Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 8655–8659, November 1990
Biochemistry

Enzymatic aminoacylation of an eight-base-pair microhelix with histidine

(tRNA identity/genetic code/RNA recognition/aminoacyl-tRNA synthetase)

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Contributed by Paul Schimmel, August 8, 1990

ABSTRACT **The major determinant for the identity of alanine tRNAs is a single base pair in the acceptor helix that is proximal to the site of amino acid attachment. A 7-base-pair microhelix that recreates the acceptor helix can be charged with alanine. No other examples of charging of small helices with specific amino acids have been reported, to our knowledge. We show here that a 13-base-pair and an 8-base-pair hairpin helix that reconstruct a domain and subdomain, respectively, of histidine tRNAs can be charged with histidine. We also show that transplantation of a base pair that is unique to histidine tRNAs is sufficient to consider histidine acceptance on a domain and subdomain of alanine tRNA. Both alanine and histidine aminoacyl-tRNA synthetases retain specficity for their cognate synthetic substrates. Alanine- and histidine-specific microhelices may resemble a system that arose early in the evolution of charging and coding.**

Within the sequence framework imposed by a conserved three-dimensional structure, all tRNA molecules possess nucleotides that permit recognition and specific aminoacylation by their cognate aminoacyl-tRNA synthetases. Recent studies suggest that, depending on the specific tRNA, determinants for discrimination by synthetases are located in the acceptor stem and, additionally or alternatively, the anticodon (1–9). A G3·U70 base pair in the acceptor stem is the major determinant for alanine identity in *Escherichia coli* and in two eukaryotes that have been investigated (3, 10, 11). Moreover, short helical hairpins that recreate the acceptor-TΨC (minihelix) or the acceptor stem (microhelix) of $tRNA^{Ala}$ are efficiently charged *in vitro* by *E. coli* alanine-tRNA synthetase (EC 6.1.1.7) (12, 13). So far, to our knowledge, this is the only system where enzymatic charging of a microhelix has been reported.

To address whether the ability to specifically aminoacylate short RNA helices is unique to alanine-tRNA synthetase, preparations of $minihelix^{Ala}$ were incubated with a mixture of *E. coli* aminoacyl-tRNA synthetases in a series of individual reaction mixtures containing one of the following radioactively labeled amino acids: alanine, leucine, valine, tyrosine, aspartate, isoleucine, glutamine, serine, or histidine. Only the mixture containing alanine showed incorporation of radioactivity above background (unpublished data). Control experiments utilizing crude *E. coli* tRNA showed the extracts to be highly active in the whole set of synthetases. Similar negative results were obtained in experiments where the crude synthetase mixture and groups encompassing all unlabeled natural amino acids were added in an attempt to depress the aminoacylation with radioactive alanine. Thus, RNA in the mini- or microhelix format *per se* is not a substrate for any of the tRNA synthetases tested. We therefore turned our attention to those tRNAs other than alanine whose aminoacylation was sensitive to specific base substitutions in the acceptor stem.

Histidine tRNAs are unique for the additional guanosine (G−1) present at their 5′ end (14, 15). This nucleotide is encoded in the gene in prokaryotes and chloroplasts, but it is added post-transcriptionally during tRNA maturation in *Drosophila* and *Schizosaccharomyces pombe* and, by inference, possibly in other eukaryotes as well (16). In *E. coli* histidine tRNAs, G−1 is paired with C73. Deletion of G−1 or substitution of G−1·C73 by G·A, G·G, or A·N base pairs decreased the relative k_{cat}/V_{max} for aminoacylation with histidine by 2–4 orders of magnitude (17). Although these experiments suggest that the G−1·C73 base pair is required for efficient aminoacylation, the influence of other nucleotides has yet to be evaluated, and transplantation experiments have not been carried out to show that G−1·C73 alone confers histidine charging on a heterologous substrate.

In view of the evidence that the 5′ end of the acceptor stem of $tRNA^{His}$ is important for aminoacylation, we investigate here whether mini- and microhelices based on the sequence of $tRNA^{His}$ are substrates for histidine-tRNA synthetase (EC 6.1.1.21). The RNAs were designed to recreate the acceptor and acceptor-TΨC stems of $tRNA^{His}$, as was done previously for $tRNA^{Ala}$ (12). We established that small helical hairpins could be aminoacylated in a sequence-dependent fashion, and then we attempted to find the minimal sequence change required to confer histidine acceptance on a heterologous RNA helix.

MATERIALS AND METHODS

***In Vitro* RNA Synthesis.** T7 RNA polymerase synthesis of RNA transcripts was carried out according to previously published methods (12, 18, 19). The DNA templates utilized an 18-base-pair duplex corresponding to the T7 promoter and a long 5′ overhang of variable length complementary to the sequence of the desired RNA product. T7 RNA polymerase was purified from the strain pAR1219/BL 21 according to Davanloo *et al.* (20) and Grodberg and Dunn (21). The transcription reaction mixtures were incubated for 4 hr at 37°C in a buffer with the following composition: 40 mM Tris·HCl (pH 8.1), 5 mM dithiothreitol, 1 mM spermidine, bovine serum albumin at 50 μg/ml, polyethylene glycol (M_r 8000) at 80 mg/ml, 20 mM $MgCl_2$, each nucleoside triphosphate (Pharmacia) at 4 mM, each DNA strand at 0.25–0.5 μM, and T7 RNA polymerase at 2.5 units/ml. To obtain transcripts with a 5′-monophosphate, a 5-fold molar excess of GMP over GTP was added, as described (19). The reactions were terminated by adding Na_2EDTA to a final concentration of 50 mM, followed by extraction with phenol/chloroform and precipitation with ethanol.

The transcripts were purified on 3-mm 20% preparative polyacrylamide gels that were run overnight at 45-mA constant current. Transcripts were visualized by UV shadowing, and then eluted from crushed gel slices by overnight diffusion into 0.5 M NH_4OAc/1 mM EDTA. The concentration of

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active RNA molecules in preparations of mini- and microhelix^{His} was determined by measuring the aminoacylation plateau under standard conditions. For purified preparations that were of homogeneous length by analytical denaturing gel electrophoresis, this value agreed closely with the concentration calculated by absorbance determinations at 260 nm (pH 7.5, 23°C).

Aminoacylation Assays. Aminoacylation of mini- and microhelices with alanine and alanine-tRNA synthetase was performed according to ref. 12 without modification. Histidine charging assays were carried out at 37°C in a buffer containing 50 mM Hepes (pH 7.5), 8 mM 2-mercaptoethanol, 10 mM $MgCl_2$, 2.5 mM ATP, 22.4 μM [^{3}H]histidine (Amersham or New England Nuclear), and various amounts of enzyme and RNA. The reactions were terminated by spotting aliquots onto Whatman 3 MM pads that had been soaked in 5% trichloroacetic acid, followed by two more washes with trichloroacetic acid and a wash with 95% (vol/vol) ethanol. After drying, bound tritium was measured by liquid scintillation counting.

Kinetic parameters for the aminoacylation of tRNA^{His} and the RNA helices were determined by measuring the initial rate of charging over the first 2.5 min (tRNA^{His}) or 4.0 min (mini- and microhelix^{His}) with catalytic (3.8 nM) amounts of histidine-tRNA synthetase. Each initial rate at a given substrate concentration was determined in duplicate, and the slope of line was derived by linear regression. The parameters were derived from Lineweaver–Burk plots. The concentration ranges of each of the RNA substrates used to measure the kinetic parameters were as follows: tRNA^{His}, 0.25–20 μM; minihelix^{His}, 1.0–50 μM; and microhelix^{His}, 5.0–250 μM. The Michaelis constant, K_m (pH 7.4, 37°C), for histidine in the aminoacylation reaction for *E. coli* histidine-tRNA synthetase has been previously reported as 6 μM, and the K_m for ATP in the pyrophosphate exchange reaction was reported to be 0.32 mM (22). The parameters for all RNA substrates studied here were determined at concentrations of ATP (4 mM) and histidine (22.4 μM) that were near saturation on the basis of the reported K_m values.

Preparation of *E. coli* Histidine-tRNA Synthetase. Details concerning strain construction and enzyme purification will be published elsewhere. In brief, a strain for the overexpression of *E. coli* histidine-tRNA synthetase was constructed by subcloning the coding sequences for the enzyme from plasmid pSE421 (23) in plasmid pKK223-3 (Pharmacia), using oligonucleotide "joining primers" and the polymerase chain reaction, according to a previously published method (24). For expression of the protein, the resulting plasmid, pHRS-1, was used to transform the bacterial strain JM109 (25) to ampicillin resistance, and then 5-liter tryptone broth cultures (26) were induced with 1 mM isopropyl β-D-thiogalactoside. After overnight induction, the cells were collected by centrifugation and then disrupted in a French press. Clarified crude extracts were applied to a Pharmacia FPLC Mono Q column that had been equilibrated with 50 mM potassium phosphate (pH 7.4)/6 mM 2-mercaptoethanol/5%

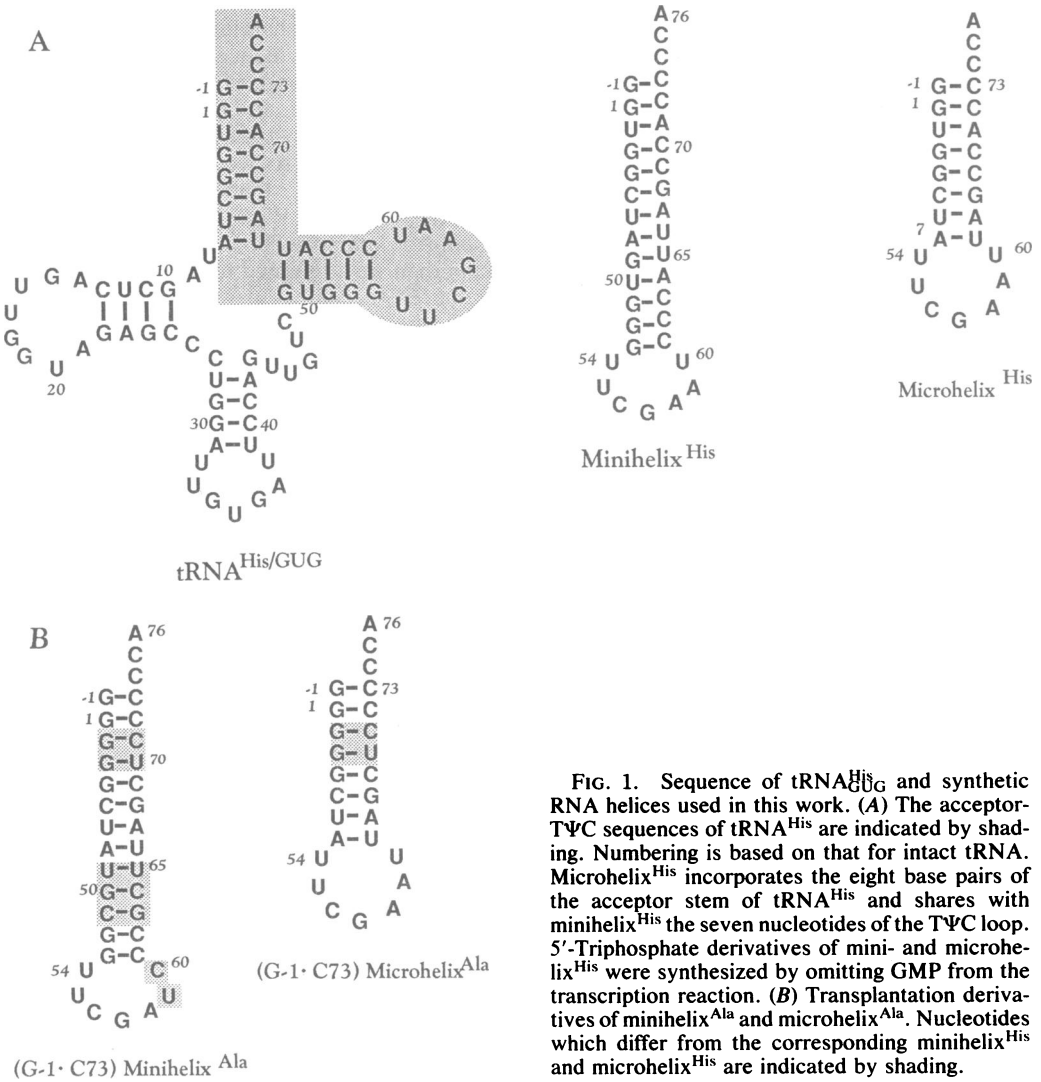

FIG. 1. Sequence of tRNA$^{His}_{GUG}$ and synthetic RNA helices used in this work. (*A*) The acceptor-TΨC sequences of tRNA^{His} are indicated by shading. Numbering is based on that for intact tRNA. Microhelix^{His} incorporates the eight base pairs of the acceptor stem of tRNA^{His} and shares with minihelix^{His} the seven nucleotides of the TΨC loop. 5′-Triphosphate derivatives of mini- and microhelix^{His} were synthesized by omitting GMP from the transcription reaction. (*B*) Transplantation derivatives of minihelix^{Ala} and microhelix^{Ala}. Nucleotides which differ from the corresponding minihelix^{His} and microhelix^{His} are indicated by shading.

(vol/vol) glycerol/0.1% phenylmethylsulfonyl fluoride. The protein was eluted with a linear gradient of 0.1–0.5 M KCl, and the peak fractions of histidine-tRNA synthetase activity (at approximately 0.25–0.3 M KCl) were pooled, concentrated, and brought to 50% (vol/vol) glycerol for storage at −20°C. The concentration of histidine-tRNA synthetase for aminoacylation assays was determined by active site titration (27).

RESULTS

Design of Mini- and Microhelix^{His} Substrates. Mini- and microhelix^{His} feature a seven-nucleotide loop (derived from the TΨC loop of tRNA^{His}) and contain the extra G−1·C73 base pair that is unique to tRNA^{His} (Fig. 1*A*). Several variants were designed to test the role of the G−1·C73 base pair. For most substrates used in this work, the addition of a 5-fold molar excess of GMP over GTP ensured that greater than 95% of the molecules initiated with a monophosphate at the 5′ end (19). GMP was omitted from transcription reaction mixtures containing minihelix^{His} or microhelix^{His} templates to synthesize (pppG−1)-mini- and -microhelix^{His}. In the last two substrates (Fig. 1*B*), G−1·C73 was transplanted into RNAs that are based on the sequences of minihelix^{Ala} and microhelix^{Ala}. The stem and loop nucleotides of G−1·C73 micro- and minihelix^{Ala} that differ from their micro- and minihelix^{His} counterparts are indicated by shading.

Minihelix^{His} and Microhelix^{His} Are Substrates for *E. coli* Histidine-tRNA Synthetase. When tested under standard aminoacylation conditions (pH 7.5, 37°C) with catalytic amounts of histidine-tRNA synthetase, both minihelix^{His} and microhelix^{His} are efficiently aminoacylated with histidine (Fig. 2). Minihelix^{Ala} and microhelix^{Ala} were not aminoacylated with histidine above background levels (Fig. 2), even with a nearly stoichiometric amount of enzyme. Similarly, the histidine tRNA helices were not aminoacylated with alanine by purified alanine-tRNA synthetase (data not shown). Thus, both alanine- and histidine-tRNA synthetases can aminoacylate small hairpin helices, and each enzyme retains specificity for its cognate mini- and microhelix.

Initial rates of charging of tRNA^{His} and of mini- and microhelix^{His} were determined over a 50-fold range of concentrations, and Michaelis–Menten kinetic parameters were obtained from double-reciprocal plots. As shown in Table 1, the relative second-order rate constant k_{cat}/K_m for minihelix^{His} and microhelix^{His} is decreased by a factor of 142 and 500, respectively, relative to that of full-length tRNA^{His}. Surprisingly, the value of k_{cat} for the microhelix is nearly twice that of the minihelix so that, while the additional sequences in the minihelix promote tighter binding (reflected in the smaller value of K_m), they also slow the rate-determining step (possibly release of the aminoacylated product from the enzyme). However, the increased K_m of the microhelix relative to tRNA^{His} (96 μM vs. 3.24 μM) corresponds to approximately 2.0 kcal·mol^{-1}, which could be accounted for by the loss of just 1 or 2 van der Waals contacts. These contacts may promote a maximal rate of aminoacylation (as shown by relative values of k_{cat} for the various substrates) but are not absolutely required for charging.

Under the standard transcription reaction conditions, T7 RNA polymerase initiates transcription with a 5′-triphosphate; substrates containing 5′-monophosphate groups were obtained by incorporating at least a 5-fold molar excess of GMP over GTP in the transcription reaction mixture (19). When preparations of minihelix^{His} and microhelix^{His} bearing either a monophosphate or triphosphate at the 5′ end were compared for extent of aminoacylation with catalytic amounts of enzyme (50 nM), the 5′-triphosphate preparations were aminoacylated to an extent significantly reduced relative to that observed with a 5′-monophosphate preparation

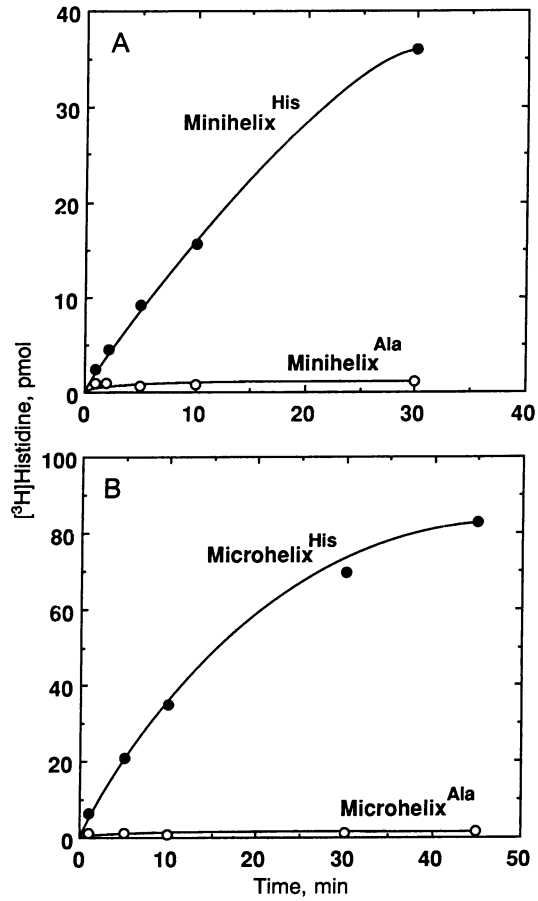

FIG. 2. Aminoacylation of mini- and microhelix substrates with histidine-tRNA synthetase. (*A*) Comparison of cognate and noncognate minihelices. (*B*) Comparison of cognate and noncognate microhelices. The assays were carried out as described in the text. RNAs were added to a final concentration of 1 A_{260} unit/ml (approximately 3.8 μM for minihelices and 5 μM for microhelices). Histidine-tRNA synthetase was added to a final concentration of 50 nM. At higher concentrations of enzyme (1 μM), aminoacylation of the noncognate substrates was still at background levels. The incorporation of histidine per 18-μl reaction aliquot is given on the ordinate.

(Fig. 3). At higher enzyme concentrations (1 μM), the pppG-(−1)-microhelix^{His} is clearly charged with histidine (Fig. 3), suggesting that the triphosphate negatively modulates but does not prevent aminoacylation with histidine. Thus, the effect of the 5′-triphosphate on charging of the minihelix is similar to that reported for full-length transcripts of tRNA^{His} (17). This further suggests that the essential features for the identity of a histidine tRNA are recapitulated in the mini- and microhelix.

Transplantation of G−1·C73 into Mini- and Microhelix^{Ala}. The marked influence of the −1·73 base pair on aminoacylation of both full-length and microhelix derivatives of tRNA^{His}

Table 1. Kinetic parameters for the aminoacylation of tRNA and synthetic substrates with histidine at pH 7.5 and 37°C

Substrate	K_m, μM	k_{cat}, sec^{-1}	k_{cat}/K_m, (sec^{-1}·M^{-1}) × 10^{-4}
tRNA^{His}	3.2	53.7	1700
Minihelix^{His}	13.7	1.7	12
Microhelix^{His}	96.0	3.3	3.4
Minihelix^{Ala}*			(0)
Microhelix^{Ala}*			(0)

*Aminoacylation of these substrates was not above background levels at substrate amounts (1 μM) of histidine-tRNA synthetase.

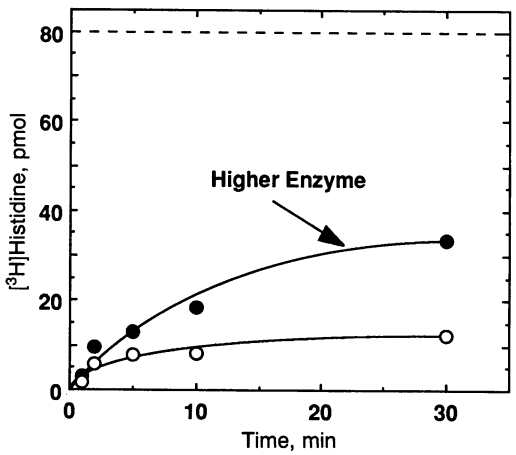

FIG. 3. Aminoacylation of (pppG−1)-microhelix^{His} with catalytic (50 nM) and higher (1 μM) enzyme concentrations. Assay conditions were as described in the legend to Fig. 2. The broken line indicates the level of charging expected for complete aminoacylation. At higher enzyme concentrations, the extent of aminoacylation is increased, but it does not reach the extents observed with the corresponding 5′-monophosphate microhelix. Similar behavior is observed for the (pppG−1)-minihelix^{His}. The incorporation of histidine per 18-μl reaction aliquot is given on the ordinate.

posed the question of whether this sequence element alone is sufficient to confer aminoacylation on heterologous sequences. Derivatives of minihelix^{Ala} and of microhelix^{Ala} were designed to add G−1 in conjunction with an A73 → C replacement. The resulting substrates are distinguished from their mini- and microhelix^{His} counterparts by five and three base pairs, respectively. In addition, minihelix^{Ala} has two base differences on the 3′ side of the loop (Fig. 1*B*). These substrates were tested in aminoacylation reactions using both catalytic and stoichiometric amounts of histidine-tRNA synthetase.

With substrate levels of enzyme, (G−1·C73)-minihelix^{Ala} and (G−1·C73)-microhelix^{Ala} are efficiently aminoacylated with histidine (Fig. 4). The initial rates of aminoacylation with *catalytic* concentrations of the enzyme for (G−1·C73)-mini- and -microhelix^{Ala} were each estimated as 2% of the initial rate of aminoacylation of the respective mini- and microhelix^{His} counterparts. The reduced efficiency of the G−1·C73 heterologous substrates suggests that, while G−1·C73 may be the major determinant recognized by histidine-tRNA synthetase, flanking nucleotides have a modulating influence. In any case, the single base pair that is unique to histidine tRNAs is sufficient to confer aminoacylation with histidine on small helical substrates that are derived from another tRNA.

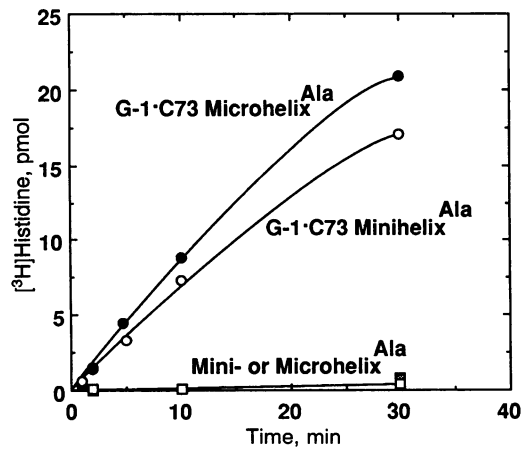

FIG. 4. Aminoacylation of (G−1·C73)-minihelix^{Ala} and of (G−1·C73)-microhelix^{Ala}. Histidine-tRNA synthetase was added to a final concentration of 500 nM. Assay conditions were in all other respects as described in the legend of Fig. 2. The incorporation of histidine per 18-μl reaction aliquot is given on the ordinate.

DISCUSSION

Histidine-tRNA synthetase is now the second example of an aminoacyl-tRNA synthetase that can charge small helical RNAs based on the acceptor stem of the cognate tRNA. In spite of the considerable deletion of tRNA structure characteristic of these substrates, the specificity of alanine- and histidine-tRNA synthetases for their cognate mini- and microhelices has been retained. This is significant, because the missing sequences could in principle have provided negative determinants that prevent misacylation with a noncognate enzyme. Also, the retention of aminoacylation specificity for these substrates is consistent with the inability of a crude *E. coli* synthetase mixture to aminoacylate minihelix^{Ala} with any of a set of at least 8 of the 20 naturally occurring L-amino acids (unpublished results).

The micro- and minihelix^{His} substrates have 8 and 14 base pairs, respectively, with a 7-base-pair loop. The sequence of this loop is unlikely to be significant for aminoacylation, because its location relative to the amino acid attachment site differs by over one-half turn of RNA helix in the two substrates. The aminoacylation of (G−1·C73)-minihelix^{Ala} with histidine is consistent with this expectation, because its loop differs at two positions from that of minihelix^{His} (Fig. 1*B*). The aminoacylation of micro- and minihelix^{Ala} is also not dependent on the loop nucleotide sequence (3, 12, 13). Thus, for both enzymes, the site for specificity is concentrated in sequences of the acceptor stem.

The ability of a single base pair (G−1·C73) substitution to confer histidine acceptance on mini- and microhelix^{Ala} is reminiscent of the ability of a single G3·U70 base pair to confer alanine acceptance on heterologous tRNA and minihelix substrates. However, in addition to G−1·C73, five base pairs of (G−1·C73)-microhelix^{Ala} are identical to base pairs in microhelix^{His} (Fig. 1*B*) and the significance of the context provided by these base pairs needs to be explored. Notwithstanding this consideration, the failure of histidine-RNA synthetase to aminoacylate microhelix^{Ala} demonstrates that these five base pairs alone cannot confer histidine acceptance. Thus it is likely that the unique position −1·73 base pair is the major determinant for recognition of micro- and minihelix^{His} and that other nucleotides play a modulating role.

Microhelix-like substrates may be analogous to early progenitors of tRNAs, such that the recognition of the anticodon sequence by some synthetases (4–8) is a later adaptation that accompanied the evolution of the full tRNA molecule. Possibly in these the determinants at the acceptor end of the molecule were translocated to the anticodon and, additionally or alternatively, interactions at the acceptor terminus now cooperate with ones at the anticodon. In the crystal structure of *E. coli* tRNA^{Gln} with glutamine-tRNA synthetase, a C-terminal β-structure in the protein interacts with the anticodon while, among other interactions at the acceptor terminus of tRNA^{Gln}, an insertion into the amino-terminal nucleotide-binding fold positions the carboxyl group of G3 of the G3·C70 base pair (8). Thus, most synthetases may have major interactions at or near the 3′ terminus of the bound cognate tRNA, and these interactions may represent ones that were sufficient to confer aminoacylation at an earlier stage of evolution.

The three-dimensional crystal structures of tyrosine-, methionine-, and glutamine-tRNA synthetases have a similar nucleotide-binding fold structural motif in the amino-terminal half of each protein (8, 28–31). Structural modeling and sequence analysis suggest that approximately half of the

aminoacyl-tRNA synthetases share this structural motif (32). In addition to methionine- and glutamine-tRNA synthetases, these enzymes include the arginine, valine, and isoleucine enzymes. For each of these synthetases, the anticodon, which is 75 Å from the amino acid attachment site, has been demonstrated to be important for tRNA identity (4, 5, 7, 8). On the other hand, alanine- and histidine-tRNA synthetases do not have the signature sequence element of the nucleotide-binding fold that is characteristic of the aforementioned enzymes (33, 34). Thus, the two enzymes now known to aminoacylate small helical substrates may also be distinguished by being members of another class of synthetases.

We thank F. William Studier for providing the strain for the overexpression of T7 RNA polymerase and Jack Parker for plasmids carrying the gene for *E. coli* histidine-tRNA synthetase. This work was supported by grant GM 15539 from the National Institutes of Health. C.F. is a National Institutes of Health Postdoctoral Fellow.

1. Schimmel, P. (1989) *Biochemistry* **28,** 2747–2759.
2. Normanly, J. & Abelson, J. (1989) *Annu. Rev. Biochem.* **58,** 1029–1049.
3. Hou, Y.-M. & Schimmel, P. (1988) *Nature (London)* **333,** 140–145.
4. Schulman, L. H. & Pelka, H. (1988) *Science* **242,** 765–768.
5. Muramatsu, T., Nishikawa, K., Nemoto, F., Kuchino, Y., Nishimura, S., Miyazawa, T. & Yokoyama, S. (1988) *Nature (London)* **336,** 179–181.
6. Sampson, J. R., DiRenzo, A., Behlen, L. & Uhlenbeck, O. C. (1989) *Science* **243,** 1363–1366.
7. Schulman, L. H. & Pelka, H. (1989) *Science* **246,** 1595–1597.
8. Rould, M. A., Perona, J. J., Soll, D. & Steitz, T. A. (1989) *Science* **246,** 1135–1141.
9. Perret, V., Garcia, A., Ebel, J.-P., Florentz, C. & Giege, R. (1990) *Nature (London)* **344,** 787–789.
10. Hou, Y.-M. & Schimmel, P. (1989) *Biochemistry* **28,** 6800–6804.
11. McClain, W. H. & Foss, K. (1988) *Science* **240,** 793–796.
12. Francklyn, C. & Schimmel, P. (1989) *Nature (London)* **337,** 478–481.
13. Shi, J.-P., Francklyn, C., Hill, K. & Schimmel, P. (1990) *Biochemistry* **29,** 3621–3626.
14. Sprinzl, M., Hartman, T., Weber, J., Blank, J. & Zeidler, R. (1989) *Nucleic Acids Res.* **17,** Suppl., r1–r172.
15. Harada, F., Sato, S. & Nishimura, S. (1972) *FEBS Lett.* **19,** 352–354.
16. Cooley, L., Appel, B. & Soll, D. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 6475–6479.
17. Himeno, H., Hasegawa, T., Ueda, T., Watanabe, K., Miura, K. & Shimuzu, M. (1989) *Nucleic Acids Res.* **17,** 7855–7863.
18. Milligan, J. F., Groebe, D. R., Witherall, G. W. & Uhlenbeck, O. C. (1987) *Nucleic Acids Res.* **15,** 8783–8798.
19. Milligan, J. F. & Uhlenbeck, O. C. (1989) *Methods Enzymol.* **180,** 51–62.
20. Davanloo, P., Rosenberg, A. H., Dunn, J. J. & Studier, F. W. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 2035–2039.
21. Grodberg, J. D. & Dunn, J. J. (1988) *J. Bacteriol.* **170,** 1245–1253.
22. Kalousek, F. & Konigsberg, W. H. (1974) *Biochemistry* **13,** 999–1006.
23. Eisenbeis, S. J. & Parker, J. (1982) *Gene* **18,** 107–114.
24. Macferrin, K. D., Terranova, M. P., Schreiber, S. L. & Verdine, G. L. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 1937–1941.
25. Yanisch-Perron, C., Vieira, J. & Messing, J. (1985) *Gene* **33,** 103–119.
26. Miller, J. H. (1972) *Experiments in Molecular Genetics* (Cold Spring Harbor Lab., Cold Spring Harbor, NY), p. 433.
27. Hill, K. & Schimmel, P. (1989) *Biochemistry* **28,** 2577–2586.
28. Bhat, T. N., Blow, D. M., Brick, P. & Nyborg, J. (1982) *J. Mol. Biol.* **158,** 699–702.
29. Blow, D. M. & Brick, P. A. (1985) *Biological Macromolecules and Assemblies,* Nucleic Acids and Interactive Proteins, eds. Jurnak, F. A. & MacPherson, A. (Wiley, New York), Vol. 2, pp. 442–469.
30. Brunie, S., Mellot, P., Zelwar, C., Risler, J.-L., Blanquet, S. & Fayat, G. (1987) *J. Mol. Graphics* **5,** 18–21, 26.
31. Blow, D. M., Bhat, T. N., Metcaffe, A., Risler, J. L., Brunie, S. & Zelwar, C. (1983) *J. Mol. Biol.* **171,** 571–576.
32. Burbaum, J. J., Starzyk, R. M. & Schimmel, P. (1990) *Proteins* **7,** 99–111.
33. Putney, S. D., Royal, N. J., de Vegvar, H. N., Herlihy, W. C., Biemann, K. & Schimmel, P. R. (1981) *Science* **213,** 1497–1501.
34. Freedman, R., Gibson, B., Donovan, D., Biemann, K., Eisenbeis, S., Parker, J. & Schimmel, P. (1985) *J. Biol. Chem.* **260,** 10063–10068.