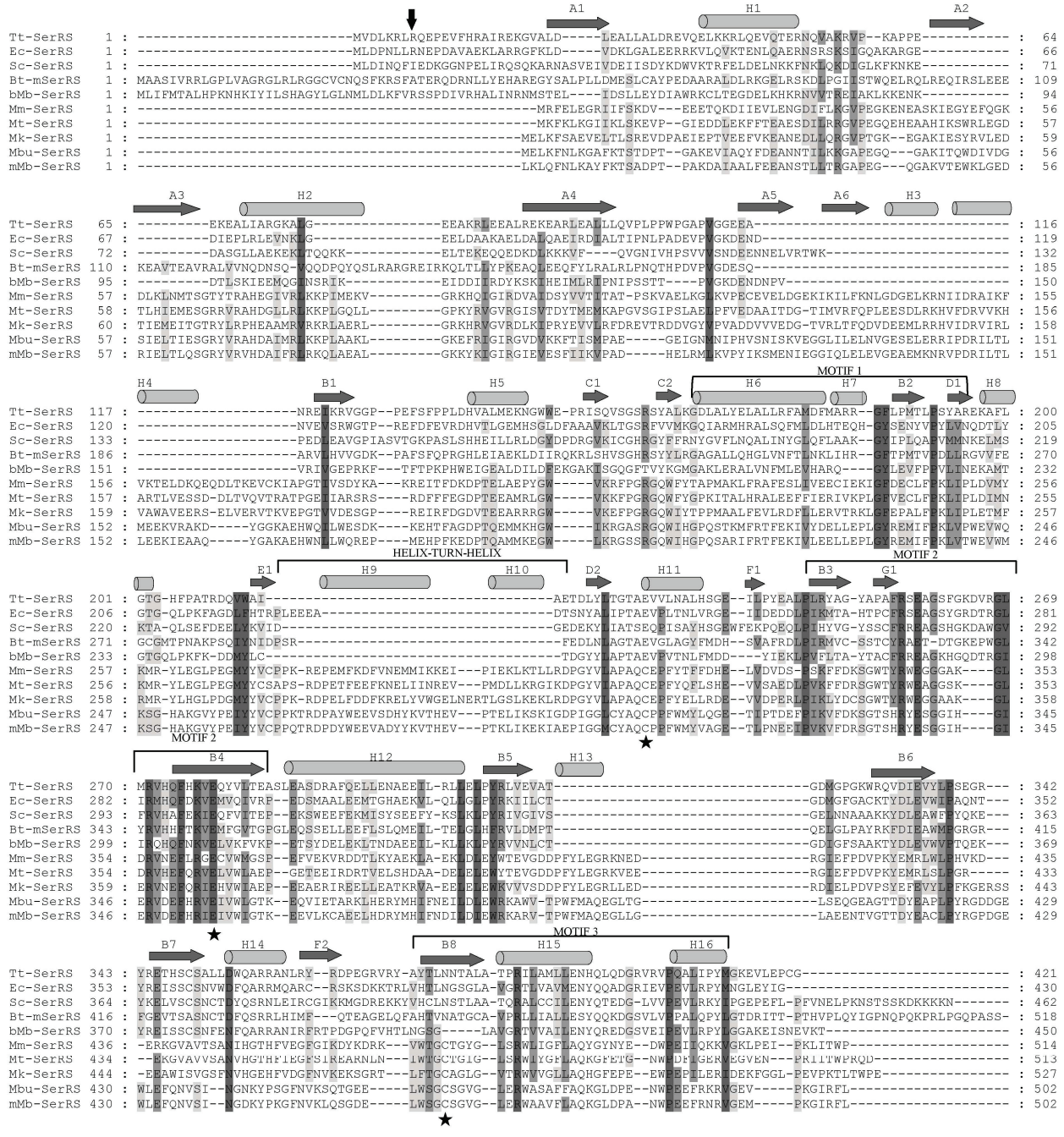

Figure 1. The structure-based sequence alignment of mMbSerRS with selected SerRS sequences from all kingdoms of life. The sequence alignment was firstly generated using the program ClustalX[54] and then manually adjusted based on structural considerations. The sequences are derived from archaea (Mm, *Methanococcus maripaludis*; Mt, *Methanothermobacter thermoautotrophicus*; Mk, *Methanopyrus kandleri*; Mbu, *Methanococcoides burtonii*; Mb, *Methanosarcina barkeri*), bacteria (Tt, *Thermus thermophilus*; Ec, *Escherichia coli*) and eukarya (Sc, *Saccharomyces cerev*isiae). Bacterial-type SerRS sequences from methanogenic archaeon (bMb, *M. barkeri*) and bovine mitochondrion (Bt, *Bos Taurus*) are also included. Completely conserved amino acids are in dark gray, while those with 80 % and 60 % conservation are in gray and light gray, respectively. Secondary structural elements are indicated above the alignment with light gray cylinders for helices and dark gray arrows for β-sheets. Residues important for zinc ion coordination in mMbSerRS are marked with black asterisks. A black arrow indicates mitochondrial signal sequence. Conserved class II motifs 1, 2 and 3, as well as methanogenic type specific HTH motif, are labeled.

various archaeal, bacterial and eukaryotic organisms, and rare methanogenic-type SerRS present only in methanogenic archaea.

The first atypical SerRS sequence was identified in the archaeal genome of *Methanococcus jannaschii*,[15] and later on in all methanogenic archaea[16–18] with the exception of *Methanosarcina mazei* and *Methanosarcina acetivorans*. These two types of SerRSs display only minimal sequence similarity, primarily within the class II conserved motifs. As revealed by the amino acid sequence alignment (Figure 1) most conspicuous differences between the two types of SerRSs are: lack of sequence similarity in the N-terminal domains of the two SerRS types, a unique ≈ 30 residue long insertion positioned between motifs 1 and 2 in the catalytic domains of atypical methanogenic-type enzymes and a deletion within the motif 2 loop, which was demonstrated to participate in both $tRNA^{Ser}$ and serine recognition in *T. thermophilus*.[19] Such sequence dissimilarity prompted us to explore the modes of substrate recognition by the two SerRS types using various biochemical approaches. Finally, in order to explain molecular basis for functional divergence between the two types of serine-charging enzymes, we determined the 3D-structure of *Methanosarcina barkeri* SerRS (mMbSerRS).[20] The structure revealed several idiosyncratic features, characterizing methanogenic-type SerRS as rather unusual and atypical SerRS.

STRUCTURAL DIFFERENCES BETWEEN THE TWO SerRS TYPES

As revealed by the crystal structures of two prokaryotic seryl-tRNA synthetases from *Escherichia coli* and *Thermus thermophilus*, each subunit of homodimeric enzyme possesses a C-terminal active site domain typical for class II aaRSs, which comprises three class II conserved signature motifs (Figures 1 and 2), whereas the first 100 N-terminal residues form an antiparallel α-helical coiled-coil, crucial for the selection and binding of tRNA.[21–24] Likewise, each mMbSerRS subunit consists of two domains linked by a short flexible oligo-peptide. The N-terminal part (residues 1–165) is a mixed α/β domain made of six stranded β-antiparallel sheet capped by a bundle of four helices (H1, H2, H3, H4) (Figures 1 and 2). This fold is quite distinct from the coiled-coil of tRNA binding domain in bacterial-type SerRS, but also interacts with variable arm of tRNA (see later). The catalytic module (residues 174–502), built upon eight antiparallel strands surrounded by three α-helices, is structurally similar to the catalytic core of bacterial enzymes with the three class II motifs. Motif 1 participates in dimer interface, while motifs 2 and 3 include residues involved in serine, ATP and tRNA 3'-end interactions.

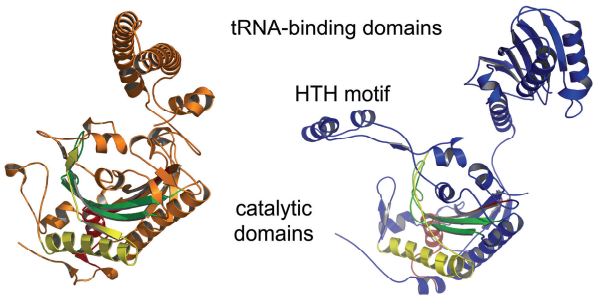

Figure 2. Comparison of Tt and mMb SerRSs. Cartoon diagrams showing single subunits of two SerRS types: bacterial-type (left, 1sry) and methanogenic-type (right; 2cim). The class II specific motifs are shown in yellow (motif 1), green (motif 2) and red (motif 3). The tRNA-binding domains are different in size and structure. The catalytic domain of mMbSerRS contains characteristic insertion, HTH fold.

Importantly, the methanogenic type enzyme is a metalloprotein, with zinc-ion in the active site, which performs a catalytic role (Figure 3). The tetra-coordinated Zn^{2+} ion interacts with three conserved amino acids (Cys306, Glu355 and Cys461, marked by an asterisks in Figure 1) and a water molecule.

RECOGNITION OF SERINE AND DISCRIMINATION AGAINST NON-COGNATE AMINO ACID SUBSTRATES

SerRS represents the only known aminoacyl-tRNA synthetase system that evolved two distinct mechanisms for the recognition of the same amino acid substrate.[20] A series of crystal structures of binary and tertiary complexes involving *T. thermophilus* SerRS (Tt-SerRS) (Figure 3), followed by a high resolution crystal structure of an *E. coli* SerRS (Ec-SerRS) arm-deletion mutant in complex with a seryl-adenylate analogue, explained the specificity and mechanism of serine activation by bacterial SerRSs.[13,25,26] In these cases serine specificity is ensured by the small size of the side-chain pocket (which permits exclusion of threonine) and by the interaction of the serine side-chain hydroxyl group with that of Thr380 in motif 3 of Tt-SerRS or Ser391 of Ec-SerRS. Furthermore, a conserved motif 2 glutamate residue (Glu279 Tt-SerRS and Glu239 in Ec-SerRS, respectively) makes simultaneous hydrogen bonds to the α-amino and hydroxyl group of the serine (Figure 3). This feature efficiently excludes other amino acids with similar structures. Therefore, bacterial SerRSs do not require an editing activity.

Upon serine binding to the active site of methanogenic type SerRS, the coordination of the active site zinc ion by amino acid side chains remains intact, while

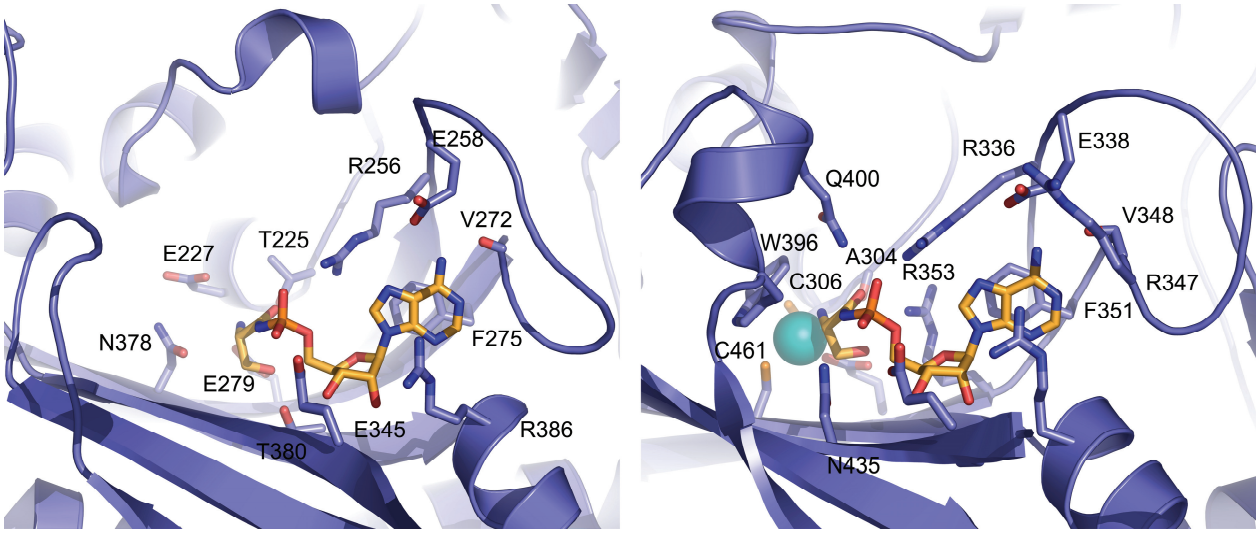

Figure 3. Active site of bacterial- and methanogenic-type SerRS. Ribbon and stick representation of the active site of TtSerRS (left, 1set) and mMbserRS (right, 2cj9) with bound analogue of seryl-adenylate. The residues interacting with the substrates are shown as sticks. Zinc ion in the active site of mMbSerRS structure is colored in cyan.

the water ligand dissociates to allow for coordination of the substrate amino group (Figure 3). The amino group of serine is additionally stabilized by hydrogen bonding with main chain oxygen of Ala304. The structure reveals that Glu355, which is conserved between the methanogenic and bacterial-type SerRSs (Figure 1), has a dual role in the methanogenic-type enzyme: in addition to being a zinc ligand, it interacts with the hydroxyl group of the serine substrate, as in the bacterial-type SerRS. Moreover, a comparison of mMbSerRS-Ser-AMS complex with the structures of the Tt-SerRS and Ec-ThrRS in complex with their respective amino-acyl adenylate analogs reveals that the hydroxyl group of the serine moiety is positioned in a way characteristic for SerRS. Thus, in the crystal structures of three SerRSs (*T. thermophilus*,[25] *Bos taurus*[27] and *M. barkeri*[20]), the amino acid hydroxyl groups are constrained by conserved glutamic acid residues in the active site (Glu279 in Tt-SerRS, Glu355 in mMb-SerRS and Glu335 in Bt-SerRS) (Figure 1).

The first direct functional evidence on mechanistic differences in serine recognition by bacterial- and methanogenic-type SerRSs was based on different sensitivity of two types of enzymes toward competitive inhibitors.[28] The elucidation of serine activation mechanism was facilitated by structure-guided mutagenesis and construction of a series of mMbSerRS mutated variants, which displayed altered kinetic parameters for both steps of aminoacylation reaction.[20,29] The binding of the serine to the mMbSerRS active site zinc ion enables direct contact between Gln400 from "serine ordering loop" (residues 394–410; Figure 1) and the carbonyl oxygen of the serine substrate,[20] positioning the carboxylate oxygen for the nucleophilic attack on the α-phosphate of ATP. Besides the essential role of zinc ion, the specificity of serine recognition by methanogenic-type SerRS is strongly affected by the size of the active site and the hydrophilic nature of serine binding pocket, as in the case of bacterial enzymes. Despite precise recognition of the cognate amino acid, functional assays have shown slight but notable misactivation of threonine (structurally similar amino acid comprising -OH group) by mMbSerRS, which does not seem to be edited *in vitro*.[20] However, although the alteration of the three residues (W396A, N435A and S437A) in the amino acid binding pocket of mMbSerRS (Figure 1) have been expected to increase the level of threonine misrecognition, no change in threonine activation was observed by mutated enzymes.[29] Thus, it appears to be far easier to abolish cognate amino acid activation than to replace it by non-cognate activation, despite redesigning the active site in terms of size, polarity and hydrogen bonding capacity.

RECOGNITION OF tRNA

Each synthetase aminoacylates only its cognate isoacceptor tRNAs, which are only a minor subset of the total cellular pool of tRNAs. In order to avoid misacylating tRNAs from any of the 19 noncognate groups, each tRNA contains identity elements that are unambiguously recognized only by a cognate synthetase.[30] tRNA recognition elements include both nucleotides that are specifically contacted by an aaRS and those with indirect effects, which dictate a proper orientation of directly interacting nucleotides or influence a particular tRNA conformation required for aminoacylation.[31]

Major recognition elements in tRNA isoacceptors specific for a particular amino acid are largely conserved among species.[32] The evolutionary changes in tRNA structure are coupled with changes in the enzyme structure, reflecting the coevolution of aaRSs and their cognate tRNAs.

Recognition Elements in tRNA^{Ser}

Identity elements required for serylation have been studied in a number of organisms, providing insights into tRNA^{Ser} recognition in different domains of life.[13] Contrary to the identity requirements of the majority of aaRSs, *E. coli* SerRS was found to recognize neither the anticodon nor the discriminator base of tRNA^{Ser}.[33] Instead, the length of the variable arm and the characteristic tRNA^{Ser} tertiary structure were shown crucial for serylation.[34–37] *In vitro* studies and footprinting experiments on yeast SerRS[38–40] revealed the discriminator base to be unimportant, while variable arm also functions as the principal identity element. Although G73 serves only as an antideterminant in bacteria[41] and lower eukaryotes,[39,40,42] it is an essential identity requirement for human tRNA^{Ser}.[43–46] In contrast to relatively conserved tRNA^{Ser} structure in majority of organisms, unusual tRNA^{Ser} isoacceptors function in mammalian mitochondria, with the T-loop as the main identity target for mitochondrial SerRS.[27,47] Taken together, the variety of tRNA^{Ser} identity determinants suggests that mechanisms of SerRS recognition have diverged during the evolution.

We have undertaken several approaches in order to elucidate the differences in recognition by methanogenic- and bacterial-type SerRSs. Kinetic analysis of variant tRNA^{Ser} transcripts by the two archaeal SerRS enzymes of different types[17] revealed that the length of the variable arm is a critical recognition element for both enzymes, as is the identity of the discriminator base (G73) and the base pair G30:C40 in the anticodon stem. However, additional determinants were identified as being required for specific serylation by the unusual methanogenic-type enzyme, which relies on G1:C72 identity and on the number of unpaired nucleotides at the base of the variable loop, unlike its bacterial-type counterpart. Additionally, observed distinction between the recognition of tRNA^{Ser} identity elements by bacterial-type *Saccharomyces cerevisiae* SerRS and methanogenic-type SerRS from archaeon *Methanococcus maripaludis*[48] further attests for their divergent evolutionary pathways.

Recognition Elements for tRNA in SerRS

In the crystal structure of *T. thermophilus* SerRS complexed with tRNA^{Ser}, the N-terminal helical arm is buried between the TψC arm and the long extra arm of tRNA^{Ser}.[19,23] Another important tRNA binding region is motif 2 loop, with Phe262 which interacts with the fifth base pair of the acceptor stem.[19] Also in the mammalian mitochondrial SerRS from *B. taurus* the N-terminal domain consists of a long α-helical arm.[27]

The N-terminal domain of the unusual archaeal mMbSerRS is significantly longer and structurally unrelated to the corresponding domain in bacterial-type tRNA synthases, and there is a significant shortening in the sequence of motif 2 loop (Figure 1). These structural differences in major tRNA recognition regions of the respective enzymes indicate that the mode of tRNA binding must also be different. Since the crystal structure of methanogenic-type SerRS:tRNA complex has not been obtained yet, we have generated the tRNA:mMbSerRS docking model (Figure 4) which suggest the involvement of highly conserved helices H1 and H2 of the N-terminal domain in tRNA extra arm recognition and the motif 2 loop in positioning of the 3'CCA end into the active site. Furthermore, our recent biochemical experiments with mutated variants, bearing the alterations at various positions in the N-terminal domain, identify Arg76 located in helix 2 as a crucial tRNA-interacting residue (J. Jarić, manuscript in prepa-

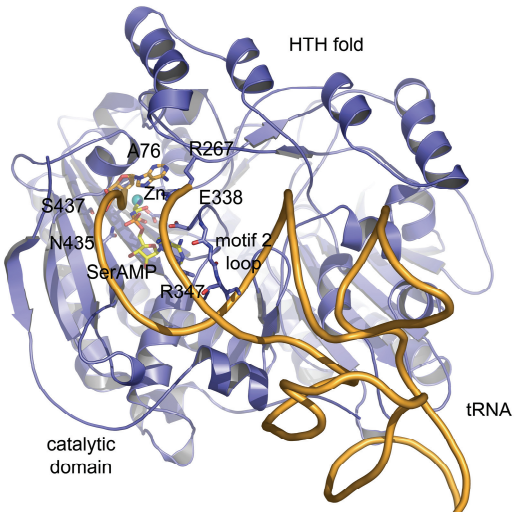

Figure 4. Proposed model of tRNA binding. A tRNA-docking model was generated by superposing the active-site domain of mMbSerRS onto that of TtSerRS in complex with cognate tRNA (1ser). As one-third of tRNA^{Ser} in the complex structure is disordered, tRNA^{Thr} (1qf6) was further superimposed onto tRNA^{Ser}. The catalytic domain of mMbSerRS in complex with SerAMS (sticks) is shown in blue. tRNA is shown in orange tube with A76 as sticks. The model is reasonable also with respect to the position of the terminal ribose where O3' is ready to accept the amino acid from the adenylate intermediate. The active-site residues for which it has been shown that are important for substrate binding are shown as sticks. The open conformations of motif 2 loop in apo-structure of mMbSerRS clashes with the modeled tRNA.

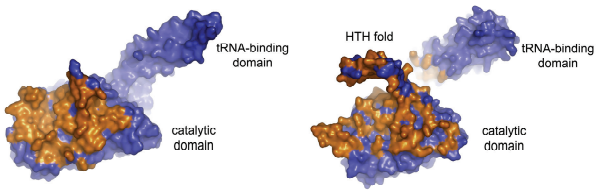

Figure 5. Dimerization interface for bacterial and methanogenic type SerRS. Surface representation of TtSerRS (left; 1sry) and mMbSerRS (right; 2cim) monomer. The residues forming the homodimeric interface are shown in orange. The motif 1 interface helices interact directly in TtSerRS, but not in mMbSerRS. HTH motif contributes to the stabilization of the dimer interface in mMbSerRS.

ration). The results of kinetic analysis with motif 2 mMbSerRS mutants[29] are in agreement with the serylation parameters obtained with altered tRNA transcripts[17] and the docking model, and support the structure-based prediction that the discriminator base (G73) and the first base pair in tRNA acceptor stem interact with motif 2 residues. It is likely that the differences in tRNA acceptor stem recognition between the two SerRS types are due to the difference in the motif 2 loop sequences and that each motif 2 loop is capable to perform different but specific interactions with cognate tRNA.

EVOLUTIONARILY PRESERVED DIMERIC NATURE OF SERYL-tRNA SYNTHETASES AND CROSS-SUBUNIT BINDING OF tRNA SUBSTRATE

All SerRSs, like most of the class II synthetases, are homodimers with the motif 1 region responsible for the dimerization. Nevertheless, the interactions between mMbSerRS monomers is not stabilized through interactions between motif 1 interface helices, as observed in TtSerRS, but by flanking residues of motif 1 region[20] (Figure 5). Interestingly, crystal structure of the mMbSerRS reveals that the HTH motif of one monomer forms extensive contacts with the catalytic domain of the other subunit (Figures 2 and 5). This contributes to the stabilization of dimers as recently revealed by comparing the denaturation of wild-type mMbSerRS and mutated variant with deleted HTH motif (S. Bilokapić, manuscript in preparation).

Dimeric SerRSs from various organisms bind one or two tRNA molecules.[13] Structural and biochemical data on bacterial-type SerRS (from *E. coli* and *T. thermophilus*) showed that tRNA molecule interacts with the N-terminal domain of one subunit and the C-terminal domain of the other subunit in the dimer.[23,49] Biochemical experiments on the unusual methanogenic-type SerRS guided by mMbSerRS:tRNA^{Ser} docking model revealed essentially the same mode of tRNA recognition despite the structural differences in the N-terminal domain. Thus, cross-subunit binding of tRNA(s) seems to be another evolutionary conserved feature of serine aminoacylation system (Figure 4).

IDIOSYNCRATIC INTERSUBUNIT CONNECTIONS IN METHANOGENIC-TYPE SerRS

The two domains of one monomer are covalently connected by a short linker sequence. However, important contacts between N-terminal domain of one monomer and C-terminal domain of another monomer is achieved by noncovalent interactions performed by the segments of HTH motif (Figures 2, 4 and 5). This is achieved by a row of salt-bridging interactions between helix 9 within the HTH fold of one subunit and helix 4 located in the N-terminal domain of the other subunit. Mutations in respective regions significantly affect serylation parameters as revealed by steady state kinetic analysis (S. Bilokapić, manuscript in preparation). Thus, it seems that without HTH motif, the N-terminal and the C-terminal domains of the tRNA synthase responsible for binding different regions of tRNA move freely and pay significant entropic penalty upon binding, which reduces the affinity of the enzyme for tRNA and impares aminoacylation (S. Bilokapić, manuscript in preparation). Our structural and biochemical data indicate that HTH motif has multiple roles: it participates in the building of the dimer interface (in addition to motif 1) and provides a hinge-like connection between the catalytic and N-terminal domains, keeping them flexible but at the same time significantly reducing their range of motion.

mMbSerRS SERYLATION MECHANISM AND THE IMPORTANCE OF SUBSTRATE INDUCED CONFORMATIONAL CHANGES

The binding of serine in the wild-type mMbSerRS is accompanied by a significant localized conformational change in serine ordering loop (SOL, residues 394–410; Figures 1 and 6). In agreement with the X-ray data, our kinetic studies showed that the alteration of Trp396 residue dramatically reduces affinity for serine, influencing adenylate synthesis and tRNA aminoacylation reaction.[29] This has been confirmed *in vivo*, by our newly developed tRNA suppression assay, which enables monitoring the efficiency of recognition between archaeal tRNA and the synthetase expressed in *E. coli* (S. Lesjak, manuscript in preparation).

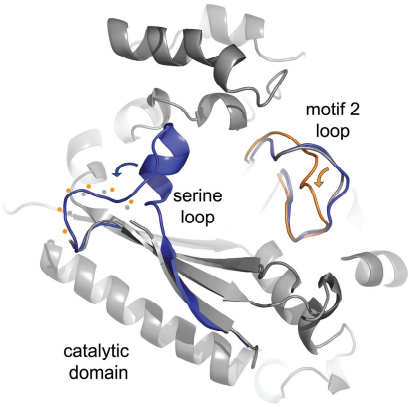

Figure 6. Superposition of catalytic domains reveal conformational changes in the motif 2 and serine ordering loops upon substrate binding. The picture shows mMbSerRS apo-structure (gray) superposed to the enzyme in complex with serine (blue). The serine loop is ordered (denoted with gray dots in apo-structure) only when serine is bound. Model of mMbSerRS active site upon $tRNA^{Ser}$ binding is shown in orange with the motif 2 loop in closed conformation. The conformational change of motif 2 loop, denoted with orange arrow, is required in order to avoid steric clashes with incoming tRNA molecule.

mMbSerRS:tRNA docking model,[20] generated using crystal structures of ThrRS in complex with the cognate tRNA (Figure 4), revealed that the positioning of the cognate tRNA in the active site of mMbSerRS requires a conformational change in motif 2 loop (Figure 6). Subsequent biochemical experiments highlighted the importance of two conserved glycines in the motif 2 loop for the flexibility of this region involved in binding 3' end of tRNA.[29] In addition, our data indicate that these amino acids contribute to the specific interactions with tRNA G1:C72 pair, an important identity element of *M. barkeri* $tRNA^{Ser}$.

The docking model also reveals that upon tRNA binding another conformational changes probably occur in the serine ordering loop to prevent clashes with the terminal adenosine of bound tRNA. The side chain of Glu338 presumably makes interactions with incoming tRNA substrate, as observed in bacterial-type SerRS, aspartyl-tRNA synthetase (AspRS) and phenylalanyl-tRNA synthetase (PheRS). The new conformation of serine ordering loop would also promote release of charged tRNA by breaking of the interactions between the substrate serine and mMbSerRS amino acids Gln400 and Trp396. Therefore, a conformational switch of the serine ordering loop between the serine-bound or tRNA-bound conformation, as well as the changes of the motif 2 loop residues in the ATP versus tRNA bound state, control the two steps of the aminoacylation reaction.

EVOLUTIONARY CONSIDERATIONS OF SERYL-tRNA FORMATION IN METHANOGENIC ARCHAEON *M. barkeri*

M. barkeri has quite unique protein biosynthesis system that presumably employs two distinct types of seryl-tRNA synthetases.[50] Both enzymes are functional *in vitro* and they recognize all three *M. barkeri* $tRNA^{Ser}$ isoacceptors although with somewhat different kinetic parameters.[17] One of the enzymes is bacterial-like, as evidenced by primary structure alignment (Figure 1) and functional complementation of a thermolabile *E. coli* SerRS at nonpermissive temperatures. The other is methanogenic mMbSerRS, which, in contrast to the bacterial-like counterpart, does not complement bacterial *ts* mutant (D. Ahel and S. Lesjak, unpublished observations). Accordingly, the two enzymes do not possess a uniform mode of tRNA and serine recognition *in vitro*.[17,29] Previously published phylogenetic analysis on SerRS suggests that these two forms may be unrelated, and is suggestive of horizontal gene transfer events.[4,51,52] If so, it is conceivable that Methanosarcineae received bacterial-type of SerRS from a Gram-positive bacterium, and subsequently lost the original, presumably methanogenic, SerRS enzyme. In that respect, *M. barkeri* might represent an evolutionary intermediate state, with two functional SerRSs. Remarkably, in the context of aminoacyl-tRNA synthetase duplicity, *M. barkeri* also possesses two unrelated LysRSs of different classes.[5,53] Our data do not suggest functional complementarity of the two SerRSs and it is plausible that one of the enzymes is dispensable.

The coexistence of two distantly related SerRS enzymes in *M. barkeri* provides a favorable system for evaluation of evolutionary aspects of tRNA discrimination. The differences in pairing patterns of the variable arm and absence of recognition of positions 2:71 and 3:70 by the two SerRSs[17] may be indicative of tRNA-driven alteration of the identity requirements. We can hypothesize adaptation of the bacterial SerRS to the methanogenic environment by modifying its identity requirements and adopting G73, pertinent to both archaeal and bacterial $tRNAs^{Ser}$, as one of the major elements of serine identity.[17]

Given the likeness of $tRNA^{Ser}$ identity requirements for two dissimilar SerRS enzymes, it can be inferred that specific mode of $tRNA^{Ser}$ recognition may have been defined before methanogenic and bacterial SerRSs diverged from the common ancestor. Furthermore, considering that methanogenic SerRS and ThrRS use a zinc ion for amino acid discrimination and that both enzymes misactivate threonine and serine, respectively, we hypothesize that the mechanism of zinc ion-

dependent amino acid recognition predates segregation of SerRS and ThrRS into enzymes with distinct specificities.

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SAŽETAK

Seril-tRNA-sintetaze: enzimi s više osobnosti

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Seril-tRNA-sintetaze (SerRS) su ključni enzimi koji kataliziraju esterifikaciju pripadnih molekula transfer-RNA serinom, koji se potom ugrađuje u proteine sintetizirane na ribosomu. Iako se sintetaze smatraju evolucijski očuvanim enzimima, filogenetske su analize ukazale na postojanje dvaju različitih tipova seril-tRNA-sintetaza; standardni ili bakterijski tip SerRS nađen je u većini organizama (prokariotima, eukariotima i arhejama), dok je bitno različiti metanogeni-tip SerRS svojstven isključivo metanogenim arhejama. U ovom ćemo kratkom pregledu prikazati naše novije biokemijske i strukturne doprinose razumijevanju različitih molekularnih mehanizama serilacije tRNA.