IDENTIFICATION OF AMINO ACIDS IN N-TERMINAL DOMAIN OF ATYPICAL METHANOGENIC-TYPE SERYL-tRNA SYNTHETASE CRITICAL FOR tRNA RECOGNITION

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Seryl-tRNA synthetase (SerRS) from methanogenic archaeon Methanosarcina barkeri, contains an idiosyncratic N-terminal domain, composed of an antiparallel beta-sheet capped by a helical bundle, connected to the catalytic core by a short linker peptide. It is very different from the coiled-coil tRNA binding domain in bacterial-type SerRS. Since the crystal structure of methanogenic-type SerRS:tRNA complex has not been obtained yet, a docking model was produced, which indicated that highly conserved helices H2 and H3 of the N-terminal domain may be important for the recognition of extra arm of tRNASer.

Based on the structural information and the docking model, we have mutated various positions within the N-terminal region and probed their involvement in tRNA binding and serylation. Total loss of activity and inability of Arg76Ala variant to form the complex with cognate tRNA identifies Arg76 located in helix H2 as a crucial tRNA-interacting residue. Alteration of Lys79 positioned in helix H2 and Arg94 in the loop between helix H2 and beta-strand A4 have pronounced effect on SerRS:tRNASer complex formation and the dissociation constants (KD) determined by surface plasmon resonance (SPR). The replacement of residues Arg38 located in the loop between helix H1 and beta-strand A2, Lys141 and Asn142 (from H3) and Arg143 (between H3 and H4) moderately affect both the serylation activity and the KD values. Furthermore, we have obtained a striking correlation between these results and in vivo effects of these mutations by quantifying the efficiency of suppression of bacterial amber mutations, after coexpression of the genes for M. barkeri suppressor tRNASer and a set of mMbSerRS variants in Escherichia coli.

The aminoacyl-tRNA synthetases (aaRSs) catalyze the activation of cognate amino acids and their transfer to the 3'-end of corresponding tRNA molecules. The aaRSs are a highly conserved family of enzymes comprised of two distinct structural groups referred to as classes I and II (1, 2, 3), with a notable exception of LysRS representatives which belong to both classes (4). While the catalytic mechanisms of various aaRSs are broadly similar (5), each enzyme has developed a high specificity in recognizing its cognate amino acid and tRNA, which is pivotal for accurate translation of the genetic code (1). The discrimination of the amino acids is based on recognizing the differences in the size and charge of the molecules (6). The specificity of tRNA selection depends on a set of identity determinants that are mostly located at two distal extremities: the anticodon loop and the amino acid accepting stem. In a few instances, identity elements are also found in the D-arm, T-arm, and variable loop. They can either act as positive determinants which enhance aminoacylation or negative ones which prevent aminoacylation. The recognition of tRNAs
by synthetases can also be affected by the modification of particular nucleotides (7, 8). AaRSs show divergent strategies for tRNA recognition. Most notably, the class I and class II aaRSs (including pyrrolysyl-tRNA synthetase, 9) approach tRNAs from the minor and major groove sides of the acceptor stem, respectively (10). Although majority of determinants are in direct contact with cognate synthetases (8), the aminoacylation fidelity is controlled by kinetic differences more than by binding affinities (11).

Seryl-tRNA synthetases (SerRSs), which catalyze the aminoacylation of several tRNA$^{\text{Ser}}$ isoacceptors and tRNA$^{\text{Sec}}$ with serine, can be divided into two structurally different groups: bacterial-type SerRSs function in a variety of archaeal, bacterial and eukaryotic organisms, whereas the methanogenic-type was found only in methanogenic archaea (12, 13). Furthermore, based on the sequence comparison (14, 15) and X-ray analyses, two subgroups of bacterial-type SerRSs were identified: one consists of the enzymes from bacterial sources, best represented by those from Thermus thermophilus (16) and Escherichia coli (3) and an archaeal/eukaryal-type, structurally related to SerRS from archaeon Pyrococcus horikoshii (17).

All seryl-tRNA synthetases (SerRSs) are functional homodimers with a C-terminal active site domain typical for class II aaRSs and an N-terminal domain that is responsible for binding of the long variable arm of tRNA$^{\text{Ser}}$ isoacceptors and tRNA$^{\text{Sec}}$ with serine, (reviewed in 18), with exception of mammalian mitochondrial enzyme (19). The long variable arm of tRNA$^{\text{Ser}}$ categorizes it as one of the type-2 tRNAs, including tRNA$^{\text{Ser}}$ and tRNA$^{\text{Leu}}_{\text{eu}}$ species (from all organisms or domains of life) and bacterial tRNA$^{\text{Tyr}}$ species (20). In bacterial-type SerRS, N-terminal domain forms an antiparallel α-helical coiled-coil structure (3, 16), while in the methanogenic-type counterpart it is significantly larger and composed of a six stranded antiparallel β-sheet capped by a bundle of three helices (H1, H2, H4) with up-down topology and an additional short helix (H3) that runs almost perpendicular to helix H4 (21) and (Figure 1). Despite pronounced structural differences between the tRNA binding domains in two SerRS types, in each case the N-terminal domain of one subunit interacts with the extra arm of tRNA$^{\text{Ser}}$, to position the 3’-end of tRNA into the C-terminal active site of another subunit (23, 24, 25). Recent crystal structure of the first archaeal/eukaryal SerRS from archaeon P. horikoshii, and structure-based model of the enzyme bound with the T. thermophilus and P. horikoshii tRNAs$^{\text{Ser}}$, suggested that the helical N-terminal domain of P. horikoshii SerRS is also involved in the binding of the extra arm of tRNA (17).

The recognition of tRNA by SerRS relies, besides the long extra arm, on identity elements in tRNA$^{\text{Ser}}$ acceptor arms, achieved by the motif 2 residues. Unlike majority of aaRSs systems, the anticodon triplet is not recognized by SerRS. The first four base pairs in the tRNA acceptor arm (G1:C72, G2:C71, A/U3:U/A70 and R4:Y69) are identity elements for bacterial SerRS, and among them, the second G2:C71 base pair is the most significant (reviewed in 18). Consistently, the crystal structure of the T. thermophilus SerRS:tRNA$^{\text{Ser}}$ complex revealed that SerRS interacts with the tRNA$^{\text{Ser}}$ acceptor stem, and Ser261 is responsible for the base-specific interaction with G2 (23, 26). Although the acceptor stem sequences are not well conserved among the eukaryal tRNA$^{\text{Ser}}$ isoacceptors, the discriminator base G73 is an essential identity requirement for human tRNA$^{\text{Ser}}$ and serves as an antideterminant in lower eukaryotes (reviewed in 18). Unlike eukaryal tRNAs$^{\text{Ser}}$, archaeal tRNAs$^{\text{Ser}}$ conserve the G1:C72, C2:G71, C3:G70 and G4:C69 base pairs in the acceptor stem. However, the tRNA specificity of the archaeal/eukaryal SerRS from P. horikoshii seems to depend mainly on the extra arm, but not on the acceptor stem. Indeed, this enzyme exhibits quite relaxed specificity for tRNA$^{\text{Ser}}$ recognition (17).

Archaeon Methanosarcina barkeri, which possesses two dissimilar SerRSs, one of a bacterial- and the other of methanogenic-type, provides an excellent system for studying the evolution of tRNA$^{\text{Ser}}$ determinants. Two enzymes recognize the same set of tRNA isoacceptors in vitro (27). We have undertaken several approaches in order to elucidate the basis for their different serylation mechanisms. Kinetic analysis of variant tRNA$^{\text{Ser}}$ transcripts by the two archaeal SerRS enzymes (27) 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. tRNA recognition pattern by two *M. barkeri* SerRS may differ in vivo, since only bacterial-type SerRS complements the function of thermolabile *E. coli* SerRS (28).

**EXPERIMENTAL PROCEDURES**

*Site-directed mutagenesis and purification of proteins* — The seryl-tRNA synthetase expression vector (pET15bmMbSerRS) has been reported previously (27). Primers listed in Supplemental table 1 and Quick Change mutagenesis kit (Stratagene) were used for site-directed mutagenesis. Point mutations were confirmed by DNA sequencing.

Wild type and mutated SerRS proteins were expressed in *E. coli*, as described previously (21). Since wt mMbSerRS and its variants are His-tagged, they were first purified by affinity chromatography on nickel-nitrilotriacetic acid-agarose (Ni-NTA agarose). Greater purity of proteins was achieved using cation exchange chromatography. Proteins were loaded on Resource S 6 ml column and eluted with linear KCl gradient (100 – 500 mM) in buffer containing 25 mM Mes, pH 6.2, 5 mM dithiotreitol (DTT) and 10 mM MgCl$_2$.

**Aminoacylation assay** — Aminoacylation was carried out at 37 °C in the reaction mixture containing 50 mM Hepes/HCl, pH 7.0, 15 mM MgCl$_2$, 4 mM DTT, 5 mM ATP, 125 µM $\text{^{14}C}$serine, 0.25 µM SerRS and 1.5 µM tRNA. Quantification of synthesized radioactive seryl-tRNA$_\text{Ser}$ was done as described (29). Relative serylization rates represent the average of at least three independent experiments.

**Electrophoretic mobility shift assay (EMSA)** — In order to check for complex formation between cognate tRNA and wild type or mutated mMbSerRS a constant amount of purified protein (8.3 pmol) was mixed with tRNA (14.8 pmol) and incubated for 15 min at 37 °C in a 13.5 µl volume containing 30 mM Hepes pH 7.0 and 6 mM MgCl$_2$ followed by cooling on ice. To test salt influence on non-covalent complex formation between protein and nucleic acid, different amount of salt (25 or 250 mM KCl) was added in the reaction mixture prior to incubation for 15 min at 37 °C. Samples were subjected to electrophoresis on a native 9% acrylamide gel containing 5% glycerol in electrophoresis buffer (25 mM Mes/25 mM Tris pH 7.6). Electrophoresis was performed at 4 °C for 2.5 h at 120 V and gels were stained with silver.

**Surface Plasmon Resonance** — Kinetic studies were performed at 25 °C using a BIACORE T100 surface plasmon resonance (SPR) instrument. Wild type protein and mutants were covalently attached to a carboxymethyl dextran-coated gold surface (CM5 sensor chip). The carboxymethyl groups of dextran were activated with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS), and seryl-tRNA synthetase, or its mutants, were attached at pH 5.0 in 10 mM sodium acetate. Any remaining reactive sites were blocked by reaction with ethanolamine and the surface was washed with 50 mM NaOH to remove any non-covalently bound ligand. Proteins were immobilized at levels of 1000 response units (RU) in one flow cell. The kinetics of association and dissociation were monitored at a flow rate of 60 µl/min. The renatured tRNA analyte was diluted in a running buffer (30 mM Hepes pH 7.0, 6 mM MgCl$_2$ and 5 mM DTT). Binding was measured for concentration ranges of 19.5 nM to 8 µM tRNA. After the end of each injection, tRNA was allowed to dissociate for 500 s and then the chip.
was regenerated with 3 M KCl at a flow of 100 µl/min for 5 min. Data reported are the difference in SPR signal between the flow cell containing the wild type or mutant mMbSerRS and the reference cell without enzyme immobilized. Duplicate injections were made for each tRNA concentration in one round of measurement and each experiment was repeated twice. The data were analyzed using Biacore T100 Evaluation Software.

**Suppression of E. coli amber mutations** — The assay is based on monitoring the suppression efficiency of bacterial amber mutations in *E. coli* strain XAC-A24 XAC-A24 (F’ ara argE(UAG) rpoB gyrA Δlac pro/F lacI(UAG)-Z proAB) (28), after co-expression of *M. barkeri* SerRS gene, encoding methanogenic-type SerRS, with the gene for cognate archaeal suppressor tRNA (supMb). Synthetic gene for supMb, bearing CGA anticodon, was inserted into pTech plasmid, behind the lpp promoter, as described (28). To obtain higher expression of genes of some SerRS mutants, NcoI –XhoI fragments containing synthetase genes with the N-terminal His tag from plasmid pET15b were then recloned into pBAD24 vector (30), where the SerRS expression is under control of arabinose inducible promoter. Suppression of argE amber mutation was tested by plating *E. coli* cells on selective M9 minimal glucose plates. The efficiency of suppression was determined by measuring the β-galactosidase activity produced from lacI-lacZ fusion harbouring a nonsense mutation in lacI portion (31).

**RESULTS**

**Selection of target sites for the site-directed mutagenesis** — Our previously published structure-based model of mMbSerRS:tRNA<sub>Ser</sub> complex (Figure 1) implies that tRNA<sub>Ser</sub> binds across two subunits of dimeric enzyme, as observed in the bacterial synthetase:tRNA complex structure. Experimental verification of predicted cross-dimer binding was provided by testing the activity of constructed SerRS heterodimers (25). In the model the long variable arm of the tRNA is positioned to interact with the N-terminal domain of mMbSerRS, in accordance with our previous biochemical experiments which identified the long variable arm of archael tRNA<sub>Ser</sub> as a major tRNA recognition determinant (27, 29, 32). Furthermore, electrostatic potential calculations of the mMbSerRS dimer show an extended area of positive surface potential on the inner bow of the N-terminal domain, supporting the idea of N-terminal domain involvement in recognition of the negatively charged tRNA backbone (21). As target sites for mutagenesis we have chosen positions encoding highly conserved amino acids in the N-terminal domain of mMbSerRS (marked by dots in Figure 1A), that are according to the docking model, in the proximity of tRNA (Figure 1B). In all constructed mMbSerRS variants (listed in Supplemental table 1) a single amino acid was replaced with alanine: arginine 38, arginine 76, arginine 78, lysine 79, lysine 87, lysine 88, tyrosine 89, lysine 90, arginine 94, lysine 141, asparagine 142 and arginine 143. Most altered residues carry basic side chains expected to interact with negatively charged tRNA. Functional characterization of mMbSerRS mutants was performed in *vitro* and in *vivo*.

A single amino acid change (R76A) causes complete loss of aminoacylation activity — Serylation propensity of all SerRS variants was tested in a standard aminoacylation assay with *in vitro* transcribed MbtRNA<sub>Ser</sub><sup>GGG</sup> as a substrate(Figure 2). Mutant R76A completely failed to serylate tRNA<sub>Ser</sub>, a notable drop in aminoacylation activity was detected for mutants K79A and R94A, while mMbSerRS variants carrying R38A, K141A, N142A and R143A replacements revealed moderately decreased initial serylation rates in comparison with wt MbSerRS. Structural integrity of the variant carrying R76A replacement was probed by circular dichroism (CD) which confirmed that observed lack of tRNA charging capacity was not caused by protein misfolding (data not shown). This is also supported by the ability of R76A, K79A, R94A and R143A mutants to catalyze amino acid activation in the pyrophosphate exchange reaction (data not shown).

Gel-retardation assay reveals amino acids crucial for non-covalent complex formation with cognate tRNA — All mMbSerRS variants were tested for the ability to participate in the non-covalent complex formation with *in vitro* transcribed MbtRNA<sub>Ser</sub><sup>GGG</sup> (Figure 3). Mutants R38A, R78A, K87A, K88A, Y89A, K90A, K141A, N142A and R143A formed complexes...
stable enough to be detected on the gel (Figure 3; lanes c, e, g-j, l-n; respectively) while variant R76A did not form a complex, as expected. Since the interactions between synthetase and tRNA are in general electrostatic, raising the salt concentration may affect the stability of the complexes. Gel mobility shift assay was thus performed in the presence of 25 mM and 250 mM KCl (Figure 4) for the wild type enzyme and mutants with moderately decreased serylation propensity (R38A, K141A and R143A). As shown in Figure 4A the complexes involving mutants R38A and R143A are much weaker in the presence of 10-fold higher salt (lanes g and i, respectively, in comparison with lanes c and e), while the complex K141A:tRNA vanishes completely (lane h in comparison with lane d). Thus, amino acids Arg38, Lys141 and Arg143 seem to be involved in non-covalent complex formation. Moreover, diminished serylation activities of these mutants in the presence of higher salt concentration emphasize their involvement in ionic interactions (Figure 4B). The activity of the wt enzyme at 25 mM KCl was used as the referent point (100% activity) and the activities of the wt SerRS, R38A and K141A enzymes were determined at 125 and 250 mM salt concentration. Evidently, the activity of mutants drops more rapidly than wt enzyme's activity. More precisely, there is a 60% drop in activity of mutated enzymes when concentration of KCl is increased from 25 mM to 125 mM, while in the case of wild type enzyme activity drops only 20% (and enzyme is still around 80% active in the presence of 125 mM KCl). Although inactive in tRNA complex formation, the capacity of mutants K79A and R94A to catalyze the formation of seryl-adenylate remained comparable to the wild-type enzyme, as revealed by PPi-exchange assay (not shown).

Determination of dissociation constants for tRNA:mMbSerRS complexes by SPR reveals the importance of Arg94 in tRNA binding — We used SPR to compare the interaction of twelve mMbSerRS proteins (wt enzyme and eleven mutants) and tRNA. The study was performed on CM5 sensor chip on which wt SerRS and the variants were independently immobilized. Different concentrations of tRNA were injected at flow rate of 60 µl/min for 210 s. The sensogram obtained for wt enzyme agreed best with a two-state binding model with conformational change, described by the following equation:

\[ A + B \overset{k_{s1}}{\underset{k_{d1}}{\rightleftharpoons}} AB \overset{k_{s2}}{\underset{k_{d2}}{\rightarrow}} AB^* \]

where \( K_A = (k_{s1}/k_{d1})(1 + k_{s2}/k_{d2}) \) and \( K_D = 1/K_A \). In this model, the analyte A (tRNA) binds to the ligand B (mMbSerRS) to form an initial complex AB. The complex then undergoes a change in conformation to form a more stable complex AB* (33). The sensorgrams of all other mutants agreed best with two-state binding model with conformational change as well, except for mutant R94A. Curves obtained for this mutant could have been interpreted only by using the steady state affinity model. In that sense, solely \( K_D \) value (without \( k_{s1} \), \( k_{d1} \), \( k_{s2} \) and \( k_{d2} \) constants) was determined for R94A variant. An example of the sensorgram, denoting tRNA binding to the R143A variant, and the kinetic constants calculated from the fitted curves, are given in Figure 5 and Table 1, respectively. SPR analysis for mutant R76A was not performed because that particular enzyme was inactive in aminacylation and did not form non-covalent complex detectable on the gel. According to the data presented in Table 1 the largest effect on binding the tRNA was achieved by altering arginine 94 to alanine. \( K_D \) for mutant R94A is almost fifty-fold higher than for the wild type enzyme (122 nM for the wt enzyme and 5.81 µM for the R94A variant). A significant effect on interaction with tRNA have mutations of lysines 79 and 141 and arginine 143. Their respective mutants K79A, K141A and R143A have between four- to seven-fold higher \( K_D \) values than the wild type enzyme. Variants R38A and N142A gave similar \( K_D \) values (422 nM and 386 nM, respectively) that are approximately 3.5-fold higher than the wild type \( K_D \). Dissociation constants for all other mutants (R78A, K87A, K88A, Y89A and K90A) ranged from 151 to 301 nM.

The first association constant, \( k_{s1} \) (constant of initial complex formation), is in the range of \( 10^4 \) M\(^{-1}\)s\(^{-1}\) for most of the mutants. Only the wild type enzyme and variants K88A and N142A have 1.175 x \( 10^5 \) M\(^{-1}\)s\(^{-1}\) ≤ \( k_{s1} \) ≤ 1.471 x \( 10^5 \) M\(^{-1}\)s\(^{-1}\) (Table 1), which is between 2- to 6-fold higher \( k_{s1} \) than for other mutated mMbSerRSs. The first-order rate constants \( (k_{d2}) \) for wt, R78A, K87A, K88A, Y89A and K90A are 2- to 6-fold larger than \( k_{d2} \); these
mutants have $K_D$ values similar to the dissociation constant of the wild type one (1.2-2.5 $K_D$ of the wild type enzyme). In the case of N142A, K79A, K141A and R143A have second dissociation rate constant $k_d$ even greater than $k_d1$. Their $K_D$ values are at least 3.5-fold larger than $K_D$ for the wt enzyme. The SPR experiment also allowed the estimation of so called "$K_{D1}$", ($K_D$ of the first reaction of the two step mechanism; $K_{D1} = k_{d1} / k_{a1}$), which is similar for all the mutants. Taken together, our results suggest that the second step of reaction predominantly determines the observed $K_D$ values.

Effects of engineered mMbSerRS amino acid alterations on serylation of tRNA in vivo — We have recently shown that the expression of the gene encoding $M$. barkeri bacterial-type SerRS (bMbSerRS) in $E$. coli complements the function of thermolabile SerRS at nonpermissive temperature, while the expression of mMbSerRS gene does not (28). However, co-expression of $M$. barkeri seryl-tRNA synthetase gene, encoding either bacterial- or methanogenic-type SerRS, with the gene for cognate archaeal suppressor tRNA leads to suppression of bacterial amber mutations, implying that $E$. coli translation machinery can use serylated tRNA from methanogenic archaea as a substrate in protein synthesis (28). Bacterial strain XAC-A24 carries two amber mutations XAC-A24 (F' ara argE(UAG) rpoB gyrA Δlac pro/F lacI(UAG)-Z proAB), suppression of which reflect recognition and aminoacylation levels of suppressor tRNA by selected aminoacyl-tRNA synthetases. The argE(UAG) mutation is suppressible by any amino acid. The other suppressible marker in strain XAC-A24, in which UAG in-frame codon has been inserted in the lac part of a lac-lacZ fusion gene, was used for quantification of suppression efficiency (35, 36). Since serylation of $M$. barkeri serine specific tRNA by endogenous $E$. coli SerRS is negligible (12, 37), suppression is entirely dependent on recognition between archael partners (mMbSerRS/suppressor tRNA$^{\text{Ser}}$). Serine-specific tRNAs are especially suitable to be used in such assays, since the anticodon is not a recognition element for interaction with the cognate synthetase, and its alteration does not change the tRNA identity (38). We have converted the tRNA$^{\text{CGA}}$ isoacceptor sequence into the tRNA$^{\text{Ser}}$ suppressor sequence (supSMb) and placed it behind the lpp promoter in pTech plasmid (pTechsupSMb, 28). Next, strain XAC-A24 was co-transformed with a pair of compatible plasmids, one of which carried the gene for a methanogenic-type synthetase variant, while the second carried the $M$. barkeri suppressor tRNA$^{\text{Ser}}$ sequence. In order to analyse the contribution of individual amino acids to tRNA binding and catalysis, enzyme variants were characterized by the ability to serylate the suppressor tRNA in vivo. Expression of wt synthetase or its mutated variants was either from pET15b plasmid, which enables constitutive expression by inefficient recognition of T7 promoter by bacterial RNA polymerase (28) or from pBAD24 where it is dependent on induction with arabinose (30). Expression of all mMbSerRS variants in XAC-A24 strain has been verified by Western blot. No suppression was obtained when mMbSerRS variants, R76A and R94A, were co-expressed with archaeal suppressor tRNA (0 and 1.65% activity, respectively; Figure 6). To exclude the possibility that the lack of suppression was caused by too low expression of two synthetase variants from pET15b plasmid, their genes were recloned to pBAD24 vector. Higher expression of mutated synthetase R94A led to production of slightly higher quantities of serylated suppressor tRNA (from 1,65% to 7% suppression efficiency relative to the wt enzyme). This increment of suppression level shows that in the case of R94A mutant a weak suppression is possible. Alteration of residue R76 resulted in an enzyme that was completely inactive in vivo (0% activity) regardless of which plasmid, pET15b or pBAD24, was used (Figure 6). These results are fully in agreement with our in vitro experiments performed with both mutants. Mutants K79A, K90A, K141A, N142A and R143A displayed reduced suppression efficiency (Figure 6) compared to the wild type mMbSerRS, confirming the contribution of these side chains in the interactions with tRNA. All other tested variants (R38A, R78A, K87A, K88A and Y89A) showed more than 40% of original suppression efficiency detected by the wt enzyme.

DISCUSSION

Idiosyncratic N-terminal domains of SerRS enzymes provide tRNA$^{\text{Ser}}$ binding capacity — The
canonical tRNA\textsuperscript{Ser} is characterized by a long variable arm (B20 bases) between the anticodon stem and the T-arm, which SerRS employs as major tRNA identity element to discriminate its cognate tRNAs from all other species. This elongated variable arm is well conserved throughout the evolutionary process from prokaryotes to eukaryotes (with the only exception being metazoan mitochondria). Accordingly, SerRS enzymes have acquired a unique N-terminal domain, mostly structured as a coiled-coil (3, 16, 17), for recognizing the variable arm. N-terminal coiled-coil of \textit{P. horikoshii} SerRS (17) comprises additional basic residues as compared with bacterial SerRSs (3, 16) and a Trp residue (Trp40) found in other archaeal/eukaryal serine specific synthetases (17). An insertion of 20 amino acids into the N-terminal sequences of metazoans and trypanosomatid SerRS sequences, at the center of the predicted coiled-coil motif, suggests that this region may extend beyond the length seen in the structures solved so far (39). On the other hand, although the coiled-coiled N-terminal domain exists in the metazoan mitochondrial SerRS, it does not bind tRNA extra arm, since its cognate tRNA structures markedly deviate from the canonical cloverleaf secondary structure with highly truncated and/or intrinsically missing arms (40). Consequently, biochemical (41) and recent structural studies (19) have revealed truly distinctive mode of tRNA binding by mammalian mitochondrial SerRS. The SerRSs from methanogenic archaea also differ markedly from their bacterial-type counterparts, most notably through the absence of N-terminal coiled-coil (21). Besides its role in tRNA binding, N-terminal domain of mMbSerRS is required to assist proper folding of the catalytic domain (25). Interestingly, archaeal SerRSs, either of archaeal/eukaryal- (17) or methanogenic-type, do not conserve the residues of \textit{T. thermophilus} SerRS that interact with tRNA extra arm (23, 26), although they can aminoacylate bacterial tRNAs\textsuperscript{Ser}, besides their homologous archaeal tRNA substrates (17, 29, 32). Therefore, it is possible that archaeal SerRSs recognize not only tRNA sequence, but also its overall 3D structure that enables recognition of different tRNAs\textsuperscript{Ser} (32).

\textbf{Identification of amino acids in N-terminal domain of mMbSerRS critical for tRNA recognition} — We have demonstrated the contribution of individual amino acid residues towards tRNA binding, since their replacement leads to clear functional defects (Table 1, Figures 2, 3, 5 and 7). The altered residues can have direct influence on tRNA binding (side chains that are in direct contact with tRNA) or indirectly affect interaction between the tRNA binding and tRNA (side chains that ensure proper positioning of amino acids involved in direct contact with tRNA). We demonstrate, by a range of methods, that the individual substitutions of R76, K79 and R94 have a pronounced effect on the enzyme’s ability to serylate cognate tRNA. The gel mobility shift assay showed that the individual substitutions of these residues decrease the stability of SerRS:tRNA\textsuperscript{Ser} complexes (Figure 3). Binding analysis using SPR revealed that wild-type archaeal mMbSerRS binds \textit{in vitro} transcribed MbtRNA\textsuperscript{Ser} with high affinity (Table 1). Determined dissociation constant (\(K_D = 0.12 \mu \text{M}\)) is similar to other synthetase:tRNA complexes (involving CysRS (42), GlnRS (43, 44) and AspRS (45). The interaction between \textit{M. barkeri} SerRS and tRNA is entirely lost after replacement of R76, located in short H2 helix, with alanine (Figures 1, 2, 3 and 6), preventing the estimation of the \(K_D\). Most affected measurable tRNA binding affinity was for mutant R94A, the \(K_D\) value of which was increased about fifty-fold relative to wt mMbSerRS. Arginine 94 is located in the loop between helix H2 and \(\beta\)-strand A4 and according to the docking model it is positioned to interact with T-loop of the tRNA\textsuperscript{Ser}. The crystal structure of the SerRS:tRNA\textsuperscript{Ser} complex from \textit{T. thermophilus} (23, 26, 46) revealed that the tRNA-binding coiled-coil of bacterial SerRS is buried between the T\(\psi\)C arm and the long extra arm of tRNA\textsuperscript{Ser}. Likewise, biochemical studies on the mammalian mitochondrial system pointed to the importance of the T-loop, which is the main identity element for two unusual tRNA\textsuperscript{Ser} isoacceptors. Previous kinetic analysis of variant tRNA\textsuperscript{Ser} transcripts by the mMbSerRS showed reduced serylation efficiency after abolishing interactions between D- and T-loop, as these alterations, presumably, affected the tertiary structure of tRNA\textsuperscript{Ser} (27). The experiments presented here with R94A mMbSerRS variant reveal the importance of T-loop for the interactions with methanogenic-type synthetase.
Alteration of K79 (positioned in H2) strongly influences tRNA binding affinity, increasing the $K_D$ five-fold. Variants R94A and K79A aminoacylated tRNA$^\text{Ser}$ with approximately one third velocity of the wild type enzyme, while the mutants carrying alanine at positions R38, K141, N142 and R143 remained moderately active, but also exhibited diminished affinity for tRNA, as expected. Arg38, with a proline and two glycines in the vicinity (Figure 1A) is located in the loop between helix H1 and $\beta$-sheet A2 (Figure 1A). The flexibility of this region may be required for correct positioning of this mMbSerRS region toward tRNA. All other conserved amino acids in that area are hydrophobic and oriented to the enzyme’s interior, implicating their involvement in maintaining the structure of the N-terminal domain. Arg143 has a dual role: besides direct involvement in tRNA binding, its main chain also interacts with Arg147, located in helix 4 that participates in HTH mediated positioning of the N-terminal domain relative to the catalytic core (25). Accordingly, R143A variant binds cognate tRNA with seven-fold lower affinity (Figure 7).

In order to assess whether selected N-terminal residues are important for aminoacylation in vivo, we tested the ability of corresponding mutant proteins to promote suppression of bacterial amber mutations. When the wild-type mMbSerRS gene was introduced with MbtRNA$^\text{Ser}$ to E. coli strain bearing lacI-lacZ fusion reporter system, robust $\beta$-galactosidase activity was produced (Figure 6). In contrast, mutated SerRS genes promoted production of detectable, but less active reporter enzyme, in agreement with the $K_D$ values estimated by SPR. Importantly, variant R76A was inactive in the suppression assay, even when coexpressed from pBAD24 plasmid behind a stronger promoter. This confirms that mutated enzyme(s) did not productively interact with tRNA$^\text{Ser}$, not only in vitro, but also in the cellular context, when competing noncognate tRNAs are present.

**mMbSerRS serylation mechanism and the importance of substrate induced conformational changes** — Our previous crystallographic studies revealed that the binding of serine to the wild-type mMbSerRS causes a significant localized conformational change in serine ordering loop (SOL, residues 394-410) of the enzyme (21). Next, the positioning of the cognate tRNA in the active site of mMbSerRS is facilitated upon the conformational change of the motif 2 loop, that participates in ATP binding and mediates the interactions with the tRNA acceptor stem (37). Our biochemical experiments point to the importance of the flexibility of tRNA 3'-end binding region (37) for avoiding a steric clash seen between the acceptor end of tRNA and the motif 2 loop Ile342 in the model of tRNA:mMbSerRS complex, and allowing hydrogen-bonding of the first base pair.

The structure of mMbSerRS enzyme shows that the orientation of N-terminal domains in two monomers differs by a rotation of ~ 20° indicating that a conformational change likely accompanies tRNA binding (21). Accordingly, the SPR sensograms obtained for tRNA binding to immobilized wt mMbSerRS and the majority of constructed variants with the mutations in the N-terminal domain agreed best with a two-state binding model implying that a conformational change occurs in the enzyme upon binding of the tRNA substrate (Table 1 and Figure 5). A two step mechanism for the complex formation of synthetase and tRNA in the serine system from yeast was postulated in mid seventies (47, 48).

Our model suggests that the first step of binding will involve formation of a bimolecular complex between the tRNA and the flexibly disposed N-terminal domain of the SerRS. In the second step of the binding reaction accommodation of the tRNA into the active site will occur, accompanied by conformational changes of the enzyme and the acceptor end of the tRNA. Nevertheless, our SPR binding experiments indicate that the second step of the binding is the rate limiting step for the reaction and that this step is directly affected for all N-terminal domain mutants. These results suggest that the initial binding of the tRNA to the N-terminal domain is fast compared to the overall binding reaction. The slow step of the binding reaction involves the delivery of the tRNA to the active site of the enzyme, accompanied by the loss of entropy due to the organization of the flexibly disposed domains of the enzyme, and the associated conformational changes in the active site. During this second stage of the binding reaction the reduction in the affinity between the tRNA and the N-terminal domain, as indicated by the kinetic data obtained here, will increase the dissociation.
rate of the complex, perhaps by reducing the precision with which the tRNA is accommodated into the active site. Consequently, this would reduce the likelihood for the associated conformational changes in the active site of the enzyme that stabilize the interaction with the tRNA resulting in an increased $k_{d2}$ of the binding reaction. A similar kinetic phenomenon was observed by Tsai and Johnson in their studies of how T7 DNA polymerase discourages the incorporation of non-cognate nucleotides (49).

The different relative orientations of the tRNA-binding and catalytic domains were also shown to be associated with tRNA binding in yeast (50) and *E. coli* AspRS (51) and in human mitochondrial PheRS (52). Therefore, although tRNA binding domains in the two SerRS types are non-homologous and evolutionarily unrelated (21), the requirement for a closing movement of the N-terminal domain upon tRNA binding has been observed in the *T. thermophilus* SerRS:tRNA co-crystal structure (23) and shown here for mMbSerRS.
REFERENCES

1. Ibba, M., and Söll, D. (2000) Annu. Rev. Biochem. 69, 617–650
2. Eriani G., Delarue, M., Poch, O., Gangloff, J., and Moras, D. (1990) Nature 347, 203-206
3. Cusack, S., Berthet-Colominas, C., Härtlein, M., Nassar, N., and Leberman, R. (1990) Nature 347, 249-255
4. Ibba, M., Morgan, S., Curnow, A. W., Pridmore, D. R., Vothknecht, U. C., Gardner, W., Lin, W., Woese, C. R., and Söll, D. (1997) Science 278, 1119-22
5. Zhang, C. M., Perona, J. J., Ryu, K., Francklyn, C., and Hou, Y. M. (2006) J. Mol. Biol. 361, 300-311
6. Ataide, S. F., and Ibba, M. (2006) ACS Chem. Biol. 1, 285-297
7. Giegé, R., Puglisi, J. D., and Florentz, C. (1993) Prog. Nucleic. Acid Res. Mol. Biol. 45, 129–206
8. Giegé, R., Sissler, M., and Florentz, C. (1998) Nucleic Acids Res. 26, 5017–5035
9. Nozawa, K., O'Donoghue, P., Gundllapalli, S., Araiso, Y., Ishitani, R., Umehara, T., Söll, D., and Nureki, O. (2009) Nature 457, 1163-1167
10. Ribas De Pouplana, L., and Schimmel, P. (2001) Cell 104, 191–193
11. Ebel, J. P., Giegé, R., Bonnet, J., Kern, D., Befort, N., Bollack, C., Fasiolo, F., Gangloff, J., and Dirheimer, G. (1973) Biochimie 55, 547-557
12. Kim, H. S., Vothknecht, U. C., Hedderich, R., Celic, I., and Söll, D. (1998) J. Bacteriol. 180, 6446-6449
13. Tumbula, D., Vothknecht, U. C., Kim, H. S., Ibba, M., Min, B., Li, T., Pelaschier, J., Stathopoulos, C., Becker, H., and Söll, D. (1999) Genetics 152, 1269–1276
14. Lenhard, B., Orellana, O., Ibba, M., and Weygand-Durasevic, I. (1999) Nucleic Acids Res. 27, 721-729
15. Woese, C. R., Olsen, G. J., Ibba, M., and Söll, D. (2000) Microbiol. Mol. Biol. Rev. 64, 202-236
16. Fujinaga, M., Berthet-Colominas, C., Yaremchuk, A. D., Tukalo, M. A., and Cusack, S. (1993) J. Mol. Biol. 234, 222-233
17. Itoh, Y., Sekine, S., Kuroishi, C., Terada, T., Shirouzu, M., Kuramitsu, S., and Yokoyama, S. (2008) RNA Biol. 5, 169-177
18. Weygand-Durasevic, I. and Cusack, S. (2005) in The Aminoacyl-tRNA Synthetases (Ibba, M., Franklyn, C., and Cusack, S., eds). pp. 177-192, Landes Bioscience, Georgetown, TX
19. Chimnarongk, S., Gravers Jeppesen, M., Suzuki, T., Nyborg, J., and Watanabe, K. (2005) EMBO J. 24, 3369-3379
20. Sprinzl, M. and Vassilenko, K. S. (2005) Nucleic Acids Res. 33, 139–140
21. Bilokapic, S., Maier, T., Ahel, D., Gruic-Sovulj, I., Söll, D., Weygand-Durasevic, I., and Ban, N. (2006) EMBO J. 25, 2498-2509
22. Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. and Higgins, D. G. (1997) Nucleic Acids Res. 25, 4876-4882
23. Biou, V., Yaremchuk, A., Tukalo, M., and Cusack, S. (1994) Science 263, 1404-1410
24. Vincent, C., Borel, F., Willison, J. C., Leberman, R., and Härtlein, M. (1995) Nucleic Acids Res. 23, 1113–1118
25. Bilokapic, S., Ivic, N., Godinic-Mikulcic, V., Piantanida, I., Ban, N., and Weygand-Durasevic, I. (2009) J. Biol. Chem. 284, 10706-10713
26. Cusack, S., Yaremchuk, A., and Tukalo, M. (1996) EMBO J. 15, 2834-2842
27. Korencic, D., Polycarpo, C., Weygand-Durasevic, I., and Söll, D. (2004) J. Biol. Chem. 279, 48780-48786
28. Lesjak, S., and Weygand-Durasevic, I. (2009) FEMS Microbiol. Lett. 294, 111-118
29. Gruic-Sovulj, I., Jaric, J., Dulic, M., Cindric, M., and Weygand-Durasevic, I. (2006) J. Mol. Biol. 61, 128-139
30. Guzman, L., Belin, D., Carson, M. J., and Beckwith, J. (1995) J. Bacteriol. 177, 4121-4130
31. Coulondre, C., and Miller, J. H. (1977) J. Mol. Biol. 117, 577–606
32. Bilokapic, S., Korencic, D., Söll, D., and Weygand-Durasevic, I. (2004) *Eur. J. Biochem.* **271**, 694-702
33. Yowler, B. C., and Schengrund, C. L. (2004) *Biochemistry* **43**, 9725-9731
34. Chenal, A., Nizard, P., Forge, V., Pugniere, M., Roy, M. O., Mani J. C., Guillain F., and Gillet D. (2002) *Protein Eng.* **15**, 383-391
35. Normanly, J., Ogden, R. C., Horvath, S. J., and Abelson, J. (1986) *Nature* **321**, 213–219
36. Polycarpo, C. R., Herring, S., Berubea, A., Wood, J. L., Söll, D., and Ambrogelly, A. (2006) *FEBS Lett.* **580**, 6695–6700
37. Bilokapic, S., Rokov-Plavec, J., Ban, N., and Weygand-Durasevic, I. (2008) *FEBS J.* **275**, 2831-2844
38. Rogers, M. J., and Söll, D. (1988) *Proc. Natl. Acad. Sci. U. S. A.* **85**, 6627-6631
39. Geslain, R., Aeby, E., Guitart, T., Jones, T. E., Castro de Moura, M., Charrière, F., Schneider, A., and Ribas de Pouplana, L. (2006) *J. Biol. Chem.* **281**, 38217-38225
40. Helm, M., Brulé, H., Friede, D., Giegé, R., Pütz, D., and Florentz, C. (2000) *RNA* **6**, 1356-1379
41. Shimada, N., Suzuki, T., and Watanabe, K. (2001) *J. Biol. Chem.* **276**, 46770-46778
42. Zhang, C. M., Perona, J. J., and Hou, Y. M. (2003) *Biochemistry* **42**, 10931-10937
43. Weygand-Durasevic, I., Schwob, E., and Söll, D. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90**, 2010-2014
44. Bullock, T. L., Sherlin, L. D., and Perona, J. J. (2000) *Nat. Struct. Biol.* **7**, 497-504
45. Frugier, M., Moulinier, L., and Giegé, R. (2000) *EMBO J.* **19**, 2371-2380
46. Yaremchuk, A. D., Tukalo, M. A., Krikliviy, I., Malchenko, N., Biou, V., Berthet-Colominas, C., and Cusack, S. (1992) *FEBS Lett.* **310**, 157-161
47. Rigler, R., Pachmann, U., Hirsch, R., and Zachau, H. G. (1976) *Eur. J. Biochem.* **65**, 307-315.
48. Riesner, D., Pingoud, A., Boehme, D., Peters, F., and Maass, G. (1976) *Eur. J. Biochem.* **68**, 71-80.
49. Tsai, Y-C. and Johnson, K. A. (2006) *Biochemistry* **45**, 9675-9687.
50. Sauter, C., Lorber, B., Cavarelli, J., Moras, D., and Giegé, R. (2000) *J. Mol. Biol.* **299**, 1313-1324
51. Rees, B., Webster, G., Delarue, M., Boeglin, M., and Moras, D. (2000) *J. Mol. Biol.* **299**, 1157-1164
52. Klipcan, L., Levin, I., Kessler, N., Moor, N., Finarov, I., and Safro, M. (2008) *Structure* **16**, 1095-1104
Footnotes
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Abbreviations
aaRS, aminoacyl-tRNA synthetase (standard amino acid abbreviations precede RS throughout); mMbSerRS, methanogenic-type Methanosarcina barkeri SerRS; MbtRNA^Ser\text{GGA}, M. barkeri tRNA^Ser with anticodon GGA; SPR, surface plasmon resonance; wt, wild type
FIGURE LEGENDS

Figure 1. Design of mMbSerRS variants based on the conserved amino acid residues in N-terminal domain of methanogenic-type SerRSs and their proximity to tRNA in mMbSerRS:tRNA<sub>Ser</sub> docking model. (A) The structure-based sequence alignment of mMbSerRS N-terminal domain with selected SerRS sequences derived from methanogenic archaea (Mk, *Methanopyrus kandleri*; Mt, *Methanothermobacter thermautotrophicus*; Mj, *Methanococcus jannaschii*; Mm, *Methanococcus maripaludis*; mMb, *Methanosarcina barkeri*). The sequence alignment was generated using the program MUSCLE (22). Amino acids that are completely conserved are in red, while those with 80% conservation are in yellow. Secondary structural elements are indicated above the alignment with gray cylinders and arrows for helices and β-sheets, respectively. Mutated residues are marked with dots. (B) mMbSerRS:tRNA<sub>Ser</sub> docking model. SerRS dimer is shown in gray and tRNA<sub>Ser</sub> is colored cyan. Conserved amino acids are indicated by the same color code as in (A).

Figure 2. Aminoacylation activity of mMbSerRS variants. Relative serylation rates of *in vitro* transcribed MbtRNA<sub>Ser</sub> by mMbSerRS variants are presented by horizontal bars. Mutated enzymes that retain the serylation ability comparable to the wt enzyme are colored black, mutants with the serylation rate lowered to 59-80% of the wt are designated with traverse lines, variants with significant drop in serylation rate (33% of the wt serylation rate) are marked black and white square pattern and one which completely loses serylation ability is highlighted white.

Figure 3. Gel mobility shift assay of the non-covalent complexes between MbtRNA<sub>Ser</sub> and various mMbSerRS mutants. MbtRNA<sub>Ser</sub> was incubated with different mMbSerRS variants (final concentration of tRNA and enzymes was 1.1 µM and 0.6 µM, respectively) and subjected to polyacrylamide gel electrophoresis (PAGE) under native conditions: wt, lane b; R38A, lane c; R76A, lane d; R78A, lane e; K79A, lane f; K87A, lane g; K88A, lane h; Y89A, lane i; K90A, lane j; R94A, lane k; K141A, lane l; N142A, lane m and R143A, lane n. Non-complexed mMbSerRS (8.3 pmol) and MbtRNA<sub>Ser</sub> (14.8 pmol) were loaded on the gel as electrophoretic mobility markers (lanes a and o, respectively). Non-covalent complexes and non-complexed tRNA are marked with black and white arrows, respectively.

Figure 4. Effect of different salt concentrations on non-covalent complex formation and serylation propensity. (A) Non-covalent complexes were made as described in Experimental procedures and the legend to Figure 3, except that in the reaction mixture the concentration of KCl was varied as indicated, and then subjected to PAGE under native conditions. Non-complexed wt mMbSerRS is visible in lane a, while non-covalent complexes between tRNA and mMbSerRS variants in the presence of 25 mM KCl were loaded into following lanes: wt, lane b; R38A, lane c; R76A, lane d; R78A, lane e; K79A, lane f; K87A, lane g; K88A, lane h; Y89A, lane i; K90A, lane j; R94A, lane k; K141A, lane l; N142A, lane m and R143A, lane n. Non-complexed mMbSerRS (8.3 pmol) and MbtRNA<sub>Ser</sub> (14.8 pmol) were loaded on the gel as electrophoretic mobility markers (lanes a and o, respectively). Non-covalent complexes and non-complexed tRNA are marked with black and white arrows, respectively. (B) Relative serylation rate of the enzymes was tested at three different KCl concentrations (25, 125 and 250 mM). Activity of the wt enzyme at 25 mM KCl was taken as the referent point (100% activity). Contrary to wt enzyme, a significant drop in serylation rate was detected for both mutants after raising KCl concentration from 25 to 125 mM.

Figure 5. Kinetic analysis of tRNA binding to immobilized mMbSerRSR143A monitored by a biosensor. Sensorgram (red) obtained for the binding of different concentrations of tRNA<sub>Ser</sub> (70.3 - 2250 nM) in 30 mM Hepes, pH 7.0, 6 mM MgCl<sub>2</sub> and 5 mM DTT to R143A mMbSerRS. Data were fit to the two-state conformational change model (black).

Figure 6. Suppression efficiency of *M. barkeri* SerRS variants. Suppression efficiency was determined by measuring the β-galactosidase activity in *E. coli* strain XAC-A24. 100% corresponds to the β-galactosidase activity of strain XAC-A24 co-transformed with pET15b plasmid, carrying the gene for a
wild type mMbSerRS, and pTech vector, carrying *M. barkeri* suppressor tRNA$^{\text{Ser}}$ sequence. Results were reported as the percentage of mutant enzyme suppression activity relative to that of the wild type enzyme.

Figure 7. **Contribution of the individual amino acids in tRNA binding.** One subunit of the mMbSerRS dimer is shown. The size and the brightness of the spheres designate the significance of the particular residue in tRNA binding according to the cumulative influence on biochemical properties of mMbSerRS. The largest and the brightest sphere (arginine 76) annotates the most important side chain in tRNA recognition.
Table 1. Kinetic constants obtained from the fit to the conformational change model of sensorgrams acquired for the binding of different concentrations of tRNA (19.5 nM - 8 µM) to various SerRS mutants. Only for mutant R94A steady state affinity model was used (see text).

| SerRS variant | $k_{a1}$ (M$^{-1}$s$^{-1}$)/10$^5$ | $k_{d1}$ (s$^{-1}$)/10$^1$ | $k_{a2}$ (s$^{-1}$)/10$^2$ | $k_{d2}$ (s$^{-1}$)/10$^3$ | $K_D$ (M)/ 10$^{-7}$ | Relative $K_D^a$ | $\chi^2b$ |
|---------------|---------------------------------|--------------------------|--------------------------|--------------------------|----------------------|-----------------|--------|
| wt            | 1.471 ± 0.010                   | 1.239 ± 0.009            | 1.482 ± 0.006            | 2.513 ± 0.003            | 1.22                 | 1.00            | 2.71   |
| R38A          | 0.442 ± 0.001                   | 0.342 ± 0.001            | 0.832 ± 0.004            | 9.949 ± 0.017            | 4.22                 | 3.5             | 0.20   |
| R78A          | 0.558 ± 0.002                   | 0.417 ± 0.002            | 1.204 ± 0.004            | 4.192 ± 0.006            | 1.93                 | 1.6             | 0.28   |
| K79A          | 0.299 ± 0.001                   | 0.231 ± 0.001            | 0.283 ± 0.002            | 7.543 ± 0.029            | 5.61                 | 4.6             | 0.18   |
| K87A          | 0.983 ± 0.013                   | 0.714 ± 0.011            | 1.345 ± 0.014            | 3.552 ± 0.011            | 1.52                 | 1.2             | 1.95   |
| K88A          | 1.175 ± 0.004                   | 0.862 ± 0.003            | 0.993 ± 0.002            | 2.703 ± 0.002            | 1.57                 | 1.3             | 0.53   |
| Y89A          | 0.568 ± 0.002                   | 0.498 ± 0.003            | 1.048 ± 0.004            | 5.494 ± 0.008            | 3.01                 | 2.5             | 0.20   |
| K90A          | 0.572 ± 0.005                   | 0.547 ± 0.006            | 1.606 ± 0.005            | 4.568 ± 0.006            | 2.12                 | 1.7             | 0.77   |
| R94A          |                                 |                          |                          |                          | 58.10 ± 1.70         | 47.6            | 0.07   |
| K141A         | 0.264 ± 0.001                   | 0.237 ± 0.001            | 0.515 ± 0.003            | 6.169 ± 0.013            | 4.89                 | 4.0             | 1.34   |
| N142A         | 1.423 ± 0.005                   | 1.212 ± 0.004            | 0.673 ± 0.002            | 5.578 ± 0.005            | 3.86                 | 3.2             | 0.77   |
| R143A         | 0.324 ± 0.001                   | 0.444 ± 0.002            | 0.605 ± 0.004            | 11.27 ± 0.03             | 8.90                 | 7.3             | 0.09   |

$^a$ Relative $K_D$ is determined as $K_D$ (mutant)/$K_D$ (wt).

$^b$ Statistical value describing the closeness of fit. Values <10 are acceptable (34)
Figure 2

![Chart showing relative serylaxation rate for different amino acids](http://www.jbc.org/Downloaded from http://www.jbc.org/)

- R143A
- N142A
- K141A
- R94A
- K90A
- Y89A
- K88A
- K87A
- K79A
- R78A
- R76A
- R38A
- wt

Relative serylaxation rate
Figure 4

A

B

25 mM KCl  260 mM KCl

a b c d e f g h i j

Relative serylation rate

[KCl] / mM

wt R38A K141A
Identification of amino acids in N-terminal domain of atypical methanogenic-type seryl-tRNA synthetase critical for tRNA recognition
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