Dissection of a Class II tRNA Synthetase: Determinants for Minihelix Recognition Are Tightly Associated with Domain for Amino Acid Activation†

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ABSTRACT: The ten class II aminoacyl-tRNA synthetases are large homo- and hetero-oligomeric proteins that share three conserved sequence motifs. Within this class, *Escherichia coli* alanyl-tRNA synthetase is the only homotetramer and is comprised of subunits of 875 amino acids. The enzyme aminoacylates sequence-specific RNA oligonucleotides that recreate as few as four base pairs of the acceptor stem of $tRNA^{Ala}$. A monomeric 461 amino acid N-terminal fragment (461N) was previously shown to have full adenylate synthesis activity. However, fragment 461N has significant, but reduced, efficiency of charging of $tRNA^{Ala}$, when compared to native enzyme [Ho, C., Jasin, M., & Schimmel, P. (1985) *Science 229*, 389–393]. We show here that, in contrast, the fragment and the native enzyme aminoacylate a 12 base pair acceptor–TΨC stem minihelix and a four base pair RNA tetraloop with the same efficiency. We also show that fragment 461N makes footprint contacts both on and outside the acceptor helix of bound $tRNA^{Ala}$. With one possible exception, the contacts observed with fragment 461N are identical to those seen with the native enzyme. In spite of contacts outside the acceptor helix, fragment 461N charges the native tRNA, minihelix, and tetraloop with similar efficiency. Thus, all minihelix contacts required for activation for charging are tightly associated with the adenylate synthesis domain and, at least in the fragment, are not influenced by additional RNA–protein contacts outside the minihelix domain. These findings and other considerations suggest that RNA interactions essential for aminoacylation may have developed as an integral part of the site for amino acid activation and are restricted to nucleotides near the amino acid attachment site.

The tRNA synthetase family of enzymes catalyze the charging of tRNA with amino acids in a two-step reaction:

enzyme + ATP + amino acid ⇌
enzyme(aminoacyl)-AMP + PP_i [adenylate synthesis]

enzyme(aminoacyl)-AMP + tRNA ⇌
aminoacyl-tRNA + AMP + enzyme [aminoacylation]

The interpretation of the genetic code depends upon the specific recognition of the correct tRNA and amino acid by the corresponding synthetase. The 20 synthetases have recently been grouped into two classes (I and II) of 10 enzymes each (Eriani et al., 1990; Cusack et al., 1991). This classification is based upon regions of primary structure held in common by the members of each class. The class I enzymes are principally monomeric proteins that have an active site Rossmann nucleotide-binding fold and share the characteristic signature sequences that end in the HIGH tetrapeptide (Webster et al., 1984; Ludmerer & Schimmel, 1987; Schimmel, 1987) and KMSKS pentapeptide (Hountondji et al., 1986; Burbaum & Schimmel, 1991). Class II enzymes, although exhibiting variability in size and oligomerization state, are characterized by three conserved sequence motifs. Motif 1 is thought to be involved in the formation of the oligomer interface, while motifs 2 and 3 contribute three of the β-strands that form the active site seven-stranded antiparallel fold, as observed in the crystal structures of *Escherichia coli* seryl-tRNA synthetase (Cusack et al., 1990) and yeast aspartyl-tRNA synthetase (Ruff et al., 1991). Alanyl-tRNA synthetase (AlaRS)[1] is an α_4 tetramer of polypeptides of 875 amino acids. The enzyme has no discernible motif 1 but has been placed in class II on the basis of the presence of motif 3 (Eriani et al., 1990) and a putative motif 2 (Cusack et al., 1991). Both motifs 2 and 3 are located within the N-terminal domain responsible for amino acid activation. Periodate-oxidized $tRNA^{Ala}$ (3′-end) cross-links to a lysine (K73) within motif 2 of AlaRS (Hill & Schimmel, 1989). A putative metal-binding sequence (^{178}Cys-X_2-Cys-X_6-His-X_2-His) is situated between motifs 2 and 3. Biochemical studies with Co^{2+}-substituted AlaRS (Miller & Schimmel, 1992) and site-specific mutagenesis (Miller et al., 1991) suggest that this region makes contacts with the acceptor stem of bound $tRNA^{Ala}$. As demonstrated by nuclease footprinting, extensive contacts are made by the enzyme on $tRNA^{Ala}$, including the acceptor stem, D loop, and TΨC/anticodon stems (Park & Schimmel, 1988). No footprint contacts were observed at the anticodon, as expected from the lack of an effect of the anticodon loop on AlaRS recognition (Hou & Schimmel, 1988; Park et al., 1989). A monomeric 699 amino acid N-terminal fragment of AlaRS binds $tRNA^{Ala}$ with the same affinity as that of the native enzyme and possesses aminoacylation activity (Regan et al., 1987). Further deletion to a monomeric N-terminal 461 amino acid fragment (461N) results in full adenylate synthesis activity, but aminoacylation activity at pH 7.4 is reduced to ~0.1% of that of the full-length enzyme (Jasin et al., 1983; Ho et al., 1985). Although binding of $tRNA^{Ala}$ at pH 5.5 to fragment 461N was decreased 20-fold compared to native enzyme, it was not possible to determine whether the reduction in charging efficiency at pH 7.4 was the result of an altered k_{cat} and/or K_m for $tRNA^{Ala}$. Further

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[1] Abbreviations: AlaRS, alanyl-tRNA synthetase; FPLC, fast protein liquid chromatography; DEAE, (diethylamino)ethyl.

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deletion of the enzyme to a 368 amino acid fragment (368N) gives a protein that also has full adenylate synthesis activity but which is unable to catalyze aminoacylation (Putney et al., 1981; Jasin et al., 1983; Regan et al., 1987).

With these considerations in mind, we sought to determine whether the C-terminal portion of AlaRS plays a role in acceptor helix recognition and aminoacylation. If, for example, the aminoacylation rate of an acceptor stem minihelix by fragment 461N was decreased compared to that of native enzyme, then a role for the C-terminal domain in acceptor helix recognition would be implicated. Alternatively, a comparable efficiency of aminoacylation of the minihelix by both the truncated and full-length enzymes would indicate that determinants for RNA oligonucleotide binding and catalysis of aminoacylation are closely integrated with those for adenylate synthesis and that the C-terminal domain does not have either a direct or indirect conformational role in determining acceptor helix aminoacylation. This conclusion, in turn, would support the idea that acceptor helix interactions evolved in close association with the catalytic site, possibly because early synthetases were small and could not extend more than 10–20 Å beyond the amino acid attachment site at the 3′-end of a bound RNA substrate.

MATERIALS AND METHODS

The construction of the plasmid encoding the 461 amino acid deletion mutant of AlaRS behind the *tac* promoter (pT461) has been described previously (Jasin et al., 1983; Regan, 1986). Fragment 461N was expressed from pT461 in *E. coli* strain W3110 (*laqI*^{q} recA Δ1 Kan^r *ala SΔ2*). This strain does not contain *alaS* on the chromosome (Jasin & Schimmel, 1984). Purification of plasmid-expressed fragment 461N was performed by DEAE-cellulose chromatography, Mono Q FPLC (Pharmacia, Piscataway, NJ), and Superose 12 (Pharmacia), size-exclusion chromatography by an adaptation of methods described previously (Hill & Schimmel, 1989). The full-length protein (AlaRS) was purified in the same manner. The 368 amino acid N-terminal fragment of AlaRS (368N) was obtained by digestion of AlaRS (2.5 mg/mL in 90 mM NH_4OAc, pH 8.3) with trypsin (25 μg/mL) for 3 h at 37 °C (Herlihy et al., 1980). Crude 368N was purified on a Superose 12 FPLC column (Pharmacia) with a buffer of 50 mM sodium phosphate/100 mM NaCl at pH 7.8. Purified proteins were concentrated to greater than 100 μM and stored at −20 °C in 40% glycerol.

Enzyme concentrations were determined by the adenylate burst assay (Fersht et al., 1975). Adenylate synthesis was measured at 25 °C in 100 mM Tris-HCl (pH 8.0), 2 mM ATP, 2 mM pyrophosphate, 10 mM KF, 2 mM alanine, 10 mM β-mercaptoethanol, and 5 mM $MgCl_2$ as previously described (Calendar & Berg, 1966). Aminoacylation activity was measured at 25 °C in 50 mM Hepes (pH 7.5), 20 μM alanine, 4 mM ATP, 20 mM KCl, 10 mM $MgCl_2$, 20 mM β-mercaptoethanol, and 0.1 mg/mL bovine serum albumin as described previously (Schreier & Schimmel, 1972; Hill & Schimmel, 1989). Measured differences in trichloroacetic acid precipitation and counting efficiencies were used to correct the specific activities of various [^{3}H]alanyl-RNA species (Shi et al., 1992).

T7 RNA polymerase was purified according to Grodberg and Dunn (1988) from *E. coli* strain BL-21/pAR 1219. Both G3·U70- and G3·C70-minihelix^{Ala} were synthesized by *in vitro* transcription as described by Francklyn and Schimmel (1989). Tetraloop^{Ala} was chemically synthesized and purified by methods described previously (Shi et al., 1992). RNA concentrations were determined by the absorbance at 260 nm using previously determined extinction coefficients (Musier-Forsyth et al., 1991; Shi et al., 1992).

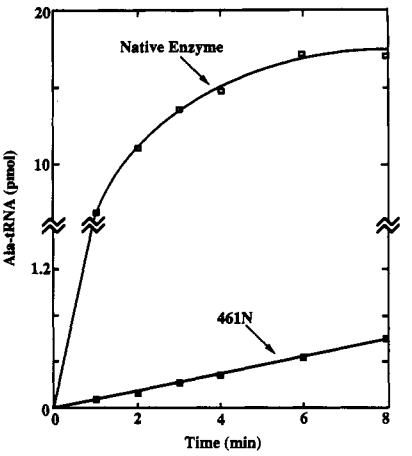

FIGURE 1: Aminoacylation with alanine of tRNA^{Ala} (2.5 μM) by AlaRS (30 nM) and fragment 461N (30 nM) at pH 7.5, 25 °C. The number of picomoles of charged tRNA in a 10-μL aliquot of a 100-μL reaction is plotted on the ordinate axis.

End labeling (5′) of tRNA^{Ala} (Subriden, Rolling Bay, WA) was carried out by established procedures (Silberklang et al., 1977). Following purification on a 12% polyacrylamide/7 M urea gel, ^{32}P-end-labeled tRNA^{Ala} was eluted from the gel by diffusion and then ethanol precipitated. Approximately 5 × 10^6 cpm was incorporated into 1 μg of tRNA. Nuclease protection experiments were performed by modifications of previously published procedures (Park & Schimmel, 1988). Typically, concentrations of 5 μM AlaRS, 25 μM fragment 461N, and 400 nM tRNA were used in pancreatic RNase A (Boehringer Mannheim, Indianapolis, IN) protection experiments, while 60 μM AlaRS, 90 μM fragment 461N, and 6.5 μM tRNA were used in experiments with cobra venom RNase V_1 (Pharmacia). Nuclease protection reaction products were electrophoresed on either 12% (RNase A) or 20% (RNase V_1) polyacrylamide/7 M urea gels. Digestion products were visualized by exposing the gels to X-ray film or phosphorimaging.

RESULTS

E. coli strain W3110 *alaSΔ2* lacking the chromosomal copy of *alaS* (Jasin & Schimmel, 1984) was used for the expression and purification of fragment 461N from recombinant plasmid pT461 (Regan, 1986). Immunoblots of purified fragment 461N confirmed the lack of contamination with full-length enzyme. Consistent with previous results (Jasin et al., 1983), fragment 461N has adenylate synthesis activity indistinguishable from native enzyme (data not shown); however, its rate of aminoacylation of tRNA^{Ala} is significantly reduced compared to that of the full-length enzyme (Figure 1). The rate is low enough that it is not possible to determine accurate values for either k_{cat} or K_m.

We examined the following substrates with fragment 461N: a 12 base pair RNA minihelix (minihelix^{Ala}) that recreates the acceptor–TΨC stem-loop of tRNA^{Ala} and a four base pair tetraloop structure (tetraloop^{Ala}) that incorporates the first three base pairs of the acceptor stem of tRNA^{Ala} with a fourth base pair (C·G) that closes the UUCG tetraloop motif (Woese et al., 1990) (Figure 2). These RNA oligonucleotides are aminoacylated provided they contain the G3·U70 base pair found in the acceptor stem of tRNA^{Ala} (Francklyn & Schimmel, 1989; Shi et al., 1992). This wobble

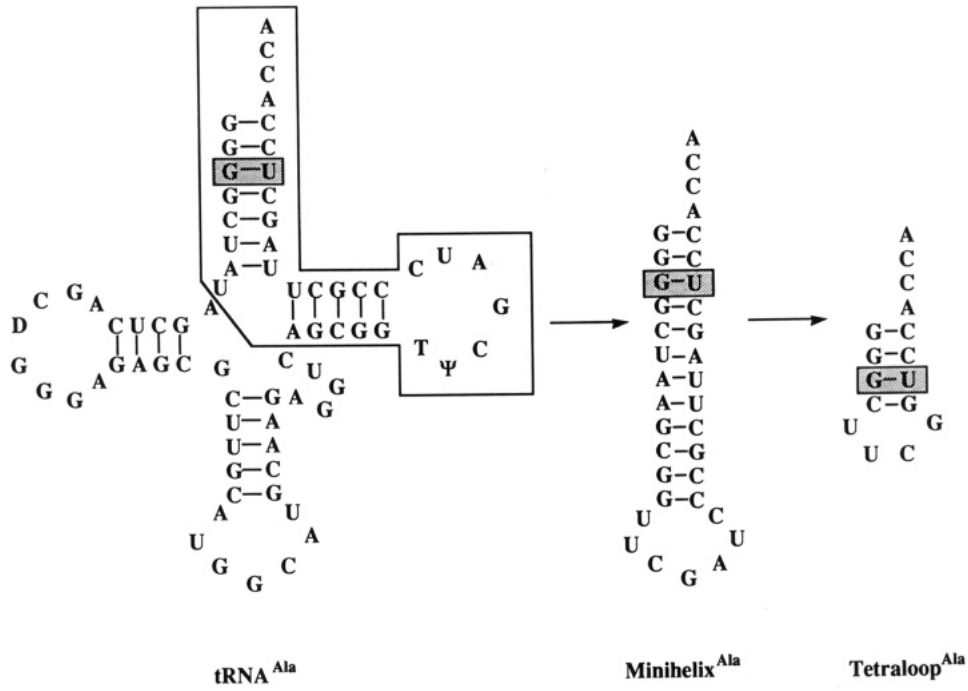

FIGURE 2: Sequences of $tRNA^{Ala}$, $minihelix^{Ala}$, and $tetraloop^{Ala}$. The sequence of $tRNA^{Ala}$ that is recreated in $minihelix^{Ala}$ is boxed. The G3·U70 base pair is boxed and shaded in each structure.

base pair presents the unpaired 2-NH_2 group of G3 in the minor groove of the RNA that is essential for aminoacylation (Hou & Schimmel, 1988, 1989; Francklyn & Schimmel, 1989; Musier-Forsyth et al., 1992). Alteration of G3·U70 to G3·C70 abolishes charging with alanine. The G3·U70 base pair and neighboring acceptor stem sequences provide the majority of the total energetic contribution to the recognition of $tRNA^{Ala}$ by AlaRS (Francklyn et al., 1992; Shi et al., 1992).

Fragment 461N aminoacylates $minihelix^{Ala}$ essentially as efficiently as the native enzyme (Figure 3A). The value of k_{cat}/K_m (at 20 μM alanine) for aminoacylation of $minihelix^{Ala}$ by the fragment and native enzyme is $k_{cat}/K_m = 1800\ M^{-1}\cdot s^{-1}$. For both 461N and AlaRS, aminoacylation is specific: changing the G3·U70 base pair to G3·C70 abolished charging (Figure 3A). In addition, the rates of aminoacylation of $tetraloop^{Ala}$ by both fragment 461N and AlaRS are similar (Figure 3B). These results suggest that the domain responsible for the specific recognition and aminoacylation of the acceptor stem of $tRNA^{Ala}$, as recreated in the oligonucleotide substrates, is fully functional in fragment 461N. The C-terminal 414 amino acids that are missing in the fragment make neither a direct nor an indirect contribution to the aminoacylation of these acceptor stem substrates.

These conclusions are further supported by comparing the aminoacylation by fragment 461N of full-length $tRNA^{Ala}$, $minihelix^{Ala}$, and $tetraloop^{Ala}$ (Figure 4). The rates of charging of all three of these substrates are similar, suggesting that only those protein–RNA contacts that are present in the fragment 461N–minihelix and fragment 461N–tetraloop complexes contribute to the efficiency of aminoacylation of full-length $tRNA^{Ala}$ by fragment 461N. Thus, the C-terminal amino acids missing in fragment 461N also make no contribution to the recognition of the acceptor–TΨC stem in the intact tRNA. It is not possible to detect charging of either $tRNA^{Ala}$ or $minihelix^{Ala}$ by a 368 amino acid N-terminal fragment of AlaRS (Figure 4), even though this fragment has full adenylate synthesis activity (Regan et al., 1987). Thus, the region of AlaRS between Arg368 and Asp461 is required, either directly or indirectly, for recognition and binding of the acceptor stem of $tRNA^{Ala}$.

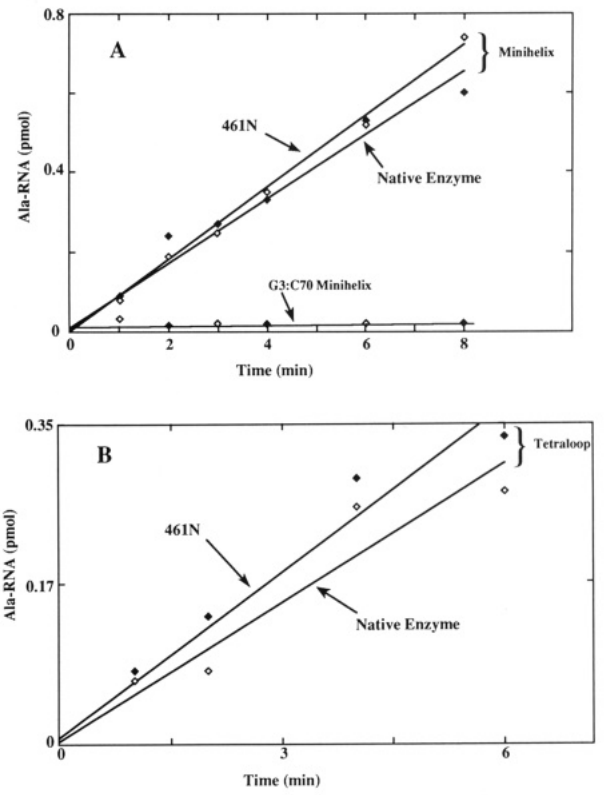

FIGURE 3: Aminoacylation of RNA oligonucleotides with alanine at pH 7.5, 25 °C. The number of picomoles of charged RNA in a 10-μL aliquot of a 100-μL reaction is plotted on the ordinate axis. (A) Aminoacylation of G3·U70 $minihelix^{Ala}$ and G3·C70 $minihelix^{Ala}$ by AlaRS and fragment 461N. Enzyme concentrations were 30 nM, and RNA concentrations were 2.5 μM. (B) Aminoacylation of $tetraloop^{Ala}$ (5 μM) by AlaRS (20 nM) and fragment 461N (20 nM).

These observations suggest that the higher overall efficiency of aminoacylation of $tRNA^{Ala}$ versus $minihelix^{Ala}$ by the full-length enzyme might be explained by protein–RNA interactions occurring outside of the acceptor–TΨC stem. The full-length enzyme, in addition to the acceptor–TΨC stem,

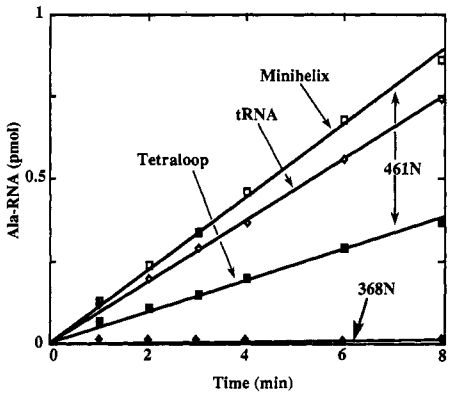

FIGURE 4: Aminoacylation of RNA oligonucleotides by fragments 461N and 368N at pH 7.5, 25 °C. Enzyme concentrations were 30 nM, and RNA concentrations were 2.5 μM. The number of picomoles of charged RNA in a 10-μL aliquot of a 100-μL reaction is plotted on the ordinate axis.

contacts the D-loop and base of the anticodon stem, but not the anticodon loop (Park & Schimmel, 1988). Because the charging rates of $tRNA^{Ala}$, $minihelix^{Ala}$, and $tetraloop^{Ala}$ by 461N were similar (Figure 4), we expected that contacts with the acceptor–TΨC domain would be the predominant ones made by fragment 461N. We performed a set of limited nuclease protection experiments using pancreatic RNase A and cobra venom RNase V_1 and compared the protection of $tRNA^{Ala}$ seen with fragment 461N to that observed with native enzyme (Figure 5). A subset of representative sites is shown in Figure 5. Other sites not shown were consistent with previous results obtained with AlaRS (Park & Schimmel, 1988) and were identical for both fragment 461N and native enzyme. As expected from the differences in K_D for $tRNA^{Ala}$ of the two enzymes (Regan et al., 1987), two to five times higher concentrations of fragment 461N were required to give protection comparable to native enzyme. The sites of protection shown in Figure 5 were specific and were not seen with BSA as a control (data not shown).

Unexpectedly, fragment 461N, like native AlaRS, protected regions outside of the acceptor–TΨC domain, including the most distal sites p30 and p42 in the anticodon stem. With one possible exception (see below), the patterns of protection for the full-length and truncated enzyme were identical. Furthermore, the contacts made by fragment 461N outside the acceptor–TΨC domain of $tRNA^{Ala}$, such as those on the anticodon stem, have no effect on the efficiency of aminoacylation of $tRNA^{Ala}$ by the fragment. This conclusion follows from the identical rates of aminoacylation by fragment 461N of $tRNA^{Ala}$ and $minihelix^{Ala}$ (Figure 4).

Of the phosphates probed in the nuclease protection experiments, position p18 in the D-loop was the only site where a difference between native enzyme and fragment 461N contacts was observed (Figure 5). Under conditions where protection was observed at the 3′-end and at other sites of $tRNA^{Ala}$, we were unable to observe protection at p18 with fragment 461N, although protection was observed with native enzyme (Figure 5). Upon increasing the concentration of 461N to greater than 100 μM, protection was observed (data not shown), even though other sites that were not protected at lower protein concentrations were not protected at the higher concentration. Although fragment 461N may contact the D-loop, such contacts appear to be considerably weaker than those with native enzyme. It has been suggested that nucleotides in the D-loop, specifically C16, U17, and G20, are minor identity elements of $tRNA^{Ala}$ (McClain & Foss, 1988; McClain et al., 1991; Tamura et al., 1991). If these contacts are not made with fragment 461N, then this may in part account for its decreased aminoacylation activity with $tRNA^{Ala}$ as substrate.

DISCUSSION

The properties of the N-terminal fragments of AlaRS are summarized in Figure 6. All of the fragments lack the domain for oligomerization and are monomeric. Each has full adenylate synthesis activity, but aminoacylation of $tRNA^{Ala}$ progressively decreases as deletions from the C-terminus are made. However, the efficiency of aminoacylation of acceptor stem oligonucleotides is essentially the same for the native tetrameric enzyme and the 461 amino acid N-terminal fragment. Deletion of amino acids between Asp461 and Arg368 abolishes aminoacylation of all RNA substrates tested.

In the class I synthetases, exemplified by the crystal structure of glutaminyl-tRNA synthetase bound to $tRNA^{Gln}$, the topologically conserved α/β active site domain contains a five-stranded parallel β-sheet dinucleotide-binding fold (Rould et al., 1989). Inserted between the two halves of this fold is a 110 amino acid domain that makes specific contacts to the tRNA acceptor stem. The class II enzymes, typified by the crystal structures of the aspartyl (Ruff et al., 1991) and seryl enzymes (Cusack et al., 1990), have an active site architecture designed around a seven-stranded antiparallel β-sheet. In the structure of the aspartyl enzyme complexed with $tRNA^{Asp}$ (Ruff et al., 1991), a loop inserted between the two β-strands of motif 2 makes sequence specific contacts with nucleotides in the acceptor stem of the tRNA. For both the class I glutaminyl- and class II aspartyl-tRNA synthetases, RNA interactions outside the acceptor stem are mediated by additional domains that can be idiosyncratic to the synthetase. It has not been determined whether adenylate synthesis and acceptor-stem interactions require these additional domains or whether the active site domain can function independently. Sequence homology of AlaRS to the aspartyl and seryl enzymes suggest that it also contains an active site domain built around the antiparallel β-sheet motif (Cusack et al., 1991). Our results indicate that in AlaRS this domain is able to function without the remainder of the protein and that acceptor-stem interactions do not require protein domains outside of the active site. Analogous to the aspartyl system, these interactions may, in part, be mediated by the loop between the β-strands of motif 2 which is proposed to be located between Lys73 and Phe89 (Cusack et al., 1991).

Ho et al. (1985) isolated a Ala409 → Val fragment 461N mutant that had a 5-fold higher k_{cat}/K_m for aminoacylation. The binding of A409V fragment 461N to $tRNA^{Ala}$ (at pH 5.5) was enhanced by 3-fold ($\sim$0.65 kcal·mol^{-1}) (Regan et al., 1988). Introduction of the A409V mutation into the full-length enzyme also resulted in a 5-fold increase in k_{cat}/K_m (Regan et al., 1988). This change resulted solely from a decrease in the K_m for $tRNA^{Ala}$. Thus, whether expressed in the context of the fragment or the full-length protein, the compensatory effect of the mutation is identical and the C-terminal oligomerization domain of AlaRS does not influence the phenotype of the A409V mutation. This is consistent with the lack of an effect of the removal of the C-terminal domain on aminoacylation of either $minihelix^{Ala}$ or $tetraloop^{Ala}$.

Deletion mutants that remove part of the C-terminal anticodon-binding domain of the class I methionyl-tRNA synthetase have no effect on either amino acid activation or aminoacylation of an RNA minihelix based on the acceptor stem of $tRNA^{fMet}$ (Kim & Schimmel, 1992). In

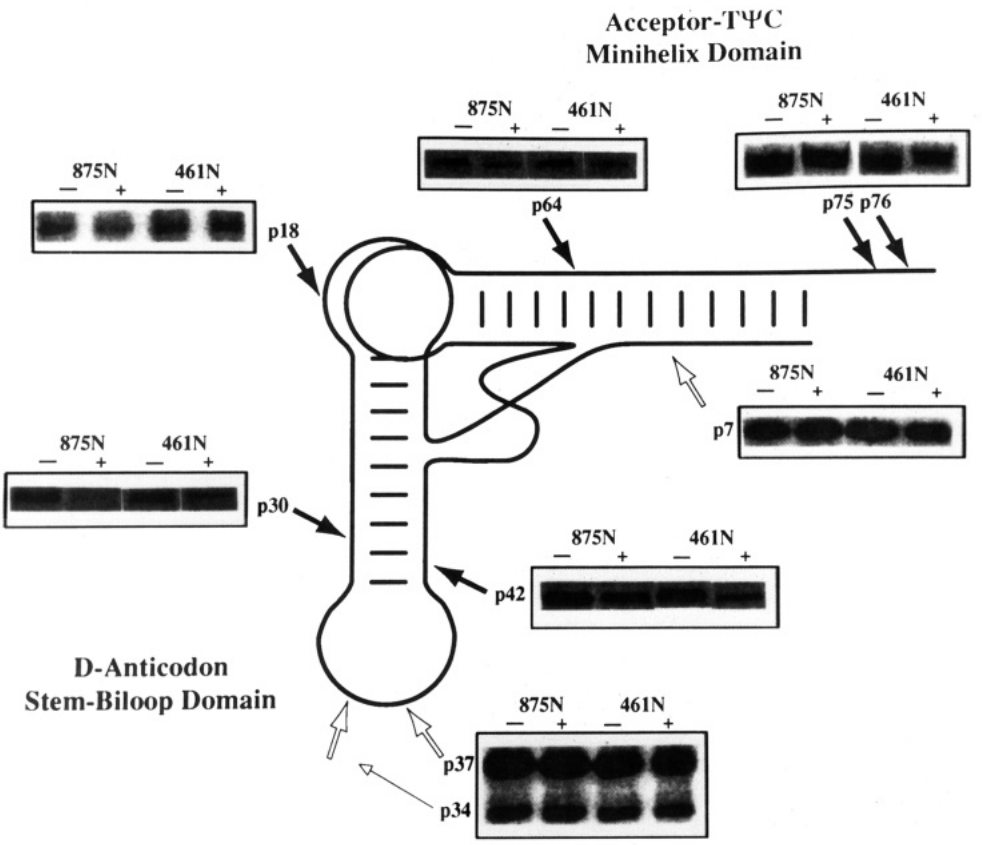

FIGURE 5: Phosphates of $tRNA^{Ala}$ probed by nuclease protection. Phosphates are numbered consecutively from the 5′-end. Protection at phosphates 30 (p30) and 42 (p42) was determined with cobra venom RNase V_1. All other sites were probed with pancreatic RNase A. Films were scanned on an Apple Scanner (Model A9M0337), and scans were used without further modification. Filled arrows indicate sites in the phosphate backbone where protection by both AlaRS and fragment 461N was seen, with the exception of p18 where differential protection was observed (see text). Open arrows indicate sites where neither enzyme showed protection. In each case, the bands shown are in the absence (–) or presence (+) of the indicated protein.

addition, Rudinger et al. (1992) showed that mutations in the acceptor stem of $tRNA^{Asp}$ that affect aminoacylation do not alter the enzyme–tRNA footprint at the anticodon loop. These examples suggest that, analogous to the alanyl system, interactions with the acceptor helix and active site are, at least under some circumstances, functionally independent of more distal interactions in the enzyme–tRNA complex.

On the basis of the results in Figure 4, acceptor-stem interactions alone are sufficient to account for the rate of charging of $tRNA^{Ala}$ by fragment 461N. Francklyn et al. (1992) and Shi et al. (1992) have estimated that interactions with the acceptor stem, which is recreated in $minihelix^{Ala}$ and in part in $tetraloop^{Ala}$, lower the free energy of activation ($\Delta G^{\circ\ddagger}$) at 37 °C for aminoacylation by >10 kcal·mol^{-1}. Nucleotides outside the minihelix domain decrease $\Delta G^{\circ\ddagger}$ by only ~3.6 kcal·mol^{-1}. Because fragment 461N aminoacylates $minihelix^{Ala}$ as efficiently as $tRNA^{Ala}$ and because full-length enzyme and fragment 461N charge $minihelix^{Ala}$ with similar efficiency (Figure 3A), this ~3.6 kcal·mol^{-1} contribution appears to result from interactions between the C-terminal region of AlaRS (beyond Asp461) and RNA sequences outside of the minihelix domain. The contribution to aminoacylation of the missing residues in fragment 461N can also be estimated by comparing the value of k_{cat}/K_m for aminoacylation of $tRNA^{Ala}$ by the fragment to that for aminoacylation by the wild-type enzyme. Using published values for k_{cat}/K_m for AlaRS (Jasin et al., 1985; Regan, 1986), a value of 4.9 kcal·mol^{-1} is calculated for this contribution. These kinetic parameters were obtained at 30 °C under assay conditions slightly different from those used by Francklyn et al. (1992) and Shi et al. (1992). Although a strict comparison is not possible, if the difference between 3.6 and 4.9 kcal·mol^{-1} is significant, then it might be attributed to weak coupling between the C-terminal and N-terminal domains of AlaRS. This analysis also implies that there is little, if any, coupling between the minihelix domain and the D-anticodon stem-biloop domain of $tRNA^{Ala}$.

Apparently specific contacts in the fragment 461N–$tRNA^{Ala}$ complex outside the acceptor stem do not influence the rate of aminoacylation of $tRNA^{Ala}$ (Figures 4 and 5). Nuclease protection outside the minihelix domain may be indirect and result from fragment 461N sterically preventing access to a site without making direct contact with the tRNA. Sites in phosphorothioate-substituted $tRNA^{Asp}$ that apparently do not make contact with AspRS have been reported to be protected against iodine cleavage (Rudinger et al., 1992). This protection presumably results from conformational changes that occur in the tRNA upon binding to the synthetase.

Although amino acids outside of the N-terminal 461 amino acids of AlaRS are important for efficiency of aminoacylation, they are not required for transfer of the activated amino acid to the 3′-end of an RNA substrate. The N-terminal domain is a core functional unit in which acceptor helix interactions required for aminoacylation are closely linked to regions involved in adenylate synthesis. This arrangement may represent a primitive tRNA synthetase and be the unit upon which additional protein components were built as needed to optimize aminoacylation and/or to fulfill other roles. Because important nucleotides in the acceptor stem of $tRNA^{Ala}$ are close to the 3′-end of the RNA, early synthetases may have been small and not able to contact more than a few nucleotides or base pairs beyond the catalytic site.

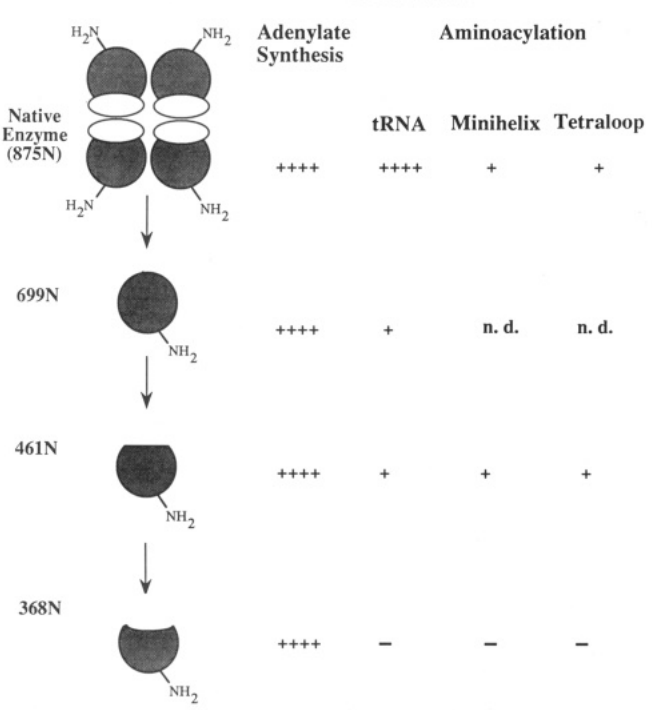

FIGURE 6: Schematic showing the relationship between various N-terminal fragments of alanine tNRA synthetase. The native tetrameric enzyme is shown with a 2-fold axis of symmetry (Putney et al., 1981), but the details of the structure are not known. Fragments 699N, 461N, and 368N are monomeric. Four pluses under "activities" indicates full activity compared to that of the native enzyme. One plus indicates a decrease in activity of approximately 3 orders of magnitude compared to the native enzyme, and a minus indicates no detectable activity (Regan et al., 1987; this work). n.d. indicates not determined.

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