An Archaeal tRNA-Synthetase Complex That Enhances Aminoacylation Under Extreme Conditions

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An Archaeal tRNA-Synthetase Complex that Enhances Aminoacylation under Extreme Conditions

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Aminoacyl-tRNA synthetases (aaRSs) play an integral role in protein synthesis, functioning to attach the correct amino acid with its cognate tRNA molecule. aaRSs are known to associate into higher-order multi-aminoacyl-tRNA synthetase complexes (MSC) involved in archaeal and eukaryotic translation, although the precise biological role remains largely unknown. To gain further insights into archaeal MSCs, possible protein-protein interactions with the atypical Methanobacterium thermotrotopicus seryl-tRNA synthetase (MtsSerRS) were investigated. Yeast two-hybrid analysis revealed arginyl-tRNA synthetase (MtaArgRS) as an interacting partner of MtsSerRS. Surface plasmon resonance confirmed stable complex formation of the two enzymes during gel filtration chromatography. The MtsSerRS-MtaArgRS complex also contained tRNAArg, consistent with the existence of a stable ribonucleoprotein complex active in aminoacylation. Steady-state kinetic analyses revealed that addition of MtaArgRS to MtsSerRS led to an almost 4-fold increase in the catalytic efficiency of serine attachment to tRNA, but had no effect on the activity of MtaArgRS. Further, the most pronounced improvements in the aminoacylation activity of MtsSerRS induced by MtaArgRS were observed under conditions of elevated temperature and osmolarity. These data indicate that formation of a complex between MtsSerRS and MtaArgRS provides a means by which methanogenic archaea can optimize an early step in translation under a wide range of extreme environmental conditions.

Aminoacyl-tRNA synthetases (aaRSs) catalyze the specific coupling of amino acids with their cognate tRNAs to produce aminoacyl-tRNAs (aa-tRNAs), which serve as starting materials for the biosynthesis of proteins. Aa-tRNA synthesis occurs in two steps: amino acid activation at the expense of ATP followed by the aminoacylation of tRNA. Although for most aaRSs the formation of aminoacyl-AMP does not require tRNA, cognate tRNA is necessary for amino acid activation by ArgRS, GlnRS, GluRS, and LysRS enzymes from many organisms. Based on structural features of their active sites, aaRSs can be divided into two classes, which comprise 10 members each (3). In addition, an unusual form of LysRS is found in class I (2), while class II also includes the noncanonical synthetases PyrRS and SepRS (4).

In all three domains of life, subsets of aaRSs have been shown to associate into higher-order multi-aminoacyl-tRNA synthetase complexes (MSCs). These complexes are distinctive compared with other macromolecular protein complexes, because their components are enzymes that carry out similar catalytic reactions simultaneously, and only some aaRSs are involved (5). In eukaryotes, MSCs tend to be larger than those discovered in bacteria and archaea and also perform a wider range of functions that include both aminoacylation and noncanonical roles beyond translation (6–9). The mammalian MSC, purified from various mammalian tissues (10–13) and from cultured Chinese hamster ovary (CHO) (14) and murine erythroleukemia cells (15), is by far the largest known MSC (16–19). This complex is composed of eleven polypeptides including the bi-functional glutamyl-prolyl-tRNA synthetase (GluProRS), seven monospecific aspartyl-, arginyl-, glutamyl-, lysyl-, methionyl-, leucyl-, and isoleucyl-tRNA synthetases (AspRS, ArgRS, GlnRS, LysRS, MetRS, LeuRS, and IleRS, respectively); and three nonsynthetase protein factors, p18, p38, and p43 (20, 21, 22). The structural organization of this complex has not yet been completely deciphered (23, 24), but the stability of some components has been shown to depend on their proximity to neighboring proteins (25). The other known multiprotein mammalian aaRS complex comprises just two monomeric subunits of valyl-tRNA synthetase and several subunits of translation elongation factor EF-1H (26). AaRS-containing complexes have also been identified in the lower eukaryote Saccharomyces cerevisiae. The most extensively studied is the yeast ternary complex that consists of MetRS, GluRS, and the nonsynthetase protein Arc1p, which has homology to the mammalian accessory protein p43 (27, 28). Association with Arc1p was shown to increase the catalytic efficiency of the two associated synthetases, enhance nuclear export of tRNA, and control the subcellular localization of the enzymes (29). The bacterial homologue of Arc1p, Trb111, was first found in the extreme thermophile Aquifex
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*Pneumocystis jirovecii* and was shown to promote tRNA binding by aaRSs (30, 31). In *S. cerevisiae* the peroxin Pex21p interacts with the characteristic C-terminal extension of SerRS, forming a complex that facilitates tRNA binding and serylation (32, 33).

In bacteria, stable complex formation between prolyl-tRNA synthetase (ProRS) and a synthetase-like protein YbaK has been shown to facilitate editing of non-cognate aa-tRNAs (34, 35). A larger bacterial *Mycoplasma pneumoniae* multi-synthetase complex, consisting of five aaRSs (PheRS, TyrRS, MetRS, GluRS, and ThrRS) has recently been discovered by the TAP-MS method (36). In the same study, many aaRSs (MetRS, GluRS, and ThrRS) has recently been discovered by the TAP-MS method (36). In this study, many aaRSs were also found to interact with other factors involved in translation, transcription, DNA replication, and metabolism, emphasizing the important and varied roles these complexes play.

An archaeal aaRS complex was first described in the extreme halophile *Haloarcula marismortui*, with many if not all of the aaRSs purified in one or possibly two large complexes (37). In *Methanocaldococcus jannaschii*, ProRS co-purified with the H$_2$-forming N$_5$-N$_{10}$-methylene tetrahydromethanopterin synthase complex, a component of the methanogenesis pathway (38). However, the cellular role of this complex is unclear, because aaRS activity remained unchanged upon complex formation. In *Methanothermobacter thermautotrophicus* (39), yeast two-hybrid analyses identified a larger archaeal complex composed of LysRS, LeuRS, ProRS, and elongation factor 1 α (EF-1α). Bioophysical analyses confirmed the formation of a stable higher order archaeal MSC, highlighting the central role LeuRS plays as a core protein, mediating interactions within the complex. In this functional archaeal complex of four translational proteins, LeuRS was found to improve the catalytic efficiency of tRNA aminoacylation by both LysRS and ProRS (40). Furthermore, EF-1α increased $k_{cat}$ for Leu-tRNA$^\text{leu}$ synthesis 8-fold, although complex formation had little effect on the activity of EF-1α itself (41).

To further investigate the extent and composition of multi-aaRS complexes in archaea we searched for proteins that interact with methanogenic-type SerRS, an atypical form of seryl-tRNA synthetase confined to certain archaea (42). The high resolution crystal structure of the SerRS enzyme from *Methanosarcina barkeri* has been recently determined, revealing a number of idiosyncratic features distinctive from the canonical and widespread bacterial-type SerRSs (43). In this work, using a yeast two-hybrid screen, we identified an interaction between methanogenic-type SerRS and *M. thermotrophicus* ArgRS (MtArgRS), the mammalian equivalent of which exists both as part of the multi-tRNA synthetase complex and as a free enzyme (44). *M. thermotrophicus* ArgRS enhances the catalytic activity of MtSerRS, resembling the functional interactions in other previously described archaeal complexes (9), and further supporting the importance of stable multi-synthetase associations in archaea.

**EXPERIMENTAL PROCEDURES**

**Media, Strains, Oligonucleotides, and Plasmid Construction**—*Escherichia coli* media were prepared by standard methods and yeast transformations were made according to the Yeast Protocols Handbook (Clontech). Vectors pDBLeu and pD-

EST22 and yeast host strain MaV203 were from Invitrogen (ProQuest Two-hybrid System). The *M. thermotrophicus* SerRS encoding gene, (serS, MTH1122), was obtained by PCR using genomic DNA as a template, *Pfu* DNA polymerase (Stratagene), upstream primer 5′-GAGAGCTAGCGATTTAACAACTC-3′ and downstream primer 5′-GAGTACTAGTTGCTCCGTGCG-3′ to generate an NheI-Spel fragment. The amplified fragment was then cloned into the yeast two-hybrid bait vector pDDBLeu and the authenticity of the plasmid was confirmed by sequencing. An N-terminally tagged His$_6$ fusion derivative of SerRS (MtSerRS) was generated by PCR using genomic *M. thermotrophicus* DNA as template and the amplified fragment was inserted into Ndel and HindIII sites of plasmid pET28 (Novagen). A GST-tagged fusion derivative of ArgRS (GST-MtArgRS) was constructed from an *argS* gene (MTH1447) PCR-amplified using genomic *M. thermotrophicus* DNA as a template, *Pfu* DNA polymerase (Stratagene), upstream primer 5′-GGGATCCATGGTTCAGGTACATTG-3′ and downstream primer 5′-CCGCTCGAGTTACTACATGGTCTC-3′, and inserted into the BamHI-Xhol sites of plasmid pGEX-6P-2 (Amersham Biosciences).

**Yeast Two-hybrid Screen**—Yeast two-hybrid vector pDBLeu containing the *serS* gene was used to transform the yeast strain MaV203 to screen for proteins that interact with MtSerRS. The cells were grown in medium without leucine (SD-Leu) and were sequentially transformed with an *M. thermotrophicus* cDNA library cloned into the vector pDEST22 (40), and all assays were performed according to the manufacturer’s instructions (Invitrogen). Potential primary positives were selected by plating transformants on medium lacking leucine, tryptophan, and histidine (SD-Leu-Trp-His) supplemented with 25 mM 3-aminotriazole (3-AT) and the plates were incubated at 30 °C for 3 days. The colonies were then replica-cleaned as described in the instructions for the Pro-Quest Two-Hybrid System. The transformation plates were then replica-plated onto SD-Leu-Trp-His + 25 mM 3-AT plates and incubated for 24 h. Transformants showing consistent growth were streaked for single colonies, and further tested for phenotypes of the other reporter genes (i.e. growth on SD-Leu-Trp-Ura and no growth on SD-Leu-Trp+0.2% 5-fluoroorotic acid). Isolation of positive clones for sequencing was done by growing the co-transformants in SD-Trp medium followed by plating on SD-Trp medium supplemented with cycloheximide to isolate colonies harboring only the prey vector. Prior to plasmid isolation from cultures and sequencing, these colonies were tested for inability to grow on medium lacking leucine. cDNA inserts were sequenced in prey vectors at Synergene Biotech GmbH (Zürich) using an oligonucleotide 5′-TATAACCGGTGTGGAATCA-3′ matching the end of the prey vector that reads into the 5′-end of the insert. A total of 23 transformants were identified by BLAST search as protein-coding interacting clones. For determination of interacting domains, plasmid pACT2 containing ArgRS was constructed by insertion a gene as a BamHI/XhoI cassette, and deletion mutants of methanogenic-type SerRS were constructed in pAB151 and used as before (45). Double transformants of strain L40 were grown in selective -Trp-Leu
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medium until later log phase (A$_{600}$ of 0.7–1.0) and serial dilutions were spotted as 4-µl aliquots on selective -Trp-Leu or -Trp-Leu-His synthetic dropout (SD) agar plates supplemented with 3 mM 3-amino-1,2,4-triazole (3AT). Transformants were also tested for β-galactosidase activity using o-nitrophenyl-β-D-galactoside (ONPG).

**Protein Production and Purification** — Production of recombinant His$_6$-MtSerRS or GST-MtArgRS was done by transforming *E. coli* BL21(DE3)pLys (Stratagene) with pET28 or pGEX-6P-2 vectors containing the relevant inserts and growing the resulting strains on LB medium supplemented with ampicillin or kanamycin. Cell-free extracts were prepared by sonication of the *E. coli* cells in lysis buffer (12.5 mM K$_2$HPO$_4$, 12.6 mM KH$_2$PO$_4$, 500 mM NaCl, 5 mM MgCl$_2$, 10% glycerol, 10 mM imidazole, 10 mM β-mercaptoethanol, and 1 mM PMSF) for MtSerRS or (12.5 mM K$_2$HPO$_4$, 12.6 mM KH$_2$PO$_4$, 500 mM NaCl, 5 mM MgCl$_2$, 10% glycerol, 0.1% NP40, 10 mM β-mercaptoethanol, and 1 mM PMSF) for GST-MtArgRS followed by centrifugation at 12,000 rpm for 1 h. The supernatant containing MtSerRS was then applied to a Ni$_2$+-nitrilotriacetic acid (Ni-NTA) resin (Qiagen) equilibrated in lysis buffer and extensively washed in the same buffer containing 30 mM imidazole. His$_6$-MtSerRS was eluted in the same buffer containing 400 mM imidazole. Fractions containing His$_6$-MtSerRS were pooled, and buffer was exchanged to storage buffer (25 mM Tris/HCl, pH 8.0, 200 mM NaCl, 5 mM MgCl$_2$, 2 mM β-mercaptoethanol, and 10% glycerol) using a PD10 desalting column (GE Healthcare). Samples were then concentrated by ultrafiltration and aliquots were stored at −80°C. Protein concentration was determined by active site titration as previously described (45). Cell-free extracts for isolation of GST-MtArgRS were prepared by sonication of the *E. coli* cells in lysis buffer as for MtSerRS. Protein extract was then applied to a glutathione-Sepharose resin (GE Healthcare) equilibrated in lysis buffer and then the resin was extensively washed with the same buffer as described by the manufacturer’s directions for GST expression and purification systems (GE Healthcare). GST-MtArgRS was eluted in the same buffer containing 20 mM reduced glutathione (Sigma). Fractions containing GST-MtArgRS were pooled, and buffer was exchanged to storage buffer (25 mM Tris/HCl pH 7.5, 150 mM NaCl, 5 mM MgCl$_2$, 2 mM β-mercaptoethanol, and 10% glycerol) using a PD10 desalting column (GE Healthcare). Samples were then concentrated by ultrafiltration and aliquots stored at −80°C. The GST tag was cleaved using 25 µg of purified protein and 20 units of PreScission™ Protease (GE Healthcare) in storage buffer. The reaction was incubated for 16 h at 4°C and then applied to equilibrated Sepharose resin substituted with glutathione followed by collection of MtArgRS in the flow through.

**Aminoacylation Assays** — Aminoacylation reactions were performed at 50°C as follows unless stated otherwise. The reaction buffer was 50 mM Hepes, pH 7.0, 15 mM MgCl$_2$, 50 mM NaCl, 4 mM DTT, 5 mM ATP, 125 µM [¹⁴C]serine, [¹⁴C]isoleucine, or [¹⁴C]arginine (PerkinElmer Life Sciences). Concentration of the proteins (MtSerRS, MtArgRS, BSA, and *E. coli* IleRS (EcIleRS) was 60–100 nM. Aliquots were removed periodically and spotted onto 3MM Whatman filter disks pre-soaked in 5% trichloroacetic acid (w/v), which were then washed and counted. For tRNA$^{Asg}$ and tRNA$^{Ser}$ Km determination, tRNA was added at concentrations varying between 0.02 and 10 times Km. Total *E. coli* tRNA (EctRNA) (Roche Applied Science) or *M. barkeri* tRNA$^{Ser}$ (MtRNA$^{Ser}$) produced in vivo were used as previously described (45). The genes encoding *M. thermautotrophicus* tRNA$^{Ser}$ and tRNA$^{Asg}$ were cloned into pUC18 and the corresponding tRNA molecules were in vitro transcribed, however both were inactive in aminoacylation assays. Similar results were reported for tRNA$^{LyS}$ from the same organism (46).

**In Vitro Pull-down Assay** — Ni-NTA resin was incubated at 4°C overnight with the *E. coli* lysates containing 10-times more MtSerRS than GST-MtArgRS in binding buffer (25 mM Tris/HCl, pH 7.5, 500 mM NaCl, 5 mM MgCl$_2$, 20 mM imidazole, 5 mM β-mercaptoethanol containing protease inhibitor mixture tablet (Complete Mini, Roche Applied Science) to a final volume of 40 ml. The cells were then passed four times through a French pressure cell and centrifuged twice at 25,000 rpm for 25 min in a Ti70 rotor (Beckman Coulter, Inc.). 5.5 mg of total protein was loaded onto a Superdex 200 HR 10/30 column (GE Healthcare). 15–30 µl aliquots were used for aminoacylation assays.

**Surface Plasmon Resonance** — Kinetic studies were performed at 20°C using a BIACORE T100 surface plasmon resonance (SPR) instrument (Biacore Inc., Uppsala, Sweden). MtSerRS was covalently attached to a carboxymethyl dextran-coated gold surface (CM5 sensor chip, Biacore Inc., Uppsala). The carboxymethyl groups of dextran were activated with injection of a mixture of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride and N-hydroxysuccinimide. Seryl-tRNA synthetase was attached to the surface 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 800 re-
sponse units in one flow cell. The kinetics of association and dissociation were monitored at a flow rate of 30 μl/min. Analyte was diluted in the running buffer (20 mM Tris/HCl, pH 7.5, 100 mM NaCl, 6 mM MgCl₂, and 5 mM DTT). Binding was monitored over a concentration range of 4.28 nM-2.3 μM GST-MtArgRS, 5.0 nM-2.8 μM MtArgRS and 35 nM-20 μM BSA. After the end of each injection, proteins were allowed to dissociate for 600 s. Data reported are the differences in SPR signal between the flow cell containing MtSerRS and the reference cell without enzyme immobilized. Duplicate injections were made for each protein concentration in one round of measurement and each experiment was repeated twice. The data were analyzed using Biacore T100 Evaluation Software and the equilibrium dissociation constant (K_d) was determined from the level of binding at equilibrium as a function of the sample concentrations.

Results

Arginyl-tRNA Synthetase Forms a Stable Interaction with seryl-tRNA Synthetase—To identify proteins interacting with the archaeal SerRS, an M. thermautotrophicus cDNA library was screened with the entire seryl-tRNA synthetase as bait. To eliminate false positives, double transformants were plated on -Trp-Leu-His+25 mM 3-AT, replica-cleaned after 48 h, and replica-plated onto both -Trp-Leu-Ura and plates containing 5-FOA (Fig. 1A), for final conformation. Yeast two-hybrid screening identified 23 potentially positive interacting proteins with the MtSerRS bait, resulting in activation of reporter genes. The majority of interacting proteins identified from the screen were found false positives with broad interaction capability (MTH412, MTH674, MTH700, MTH913, MTH1168, MTH1878) (40, 41), or other metabolic proteins (MTH846, MTH1107, MTH1134, MTH1738), based on previously published results for the same cDNA library (40, 41).

Arginyl-tRNA synthetase, which had not previously emerged from screens of this library, was also identified to associate with the archaeal SerRS. To further characterize the interaction of ArgRS with SerRS, the roles of the idiosyncratic N-terminal domain and a helix-turn-helix (HTH) motif within the C-terminal domain of methanogenic-type SerRS (43) were investigated. While the separately expressed N-terminal domain of SerRS is not sufficient for binding to ArgRS, the yeast two-hybrid assay detects a strong interaction with the C-terminal domain (Fig. 1B). Deletion of the HTH motif, which contributes to the stability of SerRS dimers (45), significantly weakens the interaction between the two synthetases.

Determination of MtSerRS-MtArgRS Complex Binding Affinities by Surface Plasmon Resonance—To confirm the interaction between MtSerRS and MtArgRS in vitro and to determine the binding affinity between the two synthetases, SPR was employed. GST-tagged arginyl-tRNA synthetase was purified and, as necessary, the GST tag was removed from MtArgRS with PreScission™ Protease. MtSerRS was covalently bound to the CM5 chip, and the interaction with either GST-MtArgRS or MtArgRS (without GST tag) was investigated. Stable complex formation with SerRS was observed between both GST-MtArgRS, as well as the untagged MtArgRS (Fig. 2). The calculated K_d for the GST-MtArgRS-MtSerRS interaction was 248 ± 52 nM and the K_d for the complex of MtArgRS-MtSerRS was 253 ± 43 nM. These almost identical K_d values suggested that the GST tag presents no conformational or kinetic barrier for association of the two enzymes. Consequently, MtArgRS and GST-MtArgRS were treated as equivalent proteins. BSA displayed no observable interaction with MtSerRS (Fig. 2B), consistent with the formation of a specific complex between MtSerRS and MtArgRS.

Ternary Complex Formation of MtSerRS-MtArgRS with tRNA<sup>Arg</sup>—The association of MtSerRS and MtArgRS was further investigated by three differently performed gel filtration analyses (Fig. 3 and supplemental Figs. S1 and S2), which all confirmed the presence of both MtArgRS and MtSerRS in

FIGURE 1. Yeast two-hybrid screen with MtSerRS as bait. A, double transformants were plated on medium without tryptophan and leucine (left panel), plates supplemented with 0.2% 5-FOA (middle panel) and plates without uracil (right panel). Controls (ProQuest Yeast Two-Hybrid System): N (no interaction control), A (weak interaction standard), B (strong interaction standard), C (moderate interaction standard). Transformants 1, 2, and 3 do not show interaction-dependent phenotypes. Transformant 4 indicates MtArgRS interacts with MtSerRS (double yeast transformant of pDBleuRS and pDEST22RS). Each patch represents an independent transformant. B, interaction of MtArgRS with MtSerRS C- and N-terminal domain mutants. Left panel, yeast L40 cells were transformed and tested in a yeast two-hybrid system with indicated combinations of prey plasmids either empty (0) or encoding MtArgRS (R) or MtSerRS deletion mutants as baits (C-domain, C-domain without HTH motif, and N-terminal domain). The positive control was a strain transformed with pAB151SES1 and pACT2pex21 (pos.). Cultures of yeast transformants were analyzed by β-galactosidase activity using ONPG. Results were calculated in Miller units and represented as average ± S.D. Right panel, transformants were spotted in serial dilutions on SD-Trp-Leu-His plates supplemented with 3 mM 3AT.
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higher molecular weight fractions. In the first experiment, purified MtArgRS and MtSerRS were either mixed or loaded separately onto a Superdex 200 HR 10/30 column. Eluted proteins were analyzed by gel electrophoresis and by measuring the corresponding aminoacylation activities using either total E. coli tRNA or MtRNA \textsuperscript{S} as a substrate. Under these conditions only 5–10% of loaded proteins appeared in the complex (supplemental Fig. S1). In contrast, gel filtration chromatography of M. thermoautotrophicus cell-free extracts (supplemental Fig. S2), showed up to 30% of SerRS and ArgRS activities co-eluting as a complex, while isoleucyl-tRNA synthetase, monitored as a control, eluted as a free protein. The main MtSerRS and MtArgRS co-elute corresponded to a molecular weight of \( \sim 300 \) kDa, with additional activity detected at 500 kDa suggesting possible formation of a larger complex with other cellular macromolecules. To facilitate more detailed analysis of the complex, it was purified from E. coli cell lysates containing either overexpressed MtSerRS or GST-MtArgRS. Lysates containing MtSerRS and GST-MtArgRS were applied to a Ni-NTA column. MtSerRS was present in a 10-fold excess to saturate the Ni-NTA resin and to ensure that all GST-MtArgRS was in the complex. After incubation, the resin was extensively washed to eliminate nonspecific binding (Fig. 3A, wells 1–10). MtSerRS and GST-MtArgRS co-eluted (Fig. 3B, wells 11–14), as confirmed by measuring corresponding aminoacylation activities (Fig. 3E). The additional band above MtSerRS could be attributed to a protein from E. coli lysate apparently interacting with the Ni-NTA resin. Eluted fractions were incubated with PreScission \textsuperscript{TM} Protease and then subjected to gel filtration (Fig. 3C). Aliquots from the eluted fractions were examined on SDS-PAGE (Fig. 3C) and denaturing acrylamide-urea gels (Fig. 3E, lower panel). The MtSerRS-MtArgRS complex was visualized as two bands of approximately equal stoichiometry in the 11th ml of the gel filtration chromatography elution profile (Fig. 3C, lane 17) and also included a small nucleic acid (Fig. 3E) indicating an apparent ribonucleoprotein complex (RNP). The identity of the unknown nucleic acid was determined by hybridization with probes specific to Ec\textsuperscript{tRNA}Arg (Fig. 3D). The molecular mass of the RNP complex was again estimated to be 300 kDa consistent with a probable stoichiometry of the complex consisting of MtSerRS dimer \( \sim 120 \) kDa, two molecules of MtArgRS \( \sim 65 \) kDa each) and two molecules of Ec\textsuperscript{tRNA}Arg \( \sim 25 \) kDa each).

**MtSerRS-MtArgRS Complex Formation Stimulates tRNA Seryl—**To investigate the functional effects of MtSerRS-MtArgRS complex formation, the kinetic parameters of MtSerRS and MtArgRS for their respective tRNAs were determined under steady-state conditions (Table 1). The addition of GST-MtArgRS had a significant effect on aminoacylation by MtSerRS (Fig. 4), resulting in an \( \sim 4 \)-fold higher
catalytic efficiency ($k_{cat}/K_m$) for MtSerRS (Table 1). This increase in catalytic efficiency was the result of a decrease in $K_m$ for tRNA$_{Ser}$ (3-fold) upon addition of GST-MtArgRS that was also accompanied by a slight increase in the $k_{cat}$ for the reaction. As negative controls, neither IleRS, which is not a component of the archaeal complex, nor BSA had an effect on aminoacylation by MtSerRS (Fig. 4). The addition of MtSerRS to MtArgRS, however, resulted in only a slight increase in the catalytic efficiency of MtArgRS, similar to that observed upon the addition of the negative control, BSA (Table 1). Taken together, MtArgRS specifically stimulated the catalytic efficiency of MtSerRS, while aminoacylation by MtArgRS itself remained unaffected by complex formation.

**The Functional Effects of Salt and Temperature on MtArgRS/MtSerRS Complex Formation**—To determine the optimum salt concentration for the seryl-tRNA synthetase in the presence of GST-MtArgRS, the impact of various concentrations of sodium chloride was investigated (Fig. 5). These data indicated the sensitivity of MtSerRS to salt concentrations, resulting in an optimal salt concentration of 200–300 mM NaCl for the seryl-tRNA synthetase reaction. Indeed, a key functional outcome of complex formation is its stimulating effect on the rate of seryl-tRNA synthetase under all ionic conditions tested. As shown in Fig. 5, higher concentrations of salt do not abolish the interaction between MtSerRS and MtArgRS as evidenced by the persistent stimulating effect of MtArgRS, leading to enhanced seryl-tRNA synthetase at 500 mM NaCl (Fig. 5).

Beside the ability to grow at concentrations of salt up to 600 mM (47), it is known that *M. thermautotrophicus* grows at temperatures as high as 65 °C (48). To further analyze the im-

**TABLE 1**

| Kinetic parameters of MtSerRS and GST-MtArgRS for tRNA$_{Ser}$ and tRNA$_{Arg}$, respectively | $K_m$ ($\mu$M) | $k_{cat}$ (s$^{-1}$) | $k_{cat}/K_m$ ($\times 10^3$) |
|---|---|---|---|
| tRNA$_{Ser}$ | GST-MtArgRS + BSA | 4.46 ± 0.45 | 0.026 | 5.8 |
| | GST-MtArgRS + MtSerRS | 4.77 ± 0.51 | 0.034 | 7.1 |
| tRNA$_{Arg}$ | MtSerRS + BSA | 5.61 ± 0.47 | 0.073 | 13 |
| | MtSerRS + IleRS | 5.74 ± 0.48 | 0.061 | 10.6 |
| | MtSerRS + GST-MtArgRS | 2.00 ± 0.22 | 0.100 | 50 |

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Portance of temperature on the MtSerRS-catalyzed reaction in the presence of GST-MtArgRS, proteins were incubated for 2 min at a range of temperatures (30–70 °C, as indicated in Fig. 6) and different salt concentrations (80 or 200 mM NaCl) prior to the start of the reaction. To ensure that any observed effects were not limited by any other condition except the change in temperature, the experiment was conducted at two concentrations of salt and different tRNA concentrations (Fig. 6, A and B). In general, the rates of reaction at 200 mM salt are, as expected, higher than at 80 mM NaCl but the temperature maximum for serylation is constant and in both cases is 65 °C. It is notable that in the presence of BSA, aminoacylation reaction rates at 40–60 °C are relatively constant, while above 60 °C an increase in the reaction rate is observed. On the other hand, the effect is different if instead of BSA, GST-MtArgRS is used. Overall, the MtSerRS-MtArgRS complex was stable at higher temperatures, and the stimulating effect of MtArgRS was more pronounced at higher reaction temperatures. MtArgRS noticeably stimulated MtSerRS at lower temperatures, but it was more efficient at higher temperatures and the stimulation by MtArgRS peaks at 65 °C (almost 3-fold stimulation) (Fig. 6C). These data indicated a wide temperature range of productive complex formation, with an optimum at 65 °C that corresponds to the optimal growth temperature of the organism (48). Taken together, these data may suggest that MtArgRS participates in complex formation with MtSerRS to optimize the serylation reaction at higher temperatures, which may be particularly advantageous for the biosynthesis of proteins in thermophilic organisms.

**DISCUSSION**

**Complex Formation between Archaeal MtSerRS and MtArgRS**—AaRS aminoacylation efficiency can be increased through interactions with N- or C-terminal extensions in cis (49, 50), and by the action of non-synthetase proteins and/or other synthetases acting in trans (21, 27, 32, 40, 41). Here we describe a new example of such an interaction, which appears to be particularly advantageous under adverse conditions; *M. thermautotrophicus* ArgRS, together with tRNA^Arg^, in a ternary complex with an atypical methanogenic-type SerRS (MtSerRS). In general, macromolecular partners of SerRS are poorly investigated and only a few interacting proteins have been identified to date (36, 51). SerRS is not a part of the larger mammalian MSC (52), nor does it participate in formation of the newly discovered bacterial (*Mycoplasma pneumoniae*) MSC (36). Arginyl-tRNA synthetase is part of a

| Salt Concentration (M) | MtSerRS + BSA | MtSerRS + GST-MtArgRS |
|------------------------|---------------|-----------------------|
| 0.108 ± 0.011          | 0.099 ± 0.011 |
| 0.105 ± 0.005          | 0.107 ± 0.010 |

* Degree of MtSerRS stimulation

**FIGURE 4.** Effect of GST-MtArgRS addition on serylation. MtSerRS activity was measured in a standard aminoacylation reaction. Reaction was performed in the presence of BSA (white bars), GST-MtArgRS (black bars), IleRS (), or without addition of any other protein (×) using MbtRNASer.

**FIGURE 5.** Influence of different salt concentrations on serylation by MtSerRS. Reactions were performed in the presence of BSA (white bars) or GST-MtArgRS (black bars). The concentration of MtRNA^Ser^ in the reaction was 6 μM, and concentration of all proteins was 100 nM. The reaction was monitored for 5 min at a temperature of 50 °C. Errors bars indicate the standard deviation from three independent experiments.

**FIGURE 6.** Serylation activity of MtSerRS monitored at different temperatures with BSA or GST-MtArgRS. Empty circles indicate MtSerRS enzyme activity in the presence of BSA, whereas full circles represent MtSerRS enzyme activity in the presence of GST-MtArgRS. A, concentrations of MtSerRS, MtRNA^Ser^ and sodium chloride were 80 nM, 3.17 μM, and 80 mM, respectively. B, concentrations of MtSerRS, MtRNA^Ser^ and sodium chloride were 80 nM, 6.34 μM, and 200 mM, respectively. C, degree of stimulation of MtSerRS by GST-MtArgRS at different temperatures with 80 mM (white vertical bars) or 200 mM NaCl (black vertical bars).
larger MSC in mammals (5) where it is bound to p43 ($K_D = 93 \text{ nM}$) and weakly associates with p38 ($K_D$ greater than 5 mm) (16). Crystallographic studies of ArgRSs from different organisms revealed their modularity and the variability of domains inserted and appended to the active site (53, 54). Based on the yeast enzyme, MtArgRS can be divided into 5 domains (53): the catalytic domain, two appended domains (Add1 and Add2) and two insertions (Ins-1 and Ins-2). Here, in a yeast two-hybrid screen, we captured a shortened MtArgRS, lacking 293 N-terminal amino acids, in a complex with MtSerRS. We determined that the C-terminal domain of SerRS is responsible for mediating interactions with MtArgRS (Fig. 1B). The interacting domain in ArgRS may be the Add2 domain, which is appended beyond the catalytic domain as a C-terminal extension, but this now needs to be further investigated in the presence of tRNAArg$^{\text{Arg}}$. The Add2 domain participates in the recognition of tRNAArg$^{\text{Arg}}$ interacting with its anticodon loop (54), and in so doing may stabilize the MtSerRS-MtArgRStRNAArg ternary complex. The other contacts with tRNAArg are predominantly realized through the Add1 domain, which recognizes dihydrouridine at position 20 in the D-loop, and the catalytic domain, which binds the CCA-end. Neither of these tRNAArg$^{\text{Arg}}$-interacting domains were implicated in the MtArgRS-MtSerRS interaction, further supporting a key role for Add2 in complex formation.

**Archael aaRS Functional Interactions under Extreme Conditions**—Many methanogenic archaea are moderate halophiles and require elevated osmolarity for normal growth and methanogenesis. For example, *M. thermautotrophicus* (strain ΔH) grows very well at concentrations of sodium chloride up to 0.6 M (47), while the cellular concentration of potassium ions in *M. thermautotrophicus* can be as high as 0.65 to 1.1 M (55). Consistent with growth under extreme conditions, the aminoacylation activity of MtSerRS was optimal at higher salt levels than normally associated with mesophilic aaRSs (56). These findings are similar to previous studies that also showed complex formation and robust aminoacylation by LysRS, LeuRS, and ProRS from *M. thermautotrophicus* in the presence of 250 mM KCl (40, 46).

MtSerRS displayed optimal activity in complex with MtArgRS at elevated salt concentrations and at a temperature of 65 °C (Fig. 6). Because of the high content of hydrogen bonds and salt bridges, thermostable proteins are often sluggish at low temperatures, but very flexible at higher temperatures (57). Thus at higher temperatures, for example, MtArgRS may modulate the flexibility of MtSerRS thereby increasing the number of ionic bridges and stabilizing the protein structure. Similarly, an increased number of ionic bridges are observed in the active site of proteins participating in the glutamate dehydrogenase complex of *Pyrococcus furiosus*, when compared with similar complexes from *Clostridium symbiosum* (58). Taken together with previous findings, our data now suggest that aaRS complexes may constitute a part of the thermo- and osmoadaptation mechanisms of thermostable methanogenic archaea, by providing an optimal microenvironment that facilitates stable tRNA aminoacylation under a range of conditions. Whether the presence of tRNAArg$^{\text{Arg}}$ in the complex also influences osmo- and thermoadaptation remains to be investigated, as does the possible association of other proteins and tRNAs as part of a larger archaeal MSC.

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