C-Terminal Peptide Appendix in a Class I tRNA Synthetase Needed for Acceptor-Helix Contacts and Microhelix Aminoacylation†

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ABSTRACT: The 10 class I tRNA synthetases have an N-terminal nucleotide-binding fold which contains the catalytic center. Insertions into the nucleotide-binding fold provide contacts for acceptor-helix interactions, which stabilize the amino acid acceptor end of the tRNA substrate in the active site. A separate and largely nonconserved C-terminal domain provides contacts with distal parts of the tRNA, including the anticodon. For *Escherichia coli* methionyl tRNA synthetase, whose structure is known, the C-terminal domain is predominantly α-helical and forms a loop which interacts with the anticodon trinucleotide located about 76 Å from the amino acid attachment site. Fused to the end of this helical domain is a peptide which curls back into the N-terminal nucleotide-binding fold and region of the active site. We show here that mutations in this peptide appendix disrupt aminoacylation and binding of a 7 base pair microhelix substrate based on the acceptor stem of $tRNA^{fMet}$, without affecting interactions with ATP or methionine or with the $tRNA^{fMet}$ anticodon. The impairment of acceptor-helix interactions by mutation of the C-terminal peptide can offset favorable anticodon interactions and severely reduce aminoacylation of $tRNA^{fMet}$. Thus, in addition to, or as an alternative to, acceptor-helix-binding insertions into the N-terminal nucleotide-binding fold, C-terminal peptide epitopes in some class I enzymes may provide a mechanism for facilitating RNA microhelix interactions with the catalytic site.

Specific recognition of cognate tRNAs and amino acids by aminoacyl tRNA synthetases is the basis of the genetic code. Aminoacyl tRNA synthetases are classified into two groups on the basis of conserved sequence motifs and their associated three-dimensional structures (Eriani *et al.*, 1990; Burbaum & Schimmel, 1991; Cusack *et al.*, 1991; Nagel & Doolittle, 1991). The class I enzymes include 10 members that are characterized by the conserved 11 amino acid signature sequence that ends in the HIGH tetrapeptide (Webster *et al.*, 1984; Ludmerer & Schimmel, 1987) and by the KMSKS pentapeptide located in the Rossman (nucleotide-binding) fold, which contains the catalytic center (Hountondji *et al.*, 1986). The conserved catalytic domain is located in the N-terminal half of the respective proteins. The class II enzymes contain three degenerate sequence motifs that are part of a seven-stranded antiparallel β-sheet that is the framework on which the active site is constructed. The conserved domain may be at either the N- or C-terminal side of the protein, depending on the specific class II enzyme.

Although the recognition of tRNAs by at least some of the class I enzymes utilizes the anticodon, sequence-specific interactions with the acceptor stem of tRNA also occur, as demonstrated by the aminoacylation of acceptor-stem-containing oligonucleotides by the class I methionyl (Martinis & Schimmel, 1992) and valyl tRNA synthetases (Frugier *et al.*, 1992). However, residues in class I enzymes involved in acceptor-helix interactions have been difficult to identify by genetic or biochemical methods because the acceptor-helix interaction is not easily separated from the interaction of the protein with the anticodon. In this work, we identified a peptide which is appended to the anticodon-binding domain of *Escherichia coli* methionyl tRNA synthetase and which interacts with the acceptor sequence of its cognate tRNA.

E. coli methionyl tRNA synthetase is a homodimer of two 676 amino acid polypeptides (Dardel *et al.*, 1984). A C-terminal truncation of this protein produced by trypsin digestion results in an approximately 550 amino acid active monomeric N-terminal fragment (Cassio & Waller, 1971) whose crystal structure has been solved at 2.5 Å resolution (Zelwer *et al.*, 1982; Brunie *et al.*, 1987, 1990). An active monomeric recombinant enzyme was constructed by introducing tandem stop codons after Lys547 (Kim & Schimmel, 1992), as described earlier (Mellot *et al.*, 1989). The N-terminal domain (1–360) contains the Rossman (nucleotide-binding) fold of alternating β-sheets and α-helices, and the C-terminal domain (361–519) is mainly α-helical (Figure 1). The conserved sequence motifs located in the N-terminal domain form structures for interactions with ATP and methionyl AMP. Cross-linking of 3′-oxidized $tRNA^{Met}$ identified peptides in the N-terminal domain as important for interactions with the acceptor end of $tRNA^{Met}$ (Hountondji *et al.*, 1990). Cross-linking between the anticodon of $tRNA^{Met}$ and the protein (Valenzuela & Schulman, 1986), as well as mutagenesis (Ghosh *et al.*, 1990) and molecular modeling (Perona *et al.*, 1991), established that the C-terminal domain interacts with the anticodon.

The N- and C-terminal domains are noncovalently linked by the C-terminal extension (L520–K547), which folds back to the N-terminal domain and overhangs the active site cleft (Figure 1). This peptide segment is important for maintaining the active site conformation (Mellot *et al.*, 1989; Brunie *et al.*, 1990). A short motif (Y531–D535) is conserved between methionyl tRNA synthetases from *E. coli*, *Bacillus stearothermophilus*, and *Saccharomyces cerevisiae* (cytoplasmic) (Figure 1). This peptide forms a part of the active site cleft and was proposed to guide docking of the tRNA acceptor end to the active site cleft (Mellot *et al.*, 1989).

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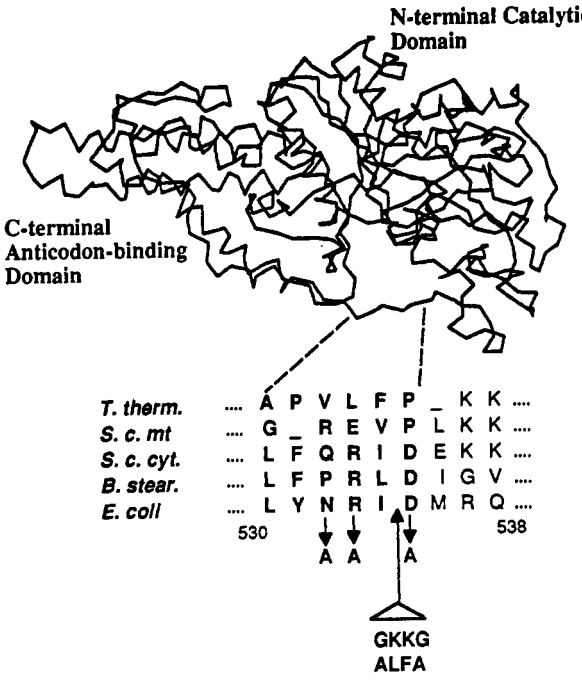

FIGURE 1: α-Carbon diagram of *E. coli* methionyl tRNA synthetase (top) and the sequences of methionyl tRNA synthetases from different species (bottom) which correspond to the L530–D535 region of *E. coli* methionyl tRNA synthetase. Arrows indicate the residues changed to alanine and the site at which the tetrapeptides (GKKG and ALFA) were inserted.

To investigate this possibility, point and insertion mutations were made, and the resulting mutants were tested for complementation of a *MetG* null strain which has an ablation of the gene (*MetG*) for methionyl tRNA synthetase from the chromosome (S. Kim and P. Schimmel, unpublished observations). These mutants were also checked for their activity with whole $tRNA^{fMet}$ and with a 7-bp microhelix based on the acceptor stem. In this way, we were able to analyze the functional significance of the appended peptide and, in particular, to investigate acceptor-helix interactions apart from interactions with the rest of the tRNA.

MATERIALS AND METHODS

Construction and Purification of Mutants. Phagemid pJB104 encoding the monomeric 547-mer of *E. coli* methionyl tRNA synthetase (Kim & Schimmel, 1992) was used to construct various mutants (Figure 1). N532A, R533A, and D535A substitutions were made by site-directed mutagenesis (Amersham) using the mutagenic primers, 5′-CCTCATATCGATGCGGGCATACAGCGCCTTGAA-3′, 5′-CTGCCTCATATCGATGGCGTTATACAGCGCCTT-3′, and 5′-ACCTGCCTCATAGCGATGCGGTT-3′, respectively. To generate insertion mutants after I534, phagemid pJB104 was cleaved with *Cla*I and ligated with the double-stranded synthetic oligonucleotide prepared by annealing 5′-CGGCAAAAAGGG-3′ and 5′-CGCCCTTTTTGC-3′, whose 5′-ends were phosphorylated. This double-stranded oligonucleotide can be ligated in two orientations, each of which encodes a tetrapeptide of sequence GKKG or ALFA. These mutant proteins were expressed in the *E. coli MetG* null strain, MN9261/pRMS615 (see below), and were purified to homogeneity according to the method previously described (Kim & Schimmel, 1992).

In Vivo Complementation Test and Western Analysis. In vivo enzyme activities of the mutants were tested by genetic complementation of *E. coli* strain MN9261/pRMS615, whose chromosome has a deletion of the *MetG* gene encoding methionyl tRNA synthetase. The viability of this strain is maintained at the permissive temperature (30 °C) by methionyl tRNA synthetase encoded from the maintenance plasmid pRMS615. The strain loses the maintenance plasmid at the nonpermissive temperature (42 °C) because its replication is temperature-sensitive, and therefore it requires functional enzyme from a second plasmid for cell growth (Jasin *et al.*, 1984).

To detect the mutant enzymes expressed in the null strain, Western blot analysis was conducted using rabbit antiserum raised against *E. coli* methionyl tRNA synthetase. Cells grown at 42 °C were lysed by ultrasonication, and proteins in the lysates were separated by 8% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE[1]). The proteins resolved by the gel were then transferred to membranes (Immobilon, Millipore), and methionyl tRNA synthetase was detected by Western blot analysis with anti-methionyl tRNA synthetase polyclonal antibodies and an enhanced chemiluminescence detection system (Amersham).

Amino Acid Activation Activities of Mutants. Amino acid activation activities of the mutants were determined by the methionine-dependent [^{32}P]pyrophosphate–ATP exchange assay using the purified mutant enzymes (Calendar & Berg, 1966). The reactions were carried out in 100 mM Tris-HCl (pH 8.0), 10 mM β-mercaptoethanol, 0.1 mM EDTA, 5 mM $MgCl_2$, 10 mM methionine, 2 mM ATP, 2 mM ^{32}P-labeled sodium pyrophosphate, 10 mM KF, and 0.1 mg/mL BSA containing 5 nM of each mutant enzyme at 37 °C. Aliquots were removed at time intervals and mixed with 15% perchloric acid containing 0.4 M sodium pyrophosphate to quench the reaction. The [^{32}P]ATP bound to the enzyme was adsorbed to charcoal, which was then filtered through the membrane pads (Schleicher and Schuell) via a vacuum manifold and washed once with 20 mM sodium pyrophosphate solution and three times with ice-cold distilled water. The membrane-containing charcoal was mixed with scintillation cocktail (Hydrofluor) and the cpm were determined.

Aminocylation of $tRNA^{fMet}$ and Methionine Microhelix. The aminoacylation reaction of $tRNA^{fMet}$ was initiated with each enzyme (1 nM) and 4 μM $tRNA^{fMet}$ (Boehringer-Mannheim), 20 μM ^{35}S-labeled methionine, and 2 mM ATP in 20 mM HEPES buffer (pH 7.5) containing 4 mM $MgCl_2$, 100 μM EDTA, 150 mM NH_4Cl, and 0.1 mg/mL BSA at 37 °C. Aliquots of the reaction were taken at time intervals and spotted onto membranes (glass microfiber filter pad, 2.3 cm diameter, Whatman) presoaked with 5% trichloroacetic acid containing 1 mM methionine. The membranes were washed in 5% ice-cold trichloroacetic acid containing 1 mM methionine, and the [^{35}S]methionine remaining on the membrane was determined by scintillation counting. To determine kinetic parameters of the wild-type and R533A mutant enzymes for aminoacylation, the reaction was carried out in the presence of 0.15–100 μM $tRNA^{fMet}$. The aminoacylation of $microhelix^{fMet}$ was conducted in the presence of 2 μM enzyme and 100 μM $microhelix^{fMet}$ under the reaction conditions used for the aminoacylation of $tRNA^{Met}$, as described above (Martinis & Schimmel, 1992; Kim & Schimmel, 1992). The aminoacylation activities for initiator and elongator $tRNA^{Met}$ were indistinguishable (S. Martinis, personal communication).

[1] Abbreviations: ACE, affinity coelectrophoresis; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid); BSA, bovine serum albumin; bp, base pair.

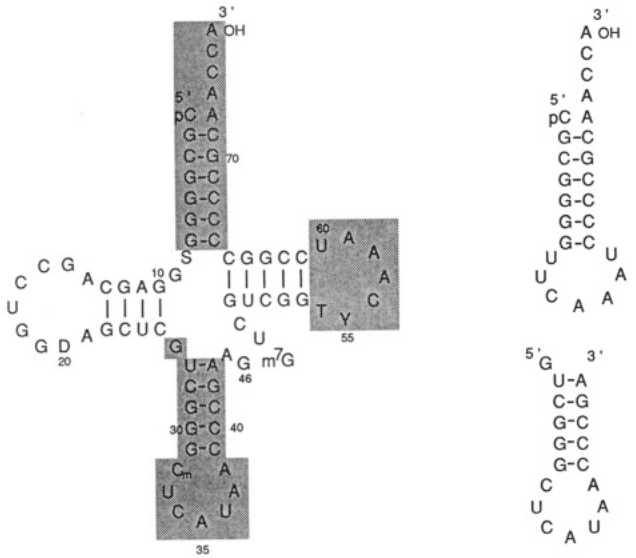

FIGURE 2: Sequence and cloverleaf structure of $tRNA^{fMet}$ (left), sequences and hairpin structures of $microhelix^{fMet}$ (top right), and anticodon stem–loop (bottom right) RNA substrates.

Affinity Coelectrophoresis. Affinity coelectrophoresis was used to investigate the binding of the wild-type and mutant enzymes to the RNA substrates which were based on anticodon stem–loop and acceptor-stem sequences of $tRNA^{fMet}$ under equilibrium conditions (Lee & Lander, 1991) (Figure 2). A 1% low melting point agarose (BRL) was prepared in 50 mM HEPES (pH 7.5), 0.1 mM EDTA, 4 mM $MgCl_2$, 1 mM β-mercaptoethanol, and 0.1 mg/mL BSA. A Teflon comb with 10 parallel bars that have a footprint of 35 × 2 mm separated by 5 mm was placed on Gelbond film (FMC BioProducts) in a Plexiglass casting tray, and a Teflon comb that made a well measuring 73 × 1 mm was placed 4 mm from one end of the parallel bars. The agarose was poured to a depth of about 2 mm. When the combs were removed, 10 parallel 35 × 2 mm wells perpendicular to a 73 × 1 mm slot resulted. The appropriate protein in a volume of 75 μL was then prepared in a series of concentrations in buffer at twice the desired final concentrations. The range of protein concentration was from 1 to 125 μM for the anticodon stem–loop experiments and from 10 to 350 μM for the microhelix experiments. These samples were mixed with an equal volume of 2% low melting point agarose and loaded into the 35 × 2 mm wells. Then [5′-^{32}P]RNA (50 000–150 000 cpm) in a volume of 35 μL was also mixed with an equal volume of 2% low melting point agarose and loaded into the 73 × 1 mm slot along with 0.02% bromophenol blue and 0.02% xylene cyanol. The gel was electrophoresed at 100–150 V for 1.5–2 h in a water-cooled circulating gel box (Hoefer Super Sub Model HE100) at 25 °C. The gel running buffer was the gel buffer without β-mercaptoethanol and BSA. Gels were dried in an open vacuum oven with low heat and exposed to a Phosphorimager screen (Molecular Dynamics).

The dissociation constants between the proteins and methionine anticodon stem–loop substrate were determined by measuring the shift of [^{32}P]RNA in each protein lane. These values are divided by the distance the free RNA traveled past the beginning of the protein lanes to give the relative shift (R). A Scatchard plot of R vs $R/[\text{protein}_{tot}]$ gave a linear plot whose slope is equal to $-1/K_d$ (Lim *et al.*, 1991).

RESULTS

In Vivo Complementation Test. The C-terminal linker peptide of a monomeric *E. coli* methionyl tRNA synthetase spans from L520 to K547. A short sequence from L530 to D535 contains conserved residues among methionyl tRNA synthetases (Figure 1). To identify whether this motif is involved in acceptor-stem interactions, alanine substitutions were made at N532, R533, and D535, and two tetrapeptides (GKKG and ALFA) were inserted between I534 and D535 (Figure 1). All of the plasmid-borne mutant proteins accumulated *in vivo,* as determined by Western blot analysis, implying that these mutations did not disrupt the native conformation of methionyl tRNA synthetase (data not shown).

Table I: Relative Amino Acid Activation and $tRNA^{fMet}$ Aminoacylation Activities of Enzymes with Substitutions and Insertions at the C-Terminal Appendix of *E. coli* Methionyl tRNA Synthetase N547[a]

proteins	amino acid activation	aminoacylation of $tRNA^{fMet}$
wild-type	100	100
N532A	78	101
R533A	65	2
D535A	104	138
^{534}IGKKGD^{535}	52	53
^{534}IALFAD^{535}	96	169

[a] Reactions for amino acid activation and $tRNA^{fMet}$ aminoacylation were carried out as described in Materials and Methods. The initial velocity of each reaction by the mutant enzyme was normalized to that of the wild-type enzyme.

In vivo $tRNA^{fMet}$ aminoacylation activities of the mutant proteins were tested in the temperature-sensitive MN9261/pRMS615 *E. coli MetG* null strain, which needs extrachromosomal methionyl tRNA synthetase activity for viability at the nonpermissive temperature (42 °C). The null strain is maintained by plasmid pRMS615, which has a temperature-sensitive replicon that is defective at 42 °C. Strain MN9261/pRMS615 consequently does not grow at 42 °C unless rescued by a compatible plasmid encoding methionyl tRNA synthetase activity. Each of these mutants complemented the tester strain, implying that the residues in this peptide region are not essential for amino acid activation and aminoacylation of $tRNA^{fMet}$ (data not shown). Some mutants containing insertions of larger than four amino acids (up to 12 amino acids) on the C-terminal side of I534 were also found to complement the tester strain, indicating that this site can accommodate relatively large peptide insertions without complete loss of activity (data not shown).

In Vitro Amino Acid Activation and tRNA Aminoacylation. The mutants were purified to determine activities for amino acid activation and aminoacylation of $tRNA^{fMet}$ and $microhelix^{fMet}$. Amino acid activation activities of the point mutants were more than 65% of the wild-type activity, suggesting that residues in this peptide are not responsible for the activation of methionine (Table I). This result is consistent with the crystal structure of the protein, which shows that this peptide epitope is at least 15–20 Å from ATP- and methionine-binding sites. The two insertion mutants also showed little difference in amino acid activation activity, suggesting that these insertions do not change the active site conformation for activation of methionine.

The $tRNA^{fMet}$ aminoacylation activities of the mutants were compared with that of the wild-type. Except for R533A, the mutant enzymes showed a less than 2-fold difference from the wild-type activity (Table I). In constrast, the activity of the R533A mutant was reduced by approximately 50-fold from the wild-type. Kinetic parameters for $tRNA^{fMet}$ aminoacylation by the R533A mutant showed that its K_m for tRNA was increased by approximately 20-fold and k_{cat} was decreased by approximately 2-fold (Table II). Therefore, its relative

Table II: Kinetic Parameters for Aminoacylation of $tRNA^{fMet}$ by Wild-Type and R533A Mutant Enzymes at pH 7.5 and 37 °C[a]

proteins	K_m (μM)	k_{cat} (s^{-1})	relative k_{cat}/K_m
N547 wild-type	2.5	1.7	1
N547 R533A	50.0	0.8	2.6×10^{-2}

[a] Aminoacylation reactions were carried out as described in Materials and Methods in the range of 0.15–100 μM $tRNA^{fMet}$.

Table III: Dissociation Constants of the Wild-Type and Mutant Enzymes for the Anticodon Stem–Loop and Microhelix RNA Substrate at pH 7.5 and 37 °C[a]

	dissociation constant (K_d)	
proteins	anticodon stem–loop (μM)	microhelix (μM)
wild-type	27.7 ± 0.5	249
R533A	5.4 ± 1.0	not detectable
Δ11(Y454–A464→S)	not detectable	not determined

[a] The anticodon stem–loop hairpin helix and microhelix were synthesized on the basis of $tRNA^{fMet}$ (Figure 2). The K_d values were determined from the Scatchard plot of the data obtained from affinity coelectrophoresis (see Materials and Methods and Figure 3).

k_{cat}/K_m is 2.5×10^{-2} that of the wild-type enzyme. Because the kinetic parameters for amino acid activation are almost unchanged, this result suggests that R533 is primarily involved in binding to $tRNA^{fMet}$.

Affinity Coelectrophoresis. It is unlikely that the R533A mutation directly affects the interaction with the anticodon that is a major recognition element of $tRNA^{Met}$, because R533 is approximately 70 Å distant from the anticodon-binding region of the protein (Brunie *et al.*, 1990). Also, the active site conformation formed by the N-terminal domain and the C-terminal appendix peptide is not perturbed by the deletion of 11 amino acids in the anticodon-binding region of the C-terminal domain (Kim & Schimmel, 1992). Binding constants of the wild-type and R533A mutant for the RNA substrates based on the anticodon stem–loop and acceptor-stem sequences of $tRNA^{fMet}$ (Figure 2) were determined by affinity coelectrophoresis (see Materials and Methods for details).

While the dissociation constant of the wild-type enzyme for the microhelix (the acceptor-stem sequence of $tRNA^{fMet}$) was determined to be 249 μM, the specific binding of R533A mutant enzyme to the microhelix was not observed (Table III). This result shows that the R533A mutant enzyme has a decreased binding affinity to the acceptor-stem sequence of $tRNA^{fMet}$. In contrast, the dissociation constant for binding of the R533A mutant enzyme to the anticodon stem–loop hairpin helix was about 5-fold lower than that for the wild-type protein (Table III), suggesting that the R533A mutant binds to the anticodon stem–loop sequence of $tRNA^{fMet}$ with an affinity comparable to, or even better than, that of the wild-type. This difference may reflect a small indirect effect of the R533A mutation on the conformation of the anticodon-binding region of the enzyme. The mutant Δ11(Y454–A464→S), which has a deletion of the anticodon-binding peptide (Kim & Schimmel, 1992), did not show detectable binding to the anticodon stem-loop RNA substrate even at a 125 μM protein concentration (Figure 3). Collectively, the results suggest that R533 is needed specifically for interaction with the acceptor helix of bound $tRNA^{fMet}$.

Microhelix Aminoacylation. In the protein structure, the side chains of Y531 and R533 project toward the active site cleft. In contrast, the side chain of N532 is directed away from the active site (Figure 4). D535 is located in the C-terminal side of the peptide loop and approximately 7 Å distant from R533 (Figure 2). The effect of the R533A mutation specifically on tRNA aminoacylation (Tables I and II) and the location of R533 in the protein structure suggest that R533 could be involved in an acceptor-stem interaction that docks the acceptor end near to bound methionyl AMP.

To investigate the effect of R533A and the other mutations on the acceptor-helix interaction, a $microhelix^{fMet}$ was synthesized and tested for aminoacylation by the various mutants (Figure 5). The methionine microhelix was previously shown to be recognized specifically by *E. coli* methionyl tRNA synthetase (Martinis & Schimmel, 1992). The aminoacylation of the methionine microhelix is sequence-specific and requires A73 and the G2-C71 and C3-G70 base pairs shared by methionine tRNA isoacceptors (Martinis & Schimmel, 1992; Meinnel *et al.*, 1993). The microhelix substrate offers the opportunity to measure acceptor-helix interactions independent of anticodon binding by the protein.

While only the R533A mutant showed a significant difference in the aminoacylation of $tRNA^{fMet}$ compared to the wild-type enzyme, the N532A, R533A, and two insertion mutants reproducibly showed little activity for aminoacylation of $microhelix^{fMet}$ (Figure 5). In contrast, the D535A mutant does not affect microhelix aminoacylation.

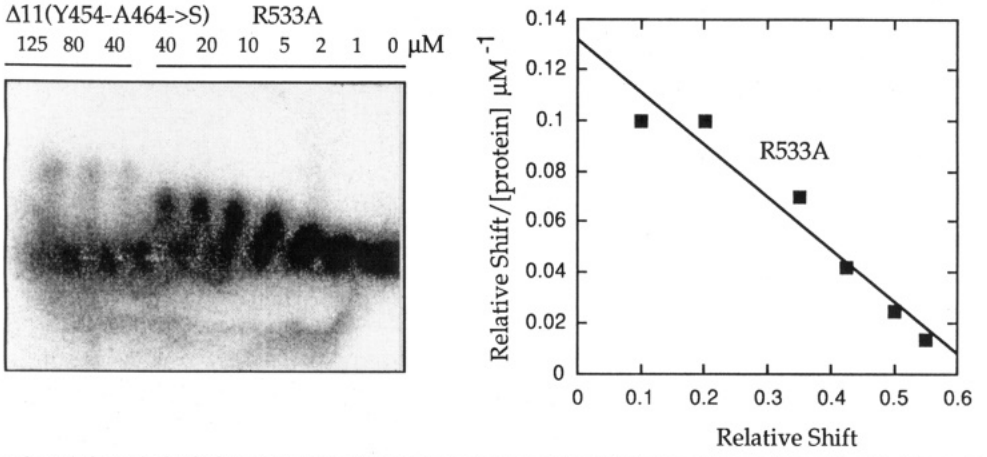

FIGURE 3: Affinity coelectrophoresis (ACE) to determine binding constants of the wild-type and mutant proteins for the anticodon stem–loop and microhelix RNA substrate (Figure 2). Gel mobility retardation of the ^{32}P-end-labeled methionine anticodon stem–loop RNA complexed with the Δ11(Y454–A464→S) and R533A mutant enzymes (left). Scatchard analysis of the data (right). The Scatchard plot for the relative shift of [^{32}P]RNA versus relative shift/[$protein_{tot}$] was made as described in Materials and Methods to determine a dissociation constant for the anticodon stem–loop RNA.

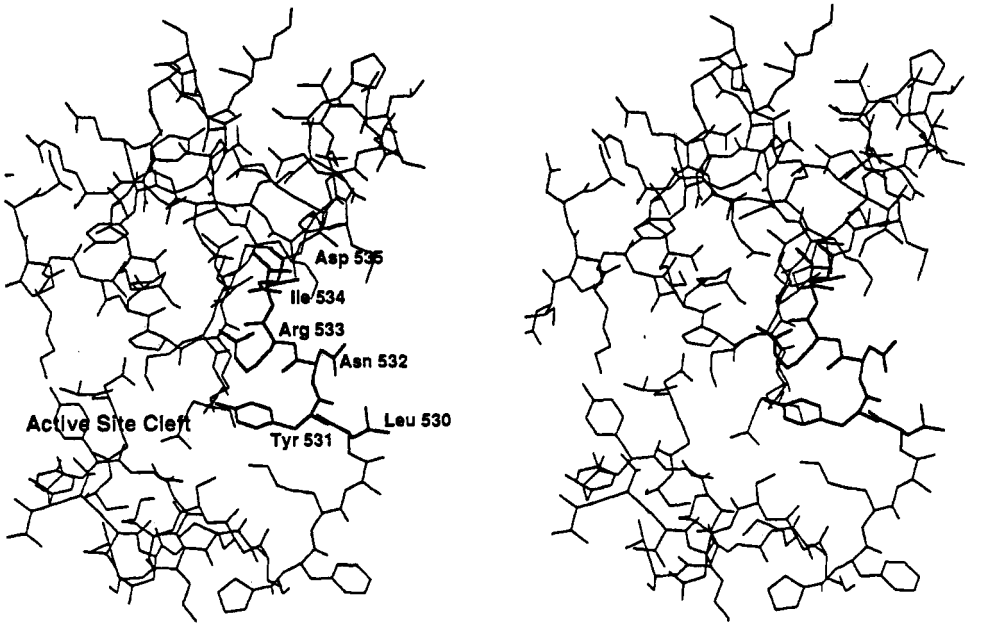

FIGURE 4: Stereoview of the residues in the C-terminal linker peptide and in the N-terminal catalytic domain, which are within 20 Å of R533. Residues from L530 to D535 in the C-terminal linker peptide are indicated.

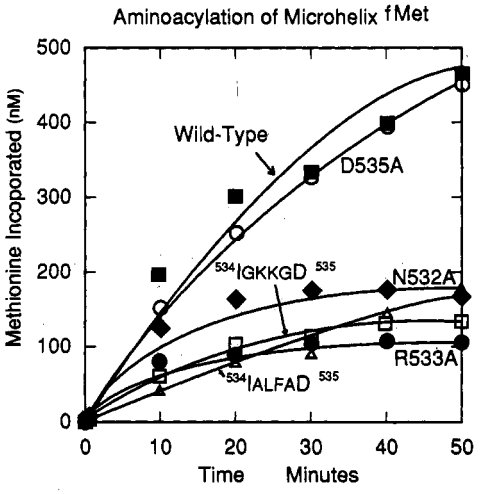

FIGURE 5: Aminoacylation of microhelix^{fMet} at pH 7.5 and 37 °C by the wild-type and various mutant enzymes.

DISCUSSION

Because the aminoacylation of the microhelix depends solely on acceptor-stem interactions, the microhelix aminoacylation reaction could be more sensitive than the tRNA aminoacylation reaction to conformational changes or mutations of a residue(s) involved in these interactions. Thus, R533 may directly contact a base or backbone phosphate in the acceptor stem. Possibly, the effects of N532A and insertion mutations result from destabilizing or misplacing the position of R533, which in turn lowers the efficiency of aminoacylation of the microhelix^{fMet}. The indirect effects of these mutations would be undetected when tRNA^{fMet} was used as a substrate for aminoacylation because the interactions with other parts of tRNA^{fMet} (in particular, with the anticodon) compensate for a slightly disturbed acceptor interaction of these mutants (Table I). However, in the case of the R533A mutant, the direct loss of binding energy resulting from the R533A mutation is not restored by the interactions with other parts of tRNA^{fMet}.

Although A73 and base pairs G2-C71 and C3-G70 in the acceptor sequence are important for the efficient aminoacylation of tRNA^{Met} (Martinis & Schimmel, 1992; Meinnel *et al.*, 1993), the protein residues that make specific contacts with the acceptor stem are unknown in methionine and most other class I aminoacyl tRNA synthetases. Sequence alignment of *E. coli* methionyl tRNA synthetase with the homologous class I tRNA aminoacyl tRNA synthetases revealed peptide insertions into the nucleotide-binding fold in the conserved N-terminal catalytic domain (Starzyk *et al.*, 1987). The insertions between the third β-strand and α-helix were designated as CP1 (connective polypeptide 1). In the crystal structure of the class I *E. coli* glutaminyl tRNA synthetase complexed with tRNA^{Gln}, residues in CP1 make contact with the tRNA^{Gln} acceptor stem (Rould *et al.*, 1989). Peptide F102–T124 in CP1 of *E. coli* glutaminyl tRNA synthetase has some structural similarity to peptide E102–I124 in CP1 of *E. coli* methionyl tRNA synthetase (Perona *et al.*, 1991). It is not known whether the residues located in CP1 of *E. coli* methionyl tRNA synthetase or in any of the other class I enzymes are actually involved in recognition of the acceptor-stem sequence.

In contrast to this structural similarity in the conserved class-defining N-terminal domain of class I tRNA synthetases, the structures of the C-terminal domains are completely varied. The anticodon-binding C-terminal domain of *E. coli* glutaminyl tRNA synthetase primarily consists of a β-barrel structure (Rould *et al.*, 1989), while that of *E. coli* methionyl tRNA synthetase is mainly α-helical (Brunie *et al.*, 1990). In the case of glutaminyl tRNA synthetase, the C-terminal end of this domain does not make contact with the acceptor stem of tRNA^{Gln}.

In evolution, the acceptor-helix-binding C-terminal peptide of *E. coli* methionyl tRNA synthetase may have been transferred from the N-terminal catalytic domain or recruited from another genetic source and added to the anticodon-binding C-terminal domain. The DNA sequence encoding the C-terminal appendix of methionyl tRNA synthetase does not have similarity to other parts of the gene, giving no support to the possibility that it was generated by gene duplication (data not shown). Alternatively, this peptide appendix may have been part of an early form of the enzyme which recognized only the sequence of the acceptor helix. Later, the anticodon-

binding domains were fused to the core protein, and in the course of this fusion, the peptide appendix was transferred to the C-terminal domain. As a consequence, strong selective pressure on retaining the nonconserved anticodon-binding C-terminal domain would be imposed.

Among the class I enzymes, methionyl tRNA synthetase is most closely related to cysteinyl, isoleucyl, leucyl, and valyl tRNA synthetases. In each of these enzymes, the C-terminal domain is predicted to be α-helical (Hou *et al.*, 1991). The 52 C-terminal amino acids of the 939 amino acid isoleucyl tRNA synthetase are required for aminoacylation activity (Shiba & Schimmel, 1992). Possibly, as in methionyl tRNA synthetase, the isoleucyl enzyme has a C-terminal appendix that curls back to the active site to contact the acceptor stem.

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