Differential Modes of Transfer RNA\textsuperscript{Ser} Recognition in Methanosarcina barkeri*

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Two dissimilar seryl-transfer RNA (tRNA) synthetases (SerRSs) exist in Methanosarcina barkeri, one of bacterial type and the other resembling SerRSs present only in some methanogenic archaea. To investigate the requirements of these enzymes for tRNA\textsuperscript{Ser} recognition, seryltransfer variant transcripts of \textit{M. barkeri} tRNA\textsuperscript{Ser} was kinetically analyzed \textit{in vitro} with pure enzyme preparations. Characteristically for the serine system, the length of the variable arm was shown to be crucial for both enzymes, as was the identity of the discriminator base (G73). Moreover, a novel determinant for the specific tRNA\textsuperscript{Ser} recognition was identified as the anticodon stem base pair G30:C40; its contribution to the efficiency of seryltransfer was remarkable for both SerRSs. However, despite these similarities, the two SerRSs do not possess a uniform mode of tRNA\textsuperscript{Ser} recognition, and additional determinants are necessary for seryltransfer specificity by the methanogenic enzyme. In particular, the methanogenic SerRS relies on G1:C72 identity and on the number of unpaired nucleotides at the base of the variable stem for tRNA\textsuperscript{Ser} recognition, unlike its bacterial type counterpart. We propose that such a distinction between the two enzymes in tRNA\textsuperscript{Ser} identity determinants reflects their evolutionary pathways, hence attesting to their diversity.

To maintain translational accuracy, aminoacyl-transfer RNA (tRNA)\textsuperscript{3} synthetases are highly selective toward their amino acid and tRNA substrates. In the process of tRNA recognition, the cognate and non-cognate substrates are discriminated according to characteristic nucleotides in certain positions of the tRNA, specific for the tRNA/synthetase system.\textsuperscript{2} Moreover, a novel determinant for the specific tRNA\textsuperscript{Ser} recognition was identified as the anticodon stem base pair G30:C40; its contribution to the efficiency of seryltransfer was remarkable for both SerRSs. However, despite these similarities, the two SerRSs do not possess a uniform mode of tRNA\textsuperscript{Ser} recognition, and additional determinants are necessary for seryltransfer specificity by the methanogenic enzyme. In particular, the methanogenic SerRS relies on G1:C72 identity and on the number of unpaired nucleotides at the base of the variable stem for tRNA\textsuperscript{Ser} recognition, unlike its bacterial type counterpart. We propose that such a distinction between the two enzymes in tRNA\textsuperscript{Ser} identity determinants reflects their evolutionary pathways, hence attesting to their diversity.

* This work was supported by grants from NIGMS, National Institutes of Health, the U.S. Department of Energy, and the National Aeronautics and Space Administration. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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\textsuperscript{3} The abbreviations used are: tRNA, transfer RNA; RS, tRNA synthetase; SerRS, seryl-tRNA synthetase.

\textsuperscript{2} Numbering of nucleotides in tRNAs was according to M. Sprinzl, K. S. Vassilenko, J. Emmerich, and F. Bauer Compilation of tRNA Sequences and Sequences of tRNA Genes (www.uni-bayreuth.de/departments/biochemie/trna/).

Received for publication, August 2, 2004, and in revised form, September 13, 2004 Published, JBC Papers in Press, September 13, 2004, DOI 10.1074/jbc.M408753200

Published, JBC Papers in Press, September 13, 2004, DOI 10.1074/jbc.M408753200

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**EXPERIMENTAL PROCEDURES**

**Materials**—Oligonucleotides were synthesized and DNAs were sequenced by the Beck Foundation Biotechnology Resource Laboratory at Yale University. [3H]Serine (26 Ci/mmol) and [14C]serine (155 mCi/ 

mmol) were from Amersham Biosciences. Restriction enzymes were from New England Biolabs. NTPs were purchased from Sigma. Expand High Fidelity polymerase and inorganic pyrophosphatase were from Roche Applied Science. pET15b vector was from Novagen. NAP-5 columns were from Amersham Biosciences. Plasmid Thy-7-911 was prepared by digestion with NsiI or BstNI, phenol/chloroform extraction, and ethanol precipitation. NAD was prepared by digestion with NsiI or BstNI, phenol/chloroform extraction, and ethanol precipitation. 50 units of pUC18. Following plasmid maxi preparation, the DNA template sequence. The genes were ligated into the BamHI/HindIII restriction sites of pUC18. Following plasmid maxi preparation, the DNA template was prepared by digestion with NsiI or BstNI, phenol/chloroform extraction, and ethanol precipitation. 50 μg of template was incubated at 42 °C for 1 h at 37 °C in a 0.5-ml reaction mixture that included 40 mM Tris–HCl, pH 8.1, 44 mM MgCl₂, 0.1% Triton, 2 mM spermidine, 10 mM dithiothreitol, and 0.1% SDS. Finally, the tRNA samples were desalted on NAP-5 columns and refolded (5 min at 95 °C followed by a gradual reduction of temperature; 5 mM MgCl₂ was added to the refolding mixture at 55 °C).

**tRNA Cloning and Preparation**—tRNA genes were identified in the preliminary M.arkeri genomic DNA sequence at the Joint Genome Institute using tRNAScan-S.E. (www.geneticwustl.edu/eddy). Wild-type and mutant tRNA genes were constructed from the synthetic oligomers carrying the tRNA gene under the T7 promoter sequence. The genes were ligated into the BamHI/HindIII restriction sites of pUC18. Following plasmid maxi preparation, the DNA template was prepared by digestion with NsiI or BstNI, phenol/chloroform extraction, and ethanol precipitation. 50 μg of template was incubated at 42 °C for 1 h at 37 °C in a 0.5-ml reaction mixture that included 40 mM Tris–HCl, pH 8.1, 44 mM MgCl₂, 0.1% Triton, 2 mM spermidine, 10 mM dithiothreitol, and 0.1% SDS. Finally, the tRNA samples were desalted on NAP-5 columns and refolded (5 min at 95 °C followed by a gradual reduction of temperature; 5 mM MgCl₂ was added to the refolding mixture at 55 °C).

**Enzyme Cloning and Preparation**—Methanogenic and bacterial type SerRS enzymes were identified in the preliminary M.arkeri genomic DNA sequence using JGI BLAST analysis (www.jgi.doe.gov). The DNA sequences were amplified by PCR using Expand High Fidelity polymerase and cloned into the pET15b vector for expression of the N-terminal His₆-tagged proteins. Purification on nickel affinity columns was performed as published (37).

**Aminoacylation Assay**—To determine the amount of the active enzyme in the preparation, active site titration was performed. The assay is based on quantifying the amount of the complex between the enzyme and radioactively labeled amino acid adenylate retained on the nitrocellulose filters (38). The reaction was performed in 0.1 ml of 0.5X EAP buffer (50 mM Tris–HCl, pH 7.5, 5 mM KCl, 5 mM MgCl₂) containing 0.5 units of inorganic pyrophosphatase, 4 mM ATP, and 20 μM [14C]serine (300 cpm/pmol) in the presence of varying concentrations of SerRSs (0.2–5 μM). After 1–10 min of incubation at 37 °C, 30-μl aliquots were spotted onto nitrocellulose filters, filtered, and washed twice with 5 ml of 0.5X EAP buffer. The filters were dried, and the radioactivity was measured by liquid scintillation counting. The amount of active sites varied between 35 and 42%.

**RESULTS**

M.arkeri Possesses Two Functional SerRS Enzymes—Both the bacterial type and the methanogenic type M.arkeri SerRS showed efficient seryltransfer activity with similar affinities for serine (or ATP) (Kₘ values of 25 ± 4 (13.6 ± 1.6) and 34 ± 4 (13.8 ± 1.5) μM for the methanogenic and bacterial type SerRS, respectively) determined in the aminoacylation reaction. Furthermore, both enzymes successfully aminoacylated transcripts of all M.arkeri tRNASer isoacceptors (tRNA₅SerGC), tRNA₅SerCGA, tRNA₅SerGCU, tRNA₅SerGAA, Fig. 1), although with different efficiencies (Table I). tRNA₅SerGC and tRNA₅SerGAA isoacceptors were similar in their kinetic properties toward the methanogenic SerRS. The same is true for the bacterial type enzyme; however, whereas the methanogenic enzyme comparatively showed a notable decrease in the efficiency of tRNA₅SerGC aminoacylation, this isoacceptor seemed to be a preferred substrate for the bacterial type SerRS.

Identity Determinants of tRNA₅Ser—To elucidate tRNA₅Ser identity determinants for the two M.arkeri SerRS enzymes, mutant tRNA₅SerCGA species were produced (Fig. 2) based on the conservation of the nucleotides among tRNA isoacceptors and on the known identity requirements of bacterial and eukaryotic serine systems. Kinetic analysis of their seryltransfer efficiency revealed that a number of mutations remarkably affected the relative k₅/k₆ values for both enzymes (Table II). Contributions of the specific positions and structural elements in different tRNA domains are discussed below.

The importance of the discriminator base and of nucleotides from the first three base pairs in the acceptor stem has been shown in E. coli by in vivo identity conversion of a leucine to a serine suppressor tRNA (6) and emphasized by work on tRNA₅Ser minihelices (18). The nucleotides in this part of the M.arkeri tRNA₅Ser species are most conserved, which suggests their involvement in specific tRNA₅Ser recognition. Accordingly,
mutational analysis shows a dramatic drop of the aminoacylation efficiencies for variants of G73 for both enzymes, as well as for the first base pair position in the case of the methanogenic SerRS (mutants 1–4). Inability of these mutants to be efficiently serylated results primarily from the decrease in their $k_{\text{cat}}$ values. However, the contribution of the acceptor stem is limited to its upper part, as mutations of the conserved base pairs C2:G71, C3:G70 and G6:C67 (mutants 5–9) either retained the wild-type activity or resulted in a slight stimulation of serylation.

The D-arm and the D-loop have been identified as elements contributing to tRNA$^{\text{Ser}}$ identity. In addition to the changes in the acceptor stem and the discriminator base, the \textit{in vivo} identity conversion from bacterial tRNA$^{\text{Leu}}$ to tRNA$^{\text{Ser}}$ required the change from G11:C24 to C:G (6). This base pair, however, is not conserved among tRNA$^{\text{Ser}}$ isoacceptors in \textit{M. barkeri}. Furthermore, bacterial tRNAs$^{\text{Ser}}$ share a specific D-loop feature, marked by a deletion at position 17 and a double insertion of the 20a and 20b nucleotides. The significance of this structural element is manifested through its involvement in the tertiary interactions with the variable arm, whose orientation is thus defined (7). On the other hand, deletion of position 17 is rather infrequent in archaeal serine tRNAs, as are the insertions at positions 20a and 20b in the D-loop. tRNA$^{\text{Ser}}$, isoacceptors from \textit{M. barkeri} do not share a uniform structure: although tRNA$^{\text{SerCGA}}$ and tRNA$^{\text{SerGGA}}$ possess deletion 17, tRNA$^{\text{SerGCU}}$ contains a double insertion at the same position. When a CC insertion was introduced into tRNA$^{\text{SerCGA}}$, the resulting variant showed a somewhat reduced serylation efficiency (mutant 12) as did the mutation of A9 (mutant 10). Possibly, these alterations affected the tertiary structure of tRNA$^{\text{Ser}}$, because the D-loop interacts with the T-loop and because position 9 participates in formation of base triplets in yeast tRNA$^{\text{Phe}}$, tRNA$^{\text{Leu}}$, and \textit{E. coli} tRNA$^{\text{Arg}}$ (7). A similar effect was observed for mutant 11, although the relative contributions of its kinetic parameters to serylation efficiency appear to differ between the two enzymes. These results imply that conservation of the G10:C25 base pair among \textit{M. barkeri} and a significant number of other archaeal tRNAs$^{\text{Ser}}$ is not coincidental.

The anticodon was expectedly shown not to contribute to serine identity (mutant 15), in accordance with the published results of other serine systems (27). However, mutation of G30:C40 in the anticodon stem resulted in a significant loss of serylation activity (mutants 13 and 14) for both enzymes with notable $K_m$ effect. Although the upper part of the anticodon stem has been suggested to possess discriminatory function by footprinting experiments in the yeast system (base pair A27:U43) (19), none of the anticodon stem nucleotides have directly been identified as recognition determinants in a mutational study. Support of such a notion comes from the fact that high conservation of nucleotides in this region of bacterial tRNAs$^{\text{Ser}}$ cannot be detected; contrary to that, the G30:C40 base pair is absolutely conserved among archaeal tRNAs$^{\text{Ser}}$ species. This position has been identified as an identity determinant for human phenylalanyl-tRNA synthetase (40). Our preliminary results have indicated that the anticodon arm of tRNA$^{\text{Ser}}$ also participates in specific recognition by \textit{M. maripaludis} SerRS.\textsuperscript{3}

\textsuperscript{3} I. Gruic-Sovulj, personal communication.

\begin{table}[h]
\centering
\caption{Serylation kinetics of tRNA$^{\text{Ser}}$ isoacceptors by the two \textit{M. barkeri} SerRSs}
\begin{tabular}{|c|c|c|c|c|c|c|}
\hline
Transcript & Methanogenic SerRS & & & Bacterial type SerRS & & \\
& $K_m$ expressed in $\mu$M; $k_{\text{cat}}$ expressed in min$^{-1}$; $k_{\text{cat}}/K_m$ expressed in min$^{-1}$.$\mu$M$^{-1}$ & & & $K_m$ & $k_{\text{cat}}$ & $k_{\text{cat}}/K_m$ \\
\hline
\text{tRNA$^{\text{SerCGA}}$} & 3 $\pm$ 0.2 & 183 $\pm$ 8.6 & 61 & 2.9 $\pm$ 0.2 & 64 $\pm$ 3.1 & 22 \\
\text{tRNA$^{\text{SerGGA}}$} & 4.7 $\pm$ 0.1 & 263 $\pm$ 17.6 & 55.9 & 2.6 $\pm$ 0.1 & 54 $\pm$ 3.1 & 20.7 \\
\text{tRNA$^{\text{SerGCU}}$} & 5.3 $\pm$ 0.4 & 68 $\pm$ 3.6 & 12.8 & 1.3 $\pm$ 0.1 & 55 $\pm$ 3.6 & 42.3 \\
\hline
\end{tabular}
\end{table}
Serylation kinetics of tRNA\textsuperscript{Sec} variants by the two SerRSs from M. barkeri

| tRNA       | Methanogenic SerRS | Bacterial type SerRS |
|------------|--------------------|----------------------|
|            | \(K_m\) \(k_{cat}\) | rel \(k_{cat}/K_m\) | \(K_m\) \(k_{cat}\) | rel \(k_{cat}/K_m\) |
| G73-U(A)   | 4.4 ± 0.2          | 4.7 ± 0.7            | 0.02 | 4.7 ± 0.6 | 0.9 ± 0.1 | 0.009 |
| G73-A(A)   | 9.2 ± 0.7          | 9.0 ± 0.8            | 0.02 | 2.1 ± 0.2 | 0.2 ± 0.009 | 0.005 |
| G73-C(A)   | 18 ± 1.6           | 6.6 ± 0.6            | 0.008 | 1.8 ± 0.1 | 0.1 ± 0.006 | 0.005 |
| G1:CC72-A(U) | 5.6 ± 0.5        | 4.7 ± 0.5            | 0.01 | 5.2 ± 0.6 | 37 ± 4.8 | 0.3 |
| C2:G71-I(U) | 3.3 ± 0.2         | 182 ± 6             | 0.9 | 3.9 ± 0.2 | 139 ± 13.6 | 1.6 |
| C3:G70-G(C) | 3 ± 0.1            | 166 ± 5.6           | 0.9 | 2.6 ± 0.1 | 95 ± 4.9 | 1.6 |
| C4:G70-A(U) | 4 ± 0.1            | 168 ± 5             | 0.9 | 3 ± 0.2 | 50 ± 6.5 | 0.9 |
| C6:G70-I(U) | 6.3 ± 0.6         | 274 ± 15.3          | 0.7 | 5.9 ± 0.3 | 85 ± 7.1 | 0.7 |
| A9-C(U)    | 2.2 ± 0.2          | 239 ± 11.5          | 1.8 | 0.8 ± 0.3 | 27 ± 1.9 | 1.5 |
| G10:C25-A(U) | 9.8 ± 1           | 135 ± 6.4           | 0.2 | 3.7 ± 0.3 | 27 ± 2.2 | 0.3 |
| C59 addition in D-loop (12) | ND*              | ND*                 | 0.05 | ND* | 34 ± 4.4 | 42 ± 1.4 | 0.06 |
| G30:C40-C(G) | 6.7 ± 0.5       | 346 ± 27.7          | 0.8 | 41 ± 0.4 | 73 ± 5.8 | 0.8 |
| Anticodon - UAG (15) | 5.4 ± 0.3       | 263 ± 28.9          | 0.8 | 3.4 ± 0.2 | 44 ± 4.2 | 0.6 |
| U addition in variable loop (18) | 21 ± 3.6        | 549 ± 49.8          | 0.4 | 3.7 ± 0.5 | 57 ± 4.6 | 0.7 |
| Ce17:G27-A(U) | 6.4 ± 0.4      | 72 ± 13             | 0.7 | 5.1 ± 0.4 | 13 ± 1.1 | 0.1 |
| C12:G22 deletion (20) | 13 ± 1.6       | 4.8 ± 0.6           | 0.006 | 26 ± 4.2 | 64 ± 4.7 | 0.1 |
| U:A addition in variable arm (22) | 7.3 ± 0.3      | 2.3 ± 0.2           | 0.005 | 5.5 ± 0.4 | 1.4 ± 0.1 | 0.01 |
| G48 substitution by UU (23) | 8.8 ± 0.8      | 163 ± 13.9          | 0.3 | 2 ± 0.1 | 68 ± 6.5 | 1.5 |
| C45-U(C) | 6.8 ± 0.8          | 63 ± 0.4            | 0.02 | 2.9 ± 0.2 | 12 ± 1.1 | 0.2 |
| C48-U(C) | 20 ± 1.6           | 329 ± 25            | 0.3 | 3.9 ± 0.3 | 118 ± 11.1 | 1.4 |
| G49:C65-G(C) | 21 ± 2           | 268 ± 18            | 0.2 | 11 ± 1.3 | 59 ± 10 | 0.2 |
| Wild-type tRNA\textsuperscript{Sec} | 3 ± 0.2         | 183 ± 8.6           | 1 | 2.9 ± 0.2 | 64 ± 3.1 | 1 |

*ND, not determined. Because of the high \(K_m\) values of mutants 11, 13, and 14 for the methanogenic SerRS, \(k_{cat}/K_m\) values were obtained from the slope of the plot of initial rates against substrate concentrations.

Itors, it makes the largest contribution to serine identity, as confirmed by different experimental approaches (9, 17, 41). Despite direct contacts that have been observed between T. thermophilus SerRS and the variable arm (7), sequence alterations of the variable arm only insignificantly affected the serylation efficiency (27). It was therefore concluded that the variable arm of tRNA\textsuperscript{Sec} is not recognized sequence specifically, but its length was shown to be most critical for efficient serylation. As many SerRSs serylate tRNASer species, whose variable arm is not recognized sequence specifically, alterations of the variable arm only insignificantly affected the aminoacylation efficiency by the methanogenic enzyme. Despite direct contacts that have been observed between T. thermophilus SerRS and the variable arm (9, 17, 41), elimination of the two unpaired U residues at the 3'-base of the variable arm would affect tRNASer recognition, G46 was replaced by a double base pair in the variable arm that is defined by the number of unpaired nucleotides between the 3'-base of the variable stem and the nucleotide at position 48 (none in tRNA\textsuperscript{Sec}, one in tRNA\textsuperscript{Leu}, and two in tRNA\textsuperscript{Ser}). Whereas deletion of one base pair in the leucine system caused only a small decrease in leucylation (9), elimination of the two unpaired U residues at the 3'-base of the variable arm in E. coli tRNA\textsuperscript{Ser} led to a 150-fold reduction in the aminoacylation efficiency (14). Unlike E. coli, M. barkeri tRNA\textsuperscript{Ser} species possess one unpaired nucleotide at the 3'-base of the variable arm, tRNAs\textsuperscript{Leu} possess two, while a long variable arm is not present in archaeal tRNAs\textsuperscript{Ser} (43). To assess whether a tRNA\textsuperscript{Leu}-like structure of the variable stem base would affect tRNA\textsuperscript{Ser} recognition, G46 was replaced by a double insertion of U (mutant 23); indeed, decrease in serylation efficiency was observed, although more conspicuously for the methanogenic SerRS. Moreover, mutation of C48 reduced relative affinity of the tRNA\textsuperscript{Ser} for the methanogenic enzyme without decrease of its ability to be serylated by the bacterial type SerRS (mutant 24). Finally, mutation of the G48:C65 pair moderately reduced serylation efficiency for both enzymes by increasing their \(K_m\) values (mutant 25).

Transplantation of Serine Recognition Elements to tRNA\textsuperscript{Leu}—As a means of affirming the entirety of the identity set determined for a given system, transplantation into a non-cognate tRNA is commonly performed. In studies dealing with serine identity, tRNA\textsuperscript{Leu} is frequently chosen as a host tRNA (23, 27). Conversion of leucine to serine identity in E. coli requires alterations of C2:G71 to G:C and G50 to A, changes in the D-loop and in the pairing pattern in the variable arm, and introduction of the tertiary base pair G15:C48 (27); the same type of conversion in yeast is achieved by a single nucleotide insertion to the long variable arm (23, 27). Our results show that adaptation of M. barkeri tRNA\textsuperscript{Ser} to a serine-accepting variant requires several changes: enlargement of the variable arm, reduction in the number of unpaired nucleotides at the 3'-base of the variable stem, mutation of A49:U65 to G:C, and change of the discriminator base from A to G (Fig. 3). Serylation of the tRNA\textsuperscript{Leu} variant containing these modifications was...
clearly detectable (Table III, variant ML3), but its efficiency was further improved in the case of the bacterial type SerRS by an additional mutation (change of C22 to U, variant ML1), which allowed formation of a D-stem consisting of four base pairs, as in tRNA^{Ser}_{CGA}. Evidently, the serylation potential of variant ML1 is comparable with that of the wild-type tRNA^{Ser}_{CGA} transcript, which substantiates the completeness of the determined tRNA^{Ser} identity set. Our results also show that omission of some of the transplantation elements from the serine-chargeable tRNA^{leu}_{CAA} variants results in either complete loss of serylation or in much less efficient serylation (variants ML2, ML4, and ML5).

**DISCUSSION**

Two SerRS Enzymes in M. barkeri—Among the Methanosarcinaceae only M. barkeri contains two SerRS enzymes, whereas the other members of this genus possess only the bacterial type SerRS. Because M. barkeri has a canonical cysteinyl-tRNA synthetase, neither SerRS is needed to participate in Cys-tRNA synthesis as was suggested for some methanogens (34, 44).

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**tRNA^{Ser} Identity in M. barkeri**—Specificity of tRNA aminoacylation is maintained both by structural and kinetic discrimination of tRNA molecules in the network of aminoacyl-tRNA synthetases. Evidently, tRNA recognition is contextually dependent and identity requirements for a given system are influenced by characteristics of other isoaccepting systems with substantially similar tRNA substrates. It is widely assumed that tRNA^{leu}_{CAA}, because of its long variable arm, presents a possible candidate for misacylation by serine, and discrimination between tRNA^{Ser}_{CGA} and tRNA^{leu}_{CAA} has received great attention (13, 25, 45). It has been suggested that the discriminator base G73 in E. coli tRNA^{Ser} serves as an anti-determinant for leucyl-tRNA synthetase and tyrosyl-tRNA synthetase, where it is most disfavored (27). Furthermore, other parts of the acceptor stem are believed to possess similar functions, because G2:C71 in tRNA^{Ser} was proposed to contribute to the rejection of the C2-G71-bearing tRNA^{leu}_{CAA} (27). The M. barkeri SerRS enzymes, however, work in a different milieu, as both tRNA^{Ser} and tRNA^{leu}_{CAA} possess the C2-G71 pair and their possible discrimination at this position is thus thwarted. However, the base pair C3-G70 is conserved among M. barkeri tRNA^{Ser}_{CGA} isoacceptors, and, as all M. barkeri tRNA^{leu} isoacceptors also have the G3-C70 base pair, the discriminatory role of this position was tested. However, none of the changes reduced serylation efficiency (Table II, mutants 6–8). Considering these observations, it may have been advantageous for the M. barkeri SerRSs to recognize the discriminator base, as our mutational study has confirmed.

Furthermore, discrimination against tRNA^{Leu} and tRNA^{Tyr} in bacteria is achieved by recognition of the variable arm orientation, specific for each of the type 2 tRNAs. The recent crystal structure of T. thermophilus TyrRS complexed to tRNA^{Tyr} shows that orientations of the variable arms in tRNA^{Ser} and tRNA^{Tyr} differ by ~50° because of the number of unpaired bases preceding nucleotide 48 (46). The specific direction of the variable arm in tRNA^{Ser} is defined by stacking of the base G20b with the first base pair in the variable stem. This insertion, although present in bacterial tRNA{^{Ser}}^{Ser}, is absent in most archaeal and cytoplasmic eukaryotic serine isoacceptors, which, together with the disparity between bacterial and archaeal tRNA{^{Ser}}^{Ser} in the number of unpaired bases at the 3'-end of the variable arm, suggests that details of the core packing of tRNA{^{Ser}} may differ between bacteria and archaea. This assumption is additionally supported by the fact that bacterial SerRSs have to discriminate between three tRNAs containing a long variable arm, whereas lack of this structural element in archaeal tRNA{^{Ser}}{^{Ser}} obviates the need for its rejection. However, our results imply that some elements of tertiary structure are pertinent to the specific tRNA{^{Ser}} recognition by both methanogenic and bacterial type SerRS.

In general, the two M. barkeri SerRS enzymes recognize their tRNAs in a similar fashion: both strongly depend on G73 identity, on the length of the variable arm, and on the G30:C40 pair in the anticodon stem for efficient serylation. However, some aspects of their identity requirements are remarkably different; whereas the methanogenic SerRS relies on G1:C72 identity, the bacterial type enzyme does not (mutant 4). Likewise, change in the number of unpaired nucleotides at the base of the variable stem dramatically affects serylation of the methanogenic, but not the bacterial, type SerRS (mutant 23). Such notable distinction could possibly be correlated to the evolutionary divergence of the two enzymes (discussed below).

Considering the structural aspects of tRNA{^{Ser}} recognition, we may infer that the N-terminal domain in the methanogenic SerRS participates in tRNA{^{Ser}} variable arm recognition, analogous to the helical arm of the bacterial SerRS counterparts. Conservation of the long variable arm in the methanogenic tRNA{^{Ser}} species and the effects of variable stem shortening on the serylation activity of the methanogenic enzyme (Table III, mutants 20 and 21) support this possibility. On the other hand, structural interpretations of acceptor stem recognition by the methanogenic SerRS are not possible, because of the divergence.
of the bacterial and methanogenic sequences within the motif 2 loop and the lack of structural data. However, sequence alignments may suggest participation of methanogenic M.arkeri SerRS Glu-338 in discriminator base recognition. As to the bacterial enzyme, its motif 2 loop shows a great degree of similarity with the loop of T.therophilus SerRS; therefore, it may be proposed that Glu-230 of the M.arkeri SerRS is involved in G73 recognition analogous to Glu-260 in the T.therophilus structure (47). Unfortunately, the available data are insufficient to comment on anticodon stem recognition.

**Evolutionary Aspects of tRNA<sup>Ser</sup> Recognition**—As patterns of tRNA recognition reflect variations in both tRNA and their corresponding aminoacyl-tRNA synthetases, their inspection is intriguing and serendipitous for preserving tRNA identity. Given the similarity of the tRNASer identity loop of the bacterial and methanogenic sequences within the motif 2 loop, one of the SerRS enzymes may possibly perform a cellulose function unrelated to direct aminoacylation of tRNA<sup>Ser</sup>.

Regarding tRNA recognition, we can hypothesize adaptation of the bacterial SerRS to the methanogenic environment: differences in recognition of the pairing patterns of the variable arm and absence of recognition of positions 2:71 and 3:70 by the two SerRSs (mutants 22 and 5–8) may be indicative of tRNA-driven alteration of the identity requirements. Supposing that M.arkeri acquired the bacterial type of SerRS from a bacterium with identity requirements similar to the ones defined for the E. coli system, it would have been challenged to recognize the archaeal tRNA substrate in a highly specific manner. The most conspicuous differences between archaeal and bacterial tRNAs<sup>Ser</sup> are located in the acceptor stem, where the G2:C71 base pair is absolutely conserved in bacteria, whereas the majority of archaeal tRNAs<sup>Ser</sup> contain a C:G pair at the same position. Furthermore, A:U is the most common base pair at position 3:70 in bacterial tRNAs<sup>Ser</sup> unlike C3:G70 in archaea. In that respect, and given the way the acceptor stem of tRNA<sup>Ser</sup> is identified in E. coli (27), the bacterial type SerRS may have modified its identity requirements and adopted G73, pertinent to both archival and bacterial tRNAs<sup>Ser</sup>, as one of the major elements of serine identity.

**Acknowledgments**—We thank Kevin Sowers for the gift of M.arkeri DNA and Constantinos Stathopoulos and Alexandre Ambrogelly for valuable help with this project.

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J. Biol. Chem. 2004, 279:48780-48786.
doi: 10.1074/jbc.M408753200 originally published online September 13, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M408753200

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