Zinc-Dependent tRNA Binding by a Peptide Element within a tRNA Synthetase†

Elizabeth Glasfeld and Paul Schimmel*

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

Received January 23, 1997; Revised Manuscript Received March 19, 1997⊗

ABSTRACT: The class I aminoacyl-tRNA synthetases are defined by an N-terminal nucleotide binding fold that contains the active site for adenylate synthesis. Insertions and additions of idiosyncratic RNA binding elements that facilitate docking of the L-shaped tRNA structure are superimposed onto this basic fold. These RNA binding elements are imagined to have been acquired during the evolution and development of the modern genetic code. The monomeric *Escherichia coli* isoleucyl-tRNA synthetase has a zinc-containing peptide at its C terminus. Removal of the zinc-containing peptide was shown previously to create a shortened enzyme with activity for adenylate synthesis but no detectable binding to $tRNA^{Ile}$. We show here that the isolated zinc-containing peptide binds to tRNA with relatively low affinity. This binding is not tRNA-specific but shows a strict requirement for zinc. In contrast, the zinc-containing peptide conferred specific and high-affinity binding when combined with the shortened enzyme. Thus, when combined with another protein, a nonspecific tRNA binding peptide is essential for formation of a high-affinity and specific tRNA binding site. These results demonstrate the feasibility of the idea that noncovalent complexes of general RNA-binding peptides with a domain for adenylate synthesis were precursors to modern tRNA synthetases. In addition, the results offer the first direct evidence of a role for zinc in the tRNA-binding activity of one of these peptide elements.

We report here a demonstration and analysis of the tRNA binding properties of a small zinc-containing peptide in a tRNA synthetase. The aminoacyl-tRNA synthetases catalyze attachment of amino acids to their cognate tRNAs and, thereby, establish the genetic code. The 20 tRNA synthetases have been grouped into two classes of 10 enzymes each on the basis of their active site structures (*1, 2*). The class I enzymes are characterized by an active site composed of a Rossmann nucleotide binding fold that encompasses the 11-amino acid signature sequence ending in HIGH and the KMSKS motif (*3, 4*). The active site of the class II enzymes is built around an antiparallel β-sheet with flanking α-helices and has three characteristic sequences known as motifs 1, 2, and 3. The earliest synthetases are thought to have consisted of the class-defining active site domains. Through evolution, RNA-binding elements have been appended to or inserted into the class-defining domains (*5, 6*). We believe that the peptide investigated here is an example of one of these elements.

The L-shaped tRNA structure is divided into two domains. One domain contains the acceptor stem and amino acid attachment site. This domain is believed to be the historical, early form of a tRNA (*5, 7*−*9*). The second domain contains the template reading head with the anticodon trinucleotide. Whereas the C-terminal domains of the class I synthetases bind the anticodon arm of the tRNA, the N-terminal domains have RNA binding elements that interact with the acceptor stem. Studies with minihelix and microhelix models of this part of the tRNA structure have demonstrated that the acceptor stem contains sufficient determinants to allow for specific aminoacylation by at least 11 aminoacyl-tRNA synthetases (*10*−*13*). In the cocrystal structure of glutaminyl-tRNA synthetase complexed with $tRNA^{Gln}$, contacts with the acceptor stem occur via a subdomain inserted into the nucleotide binding fold (*14*). This acceptor stem binding subdomain, also known as connective polypeptide 1 (CP1),[1] links together the two halves of the nucleotide binding fold. The structures and sequences of the CP1 insertions appear to be idiosyncratic among the class I enzymes (*15*). The limited extent of redundancy of RNA binding motifs among the aminoacyl-tRNA synthetases makes assignment of important protein contacts to the tRNA difficult in synthetases of unknown structure.

The analysis of the N-terminal catalytic domain of the class I *Escherichia coli* isoleucyl-tRNA synthetase benefits from the enzyme's sequence homology to *E. coli* methionyl-tRNA synthetase (*3*), whose crystal structure has been solved (*16*). The Rossmann nucleotide binding fold of the isoleucyl-tRNA synthetase monomer has been modeled on the basis of this relationship (Figure 1) (*17, 18*). The larger size of the isoleucine enzyme (939 amino acids) compared to the methionine enzyme (676 amino acids) is due in part to the larger CP1 insertion (approximately 300 amino acids) into the active site of isoleucyl-tRNA synthetase. The similarity between the two enzymes also extends to at least parts of their C-terminal domains which provide the anticodon binding sites. For example, anticodon recognition can be switched from that of $tRNA^{Met}$ to $tRNA^{Ile}$ (and vice versa) by a single amino acid replacement in a designed peptide transplanted into the C-terminal domain of either methionyl- or isoleucyl-tRNA synthetase (*19*). This result suggests that,

† This work was supported by Grant GM15539 from the National Institutes of Health. E.G. is an NIH postdoctoral fellow.

* To whom correspondence should be addressed at Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139, Telephone: 617-253-6739. Fax: 617-253-6636.

⊗ Abstract published in *Advance ACS Abstracts,* May 1, 1997.

[1] Abbreviations: CP1, connective polypeptide 1; ACE, affinity coelectrophoresis; 886N, N-terminal fragment of isoleucyl-tRNA synthetase (amino acids 1−886); 53C, the 53-amino acid C-terminal peptide of isoleucyl-tRNA synthetase; GST, glutathione *S*-transferase.

S0006-2960(97)00151-7 CCC: $14.00

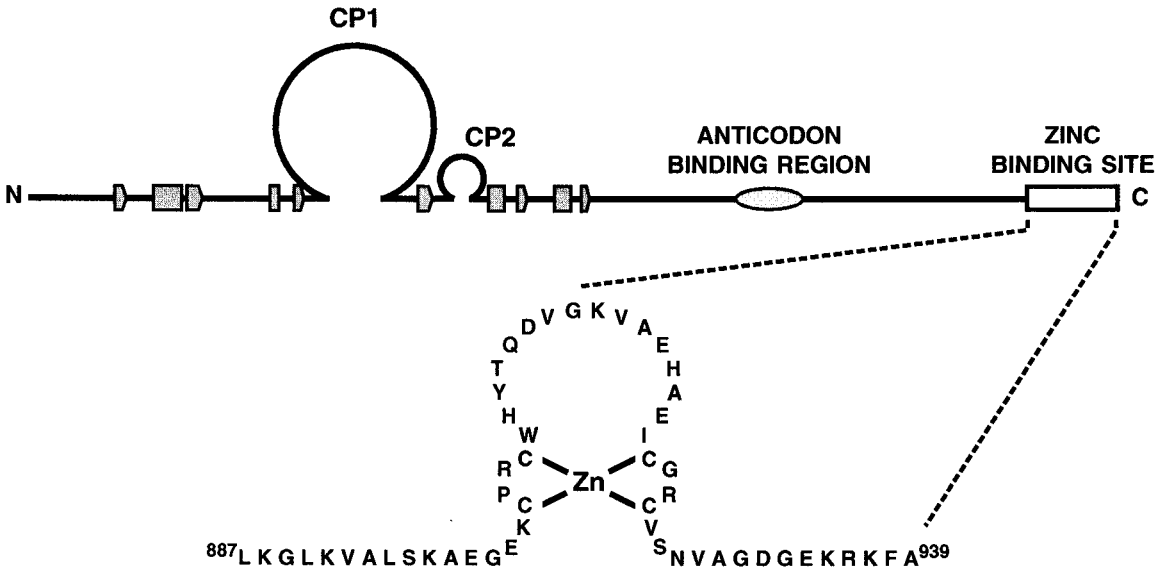

FIGURE 1: (Top) Model of the structure of *E. coli* isoleucyl-tRNA synthetase (*17*, *18*). The β-sheets and α-helices of the class-defining Rossmann fold are represented as pentagons and squares, respectively. The locations of the anticodon-binding region and the C-terminal zinc-binding site are indicated. (Bottom) Sequence of the C-terminal zinc-binding peptide Zn^{2+}-53C of isoleucyl-tRNA synthetase (residues 887−939) with zinc ligated to the four cysteines in this peptide.

in spite of little sequence similarity, the anticodon binding regions of the two enzymes are also highly homologous.

The *E. coli* isoleucyl-tRNA synthetase is unique among the synthetases in that it binds two zincs per monomer (*20*−*22*). EXAFS (extended X-ray absorption fine structure) analysis of the enzyme showed that each zinc is bound by four cysteines (*23*). One zinc is found within the N-terminal domain. The second zinc binding site is located within the last 40 amino acids of the C terminus (Figure 1) (*24*). The four cysteines which bind the C-terminal zinc [Cys902, Cys905, Cys922, and Cys925 in the *E. coli* enzyme (see Figure 1)] are conserved among most prokaryote and mitochondrial isoleucyl-tRNA synthetases (*25*). Deletion of the zinc-containing peptide leads to a truncated enzyme that does not complement an *ileS* null allele (*26*). *In vitro* studies showed that, though the adenylate synthesis activity of the shortened enzyme was unaffected by the deletion, the 886-amino acid N-terminal fragment (886N) could not catalyze the transfer of the amino acid to the tRNA. This deficiency arises from the inability of fragment 886N to bind $tRNA^{Ile}$ (*25*). A free zinc-containing peptide incorporating the C-terminal 53 amino acids (Zn^{2+}-53C) restored activity to fragment 886N when it was either coexpressed *in vivo* or added to 886N *in vitro* to produce the reassociated form of the enzyme. On the basis of this result, we imagined that Zn^{2+}-53C either had or conferred tRNA binding, although this possibility was not proven.

In methionyl-tRNA synthetase, a peptide extends from the end of the C-terminal anticodon binding domain to fold against the active site cleft (*16*). Mutations within this C-terminal extension lead to a reduced affinity for the microhelix mimic of the acceptor stem of $tRNA_f^{Met}$ (*27*). Thus, the C-terminal extension may have been incorporated into the methionyl-tRNA synthetase to work with the CP1 to provide acceptor stem recognition and binding. By analogy to the methionyl-tRNA synthetase structure, we propose that the zinc-peptide of isoleucyl-tRNA synthetase extends back to the N-terminal domain to assist in binding the acceptor stem of $tRNA^{Ile}$.

In studies presented here, we sought to clarify the role the C-terminal zinc-containing region plays in the binding of $tRNA^{Ile}$. For this purpose, we sought to determine whether the peptide alone had zinc-dependent RNA binding activity. We also wanted to establish directly whether addition of peptide 53C to fragment 886N restored tRNA binding and, if so, whether that restoration was zinc-dependent. In the course of these studies, we were able to evaluate the physical interaction energy between the peptide and the rest of isoleucyl-tRNA synthetase. This analysis suggested that at least part of this interaction energy is used to shape a binding site that is specific for $tRNA^{Ile}$.

EXPERIMENTAL PROCEDURES

Protein Purifications. The N-terminal fragment of isoleucyl-tRNA synthetase (amino acids 1−886, 886N) was expressed from the plasmid pJL22 (*25*) transformed into the *E. coli* strain ER2508 [*lon*::Tn*10*Δ*16*Δ*17* Δ*(malB) zkb*::Tn*5* Δ*(arg F-lac)U169* Δ*(mcrC-mrr)20 ara-14 galK*2 *rpsL*20 *xyl-5 mtl*1 *supE*44 *leuB*6 *fhuA*2] (New England Biolabs, Beverly, MA). Expression and purification of 886N were done as described earlier (*25*).

The glutathione *S*-transferase (GST) fusion of the C-terminal 53 amino acids of isoleucyl-tRNA synthetase was expressed from the plasmid pJL35 (*25*) in the *E. coli* strain JM109 [*recA*1 *supE*44 *endA*1 *hsdR*17 *gyrA*96 *relA*1 *thi* Δ*(lac-proAB)*/F′ (*traD*36 *proAB*$^+$ *lacI*^{q} *lacZ*Δ M15)] (*28*). The GST−peptide fusion was expressed and purified, and the peptide was cleaved from GST using thrombin and purified as described in Glasfeld et al. (*25*). The peptide was further purified on a Pharmacia Biotech (Uppsala, Sweden) Superose 12 HR 10/30 column equilibrated in 20 mM Hepes (pH 7.0), 0.1 M NaCl, and 10 mM 2-mercaptoethanol. Reduction of the four cysteines in the peptide and separation of the peptide from reductant were done as in Glasfeld et al. (*25*). $ZnCl_2$ was added to a final concentration of 0.5 mM with peptide concentrations of approximately 200−300 μM. The apo form of the peptide was prepared by reduction as described above and then passed through a 1.8 mL Sephadex G25 column at 4 °C equilibrated in 20 mM Hepes (pH 7.0), 0.1 mM EDTA, and 10 mM 2-mercaptoethanol.

Native *E. coli* IleRS was overexpressed from the phagemid pKS21 in MV1184 [*ara*, Δ(*lac-proAB*), *rspL*, *thi*, (*ϕ*80 *lacZ*ΔM15), Δ(*srl-recA*), 306::Tn*10*(*tet*^{r})/F′(*traD36*, *proAB*$^+$, *lacI*^{q}, *lacZ*ΔM15)] cells and purified as previously described (*29*).

Purification and Radioactive Labeling of tRNA. The major isoacceptor of *E. coli* tRNA^{Ile} was expressed from plasmid pES300 (*30*) transformed into the *E. coli* strain MV1184. The tRNA^{Ile} was purified from IPTG-induced cells according to the protocols described elsewhere (*30*). *E. coli* tRNA^{Glu} was purchased from Sigma (St. Louis, MO). The tRNA^{Ile} and tRNA^{Glu} were treated with calf intestine phosphatase and then 5′-labeled with ^{32}P as described elsewhere (*31*, *32*).

Affinity Coelectrophoresis. Affinity coelectrophoresis was performed as detailed previously (*33*). For the gels in which Zn^{2+}-53C was used, EDTA was omitted from the gel and running buffers. In addition, for the ACE gel in which apo-53C was added to 886N (Figure 4A), the low-melting point agarose was treated with Chelex 100 (Bio-Rad, Hercules, CA) to remove adventitious metals. This was accomplished by first melting the agarose in the gel buffer, adding Chelex, and stirring at 60 °C for 1 h. The melted agarose was then filtered through glass wool, and the gel was prepared in the usual manner. Each of the gels shown in Figures 3 and 4 was prepared in this way.

Gel Mobility Shift Assays. The gel mobility shift assay was used to examine complex formation between the zinc-peptide and tRNA^{Ile}. In a volume of 10 μL, the peptide at various concentrations was incubated with 5′-^{32}P-labeled tRNA^{Ile} in 20 mM Hepes (pH 7.0), 50 mM NaCl, 5 mM $MgCl_2$, 0.02% Nonidet P 40 (Fluka, Switzerland), and 2.5% Ficoll 400 (Pharmacia Biotech) on ice for 10 min. The complexes were electrophoresed through 10% acrylamide (60:1 acrylamide:bisacrylamide), 134 mM Tris-borate, and 5 mM $MgCl_2$ in the cold room (4 °C). The running buffer was 134 mM Tris-borate. The gels (7 cm long, 1.5 mm wide) were prerun at 160 V for 45 min. The samples were applied to a running gel at 160 V and electrophoresed for 50 min. After drying, the gels were visualized using a Molecular Dynamics (Sunnyvale, CA) phosphorimager, and complex formation was analyzed with ImageQuant software.

RESULTS

Zinc-Dependent RNA Binding by the C-Terminal Peptide. Removal of the C-terminal zinc-containing peptide (Zn^{2+}-53C) from isoleucyl-tRNA synthetase results in an N-terminal fragment (886N) which can no longer bind tRNA^{Ile} and is, therefore, unable to aminoacylate tRNA^{Ile}. Addition of Zn^{2+}-53C *in trans* to the fragment 886N rescues the aminoacylation activity of the fragment, whereas the apo form of the peptide does not (*25*). This ability of the zinc-peptide led us to investigate whether the zinc-peptide itself might have any intrinsic RNA binding properties.

Affinity coelectrophoresis (see below) could not be used to study RNA binding by the peptide, because the peptide is too small (5900 Da) to produce a detectable shift in the migration of tRNA. Therefore, we used the gel mobility shift assay to study complex formation between the zinc-peptide and tRNA^{Ile}. With this method, free tRNA^{Ile} is rapidly separated from the slower migrating Zn^{2+}-53C-bound tRNA^{Ile} upon electrophoresis through the gel.

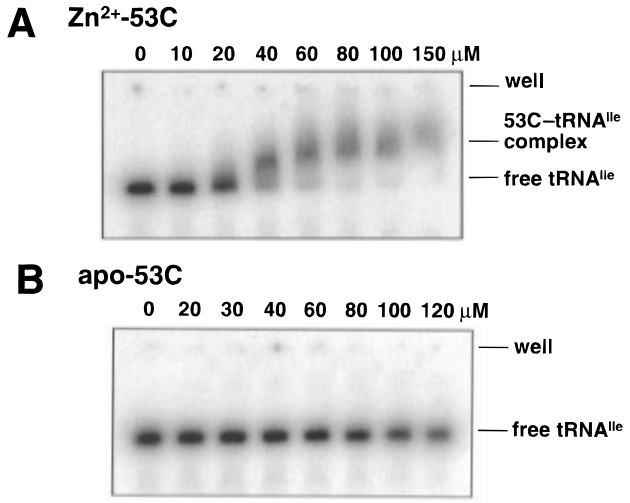

FIGURE 2: The zinc−peptide binds tRNA. (A) Gel mobility shift assay of zinc-peptide−tRNA^{Ile} complex formation at pH 7.0 and 4 °C. The concentration of peptide is indicated at the top of each lane. The details of the experiment are described in Experimental Procedures. (B) A gel mobility shift assay with the apopeptide and tRNA^{Ile}. No tRNA binding could be detected.

The zinc-peptide clearly binds tRNA^{Ile} (Figure 2A). The Zn^{2+}-53C−tRNA^{Ile} complex appears to be weak and to dissociate within the gel, giving rise to a band which is shifted yet broad. More importantly, no shift is detected with the apo form of the peptide (Figure 2B). Thus, binding is zinc-dependent.

Analysis of the binding data indicates that the apparent dissociation constant for the zinc-peptide−tRNA complex is 30 μM (pH 7 and 4 °C). However, similar affinity is also seen between the zinc-peptide and *E. coli* tRNA^{Glu} (data not shown), which does not share any sequence identity with tRNA^{Ile} at the nucleotide positions known to be strong determinants for aminoacylation by isoleucyl-tRNA synthetase (*34*, *35*). In addition, a 10-fold excess of poly(A) is sufficient to inhibit the shift seen with tRNA^{Ile} (data not shown).

These results show that Zn^{2+}-53C has nonspecific RNA binding properties. The lack of specificity and the relatively weak RNA binding do not by themselves explain the ability of Zn^{2+}-53C to reconstitute tRNA^{Ile} aminoacylation when Zn^{2+}-53C is reassociated with 886N. On the other hand, the requirement of zinc for nonspecific tRNA binding by the peptide is consistent with the requirement of zinc for the aminoacylation activity of the reassociated enzyme. These considerations motivated us to investigate the tRNA binding properties of the reassociated enzyme, and the dependence on zinc for binding.

Reconstitution of tRNA^{Ile} Binding with Fragment 886N and the C-Terminal Peptide. With the use of affinity coelectrophoresis (ACE) (*33*, *36*, *37*), we were able to investigate whether incubation of 886N with Zn^{2+}-53C would restore tRNA binding in a zinc-dependent manner. As mentioned above, previous work using the ACE procedure showed that 886N by itself does not bind to tRNA^{Ile} (*25*). Fragment 886N was incubated with either the zinc or apo form of the C-terminal peptide and imbedded at increasing concentrations in consecutive lanes of a horizontal agarose gel. The 5′-^{32}P-labeled tRNA^{Ile} was then electrophoresed through the gel and the reassociated protein. As the protein formed a complex with the tRNA, a shift in the gel mobility of the tRNA could be detected. By measurement of the shifts

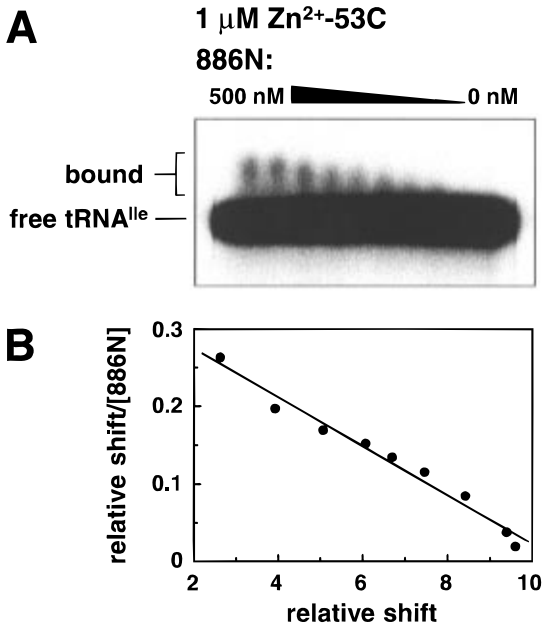

FIGURE 3: Binding of tRNA by fragment 886N was reconstituted upon addition of Zn^{2+}-53C. (A) Affinity coelectrophoresis was used to examine the restoration of tRNA binding to 886N upon addition of Zn^{2+}-53C at pH 7.5 and 25 °C. The gel has 10 parallel lanes with protein. The concentrations of 886N in the 10 lanes are, from left to right, 500, 250, 100, 65, 50, 40, 30, 20, 10, and 0 nM. Zn^{2+}-53C was included in all 10 protein lanes to a final concentration of 1 μM. (B) Scatchard analysis of a data set averaged from three gels. The relative shift of the tRNA divided by the concentration of 886N is plotted versus the relative shift.

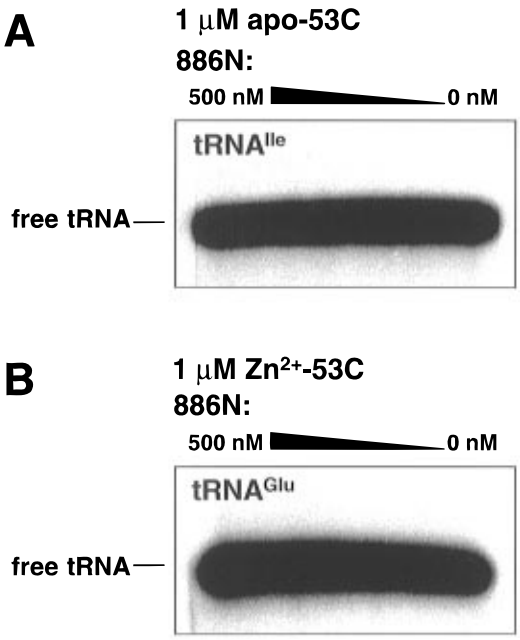

FIGURE 4: Reconstitution of tRNA binding of 886N by 53C is zinc-dependent and specific. (A) An ACE gel of 886N (at the same concentrations as in Figure 3A) combined with 1 μM apo-53C shows that tRNA binding is not restored (pH 7.5 and 25 °C). (B) The 886N−Zn^{2+}-53C complex does not bind $tRNA^{Glu}$. The protein lanes are as in Figure 3A.

observed under various concentrations of protein, the dissociation constant was determined (*36*).

The ACE gel of Figure 3A shows that, when fragment 886N was combined with Zn^{2+}-53C, $tRNA^{Ile}$ binding was reconstituted. The concentration of Zn^{2+}-53C was held constant at 1 μM throughout the 10 protein lanes, while the concentration of 886N was decreased from 500 to 10 nM. The dissociation constant measured between the $tRNA^{Ile}$ and the 886N−Zn^{2+}-53C complex was approximately 35 nM (pH 7.5 and 25 °C), compared to a K_d of about 14 nM detected between $tRNA^{Ile}$ and native isoleucyl-tRNA synthetase (*25*).

Reconstitution of $tRNA^{Ile}$ binding to 886N was dependent upon the zinc-containing form of the peptide. The apo form of 53C did not rescue tRNA binding (Figure 4A). In addition, the 886N−Zn^{2+}-53C complex maintains specificity for $tRNA^{Ile}$. In particular, binding of $tRNA^{Glu}$ was not detected (Figure 4B).

These results contrast with those of the gel mobility shift assays where zinc-dependent peptide binding to tRNA is nonspecific. On the other hand, the zinc dependency of tRNA binding in both systems suggests that the general RNA binding properties of the peptide are responsible for the zinc sensitivity of $tRNA^{Ile}$ binding in the reconstituted enzyme.

Estimation of the Interaction Energy between Fragment 886N and the C-Terminal Peptide. In addition to showing that $tRNA^{Ile}$ binding can be restored to 886N by addition of the zinc-peptide, affinity coelectrophoresis also permits estimation of the dissociation constant between the zinc-peptide and the N-terminal fragment. Upon addition of increasing amounts of the zinc-peptide to 886N, an increasing fraction of 886N is in the 886N−zinc-peptide complex, and higher affinities for $tRNA^{Ile}$ can be measured. In Figure 5A, the 886N concentration was held constant at 50 nM while the zinc-peptide concentration was increased from 0.1 to 10

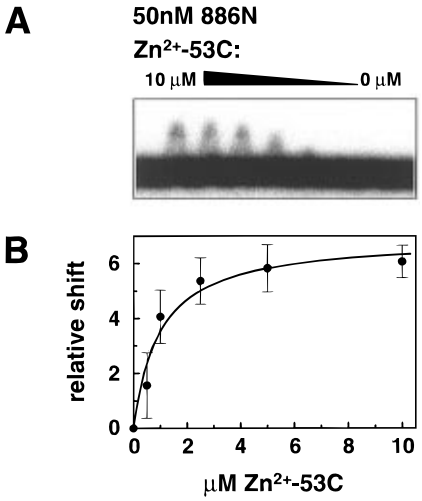

FIGURE 5: Affinity between 886N and the zinc-peptide as measured by restoration of $tRNA^{Ile}$ binding to 886N. (A) As increasing amounts of Zn^{2+}-53C are added to a constant amount of 886N at pH 7.5 and 25 °C, increased 886N−zinc-peptide complex formation can be measured as an increase in the shift in $tRNA^{Ile}$ mobility. The concentration of 886N in seven lanes is 50 nM, while the concentrations of Zn^{2+}-53C are, from left to right, 10, 5.0, 2.5, 1.0, 0.5, 0.1, and 0 μM. (B) The Zn^{2+}-53C-dependent shift is used to determine the dissociation constant of the 886N−Zn^{2+}-53C complex. The relative shift of the $tRNA^{Ile}$ is plotted versus the Zn^{2+}-53C concentration. The data represent the averages of three experiments and are fit with an equation for a simple bimolecular process.

μM. Analysis of these data (Figure 5B) gives a dissociation constant of 1 μM (pH 7.5 and 25 °C) for the interaction between the two isoleucyl-tRNA synthetase fragments when they associate *in trans*. This dissociation constant corresponds to an interaction free energy of −8.3 kcal mol^{-1}. To estimate the interaction free energy when the two components are covalently joined *in cis* (as in the native protein), the cratic entropy contribution of −2.4 kcal mol^{-1} (*38*) must be

subtracted. Thus, the interaction energy in the native protein is estimated to be -10.7 kcal mol^{-1}.

DISCUSSION

Though several class I aminoacyl-tRNA synthetases are known to have incorporated zinc-binding sites, including *E. coli* and *Thermus thermophilus* methionyl- and *E. coli* glutamyl-tRNA synthetases, isoleucyl-tRNA synthetase may be unique in that a zinc-containing peptide element contributes to tRNA binding per se. In the case of *T. thermophilus* and *E. coli* methionyl-tRNA synthetase, a role for zinc in catalytic activity was clearly established with zinc-deficient mutant enzymes (*20, 39*). The zinc-binding site of *E. coli* methionyl-tRNA synthetase is a Cys-X_2-Cys-X_9-Cys-X_2-Cys motif located within connective polypeptide 1 (*39*−*41*). Site-directed mutagenesis of any one of the four cysteines to alanine weakened zinc affinity significantly and produced a strong decrease in both adenylate synthesis and aminoacylation activities, yet the enzyme−tRNA^{Met} dissociation constant was essentially unaffected, even in the presence of EDTA (*39*). Mutations of neighboring residues did not greatly affect tRNA affinity, although effects on the K_m for tRNA^{Met} in the aminoacylation reaction were observed for some mutants (*42*).

Comparable results have been noted with the zinc-binding site of *E. coli* glutamyl-tRNA synthetase. In this enzyme, the zinc is bound by three cysteines and one histidine also located in the CP1 domain (*43*). Removal of the zinc by dialysis against 1,10-phenanthroline resulted in a change of conformation and a loss of aminoacylation activity, but tRNA^{Glu} affinity remained strong (*44*). The conclusion drawn from analyses of zinc binding to both methionyl- and glutamyl-tRNA synthetases is that zinc is necessary to maintain the correct secondary structures for positioning of the 3′-end of the acceptor stem during amino acid transfer but is not essential for tRNA binding (*42, 44*).

More generally, zinc may be required to stabilize the folding of the CP1 domain. Because this domain is inserted into the catalytic domain, activity is expected to be sensitive to perturbations of CP1. *E. coli* isoleucyl-tRNA synthetase has two zinc-binding sites, one within CP1 in addition to the other at the C terminus. Consistent with the observations for methionyl- and glutamyl-tRNA synthetases, perturbations of zinc binding to CP1 are known to affect activity of the isoleucine enzyme (*23*). However, isoleucyl-tRNA synthetase is distinct in that it has a second zinc-binding site that is outside of the catalytic domain, and this site appears to play an important role in tRNA binding, as shown here. The tRNA-modifying enzyme tRNA-guanine transglycosylase is the only other tRNA-related enzyme identified which also has a zinc site which is essential for tRNA binding, though it shares no homology with that of isoleucyl-tRNA synthetase (*45, 46*).

The zinc-dependent but nonspecific binding of peptide Zn^{2+}-53C to tRNA^{Ile} has an apparent dissociation constant of 30 μM, while no binding of fragment 886N to tRNA^{Ile} can be detected (*25*). In contrast, when the two fragments are combined, the dissociation constant for tRNA^{Ile} is lowered to 35 nM (Figure 3B). Moreover, while Zn^{2+}-53C binds to tRNA^{Glu} as well as to tRNA^{Ile}, no binding of the reassociated fragments to tRNA^{Glu} was detected (Figure 4B). These results suggest that the two components together are required for both a high-affinity and specific tRNA^{Ile} binding site. Our data show that this site is generated with an interaction energy of greater than 10 kcal mol^{-1} in the native enzyme.

The C-terminal peptide has a net positive charge of +3 (cf. Figure 1). This positive charge of Zn^{2+}-53C could provide part, if not all, of the electrostatic attraction necessary for initial binding of the tRNA, with the appropriate positioning and discrimination occurring in succeeding events (*47*). In addition, the binding of the two arms of the L-shaped tRNA could be a cooperative event, with binding of the acceptor stem facilitating binding of the anticodon loop (*48*). Previous kinetic studies of isoleucyl-tRNA synthetase with RNA substrates are consistent with most of the binding energy arising from the enzyme−acceptor arm interaction. Microhelix and minihelix models of the acceptor stem of tRNA^{Ile} have K_ms nearly identical to that of the full length transcript of tRNA^{Ile} (*34*). Thus, by analogy to methionyl-tRNA synthetase, where the C-terminal peptide appendix appears to provide acceptor helix contacts, Zn^{2+}-53C may be an essential part of the acceptor helix binding pocket of isoleucyl-tRNA synthetase. This role would explain why detectable binding of tRNA^{Ile} to isoleucyl-tRNA synthetase is completely dependent on Zn^{2+}-53C (Figures 3 and 4).

Interestingly, binding of tRNA to Zn^{2+}-53C is not specific. However, other general RNA binding domains have been identified among the aminoacyl-tRNA synthetases. The yeast glutaminyl-tRNA synthetase has a large N-terminal extension which is not present in the *E. coli* enzyme (*49*−*51*). *E. coli* glutaminyl-tRNA synthetase does not aminoacylate or bind yeast tRNA^{Gln}, yet addition of this extension to the N terminus of the *E. coli* enzyme bestowed upon it the ability to aminoacylate yeast tRNA^{Gln}. Analysis of the chimeric enzyme indicated that addition of the N-terminal extension not only enabled the *E. coli* enzyme to bind and aminoacylate the yeast tRNA^{Gln} but also enabled it to bind other noncognate tRNAs, such as *E. coli* tRNA^{Ile}, which were not glutaminylated (*52*). The yeast N-terminal extension appears to have a general RNA-binding site which facilitates correct positioning of yeast tRNA^{Gln} on *E. coli* glutaminyl-tRNA synthetase (for the aminoacylation step).

Yeast methionyl- and glutamyl-tRNA synthetases also have N-terminal extensions which are absent from their prokaryote counterparts. However, in these two instances, the extensions appear to act in a manner different from that of the glutaminyl enzyme and, instead, anchor a separate cofactor which has general tRNA binding properties. When the 43 kDa protein Arc1p binds to the N-terminal extension of yeast methionyl-tRNA synthetase, the catalytic efficiency of the synthetase is increased 500-fold, mostly due to a significant decrease in the Michaelis constant K_m (*53*). Studies of isolated Arc1p demonstrated that it binds preferentially to tRNA rather than to unstructured RNA but shows equal affinity for tRNA^{Met} and tRNA^{Phe}. Further analysis using competition binding assays demonstrated that Arc1p appears to bind to the acceptor-TYC arm of tRNA and not to the anticodon arm. The most striking feature of this system is that a 100-amino acid stretch of Arc1p is highly homologous to the C terminus of prokaryote methionyl-tRNA synthetases (*53*).

Thus, the C-terminal zinc-peptide of isoleucyl-tRNA synthetase may be part of a general group of RNA binding elements that, in themselves, do not have RNA binding specificity but, when combined with other parts of a synthetase, form a specific RNA binding pocket. The

formation of this pocket might be an example of a system in which the RNA ligand induces a proper fit between itself and the protein−peptide complex. If so, and given the role of induced fit in protein−nucleic acid recognition (*54*, *55*), then induced fit may have influenced the selection of RNA binding peptides during the development of tRNA synthetases. Indeed, we have been able to mimic this process by demonstrating that the zinc-peptide is able to form a stable, noncovalent complex with an adenylate-forming fragment of isoleucyl-tRNA synthetase. With time, coding sequences for these cofactors may have been incorporated into some of the synthetase genes.

ACKNOWLEDGMENT

We thank Dr. Magali Frugier for helpful discussions and Dr. Rebecca Alexander for comments on the manuscript.

REFERENCES

1. Eriani, G., Delarue, M., Poch, O., Gangloff, J., and Moras, D. (1990) *Nature 347*, 203−206.
2. Cusack, S., Berthet-Colominas, C., Hartlein, M., Nassar, N., and Leberman, R. (1990) *Nature 347*, 249−255.
3. Webster, T. A., Tsai, H., Kula, M., Mackie, G. A., and Schimmel, P. (1984) *Science 226*, 1315−1317.
4. Hountondji, C., Dessen, P., and Blanquet, S. (1986) *Biochimie 68*, 1071−1078.
5. Schimmel, P., Giegé, R., Moras, D., and Yokoyama, S. (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*, 8763−8768.
6. Schimmel, P., and Ribas de Pouplana, L. (1995) *Cell 81*, 983−986.
7. Weiner, A. M., and Maizels, N. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 7383−7387.
8. Noller, H. F. (1993) in *The RNA World* (Gesteland, R. F., and Atkins, J. F., Eds.) pp 137−156, Cold Spring Harbor Laboratory Press, Plainview, NY.
9. Weiner, A. M., and Maizels, N. (1994) *Curr. Biol. 4*, 560−563.
10. Hou, Y.-M., Sterner, T., and Bhalla, R. (1995) *RNA 1*, 703−713.
11. Martinis, S. A., and Schimmel, P. (1995) in *tRNA: Structure, Biosynthesis and Function* (Soll, D., and RajBhandary, U., Eds.) pp 349−370, American Society for Microbiology, Washington, DC.
12. Quinn, C. L., Tao, N., and Schimmel, P. (1995) *Biochemistry 34*, 12489−12495.
13. Saks, M. E., and Sampson, J. R. (1996) *EMBO J. 15*, 2843−2849.
14. Rould, M. A., Perona, J. J., Söll, D., and Steitz, T. A. (1989) *Science 246*, 1135−1142.
15. Nureki, O., Vassylyev, D. G., Katayanagi, K., Shimizu, T., Sekine, S., Kigawa, T., Miyazawa, T., Yokoyama, S., and Morikawa, K. (1995) *Science 267*, 1958−1965.
16. Brunie, S., Zelwer, C., and Risler, J. (1990) *J. Mol. Biol. 216*, 411−424.
17. Starzyk, R. M., Webster, T. A., and Schimmel, P. (1987) *Science 237*, 1614−1618.
18. Shepard, A., Shiba, K., and Schimmel, P. (1992) *Proc. Natl. Acad. Sci. U.S.A. 89*, 9964−9968.
19. Auld, D. S., and Schimmel, P. (1995) *Science 267*, 1994−1996.
20. Nureki, O., Kohno, T., Sakamoto, K., Miyazawa, T., and Yokoyama, S. (1993) *J. Biol. Chem. 268*, 15368−15373.
21. Schimmel, P., Landro, J. A., and Schmidt, E. (1993) *J. Biomol. Struct. Dyn. 11*, 571−581.
22. Xu, B., Trawick, B., Kaudy, G. A., Phillips, R. M., Zhou, L., and Rosevear, P. (1994) *Biochemistry 33*, 398−402.
23. Landro, J. A., Schmidt, E., Schimmel, P., Tierney, D. L., and Penner-Hahn, J. E. (1994) *Biochemistry 33*, 14213−14220.
24. Landro, J. A., and Schimmel, P. (1994) *J. Biol. Chem. 269*, 20217−20220.
25. Glasfeld, E., Landro, J. A., and Schimmel, P. (1996) *Biochemistry 35*, 4139−4145.
26. Shiba, K., and Schimmel, P. (1992) *Proc. Natl. Acad. Sci. U.S.A. 89*, 1880−1884.
27. Kim, S., Landro, J. A., Gale, A. J., and Schimmel, P. (1993) *Biochemistry 32*, 13026−13031.
28. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory Press, Plainview, NY.
29. Schmidt, E., and Schimmel, P. (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*, 6919−6923.
30. Schmidt, E. (1996) Ph.D. Thesis, p 119, Massachusetts Institute of Technology, Cambridge, MA.
31. Silberklang, M., Gillum, A. M., and RajBhandary, U. L. (1977) *Nucleic Acids Res. 4*, 4091−4108.
32. Park, S. J., and Schimmel, P. (1988) *J. Biol. Chem. 263*, 16527−16530.
33. Gale, A. J., and Schimmel, P. (1995) *Biochemistry 34*, 8896−8903.
34. Nureki, O., Niimi, T., Muramatsu, T., Kanno, H., Kohno, T., Florentz, C., Giegé, R., and Yokoyama, S. (1994) *J. Mol. Biol. 236*, 710−724.
35. Sprinzl, M., Steegborn, C., Hübel, F., and Steinberg, S. (1996) *Nucleic Acids Res. 24*, 68−72.
36. Lim, W. A., Sauer, R. T., and Lander, A. D. (1991) *Methods Enzymol. 208*, 196−210.
37. Gale, A. J., Shi, J. P., and Schimmel, P. (1996) *Biochemistry 35*, 608−615.
38. Cantor, C. R., and Schimmel, P. R. (1980) *Biophysical Chemistry*, Part I, pp 283−285, W. H. Freeman and Company, New York.
39. Fourmy, D., Meinnel, T., Mechulam, Y., and Blanquet, S. (1993) *J. Mol. Biol. 231*, 1068−1077.
40. Landro, J. A., and Schimmel, P. (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*, 2261−2265.
41. Xu, B., Krudy, G. A., and Rosevear, P. R. (1993) *J. Biol. Chem. 268*, 16259−16264.
42. Fourmy, D., Mechulam, Y., and Blanquet, S. (1995) *Biochemistry 34*, 15681−15688.
43. Liu, J., Gagnon, Y., Gauthier, J., Furenlid, L., L'Heureux, P.-J., Auger, M., Nureki, O., Yokoyama, S., and Lapointe, J. (1995) *J. Biol. Chem. 270*, 15162−15169.
44. Liu, J., Lin, S.-X., Blochet, J.-E., Pezolet, M., and Lapointe, J. (1993) *Biochemistry 32*, 11390−11396.
45. Chong, S., Curnow, A. W., Huston, T. J., and Garcia, G. A. (1995) *Biochemistry 34*, 3694−3701.
46. Romier, C., Reuter, K., Suck, D., Ficner, R., Chong, S., Curnow, A. W., Huston, T. J., and Garcia, G. A. (1996) *EMBO J. 15*, 2850−2857.
47. Schreiber, G., and Fersht, A. R. (1996) *Nat. Struct. Biol. 3*, 427−431.
48. Pütz, J., Puglisi, J. D., Florentz, C., and Giegé, R. (1993) *EMBO J. 12*, 2949−2957.
49. Ludmerer, S. W., and Schimmel, P. (1987) *J. Biol. Chem. 262*, 10801−10806.
50. Ludmerer, S. W., and Schimmel, P. (1987) *J. Biol. Chem. 262*, 10807−10813.
51. Mirande, M. (1991) *Prog. Nucleic Acid Res. Mol. Biol. 40*, 95−142.
52. Whelihan, E. F., and Schimmel, P. (1997) *EMBO J.* (in press).
53. Simos, G., Segref, A., Fasiolo, F., Hellmuth, K., Shevchenko, A., Mann, M., and Hurt, E. C. (1996) *EMBO J. 15*, 5437−5448.
54. Spolar, R. S., and Record, M. T. J. (1994) *Science 263*, 777−784.
55. Ribas de Pouplana, L., Auld, D. S., Kim, S., and Schimmel, P. (1996) *Biochemistry 35*, 8095−8102.

BI970151N