An RNA Binding Site in a tRNA Synthetase with a Reduced Set of Amino Acids†

Sunghoon Kim, Lluis Ribas de Pouplana, and Paul Schimmel*

Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Received May 19, 1994; Revised Manuscript Received July 5, 1994®

ABSTRACT: A 30 amino acid helix-loop of known structure on the surface of the C-terminal domain of the class I *Escherichia coli* methionine tRNA synthetase is essential for methionine tRNA anticodon discrimination. Replacing this 30 amino acid peptide with a previously described sequence containing residues from the wild-type protein imbedded in a sequence matrix of mostly alanines and serines, we used a combinatorial mutagenesis and selection strategy to define residual wild-type residues that are not replaceable with alanine or serine, because they are needed for specific recognition of methionine tRNA. Four were identified, of which three have functional side chains (Asn, Arg, Lys). These four and a fifth (Trp) that was previously identified are located at the end of the helix and within the loop, lie on the same side of the structure, and span a distance of about 20 Å. We conclude that, within the alanine, serine sequence matrix, only a few non-alanine, non-serine residues in the specificity-determining part of the structure are essential.

To a first approximation, tRNA synthetases in each of the two classes have two major domains that are important for catalysis and tRNA interactions (Cusack et al., 1991; Burbaum & Schimmel, 1991b; Moras, 1992). One is the class-defining active site domain, which is believed to be the earliest form of a synthetase and which incorporates determinants for acceptor–helix interactions. These interactions are necessary for aminoacylation of RNA oligonucleotides that recapitulate the acceptor helix of the tRNA molecule, and the amino acid/RNA sequence relationships manifested by these reactions have suggested an operational RNA code for amino acids that is related in some way to the genetic code (Schimmel et al., 1993). For a given synthetase, the sequence of the class-defining catalytic domain is reasonably conserved through evolution, from prokaryotes or lower eukaryotes to humans (Hou et al., 1991; Shiba et al., 1994). Fused to the conserved domain is a second domain, which typically is idiosyncratic to the synthetase even within the same class and, in addition, for a given synthetase is poorly conserved. This domain provides for interactions with parts of the tRNA outside of the acceptor helix, including in some instances the anticodon or the variable loop (Brunie et al., 1990; Rould et al., 1991; Perona et al., 1991; Cavarelli et al., 1993; Cusack et al., 1993; Biou et al., 1994). The high sequence divergence of this domain, even for the same synthetase, suggests a much greater selective pressure on the domain needed for catalysis and acceptor–helix interactions than on that needed for anticodon binding (Shiba et al., 1994).

With these considerations in mind, we sought to investigate further the potential for variability of the nonconserved domain of a tRNA synthetase which makes contact with the anticodon, and see whether it was possible to vary a limited region within this domain in a way that generates synthetases capable of different anticodon sequence specificities. Related to this objective was our interest in exploring the design of binding sites that have a reduced set of amino acids, if for no other reason than to reduce the number of variables needed to analyze and define the essential features needed for a specific conformation as well as for specific contacts with a ligand.

In this work, we investigated the anticodon binding domain of the class I *Escherichia coli* methionyl-tRNA synthetase. The three-dimensional structure of a 547 amino acid active N-terminal fragment has been determined (Brunie et al., 1990) and, while a co-crystal with bound $tRNA^{Met}$ has not been obtained, biochemical and genetic data, together with computer modeling, have defined a 30 amino acid helix-loop in the nonconserved domain as critical for anticodon contacts (Meinnel et al., 1990; Ghosh et al., 1990; Perona et al., 1991; Kim et al., 1993a). This motif extends from Lys439 to Glu468 and encompasses Trp461, which has been implicated as making direct contact with C^{34} of the CAU anticodon (Ghosh et al., 1990), while other residues are thought to lie along the anticodon stem-loop structure.

In an earlier study, we made a sequence library with binary combinations of either alanine (hydrophobic)/serine (hydrophilic) or the wild-type residue, at most positions in the 30 amino acid motif (Kim et al., 1993b). From a genetic selection we obtained a variant designated AV16 (active variant 16) in which 10 of the first 13 residues (positions 439–451) in the helix consisted of alanine or serine and, of the remaining 17 residues, seven were alanine or serine (Figure 1). We also established that the remaining three non-alanine/serine residues (Val, Ile, Leu) in the first 13 were not essential for contact with the anticodon, but rather could be replaced in other active variants that were obtained. We concluded that functional contacts with the anticodon stem-loop motif were made with residues in the C-terminal 17 amino acids of the helix-loop motif.

Of these 17 amino acids, seven had not been tested for the possibility of an alanine or serine replacement. One of them, Trp461, was not varied because previous work by others and by us suggested it was essential (see below). The remaining six positions (N452, R453, E457, P460, K465, and E467) flank either side of the critical W461 and are well conserved among *E. coli*, *Saccaromyces cerevisiae* (mitochondrial), *Thermus thermophilus*, and *Bacillus stearothermophilus* methionyl-tRNA synthetases (Kim et al., 1993b). Five of these six residues bear functional side chains and, at least superficially, are candidates for direct interactions with bases or the backbone of the bound RNA. We thought that an

† This work was supported by Grant No. GM 23562 from the National Institutes of Health and by an award from the National Foundation for Cancer Research.

* To whom correspondence should be addressed.

® Abstract published in *Advance ACS Abstracts,* September 1, 1994.

0006-2960/94/0433-11040$04.50/0

attempt to replace some or all of these residues with alanine/serine could lead to the identification of which functional groups were essential. In addition, based upon the spatial locations of the replacements in active versus inactive variants, we thought that the side or face of the helix-loop structure needed for interactions with the anticodon stem-loop motif might be identified. For this purpose, we created a sequence library of binary combinatorial variants which allowed for alanine/serine or the wild-type residue at each of the aforementioned six positions and selected for active variants. This new combinatorial library was created in the context of the previously studied AV16 active variant (see above) which already had 17 alanine or serine residues in the 30 amino acid motif. By using AV16 as a starting structure, we were able to focus more sharply on that subregion of the 30 amino acid motif which most likely contained residues making direct contact with the RNA.

MATERIALS AND METHODS

Construction of Ala/Ser Combinatorial Sequence Library. Phagemid pJB104 encodes a monomeric form of *E. coli* methionyl-tRNA synthetase containing 547 amino acids (Kim & Schimmel, 1992). Substitutions for alanine and serine at each of the six variable positions (N452, R453, E457, P460, K465, E467) were mostly chosen so as to make only one change between the codon for the wild-type residue and that for alanine or serine. At the AAA codon for K465, a total of four different codons (AAA for lysine, AGC for serine, AGA for arginine, and AAC for asparagine) were generated, because all of the codons for alanine and serine would have at least two changes from the AAA codon for K465. At the GAA codon for E457 and E467, a GCA codon for Ala was introduced, because none of the six codons for Ser were accessible by a single substitution. The 74-mer oligonucleotide synthesized for combinatorial mutagenesis was designed to have an equal amount of the nucleotides for the wild-type and non-wild-type residues. Another oligonucleotide (48-mer) was synthesized to have 15 bases at its 3′ end antiparallel complementary to the 15 bases on the 3′ end of 74-mer mutagenic oligonucleotide. These two oligonucleotides were annealed in their complementary region and thus mutually prime their complementary strands for DNA synthesis by incubation at 37 °C with T7 DNA polymerase in 40 mM Tris-HCl buffer (pH 7.5), 20 mM $MgCl_2$, 50 mM NaCl, 5 mM dithiothrietol, and 1 mM dNTPs. The ends of these double-stranded oligonucleotides (107-mer) contained artificially designed *Nar*I and *Bss*HII restriction sites, so that the ends of the double-stranded oligonucleotides could be then digested with *Nar*I and *Bss*HII restriction enzymes.

Phagemid pSKNB was derived from phagemid pJB104 which expresses the monomeric form (547-mer) of methionine tRNA synthetase and activity for ampicillin resistance, and contains *Nar*I and *Bss*HII sites in the *metG* gene resulting from K439A (GGTAAA → GGC GCC) and G468A (GGC → GCG) substitutions. Phagemid pSKNB was cleaved with *Nar*I and *Bss*HII, and the gene encoding the 30 amino acid peptide motif was replaced with the *Nar*I-*Bss*HII digested oligonucleotides encoding the mutant sequences. The ligation mixture was then transformed into the *E. coli metG* null strain MN9261/pRMS615 which is a tester strain for genetic complementation (Kim et al., 1993b). Strain MN9261[V355/ F^{-}I^{-} *lacZ*$^{-}$ *galK2 galT22 rpsL179 recD1014* Δ*metG*] has an ablation of the *metG* gene encoding methionyl-tRNA synthetase. Cell viability is normally maintained by plasmid pRMS615 which has a temperature-sensitive replicon and expresses functional methionyl-tRNA synthetase and activity for chloramphenicol resistance. Because of the temperature sensitivity of the pRMS615 maintenance plasmid for replication, strain MN9261/pRMS615 loses the maintenance plasmid at 42 °C and cannot grow at this temperature unless an active methionyl-tRNA synthetase is provided from a second compatible plasmid.

Selection/Screen for Active and Inactive Variants. The phagemid pSKNB recombinants containing the *Nar*I–*Bss*HII mutagenic oligonucleotides were transformed into strain MN9261/pRMS615, the cells were allowed to recover at 30 °C for 1 h under no selective pressure in Luria Broth, and the transformants were then plated (at appropriate dilutions to give single colonies) at 30 °C onto Luria Broth plates containing 40 μg/mL of ampicillin. (This procedure was followed to assure that any colonies subsequently isolated were independent.) Single colonies of ampicillin-resistant transformants were then randomly picked and transferred to 42 °C to find enzyme variants which did or did not complement the *metG* null strain. Loss of the pRMS615 maintenance plasmid was verified by checking for sensitivity to 20 μg/mL of chloramphenicol. After the analysis for the complementation phenotype at 42 °C, individual complementing and noncomplementing *metG* genes encoded by plasmid pSKNB were sequenced through the oligonucleotide cassette which had been transplanted into the unmutagenized gene *via* the created *Nar*I and *Bss*HI restriction sites. In all cases, the presence of these restriction sites was confirmed.

Protein Purification and Enzyme Assay. In vivo stability of the enzyme variants was determined by Western blot analysis. Proteins in each cell lysate were resolved by SDS–polyacrylamide gel electrophoresis and were transferred onto an Immobilon-P membrane using a Milliblot-SDE System (Millipore). The methionyl-tRNA synthetase on the membrane was complexed with rabbit anti-methionyl-tRNA synthetase polyclonal antibodies and then detected using the enhanced chemiluminescence (ECL) system (Amersham) (Burbaum & Schimmel, 1991a). The wild-type and mutant enzymes were purified by fast protein liquid chromatography (FPLC) on a MonoQ HR10/10 column (Pharmacia) following the procedure previously described (Kim & Schimmel, 1992). Purified proteins were used to determine the kinetic parameters for aminoacylation of $tRNA^{fMet}$ at pH 7.5, 37 °C, over a concentration range for $tRNA^{fMet}$ of 0.25–32 μM (Kim & Schimmel, 1992).

RESULTS

Ala/Ser Combinatorial Sequence Library in Anticodon-Binding Motif. AV16 is one of the active derivatives of *E. coli* methionine tRNA synthetase containing a total of 17 alanines and serines in the 30 amino acid anticodon-binding peptide motif which extends from position K439 to G468 (Figure 1). This variant was selected from the alanine/serine combinatorial substitution library. The estimated sequence complexity of this library was 1.9×10^7, and the active sequence variants were found at a frequency of approximately 1% (Kim et al., 1993b).

In the present work, we varied the six positions flanking the critical W461 in the C-terminal half of the 30 amino acid helix-loop which we held fixed in the previous study. W461 was also held fixed in this work because of previous results demonstrating its importance (Ghosh et al., 1990; Meinnel et al., 1991; Schulman, 1991) and because we found that W461A and W461S mutants did not complement the MN9261/pRMS615 null strain (S. Kim and P. Schimmel, unpublished

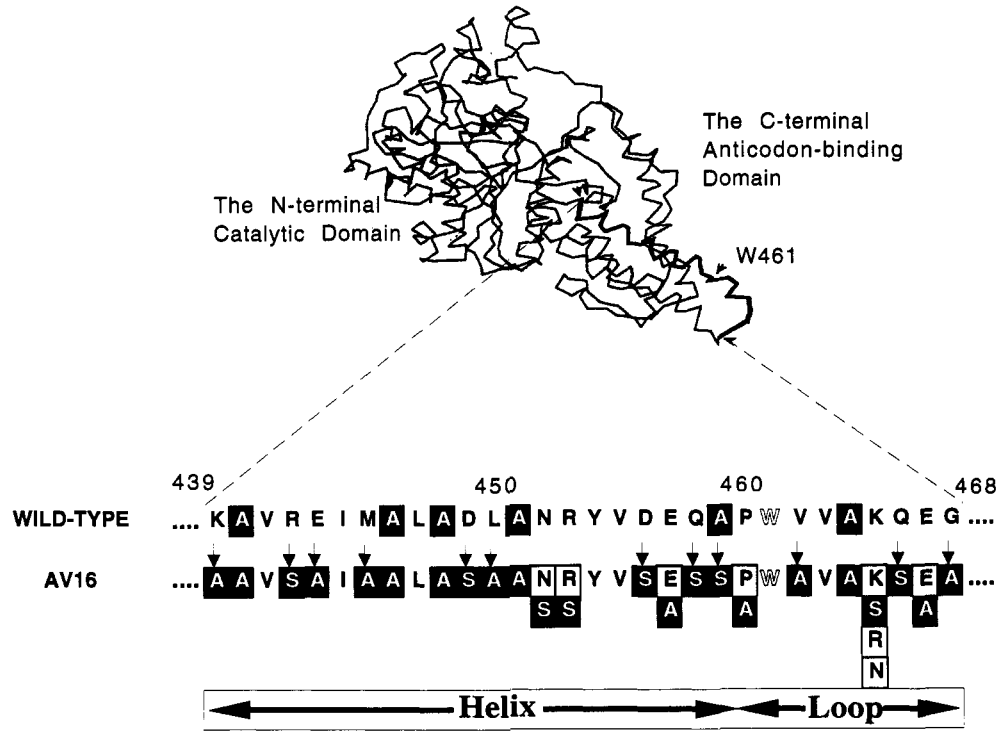

FIGURE 1: (Top) Schematic illustration of the C^{α} backbone of *E. coli* methionyl-tRNA synthetase (Brunie et al., 1990), with the anticodon-binding helix-loop peptide motif from K439 to G468 highlighted. (Bottom) Amino acid sequences of the wild-type and active variant AV16 helix-loop motif. Alanine and serine residues are highlighted in black, and the arrows indicate positions of alanine and serine replacements in the wild-type enzyme to create AV16. The amino acid sequence of AV16 (Kim et al., 1993b) was used as a starting sequence to replace previously fixed residues with alanine or serine. These residues are N452, R453, E457, P460, K465, and E467, and the substitutions introduced in a combinatorial fashion in this work are boxed with the AV16 residues.

data). The combinatorial sequence library based on substitutions of the remaining six residues (see Materials and Methods for details) generated a total of 128 different peptide sequences (two codons for each of positions 452, 453, 457, 460, and 467 and four codons for position 465) which ranged from no to all six amino acid substitutions (Figure 1). The *Nar*I–*Bss*HII cleaved oligonucleotides encoding these substitutions were transplanted into *Nar*I and *Bss*HII sites of phagemid pSKNB and then introduced into strain MN9261/pRMS615 for analysis by complementation of the *metG* null allele.

Active and Inactive Variants. The *E. coli metG* null strain (MN9261/pRMS615) lacks the chromosomal copy of *metG* which encodes methionine tRNA synthetase (Kim et al., 1993b). However, this strain grows at 30 °C because it contains a copy of *metG* in plasmid pRMS615 which has a temperature-sensitive replicon. Because of the temperature sensitivity of plasmid maintenance, the gene for methionine tRNA synthetase is lost at 42 °C so that cells harboring the null allele cannot grow at this temperature. If methionyl-tRNA synthetase expressed from phagemid pSKNB containing the sequence variants is functional *in vivo*, then growth at 42 °C is rescued.

The sequence variants were classified into two groups on the basis of their ability to complement the MN9261/pRMS615 strain at 42 °C. Six different active sequence variants (including AV16) were found by their ability to complement cell growth at 42 °C (Figure 2A). These randomly sequenced active variants were independently scored two to four times. The repetitive and independent scoring of the same six variants suggests that all active enzymes in the library of 128 variants have been identified.

Considering next a sample of 16 variants which did not complement the *metG* null strain, wild-type and substituted residues were found at each of the the variable positions (Figure 2B). Also, all but the inactive SIV6 variant (found twice) was found only once. Thus, the sequence library had the expected high diversity of sequences and did not have a codon bias introduced during DNA synthesis or during the construction of the sequence library in phagemid pSKNB. The number of substitutions among the characterized inactive variants ranged from a single K465N substitution in SIV2 to five mutations in SIV15. Although the inactive variants did not complement the *metG* null strain, each accumulated *in vivo*, as shown by our ability to detect them by immunoblots of crude extracts resolved by gel electrophoresis (data not shown). Thus, the inactivity of these mutants suggests that at least some of the varied residues may be involved in tRNA recognition and are not simply to stabilize the folding of the protein so as to prevent its digestion by proteases.

The collective pattern of substitutions in the active and inactive variants seemed to be random, without bias for a particular combination of residues. This observation confirmed that the mutagenesis was as intended. However, in contrast to the sequences of the inactive variants, the active sequences showed a strong resistance to multiple mutations. Active variant SAV1 had a conservative K465R substitution (while inactive SIV2 had a single K465N replacement), SAV2 and SAV3 had single E467A and E457A substitutions, respectively, and SAV5 had the double E457A/E467A substitution. SAV4 also had the E457A change, combined with the conservative K465R. Thus, ignoring the conservative K465R substitution, N452, R453, P460, and K465 were all maintained among the active variants.

Aminoacylation Activity. Determination of the aminoacylation activity of the mutant enzymes is one way to monitor the interaction with $tRNA^{Met}$. To investigate how alanine substitutions at E457 and E467 affect the aminoacylation of $tRNA^{Met}$, one of the active variants, SAV5 (containing E457A

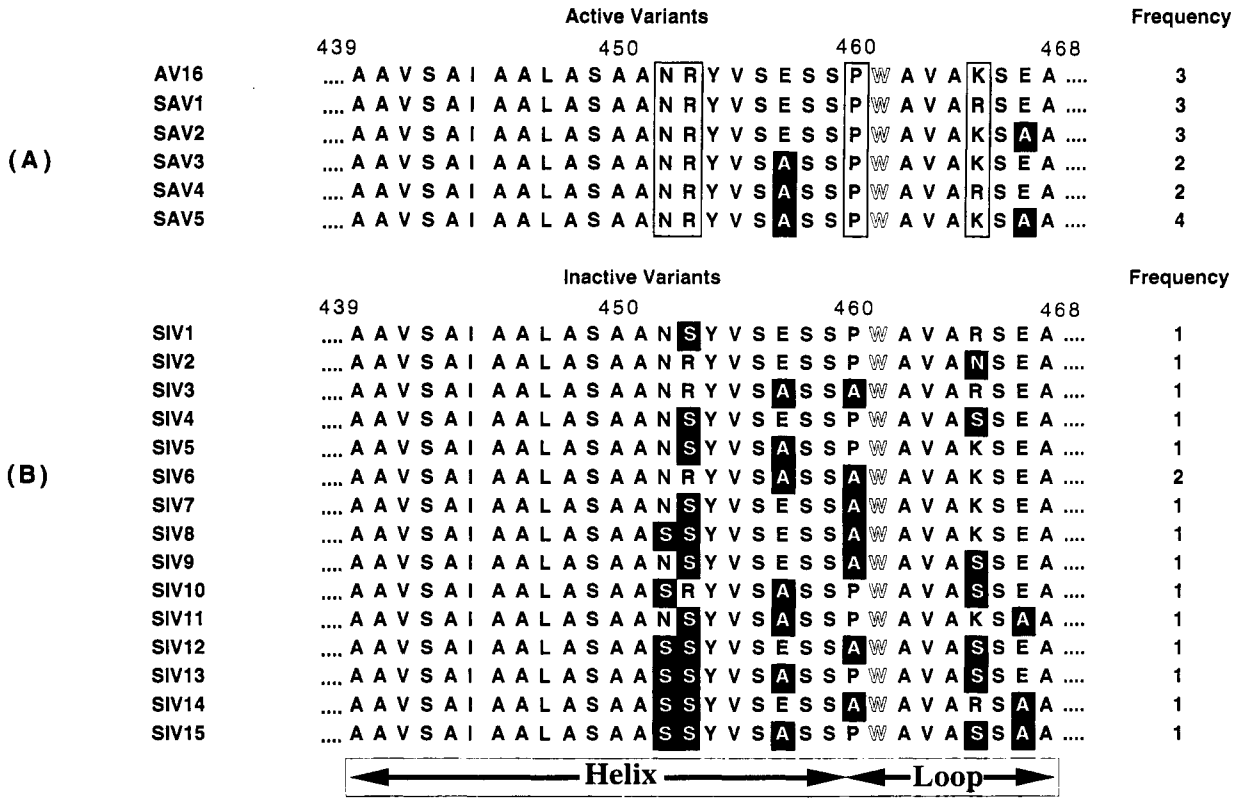

FIGURE 2: (A) Sequences of active variants selected from the combinatorial library. The rectangular boxes enclose positions which were subjected to mutagenesis but were not varied in any of the active variants. Black boxes highlight positions which were substituted with alanine. The invariant W461 is highlighted with an open letter. The frequency at which each variant was independently scored in a random selection of mutagenized plasmids which complemented the null strain are indicated on the right. (B) Sequences of 15 inactive variants with substitutions (other than K465R) in AV16 highlighted in black, and the frequency at which each was independently obtained in a random selection of inactive clones is indicated on the right. Each of these mutants is stable *in vivo* and can be detected by Western blot analysis of crude cell extracts.

Table 1: Kinetic Parameters

	K_m (tRNA^{fMet}) (μM)	k_{cat} (s^{-1})	k_{cat}/K_m (M^{-1} s^{-1})
wild-type N547	2.5	1.7	0.68
SAV5	5	0.8	0.16

and E467A substitutions) was purified and its enzyme activity was compared with that of the wild-type enzyme (Table 1). SAV5 showed an approximately 2-fold increase in K_m for tRNA^{Met} and a comparable decrease in k_{cat}. Because SAV5 can serve as the sole source or methionyl-tRNA synthetase *in vivo*, we also surmise that this variant is highly specific for tRNA^{Met}. Presumably, a significant breakdown in specificity would lead to charging of methionine to tRNAs other than tRNA^{Met}, and this, in turn, would be toxic. Thus, because the activity and apparent specificity of the SAV5 variant is comparable to that of the wild-type enzyme, we imagine that the helix-loop epitope has the same or a closely similar conformation to the part of the wild-type protein that is critical for RNA contacts.

DISCUSSION

A potential limitation to the application of combinatorial libraries to generate multisubstitutions in ligand binding sites is the likelihood of generating proteins which are defective in folding or stability so that, because they do not accumulate *in vivo*, they cannot be tested for function, either by enzyme assays *in vitro* or by complementation assays *in vivo*. All 15 of the randomly isolated inactive variants investigated here (Figure 2B) accumulated *in vivo*, suggesting that the protein structure *per se* was not greatly perturbed by placing substitutions in the chosen region. For this reason, we believe that the failure to generate active variants containing alanine or serine at positions N452, R453, P460, and K465 reflects the importance of these residues for the anticodon stem-loop interaction *per se* and not for the global stability of the protein.

Figure 3 displays the region of interest in a ribbon format, where the α-carbons of the alanine/serine residues in SAV5 are shown by dark cross-hatched circles, the residues varied in this study are marked with arrows, and the black circles indicate the α-carbon positions of residues found to be not replaceable. In this figure, the immutable N452, R453, P460, W461, and K465 are seen to lie on the same side of the helix-loop motif which faces outward, away from the core of the protein, with the five residues spanning a distance of about 20 Å. The replaceable E467 is outside of the boundaries of the face defined by these five residues. Because they bear functional side chains, N452, R453, and K465, in addition to W461, are likely candidates for a direct interaction with the backbone and bases of the RNA. On the other hand, P460 is most likely required to terminate the α-helix, which ends at position 458–459. Because alanine is a strong helix former, and because there are not strong helix breakers after P460, we imagine that a P460A substitution extends the helix and that this extension, in turn, prevents W461 and K465 from making a required contact with the bound tRNA.

In a recently reported randomized mutagenesis of the same region of methionyl-tRNA synthetase investigated here, Schmitt et al. (1993) noted the importance of N452 for the activity of the enzyme. While their studies were not intended to investigate the importance of individual residues such as N452 within the context of an alanine/serine sequence matrix,

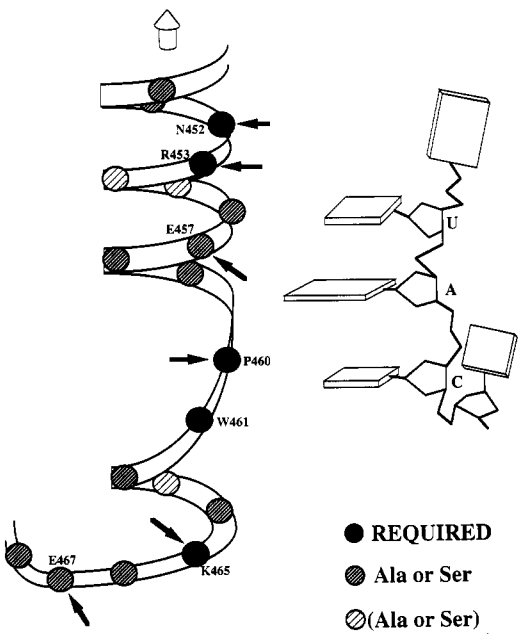

FIGURE 3: (Left) Ribbon representation of the C-terminal region (from position 450 to 468) of the 30 amino acid helix-loop motif of the active SAV5 variant of *E. coli* methionyl-tRNA synthetase, based on the structure determined by Brunie et al. (1990). The positions of the α-carbon atoms are shown as circles. Arrows designate the six residues subjected to combinatorial mutagenesis in this work. Black circles designate amino acids that were found to be not replaceable. Densely cross-hatched circles are alanine or serine; lightly cross-hatched circles are based on the wild-type enzyme sequence, but in other variants were found to be replaceable with alanine or serine (Kim et al., 1993b). (Right) The anticodon and flanking bases represented as boards, based on the structure of Basavappa and Sigler (1991). The dimensions of the peptide backbone on the left and of the anticodon motif on the right are approximately to scale.

they noted the significance of N452 in the context of the wild-type enzyme's sequence. In particular, a single N452A substitution reduced k_{cat}/K_m for aminoacylation to under 3% of that of the wild-type enzyme, and a N452F substitution reduced activity to under 0.05%.

Apart from the five residues highlighted in Figure 3, there are three additional positions which do not contain alanine or serine (light cross-hatched circles). These are Y454, V455, and V463, and each is contained in the AV16 progenitor of SAV5. In our previous study, we reported the isolation of active variants which contained substitutions at these positions, but no variant which had substituted all three of these residues. Y454 and V455 face the interior of the protein and are believed important for stabilizing the helix of AV16, while energy calculations suggest that V463 is needed for stabilizing a particular loop structure in AV16 (Kim et al., 1993b). Thus, it seems unlikely that side chains of residues other than N452, R453, W461, and K465 make essential direct contacts with the RNA. Although we cannot exclude the possibility that new specific interactions are generated by the multiple substitutions that are present in SAV5, we believe that the sensitivity of the activity to the conformation of this part of the structure severely limits the likelihood for these kind of compensations.

Of the 19 alanine and serine residues in the SAV5 30 amino acid RNA binding motif that extends from position 439 to 468, 10 are located between 439 and 451, and three occur after the important K465, at positions 466–468. Thus, the 16 positions collectively encompassed by 439–451 and 466–468 have 13 of the alanine/serine substitutions of SAV5. The higher mutability of the two sides which flank 452–465 provide evidence that they are not directly involved in RNA contacts but rather contribute to the framework which supports the 452–465 region. In the 452–465 segment, there are a total of six alanine/serine residues in SAV5 so that, to achieve a functional and selective RNA binding determinant, about half of the residues in the specificity-determining part of the structure had to be residues other than alanine or serine.

Ghosh et al. (1991) and Kim et al. (1993) presented evidence that a separate peptide from Asn387 to Arg395, which is close in space to the 452–465 segment investigated here, also contributes to the anticodon binding site of methionyl-tRNA synthetase. This segment was not varied in the present work, and yet the multiple alanine, serine substitutions in SAV5 apparently do not perturb the Asn387–Arg395 peptide in a way that affects its interaction with the anticodon. The success of the present experiments may depend in part on an inherent design in at least some class I tRNA synthetases which enables them to evolve different anticodon binding specificities through major variations in the sequence of the anticodon binding motif, without at the same time perturbing the active site domain. Thus, the protrusion of the 30 amino acid helix-loop motif from the surface of the C-terminal domain (Figure 1) may provide a design more accommodating to sequence variations than a design where the anticodon binding site would be in a deep cavity.

ACKNOWLEDGMENT

We thank Drs. R. Basavappa and P. Sigler for providing us with the coordinates of *E. coli* $tRNA^{fMet}$.

REFERENCES

Basavappa, R., & Sigler, P. B. (1991) *EMBO J. 10*, 3105–3111.

Biou, V., Yaremchuk, A., Tukalo, M., & Cusack, S. (1994) *Science 263*, 1404–1410.

Brunie, S., Zelwer, C., & Risler, J. (1990) *J. Mol. Biol. 216*, 411–424.

Burbaum, J. J., & Schimmel, P. (1991a) *Biochemistry 30*, 319–324.

Burbaum, J. J., & Schimmel, P. (1991b) *J. Biol. Chem. 266*, 16965–16968.

Cavarelli, J., Rees, B., Ruff, M., Thierry, J.-C., & Moras, D. (1993) *Nature 362*, 181–184.

Cusack, S., Hartlein, M., & Leberman, R. (1991) *Nucleic Acids Res. 19*, 3489–3498.

Cusack, S., Berthet-Colominas, C., Biou, V., Borel, F., Fujinaga, M., Hartlein, M., Krikliviy, I., Nassar, N., Price, S., Tukalo, M. A., Yaremchuk, A. D., & Leberman, R. (1993) in *The Translation Apparatus* (Nierhaus, K. H., Franceschi, F., Subramanian, A. R., Erdmann, V. A., & Wittmann-Liebold, B., Eds.) pp 1–12, Springer-Verlag, Berlin.

Ghosh, G., Pelka, H., & Schulman, L. H. (1990) *Biochemistry 29*, 2220–2225.

Ghosh, G., Kim, H. Y., Demaret, J.-P., Brunie, S., & Schulman, L. H. (1993) *Biochemistry 30*, 11767–11774.

Hou, Y.-M., Shiba, K., Mottes, C., & Schimmel, P. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 976–980.

Kim, H. Y., Pelka, H., Brunie, S., & H., S. L. (1993a) *Biochemistry 32*, 10506–10511.

Kim, S., & Schimmel, P. (1992) *J. Biol. Chem. 267*, 15563–15567.

Kim, S., Ribas de Pouplana, L., & Schimmel, P. (1993b) *Proc. Natl. Acad. Sci. U.S.A. 90*, 10046–10050.

Meinnel, T., Mechulam, Y., Dardel, F., Schmitter, J.-M., Hountondji, C., Brunie, S., Dessen, P., Fayat, G., & Blanquet, S. (1990) *Biochimie 72*, 625–632.

Meinnel, T., Mechulam, Y., Le Corre, D., Panvert, M., Blanquet, S., & Fayat, G. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 291–295.

Moras, D. (1992) *Trends Biochem. Sci. 17*, 159–164.

Perona, J. J., Rould, M. A., Steitz, T. A., Risler, J.-L., Zelwer, C., & Brunie, S. (1991) *Proc. Natl. Acad. Sci. U.S.A. 88*, 2903–2907.

Rould, M. A., Perona, J. J., Söll, D., & Steitz, T. A. (1991) *Nature 352*, 213–218.

Schimmel, P., Giege, R., Moras, D., & Yokoyama, S. (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*, 8763–8768.

Schmitt, E., Meinnel, T., Panvert, M., Mechulam, Y., & Blanquet, S. (1993) *J. Mol. Biol. 233*, 615–628.

Schulman, L. H. (1991) *Prog. Nucleic Acids Res. 41*, 23–87.

Shiba, K., Suzuki, N., Shigesada, K., Namba, Y., Schimmel, P., & Noda, T. (1994) *Proc. Natl. Acad. Sci. U.S.A.* (in press).