Mutational Analysis Suggests the Same Design for Editing Activities of Two tRNA Synthetases†

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ABSTRACT: Although the structural basis for amino acid activation by class I tRNA synthetases is known, that for their editing activities has remained elusive. Two class I tRNA synthetases discriminate closely similar amino acids by RNA-independent and RNA-dependent mechanisms. In the absence of tRNA, isoleucyl-tRNA synthetase misactivates valine, while valyl-tRNA synthetase misactivates threonine. Both enzymes improve amino acid discrimination by tRNA-dependent hydrolytic editing reactions. Recent mutational analysis of an isoleucyl-tRNA synthetase showed that discrimination of valine from isoleucine by amino acid activation was functionally independent of discrimination by editing. In this work, we used mutational analysis to test whether the two types of amino acid discrimination were functionally independent in valyl-tRNA synthetase. We obtained four mutations in the valine enzyme which severely affected amino acid activation. The two most defective enzymes reduced k_{cat}/K_m for activation of valine by more than 4 orders of magnitude and were essentially inactive for aminoacylation. These two defective enzymes were tested and found to be unaltered in catalysis of rapid and selective removal of threonine misacylated onto valine tRNA. On the basis of these data, and in spite of there being few residues conserved between the two proteins in a region believed important for editing, we propose that the valine and isoleucine enzymes share a global design which functionally separates amino acid editing from amino acid activation.

The genetic code is established by the aminoacylation reactions catalyzed by aminoacyl-tRNA synthetases, whereby specific amino acids are attached to tRNAs bearing the anticodon triplets of the code (Schimmel, 1987; LaPointe & Giegé, 1991; Carter, 1993). To discriminate by direct binding interactions a given amino acid from the other 19 with an accuracy of better than 1 part in 10^4 is difficult only when amino acids are closely similar in structure (Fersht, 1986). Two of the inherently difficult discriminations are isoleucine *versus* valine, for isoleucyl-tRNA synthetase (IleRS), and valine *versus* threonine, for valyl-tRNA synthetase (ValRS). In the former case, the removal of a β-methylene group makes valine fit easily into any pocket that binds isoleucine. In the latter case, the β-hydroxyl group of threonine is isosteric with a β-methyl group of valine, and therefore, threonine sterically fits into any binding site for valine. Consequently, the misactivation of valine (by isoleucyl-tRNA synthetase) and of threonine (by valyl-tRNA synthetase) occurs with a frequency of one part in 200−400, and yet the overall error rate in protein biosynthesis is in the range of 1 mistake per 3000 amino acids (Loftfield, 1963; Loftfield & Vanderjagt, 1972). (The latter number includes the cumulative errors in transcription, aminoacylation of tRNAs, and codon−anticodon pairing on the ribosome.) Thus, to achieve the high fidelity required for accuracy of the genetic code, some synthetases have evolved tRNA-dependent editing activities which are sensitive to the subtle differences between closely similar amino acid pairs (Schimmel & Söll, 1979; Fersht, 1985; Freist, 1989; Jakubowski & Goldman, 1992).

Two distinct editing activities have been characterized (Fersht, 1985). One activity is designated as "pre-transfer" editing, and involves the tRNA-induced hydrolysis of the misactivated aminoacyl adenylate (Norris & Berg, 1964). The other activity is "post-transfer" editing and occurs after the misactivated enzyme-bound amino acid has reacted with tRNA to create a mischarged species such as Val-tRNA^{Ile} or Thr-tRNA^{Val} (Eldred & Schimmel, 1972; Yarus, 1972). In these instances, isoleucyl-tRNA synthetase catalyzes hydrolysis of Val-tRNA^{Ile} and the valine enzyme stimulates hydrolytic cleavage of Thr-tRNA^{Val}. These tRNA-dependent pre- and post-transfer editing reactions act together to improve amino acid discrimination by more than an order of magnitude (Fersht & Kaethner, 1976; Fersht, 1977).

Valyl- and isoleucyl-tRNA synthetases are examples of class I enzymes which characteristically have a Rossmann nucleotide binding fold in an N-terminal domain (Webster et al., 1984; Brick et al., 1988; Brunie et al., 1990). The alternating β-strands and α-helices of the Rossmann fold make up the catalytic center for aminoacyl adenylate synthesis and, together with insertions which divide the fold, for docking of the 3′-end of the tRNA. X-ray structures for class I enzymes include those for tyrosyl- (Brick et al., 1988), methionyl- (Brunie et al., 1990), glutaminyl- (Rould et al., 1989), glutamyl- (Nureki et al., 1995), and tryptophanyl-tRNA synthetases (Doublié, 1995). These structures include a cocrystal with tRNA for the glutamine enzyme (Rould et al., 1989). Because valyl- and isoleucyl-tRNA synthetases are part of a subgroup of class I enzymes which are closely related to methionyl-tRNA synthetase (Eriani et al., 1991; Hou et al., 1991), secondary structures of the valine and isoleucine enzymes can be modeled with some degree of

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confidence (Starzyk et al., 1987). In spite of the structures and models of these five class I tRNA synthetases, however, the structural basis for editing remains obscure.

That tRNA-dependent discrimination by editing is functionally independent of initial amino acid recognition was established for *Escherichia coli* isoleucyl-tRNA synthetase by mutational isolation of the "sieve" for editing (Schmidt & Schimmel, 1994). In that work, a mutant enzyme was isolated which eliminated the discrimination between valine and isoleucine in the initial binding and activation step. Although each amino acid was activated at equal rates, the mutation had no effect on tRNA-dependent discrimination of valine *versus* isoleucine. Thus, for example, the rate of hydrolysis of Val-tRNA^{Ile} by the mutant enzyme was not reduced compared to that of the wild-type protein. This observation demonstrated functional independence of amino acid binding and activation from tRNA-dependent amino acid recognition as manifested in the editing reactions.

Because valyl- and isoleucyl-tRNA synthetases appear to have developed from a common ancestor (Borgford et al., 1987; Heck & Hatfield, 1988), and because these two enzymes have two of the most prominent editing activities among all tRNA synthetases, we were interested to see whether a common design underlay the editing activities. The chemical nature of the tRNA-dependent discrimination achieved by the two enzymes is quite different. In particular, isoleucyl-tRNA synthetase discriminates two hydrophobic amino acids based on the steric difference of a single methylene group, while valyl-tRNA synthetase selectively eliminates activated threonine on the basis of a polar hydroxyl group being chemically distinct from an isosteric and nonpolar methyl group. Given that the structural basis for tRNA-dependent discrimination is unknown, and that the chemical nature of the discrimination achieved by the two enzymes was quite different, we thought that it would be interesting to see whether the two enzymes shared a common or had an idiosyncratic design for tRNA-dependent amino acid editing component. As a step toward this goal, we investigated whether the two systems of amino acid discrimination are functionally independent in the valine enzyme, as they are in isoleucyl-tRNA synthetase.

MATERIALS AND METHODS

A gene fragment from plasmid pTB8 which encodes the *valS* gene from *Bacillus stearothermophilus* (Borgford et al., 1987) was subcloned into phagemid pBluescript II (KS+) (Stratagene, La Jolla, CA) and used as a template for subsequent mutagenesis with the Sculptor™ *in vitro* mutagenesis system (Amersham, Arlington Heights, IL). The mutations were confirmed by dideoxy sequencing of the DNA templates (Sanger et al., 1980). Wild-type and mutant enzymes were expressed in *E. coli* strain MV1184 (*ara* Δ(*lac-proAB*) *rspL thi* (*ϕ80 lacZ* Δ*M15)* Δ(*srl-recA*) *306*:: *Tn10 (tet^r)/F′ (traD36 proAB+ lacI^q lacZ* Δ*M15*)) (Schmidt & Schimmel, 1993) and purified to homogeneity as described (Borgford et al., 1987). *E. coli* threonyl-tRNA synthetase (ThrRS) was purified from an overproducing strain pBG-SUB4+/IBPC6183 (a generous gift from Dr. Mathias Springer, Institut de Biologie Physico-Chemique, Paris, France) and was used to prepare mischarged Thr-tRNA^{Val} (Igloi et al., 1977). Phagemid pLL101 carrying the gene for *B. stearothermophilus* tRNA$^{Val/GAC}$ was constructed by inserting the synthetic DNA oligonucleotides encoding the T7 promoter and tRNA gene sequence into the expression vector pGFIB-1 (Masson & Miller, 1986). The tRNA^{Val} was then overexpressed in *E. coli* strain JM109 (Yanisch-Perron et al., 1985) and purified through high performance liquid chromatography on a Nucleogen DEAE column (The Nest Group, Southboro, MA) followed by a reverse-phase C4 column. The resulting tRNA gave valine acceptance of 1450 pmol/A_{260}.

The aminoacylation reactions were performed using the standard procedure (Shepard et al., 1992) at 25 °C. The amino acid activation activities were measured at 37 °C according to the conditions described by Kim and Schimmel (1992) . Deacylation of Thr-tRNA^{Val} was performed in the presence of 150 mM Tris-HCl (pH 7.5), 150 mM KCl, 10 mM $MgCl_2$, 2 μM aminoacyl-tRNA, 4 U/mL inorganic pyrophosphatase (Boehringer Mannheim, Indianapolis, IN), and 1−30 nM purified ValRS. Tritiated threonine (Amersham, Arlington Heights, IL) was used to monitor the reaction. Aliquots of the reaction mixture were quenched onto Whatman 3MM filter pads presoaked in 5% trichloroacetic acid, and then washed and quantified by liquid scintillation counting in Bataflor (National Diagnostics, Manville, NJ).

RESULTS

To address the question of how valyl-tRNA synthetase discriminates activated valine *versus* threonine and the relationship of this activity to the isoleucine *versus* valine discrimination of isoleucyl-tRNA synthetase, we focused on the mutational analysis of isoleucyl-tRNA synthetase which demonstrated functional independence of the two kinds of amino acid discrimination. Figure 1 shows a schematic illustration of the nucleotide binding fold and class-defining catalytic domain of valyl- and isoleucyl-tRNA synthetase, and an alignment of 13 sequences of these enzymes using the PILEUP program (Genetics Computer Group, Madison, WI) in a region previously studied in the *E. coli* isoleucine enzyme. The fold is comprised of alternating α-helices and β-strands in a $\beta_6\alpha_4$ structure which is split into two $\beta_3\alpha_2$ halves by an insertion known as connective polypeptide 1 (CP1) (Starzyk et al., 1987). A second insertion designated as CP2 occurs after the first β-strand of the second half of the fold.

A region of 59 amino acids extends from the beginning of the first β-strand to the end of the second strand and encompasses the 11 amino acid signature sequence which ends in the "HIGH" tetrapeptide (Webster et al., 1984) which is part of the motif which interacts with ATP (Brick et al., 1988; Rould et al., 1989). Among the 59 amino acids in this region, only 9 are conserved across all 13 sequences (shown as shaded in Figure 1). In addition, near the beginning of the aligned sequences, a proline occurs in all valyl-tRNA synthetases which is replaced by a glycine in all isoleucyl-tRNA synthetases (shown as boxed). This glycine occurs at position 56 in *E. coli* isoleucyl-tRNA synthetase. A G56A substitution in *E. coli* isoleucyl-tRNA synthetase eliminates the ability of the enzyme to discriminate between isoleucine *versus* valine in initial amino acid activation (identical k_{cat} and K_m for amino acid activation with either amino acid). The G56A isoleucyl-tRNA synthetase was not defective, however, in its tRNA^{Ile}-dependent discrimination of valine *versus* isoleucine. The behavior of

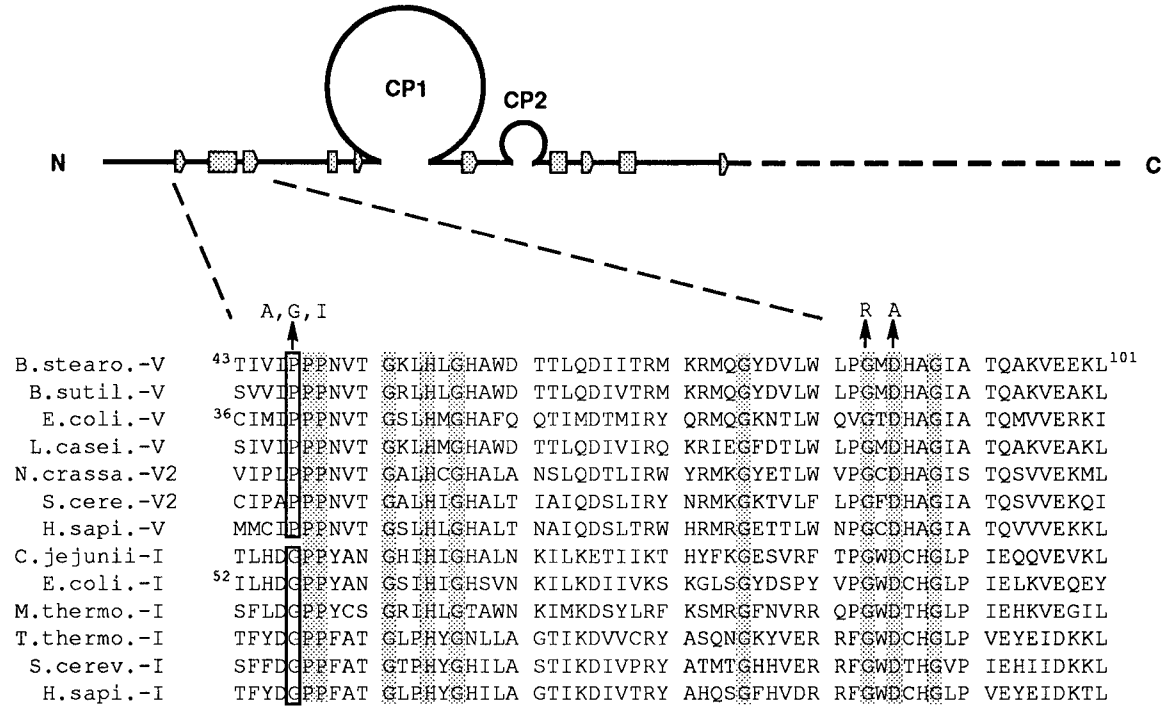

FIGURE 1: The partial sequence alignment for 13 valyl- and isoleucyl-tRNA synthetases along with the predicted secondary structure for ValRS. Only the region subjected to mutagenesis is shown. The alignment was made using the multiple sequence alignment program PILEUP (UWGCG package) (Devereaux et al., 1984). The conserved residues are indicated as shaded. Seven sequences of ValRS are from *B. stearothermophilus* (Borgford et al., 1987), *Bacillus subtilis* (Genebank accession no. x77239), *E. coli* (Härtlein et al., 1987), *Lactobacillus casei* (Taylor et al., 1993), *Neurospora crassa* (Kubelik et al., 1991), *Saccharomyces cerevisiae* (Jordana et al., 1987), and *Homo sapiens* (Hsieh & Campbell, 1991), and six IleRS sequences are from *Campilobacter jejunii* (Genebank accession no. u15295), *E. coli* (Webster et al., 1984), *Methanobacterium thermoautotrophicum* (Jenal et al., 1991), *Tetrahymena thermophila* (Csank & Martindale, 1992), *S. cerevisiae* (Englisch et al., 1987; Martindale et al., 1989), and *H. sapiens* (Shiba et al., 1994). V2 represents the mitochondrial sequence.

this and other mutant enzymes established the functional independence of initial amino acid recognition from tRNA-dependent editing (Schmidt & Schimmel, 1994).

With these results in mind, we investigated *B. stearothermophilus* valyl-tRNA synthetase. The *B. stearothermophilus* enzyme is a 880 amino acid monomer. It was chosen because of its stability, ease of purification, and well-characterized editing activity (Fersht & Kaethner, 1976). Three sites chosen for mutations in this study are indicated by arrows in Figure 1. These include P47, G85, and D87. P47 was chosen because it is at the same position as the aforementioned G56 in *E. coli* isoleucyl-tRNA synthetase. The other two sites were chosen because they are highly conserved among a subgroup of five class I tRNA synthetases (Hou et al., 1991), and previous studies of *E. coli* IleRS and MetRS indicated that these are critical residues for amino acid binding and activation (Clarke et al., 1988; Ghosh et al., 1991; Schmidt & Schimmel, 1993). Thus, mutation of any one of these residues was expected to have some effect on amino acid recognition, and the question was whether there would be a concomitant effect on tRNA-dependent amino acid discrimination.

In Vivo Expression and Purification of the Mutant Enzymes. The expressed mutant proteins accumulated in *E. coli in vivo* and could be purified to homogeneity as judged by SDS−PAGE. Because the *B. stearothermophilus* enzyme is a thermally stable protein, endogenous *E. coli* valyl-tRNA synthetase was removed through a heat-treatment step by incubating the enzyme at 70 °C for an hour during the course of purification. Although the mutational sites chosen in this study were at putative amino acid binding sites, none of the mutant enzymes produced toxicity toward the host *E. coli* cells. The lack of toxicity could be explained by the presence of endogenous *E. coli* ValRS to sustain normal cell growth, and by the expression level of *B. stearothermophilus* enzyme being not high enough to give dominant lethal phenotype from sequesting host cell $tRNA^{Val}$ (Schmidt & Schimmel, 1993).

Mutations of Conserved Residue Yield Defects in Amino Acid Activation. Substitutions at P47, G85, and D87 were selected with the deliberate intention of substantially altering the efficiency and, additionally or alternatively, the specificity of amino acid activation. The most severely affected mutant enzymes were then tested to see whether they were altered in their ability to edit misactivated threonine. As it turns out, most of the editing by *B. stearothermophilus* valyl-tRNA synthetase occurs after transfer of the misactivated amino acid to $tRNA^{Val}$, that is, through deacylation of Thr-$tRNA^{Val}$ (Fersht & Kaethner, 1976). In addition, some of the mutant enzymes were so defective in amino acid activation and aminoacylation that only "post-transfer" and not "pre-transfer" editing could be measured in any case.

Proline 47 was substituted with glycine so that this position would have the amino acid that is found in all of the isoleucyl-tRNA synthetases (Figure 1). In addition, we also made alanine and isoleucine substitutions at this position. The idea was that, if P47 is in the amino acid binding pocket, then P47G and P47I mutations would give two extremes in the resulting pocket size without greatly changing the polarity of the side chain.

In the case of position 85, a G85R substitution was made. We chose this particular substitution because earlier work showed that a G→R substitution at this position in *E. coli* isoleucyl-tRNA synthetase gave a 6000-fold increase in the

Table 1: Amino Acid Activation by ValRS Mutants at pH 7.5, 37 °C

	Val			Thr			specificity
ValRS	K_m (μM)	k_{cat} (s^{-1})	k_{cat}/K_m (M^{-1} s^{-1})	K_m (mM)	k_{cat} (s^{-1})	k_{cat}/K_m (M^{-1} s^{-1})	$(k_{cat}/K_m(Val))/(k_{cat}/K_m(Thr))$
WT	59	18.1	3.1×10^5	16	12.8	8.0×10^2	388
P47A	189	26.7	1.4×10^5	19	8.43	4.4×10^2	318
P47G	140	6.45	4.6×10^4	38	0.583	1.5×10^1	3067
P47I	110 000	(0.024)[a]	ND[b]	>200	(0.027)	ND	
G85R	186	0.92	4.9×10^3	26	0.173	6.7	731
D87A	6 400	0.067	1.1×10^1	>200	(0.019)	ND	

[a] The numbers in parentheses are apparent values due to the elevated K_m. [b] Not done. Attempts to determine the k_{cat}/K_m for the P47I and D87A mutant enzymes were unsuccessful due to the slow rates and high K_ms.

K_m for isoleucine (Clarke et al., 1988). As for position 87, a D87A mutation was created because a D→A substitution at the analogous position in either *E. coli* isoleucyl- or methionyl-tRNA synthetase caused a significant reduction in the efficiency of amino acid activation (Ghosh et al., 1991; Schmidt & Schimmel, 1993).

The effects of mutations on tRNA-independent amino acid activation were investigated by measuring the valyl-tRNA synthetase catalyzed rate of valine- and threonine-dependent exchange of ^{32}P-labeled pyrophosphate into ATP (Calendar & Berg, 1966). Wild-type enzyme showed a 388-fold preference for activating valine over threonine, in agreement with previous observations (Fersht & Kaethner, 1976). With the exception of the P47A mutation, all other substitutions significantly reduced the efficiency of amino acid activation (k_{cat}/K_m) with either valine or threonine (Table 1).

The effect of substitution at position 47 was highly sensitive to the nature of the substituted amino acids. For the P47G mutation, the specificity $(k_{cat}/K_m(Val))/(k_{cat}/K_m(Thr))$ was increased about 8-fold. For two others (P47I and D87A), the amino acid K_m values were raised so much that accurate kinetic and specificity parameters could not be obtained. However, for these two mutant enzymes, we were able to estimate that k_{cat} values for both amino acids were reduced by more than 100-fold and that the K_m for valine was raised in one case (P47I) by more than 100-fold.

In the case of *E. coli* isoleucyl-tRNA synthetase, a G56A substitution reduced the specificity ratio $(k_{cat}/K_m(Ile))/(k_{cat}/K_m(Val))$ from 180 to 1 (Schmidt & Schimmel, 1994). At position 47 of *B. stearothermophilus* valyl-tRNA synthetase, one mutant (P47G) increased the specificity ratio $(k_{cat}/K_m(Val))/(k_{cat}/K_m(Thr))$ by about 8-fold (Table 1). Another mutant (P47A) had little effect on specificity. For a third mutant (P47I), the specificity ratio could not be determined because of the high K_m for threonine of the mutant enzyme. Thus, while the alignment between valyl- and isoleucyl-tRNA synthetases in the first half of the nucleotide binding fold (Figure 1) shows the clear relationship between these enzymes in this part of the amino acid binding pocket, the details of how residues at specific positions affect amino acid specificity cannot be predicted or explained in any simple way. These details may be quite different for the two enzymes.

Enzymes Defective for tRNA-Independent Amino Acid Discrimination and Aminoacylation Are Completely Active for tRNA-Dependent Discrimination. The above data established the importance of the selected positions in *B. stearothermophilus* valyl-tRNA synthetase for amino acid recognition. For the purpose of seeing whether enzymes severely affected in amino acid recognition were also affected in their capacity to deacylate Thr-tRNA^{Val}, we chose the P47I and D87A mutations as the two most deficient (for amino acid activation) of those that are listed in Table 1. When assayed for their ability to charge tRNA^{Val}, these mutant enzymes showed no activity (Figure 2, left panel). Given that amino acid activation precedes amino acid attachment to tRNA, this lack of activity for aminoacylation was unsurprising.

The P47I and D87A mutant enzymes were compared with wild-type valyl-tRNA synthetase for ability to deacylate Thr-tRNA^{Val}. In contrast to the large difference between the mutant enzymes and the wild-type protein in their aminoacylation activities, the three enzymes were indistinguishable in their hydrolytic editing activities (Figure 2, right panel). The measured k_{cat} parameters for the wild-type, P47I, and D87A enzymes were 1.47 s^{-1}, 1.25 s^{-1}, and 1.36 s^{-1}, respectively. Thus, the P47I and D87A mutations clearly segregate amino acid recognition during *activation* from recognition during *editing*.

DISCUSSION

The locations of the residues which make up the site for tRNA-dependent amino acid discrimination are unknown. After transfer of the activated amino acid to bound tRNA on the surface of the enzyme, the position of the amino acid must change. Surprisingly, the data presented here for the valine enzyme and the previous results on the isoleucine system (Schmidt & Schimmel, 1994) suggest that this change is of such a nature that a severe mutational perturbation of the site for amino acid activation is not "felt" by the aminoacyl moiety in the editing site. By uncoupling the structure of the initial recognition site from that for editing in these two enzymes, overall amino acid discrimination is the product of the discrimination achieved in each site, even for mutants where initial recognition in the activation step has been impaired.

Of the ten class I tRNA synthetases, isoleucyl- and valyl-tRNA synthetases are most closely related to cysteinyl-, leucyl-, and methionyl-tRNA synthetases (Eriani et al., 1991; Hou et al., 1991). In the case of methionyl-tRNA synthetase, homocysteine is misactivated. The misactivated amino acid is not coupled to tRNA^{Met} (Fersht & Dingwall, 1979), but instead is enzymatically converted to and then released as a thiolactone. This reaction occurs in the absence of tRNA^{Met} and thus is an example of "tRNA-independent" editing (Jakubowski & Fersht, 1981), which bears no relationship to the tRNA-dependent processes catalyzed by isoleucyl- and valyl-tRNA synthetases. For this reason, the use of the structure of methionyl-tRNA synthetase to decipher the site for editing in isoleucyl- and valyl-tRNA synthetases is difficult to justify, even though the amino acid activation

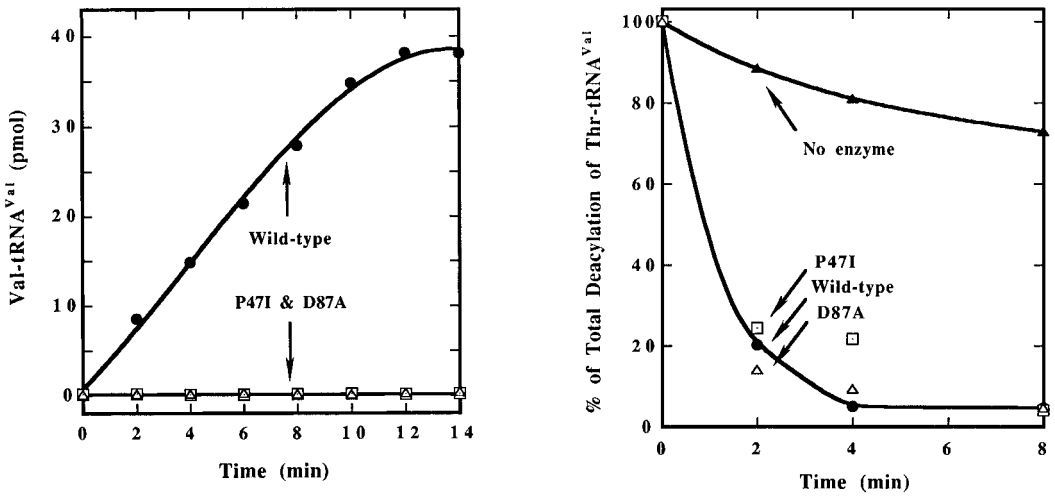

FIGURE 2: Left panel: Valylation of tRNA^{Val} by wild-type and ValRS mutants at pH 7.5 and 25 °C. The number of picomoles of charged tRNA in an 18 μL aliquot of a 200 μL reaction is plotted against time. The enzyme concentrations were 2 nM, and tRNA concentrations were 4 μM. Right panel: Deacylation of Thr-tRNA^{Val} by wild-type and mutant ValRS in the absence of AMP and PP_i. The enzyme-induced hydrolysis contained 2 μM Thr-tRNA^{Val} and 30 nM ValRS. The reaction was carried out at pH 7.5 and 25 °C. Upon mixing the enzymes with Thr-tRNA^{Val}, about 60% of the starting material was hydrolyzed rapidly while the remaining 40% was hydrolyzed at a much slower rate. (A similar phenomena was also observed by others (Fersht, 1977).) The reason for this behavior is unknown, and is possibly due to the nature of the conditions used to prepare mischarged aminoacyl-tRNA which requires the presence of organic solvent. Therefore, the percentage of deacylation in this figure was normalized to the total material that was rapidly hydrolyzed by the wild-type enzyme.

sites in these enzymes are believed to be closely similar. As for cysteinyl- and leucyl-tRNA synthetases, editing reactions of these enzymes have not been well characterized.

The assignment of the isoleucine and valine enzymes to the class I grouping of tRNA synthetases is based on their having sequence elements associated with the nucleotide binding fold of the catalytic domain (Webster et al., 1984; Schimmel, 1987; Cusack et al., 1990; Eriani et al., 1990). The fold is interrupted by the CP1 insertion, and in the case of *E. coli* glutaminyl-tRNA synthetase, this insertion provides for contacts with the acceptor helix of bound tRNA^{Gln} (Rould et al., 1989). The same CP1 insertion is not shared by all members of class I, as it varies in size from about 69 to 300 amino acids among the *E. coli* enzymes (Hou et al., 1991; Shiba & Schimmel, 1992). Possibly, this insertion was recruited into a primitive tRNA synthetase on an idiosyncratic basis to facilitate docking of an RNA molecule next to a bound activated amino acid.

We noted that, while the sequences and sizes of CP1 for many of the class I enzymes are different, those found in isoleucyl- and valyl-tRNA synthetases are similar in size (about 300 amino acids). Internal deletions of *E. coli* isoleucyl-tRNA synthetase showed that part of the CP1 was dispensable for amino acid activation and charging (Starzyk et al., 1987). A recent analysis on the indispensable region of CP1 of *E. coli* isoleucyl-tRNA synthetase showed that the relative tRNA-dependent discrimination of valine *versus* isoleucine, as measured by the enzymatic deacylation of Val-tRNA^{Ile} and Ile-tRNA^{Ile}, could be changed substantially by mutation of specific residues on the carboxyl-terminal side of CP1 (Schmidt & Schimmel, 1995). As mentioned earlier, the editing function of methionyl-tRNA synthetase does not require the presence of tRNA^{Met}, and yet the CP1 sequence of this enzyme is relatively small (126 amino acids in *E. coli* MetRS).

On the basis of the results presented here showing the functional independence of amino acid activation from amino acid editing, we propose that isoleucyl- and valyl-tRNA synthetases have the same global design for incorporation of structural motif(s) needed for editing. Possibly, this common design is based in part on their developing with the same historical CP1 element. However, multiple sequence alignments of the CP1 regions between these two synthetases show only nine conserved residues. These nine are among the fifty-eight residues which are conserved in the sequence alignment of the CP1 regions of the valine enzymes alone. Because isoleucyl- and valyl-tRNA synthetases have evolved to discriminate different sets of amino acids, the sequence differences in their CP1 regions are not surprising. Still, it remains to be seen whether these conserved residues in valyl-tRNA synthetase play any role in tRNA-dependent amino acid recognition. Moreover, as suggested with the specificity of amino acid recognition during amino acid selection (see above), the details of how specific motifs act in editing may be quite different in the two proteins.

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