Mechanism of an Aminoacyl Transfer Ribonucleic Acid Synthetase

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The aminoacyl transfer RNA synthetases are enzymes which catalyze the first steps in protein biosynthesis.[1-3] These enzymes attach amino acids to transfer RNA (tRNA) chains. For each amino acid there is a corresponding tRNA synthetase and tRNA species, and each enzyme specifically esterfies its amino acid to the ribose hydroxyl group at the 3′ terminus of its cognate tRNA.

The energy required to make the aminoacyl linkage is derived from adenosine triphosphate (ATP) which is hydrolyzed to adenosine monophosphate (AMP) and inorganic pyrophosphate (PP_i) in the esterfication process. The overall reaction may thus be written

$$\text{ATP} + \text{amino acid} + \text{tRNA} \rightleftarrows \text{aminoacyl-tRNA} + \text{AMP} + \text{PP}_i \quad (1)$$

The transfer RNAs are nucleic acid chains of about 80 nucleotide units.[4] The sequence of nucleotide bases is distinct for each amino acid specific tRNA, although some common short sequences do occur. A particularly important sequence on each tRNA is the anticodon, a triplet of bases which is complementary to the codon—the three-base sequence which specifies a particular amino acid. In protein synthesis there is a matching (by hydrogen bonding) of the tRNA's anticodon with a complementary triplet codon sequence on messenger RNA. This interaction determines the insertion point of the amino acid in a growing polypeptide chain. Hence, once an amino acid is stably attached to its tRNA, its position in a polypeptide is determined by the anticodon of the tRNA, and not by the amino acid.[5] For this reason, the tRNA synthetases must catalyze their reactions with a high degree of fidelity in order to prevent altered proteins from occurring.

Research has been carried out on synthetases from several different sources. Although the reactions catalyzed by these enzymes involve the same esterification process in all cases, the enzymes themselves show some differences in such characteristics as their molecular weights and subunit makeup (as examples, see ref 6-12). On the other hand, the synthetases appear to share certain specific features in common, *e.g.*, virtually all of them appear to have a reactive sulfhydryl group,[13] and they each appear to associate strongly with their cognate tRNAs.[3,14]

This Account focuses attention on the mechanism of the tRNA synthetase specific for isoleucine (Ile), usually called Ile-tRNA synthetase (*E. coli* B). This enzyme is one of the most extensively studied of the tRNA synthetases. Many of the questions raised in connection with the mechanism of action of an aminoacyl tRNA synthetase have been attacked from one or more directions in the case of this enzyme. These studies are, therefore, typical of the kinds of investigations and approaches which have been or can be used to understand the mechanism of the synthetases. The results of these investigations have also raised new questions and in some cases failed to answer old ones, so that they serve to point out areas in which new approaches are desirable as well as probable future directions of research.

Amino Acid Activation and Transfer

The Ile-tRNA synthetase can be purified to apparent homogeneity by a series of chromatographic steps.[15,16] A yield of 50 mg of purified enzyme from 1 lb. of *E. coli* B cells has been obtained.[16] The enzyme has a molecular weight of *ca.* 110,000[15] and consists of a single polypeptide chain.[17] This chain contains one site for each of the substrates.[18-22]

The overall reaction catalyzed by the enzyme is given by eq 1 with isoleucine and isoleucine-specific tRNA ($tRNA^{Ile}$) as the amino acid and tRNA species, respectively. The equilibrium constant of the reaction is of the order of unity.[1] Equation 1 may be

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split into two parts, each of which may be studied independently[1,2] (eq 2 and 3, where Ile-tRNA^{Ile} denotes the isoleucine esterified form of tRNA^{Ile}.

$$\text{Ile-tRNA synthetase} + \text{ATP} + \text{Ile} \rightleftharpoons \text{Ile-tRNA synthetase·Ile-AMP} + \text{PP}_i \quad (2)$$

$$\text{Ile-tRNA synthetase·Ile-AMP} + \text{tRNA}^{Ile} \rightleftharpoons \text{Ile-tRNA}^{Ile} + \text{AMP} + \text{Ile-tRNA synthetase} \quad (3)$$

Equation 2 is the amino acid activation reaction in which ATP and Ile condense to form a tightly bound enzyme–isoleucyladenylate (Ile-AMP) complex. This reaction is typically studied by ATP–PP$_i$ isotope exchange. It cannot be demonstrated to occur in the absence of divalent cation (*e.g.*, Mg^{2+}),[21,23,24] which is required for complexation with ATP and PP$_i$.[24] Equation 3 is a transacylation reaction (or transfer) reaction in which the aminoacyl moiety is transferred from AMP to the 3′-terminal hydroxyl of tRNA^{Ile}. This reaction appears to occur in either the presence or the absence of divalent cations.[16,18,25,26]

The overall rate of aminoacylation, starting from ATP, Ile, and tRNA, is about 10- to 100-fold slower than the independently measured rate of eq 2.[16,27] This implies that the rate-determining step occurs in eq 3, the transfer reaction. However, when the Ile-tRNA synthetase·Ile-AMP complex is isolated from a Sephadex column and allowed to react with tRNA, the rate of the "one-shot" reaction (eq 3) is approximately ten times more rapid than the overall rate of aminoacylation.[16] Thus, both eq 2 and 3 when studied individually appear to have rates which greatly exceed that of the overall reaction, eq 1.

How is this paradox resolved? Using a nitrocellulose filter assay, Yarus and Berg found that the rate of dissociation of acylated tRNA from the enzyme is very slow.[27] Their assay involved trapping the protein–nucleic acid complex on filter pads. The assumption made is that what is trapped on the pad accurately represents all complexes which preexisted in solution at the time of filtration. Admittedly, this assumption appears hazardous. The authors studied the exchange on the enzyme in solution of prebound labeled tRNA with unlabeled, free tRNA, the amount of exchange being monitored by trapping the complex at various times on the filter pads. From this study, they found that dissociation of tRNA from the enzyme is extremely slow; its rate appears to correlate well with the overall rate of aminoacylation.[27]

In measuring the rate of the "one-shot" transfer reaction (eq 3), the experiment makes no distinction between newly synthesized Ile-tRNA which is enzyme bound and that which is free. The incorporation of radioactive isoleucine into tRNA is monitored by acid-precipitable radioactivity. Thus, in studying eq 3 the release step is not explicity monitored, since once isoleucine is attached to the tRNA it is assayed regardless of whether or not Ile-tRNA is bound to the enzyme.

To test the hypothesis that release is the rate-determining step, the transfer reaction must be studied through more than one cycle, *viz.*, an excess of Ile-AMP over enzyme must be used in order to drive the enzyme through a catalytic cycle. The mechanism of the transfer reaction might be envisioned as shown in Scheme I.

Scheme I

$$\text{Ile-tRNA synthetase·Ile-AMP} + \text{tRNA}^{Ile} \rightarrow \text{Ile-tRNA synthetase·Ile-tRNA}^{Ile} + \text{AMP} \quad \text{(cycle 1)}$$

$$\text{Ile-tRNA synthetase·Ile-tRNA}^{Ile} + \text{Ile-AMP} \rightarrow \text{Ile-tRNA synthetase·Ile-AMP} + \text{Ile-tRNA}^{Ile} \quad \text{(release)}$$

$$\text{Ile-tRNA synthetase·Ile-AMP} + \text{tRNA}^{Ile} \rightarrow \text{Ile-tRNA synthetase·Ile-tRNA}^{Ile} + \text{AMP} \quad \text{(cycle 2)}$$

etc.

According to Scheme I, the production of Ile-tRNA should be biphasic if the release step is the slowest step in the reaction sequence. That is, there should be a rapid initial production of Ile-tRNA^{Ile} in an amount equal to the enzyme concentration followed by a slow phase as additional molecules of Ile-tRNA^{Ile} are made. On the other hand, if the release step is not rate limiting the biphasic reaction kinetics will not be evident.

Figure 1a shows the results of a multicycle transfer reaction. (These experiments were done at pH 6, 3°, in order to slow down the reaction and to stabilize the adenylate complex.) Biphasic reaction kinetics are observed: there is a burst of Ile-tRNA^{Ile} formed in an amount about equal to enzyme, after which the production of Ile-tRNA^{Ile} is much slower. The rate of the second phase correlates well with that of the overall rate of aminoacylation under similar conditions.[16]

An even more direct way of demonstrating the marked difference in the rate of the first cycle as opposed to subsequent ones is to perform a double-label experiment in which Ile-tRNA synthetase·[^{14}C]Ile-AMP is mixed with [^{3}H]Ile-AMP and tRNA.[16] In the time scale of the experiment there should be no exchange between enzyme-bound [^{14}C]Ile-AMP and [^{3}H]Ile-AMP. Therefore, the production of [^{14}C]Ile-tRNA^{Ile} monitors the first cycle and that of [^{3}H]Ile-tRNA^{Ile} monitors the subsequent cycles. The results of this kind of experiment are shown in Figure 1b. Again the first cycle, as followed by ^{14}C, is considerably more rapid than subsequent ones.

In summary the kinetic results obtained on the various reactions—amino acid activation (eq 2), overall aminoacylation (eq 1), and the individual cycles of the transfer reaction (eq 3)—show that only the second cycle of the transfer reaction (see Scheme I) occurs at the same rate as overall aminoacylation. Thus, the rate-determining step is pinpointed. Although these data apply to pH 6, 3°, other results suggest a similar relationship between the various rates under different conditions.[16]

What is the mechanism of the release step? In Scheme I, the release of the Ile-tRNA and the binding of a new molecule of the Ile-AMP are depicted as occurring in a single step. There are at least two ways in which this overall process can occur. One is that Ile-tRNA must be released from the synthetase before a new molecule of tRNA or of Ile-AMP can

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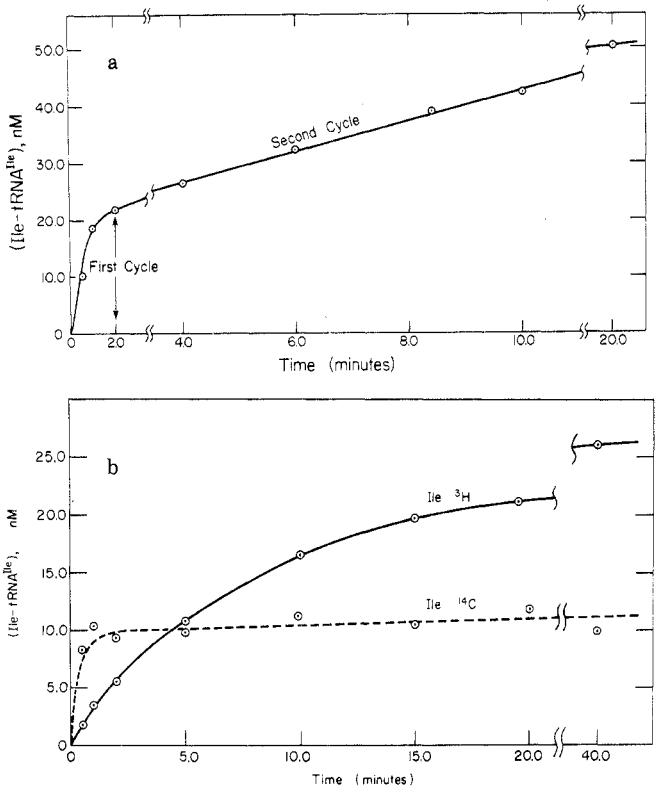

Figure 1. (a) Time course of production of Ile-tRNA^{Ile} in a multicycle transfer reaction at pH 6.0, 3°. Reaction mixture contained (in 1 ml) about 22 pmol of Ile-tRNA synthetase, 58 pmol of [^{3}H]Ile-AMP, and 1 nmol of tRNA^{Ile}. Further details are in ref 16. (b) Time course of production of [^{14}C]Ile-tRNA^{Ile} and [^{3}H]Ile-tRNA^{Ile} in a multicycle transfer reaction. Reaction mixture contained (in 1 ml) about 12 pmol of Ile-tRNA synthetase·[^{14}C]Ile-AMP complex, 31 pmol of [^{3}H]Ile-AMP, and 1 nmol of tRNA^{Ile}. Further details are in ref 16.

bind (or be synthesized). Another is a scheme involving the binding of a new Ile-AMP molecule prior to the release of the just synthesized Ile-tRNA. In this mechanism, two isoleucyl moieties would be simultaneously bound to the enzyme in an intermediate complex.

That the latter mechanism prevails was first suggested by nitrocellulose filter studies which indicated that dissociation of acylated tRNA from enzyme was accelerated by isoleucine, but not by ATP.[27] This implies that the amino acid is binding to the enzyme–nucleic acid complex and thereby promoting an accelerated dissociation. Similar results were obtained with mixtures of ATP and isoleucine, which raised the possibility that the adenylate was also capable of promoting release, although this was not really proven to be the case.[27] However, by an adaptation of the transfer reaction, it was possible to show in a kinetic study that Ile-AMP binds to the enzyme·Ile-tRNA complex prior to dissociation of the complex. This molecule of Ile-AMP now promotes release of the newly synthesized Ile-tRNA. This acceleration in release is about tenfold.[16] Therefore, the rate of the second cycle of the transfer reaction and of overall aminoacylation actually represents the rate of the accelerated release step, so that, in spite of the acceleration, release remains rate limiting. The major part of these results is further confirmed by entirely different kinds of experiments.[28]

To summarize, the synthesis of aminoacyl-tRNA is envisioned to occur by formation of an aminoacyl adenylate, esterification of the amino acid to the tRNA, synthesis of a new adenylate molecule, and adenylate-stimulated release of the aminoacyl-tRNA.[16,27] It is likely that during catalysis the adenylate rather than the isoleucine actually stimulates the release. This is based on the fact that the adenylate and aminoacyl-tRNA can simultaneously combine with enzyme, and the fact that even in the presence of tRNA adenylate appears to be synthesized at a rate which is more rapid than the stimulated release of the aminoacyl-tRNA.[16]

The proposal that in the presence of tRNA the adenylate is not formed, but rather that a concerted mechanism prevails for the formation of aminoacyl-tRNA,[29] is contested by the following observation. When ATP, isoleucine, tRNA, and enzyme are mixed together there is an initial burst production of Ile-tRNA in an amount corresponding to the amount of enzyme present. Subsequent cycles of production of aminoacyl-tRNA are much slower.[16] Thus, the production of aminoacyl-tRNA in the overall aminoacylation reaction is biphasic just as it is in the transfer reaction. The crucial point, however, is that the rate at which the initial burst of overall aminoacylation proceeds (eq 1) is the same as the rate of the "one-shot" transfer reaction (eq 3). This argues that the same limiting step (prior to release) is being monitored in each case and thus strongly implicates the presence of the adenylate intermediate.

Deacylation in the Absence of AMP and PP_i

Although the aminoacyl linkage is somewhat labile, especially at alkaline pH values, it came as a surprise to find that in the presence of the tRNA synthetase this lability is substantially enhanced.[30]

The reaction catalyzed is

$$\text{Ile-tRNA synthetase} + \text{Ile-tRNA}^{Ile} \rightleftarrows \text{Ile} + \text{tRNA}^{Ile} + \text{Ile-tRNA synthetase} \quad (4)$$

It also occurs with other synthetases.[30-33] This reaction is only evident when approximately stoichiometric concentrations of enzyme are employed. The turnover number is about 1 min^{-1} at pH 7, 37°, and the reaction is strongly pH dependent.[30] The rate decreases sharply with decreasing pH, and the reaction is barely observable at pH 5.5. This deacylation reaction appears to occur during the aminoacylation of tRNA, so that a greater amount of ATP can be hydrolyzed to AMP than aminoacyl-tRNA is produced.[30]

Is this reaction telling us something about the mechanism of action of the enzyme? For example, one can imagine that eq 4 is a side reaction which occurs as a result of the absence of AMP and PP_i to complete the reverse of aminoacylation. We can conjecture that an enzyme–Ile "bond" (*e.g.*, a thioester) is made as the Ile-tRNA^{Ile} binds to the synthetase.

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In the presence of AMP, this species could react to yield adenylate; in the absence of AMP, a slow reaction with water could occur which would release the amino acid from the enzyme. Thus aminoacylation might be envisioned as proceeding to the stage of the bound adenylate intermediate which could then react with a group on the enzyme to yield an enzyme–amino acid linkage which in turn would react with tRNA to give aminoacyl-tRNA. This kind of mechanism is analogous to the manner in which cyclic polypeptide antibiotics are synthesized by an enzyme complex from *B. brevis*.[34] Aminoacyl adenylates are first formed and subsequently react with sulfhydryls on the enzyme to yield aminoacyl thioesters. These in turn react to yield cyclic polypeptides.

In spite of the attractiveness of this kind of mechanism, no evidence could be found in support of it.[30] Approaches used included attempts to isolate a covalent enzyme–isoleucine intermediate as well as some isotope exchange experiments. Although these experiments did not demonstrate the existence of the proposed intermediate, neither did they rule it out.[30]

Does the reaction of eq 4 have any physiological significance? Because of the many poorly understood roles which tRNA may play in the cell, it is not profitable to speculate too freely on this question. On the other hand, there is one physiological function which immediately suggests itself. Perhaps the slow deacylation observed with the cognate amino acid would be much more rapid if the incorrect amino acid were attached to the tRNA. The logical incorrect amino acid is valine, since the enzyme is known to activate valine.[18,23,35] However, when presented with the $tRNA^{Ile}$, the enzyme-bound Val-AMP is rapidly hydrolyzed, *viz.*, valine is not stably attached to $tRNA^{Ile}$.[35] This raises the possibility that Val-$tRNA^{Ile}$ is formed as a transient intermediate which is rapidly hydrolyzed by the enzyme's deacylation activity. Support for this notion comes from the observation that a modification destroying the amino acid acceptor function of $tRNA^{Ile}$ also eliminates its ability to induce hydrolysis of enzyme-bound Val-AMP.[35]

The crucial experiment, of course, is to test the enzyme's deacylation activity with Val-$tRNA^{Ile}$. This mischarged tRNA was enzymatically synthesized according to a novel procedure.[36,37] As an additional control, Phe-$tRNA^{Ile}$ was also made. Figure 2 displays the rate of enzymatic deacylation of Ile-$tRNA^{Ile}$, Val-$tRNA^{Ile}$, and Phe-$tRNA^{Ile}$, in the absence of AMP and PP_i. It is clear that most of the Val-$tRNA^{Ile}$ is almost instantaneously deacylated, whereas the deacylation of Ile-$tRNA^{Ile}$ is much slower; in the case of Phe-$tRNA^{Ile}$, deacylation is negligible. Thus, when the logical candidate—valine—for the mischarged amino acid is attached to $tRNA^{Ile}$, isoleucyl-tRNA synthetase's deacylation activity appears greatly enhanced.[37]

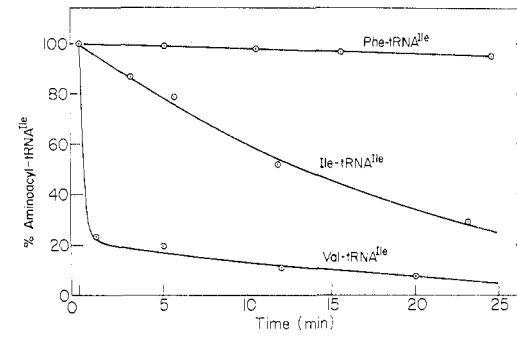

Figure 2. Deacylation of Phe-$tRNA^{Ile}$, Val-$tRNA^{Ile}$, Ile-$tRNA^{Ile}$ by Ile-tRNA synthetase in the absence of AMP and PP_i at pH 7.0, 15°. Data are corrected for spontaneous deacylation occurring in absence of enzyme. Reaction mixtures contained (in 0.5 ml) *ca.* 25 pmol of the appropriate aminoacyl-tRNA and 0.2 nmol of Ile-tRNA synthetase. Further details are in ref 37.

If Val-$tRNA^{Ile}$ is a transient intermediate in the $tRNA^{Ile}$-induced hydrolysis of the Ile-tRNA synthetase·Val-AMP complex, then the rate of enzymatic hydrolysis of Val-$tRNA^{Ile}$ must be at least as fast as that of the $tRNA^{Ile}$-induced valyl adenylate hydrolysis. Thus, the maximal velocities of these two reactions must be compared. When this is done, enzymatic deacylation of Val-$tRNA^{Ile}$ is far more rapid than that of $tRNA^{Ile}$-induced hydrolysis of Val-AMP at pH 7, 5 m*M* Mg^{2+}, 3°.[37] This result is consistent with the idea that Val-$tRNA^{Ile}$ is a transient intermediate in the $tRNA^{Ile}$-promoted hydrolysis of synthetase-bound Val-AMP.[38] However, some caution in interpretation is necessary.[37]

It should also be mentioned that the Ile-tRNA synthetase can aminoacylate $tRNA^{Phe}$(*E. coli B*) with isoleucine to yield Ile-$tRNA^{Phe}$.[39,40] When this mischarged species is presented to the phenylalanyl tRNA synthetase, it is rapidly deacylated.[40] Thus, the weak deacylation activity exhibited by the synthetases toward their properly aminoacylated cognate tRNAs may indeed function as a powerful error-correcting activity when certain improper amino acids are attached to their respective tRNAs. However, this "error correction" exhibited by a synthetase may be selective, as evidenced by the fact that the Ile-tRNA synthetase does not deacylate Phe-$tRNA^{Ile}$ (*vide supra*).[37]

Synthetase–tRNA Interaction

The association between Ile-tRNA synthetase and $tRNA^{Ile}$, like all synthetase–tRNA interactions, is strong. The association constant at pH 5.5, around ambient temperatures, can fall in the range of 10^8–10^9 M^{-1}.[27,41] This strong association is quite sensitive to ions, pH, and solvent composition, however.[41,42] For example, under a given set of conditions the association constant is at least two orders of magnitude lower at pH 7 than at pH 5.5.[42] On the other hand, the association constant is reported to increase

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(35) A. N. Baldwin and P. Berg, *J. Biol. Chem.*, 241, 839 (1966).
(36) R. Giege, D. Kern, J.-P. Ebel, and R. Taglang, *FEBS (Fed. Eur. Biochem. Soc.) Lett.*, 15, 281 (1971).
(37) E. W. Eldred and P. R. Schimmel, *J. Biol. Chem.*, 247, 2961 (1972).
(38) If we think in terms of an enzyme–amino acid covalent intermediate, one must argue that the positions of the catalytic groups at the active site are such as to render the enzyme–valine intermediate as more labile than the isoleucine intermediate.
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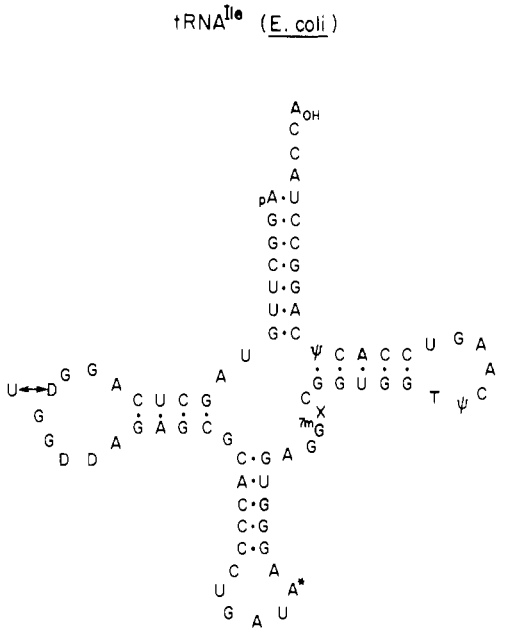

Figure 3. The cloverleaf representation of the sequence of the two major species of tRNA^{Ile}(*E. coli* B). See ref 50 for details.

by a factor of 10 or so at pH 5.5 when the solvent includes 10% dioxane.[41] Unfortunately, large amounts of data as a function of pH and temperature have not been obtained. This is largely due to the fact that most studies have been conducted by the nitrocellulose filter assay. The efficiency and practicality of this method drop sharply with increasing pH above pH 5.5.[19,43] Fluorescence studies of the synthetase–tRNA interaction are not subject to this limitation and should yield more useful information.

There is crucial need to understand the physical basis underlying the marked specificity and strength of the protein–nucleic acid association. Although only sparse quantitative data are available, in one case it has been shown that at pH 5.5 the association of a noncognate tRNA (tRNA^{Phe}) is several orders of magnitude weaker than that of the cognate tRNA.[39] Thus, the specificity is pronounced, in spite of the fact that all tRNA chains are of similar length and are believed to be folded in a basically standard conformation.[4]

How does one attack the question of specificity of protein–nucleic acid interactions? One obvious tactic is to study the interaction of tRNA fragments or of modified tRNAs with the synthetase. If a strongly binding fragment is found, for example, it is logical to conclude that it must contain a significant portion of the locus (or loci) for the synthetase interactions. Although this approach has obvious appeal, a large amount of work with a variety of tRNAs and their respective synthetases has not been successful in pinning down the tRNA recognition locus (as examples see ref 44–47). However, in the case of the isoleucine system, it must be admitted that experiments of this kind have been rather limited.

In an alternate approach to the problem, one can ask what regions on the tRNA are covered up or shielded by the enzyme and what regions are not in contact with or shielded by the enzyme. With this information in hand, one can certainly exclude certain segments of the tRNA from consideration as far as recognition is concerned, and focus attention on those areas of the nucleic acid which are in close association with the enzyme.

This kind of approach was used in a recent investigation in which the oligonucleotide hybridization technique[48] was extended to the Ile-tRNA synthetase·tRNA^{Ile} complex.[49] This technique involves determining by equilibrium dialysis the binding of tri- and tetranucleotides to the unpaired and unshielded regions on tRNA. Once the free tRNA has been "mapped" as to its oligonucleotide binding pattern, enzyme is added and the synthetase-bound tRNA is mapped in order to learn which regions can still accept their complementary oligonucleotides and which can not.

The cloverleaf representation of tRNA^{Ile} is given in Figure 3.[50] Only single-stranded regions in the cloverleaf are possible candidates to bind complementary oligonucleotides. In the case of free tRNA^{Ile}, strong binding of AUCA and AUCG to the anticodon sequence UGAU was observed.[49] (AUCG binds well because of the possibility of a G–U wobble interaction.[51]) Also, GGU hybridizes well to the ACC sequence of the 3′ terminus.[49] Unfortunately, strong binding to other regions was not observed. However, the fact that the 3′ terminus and the anticodon section can both be probed is very attractive inasmuch as those two regions are presumably well separated in space.[52]

The effect on oligonucleotide binding of adding variable amounts of enzyme to the tRNA was subsequently studied. Figure 4 gives the results obtained with GGU. The data are plotted as R *vs.* the ratio of enzyme to tRNA^{Ile}, where R = (free + bound GGU)/(free GGU). Thus, R is merely the ratio of total GGU on the tRNA side of the dialysis membrane to that on the opposite side. In the absence of enzyme and for the particular tRNA^{Ile} concentration employed, R = 3.7. As enzyme is added, however, R steadily drops until there is somewhat of an excess of enzyme to tRNA^{Ile}. At this point R = 1, which indicates that GGU cannot bind to the ACC sequence on the synthetase-bound tRNA. This result is not overly surprising in view of the fact that the enzyme attaches amino acid to the 3′ end of the tRNA and thus probably interacts with this portion.

The effects of enzyme on the hybridization of AUCA and AUCG to the anticodon are shown in Table I. Here values of R are given for various

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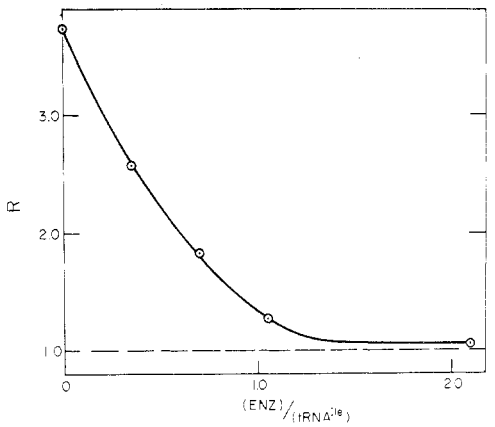

Figure 4. Effect of Ile-tRNA synthetase (ENZ) on hybridization of GGU to $tRNA^{Ile}$ at 0°. The $tRNA^{Ile}$ concentration varied between about 5 and 12 μM. Further details are in ref 49.

$tRNA^{Ile}$ concentrations in the presence and absence of varying amounts of enzyme. The enzyme completely eliminates the ability of these oligomers to hybridize to the anticodon. Thus, both the 3′ terminus and the anticodon section appear to be at least partly covered up in the complex. Since the distance between these two portions of tRNA is perhaps *ca.* 80 Å,[52] the results suggest that the enzyme–tRNA interaction encompasses or spans a rather large region.

Other questions can also be raised in connection with the synthetase–tRNA interaction. For example, does the tRNA undergo some sort of conformational change when it is bound to the enzyme? One way to investigate this question is to study the exchange rate of the relatively slow exchanging hydrogens on $tRNA^{Ile}$ in the presence and absence of the Ile-tRNA synthetase. These slow exchanging hydrogens are largely those participating in hydrogen bonds.[53] If some tRNA hydrogen bonds are broken upon binding to the enzyme, or if the enzyme shields base-paired nucleotides, one expects the exchange rate to be affected. However, there is apparently no effect of the enzyme on the rate of exchange of the hydrogen-bonded hydrogens on $tRNA^{Ile}$.[54]

Looking Ahead

There are many future directions and areas to which research on the Ile-tRNA synthetase and other synthetases will be carried. A major problem which remains unsolved is the mechanism of protein–nucleic acid recognition. Badly needed are careful studies which quantitate the association constants for various mutant or modified tRNAs and fragments, as opposed to testing simply their ability to be aminoacylated or to be trapped with enzyme on a nitrocellulose filter. Fluorescent probes covalently attached to enzyme and/or nucleic acid may provide a sensitive means for investigating complex formation.

Techniques are also needed to map more accurately the free and shielded portions of a synthetase-bound tRNA. One possibility being explored is C-8 tritium labeling of purine bases. The C-8 hydrogens

Table I
Effect of Ile-tRNA Synthetase on the Hybridization of Oligonucleotides to the Anticodon of $tRNA^{Ile}$ [a]

Ile-tRNA synthetase / $tRNA^{Ile}$	*R*, AUCA		*R*, AUCG			
0	2.6[b]	1.7[c]	2.5[b]	2.7[d]	1.8[c]	2.8[b]
0.6	1.5[b]		1.7[b]			
1.0				1.2[d]		
1.3		1.1[c]			1.2[c]	
2.1						1.0[b]

[a] Data are taken from ref 49. [b] ($tRNA^{Ile}$) ≃ 11 μM. [c] ($tRNA^{Ile}$) ≃ 5.6 μM. [d] ($tRNA^{Ile}$) ≃ 12 μM.

very slowly exchange and thus provide a means of stably labeling the purines with ^3H.[55,56] One can investigate whether or not certain positions which exchange in the free tRNA no longer exchange (or more slowly exchange) when the tRNA is complexed with the synthetase. The aberrant bases may be directly identified by radioactive fingerprint analyses. Preliminary data in this laboratory indicate that bases which are partially shielded from solvent do in fact incorporate H at C-8 more slowly.

Further investigation into the synthetase-catalyzed deacylation of mischarged tRNA is also required. Ile-tRNA synthetase will rapidly deacylate Val-$tRNA^{Ile}$, but not Phe-$tRNA^{Ile}$.[37] On the other hand, the Phe-tRNA synthetase deacylates Ile-$tRNA^{Phe}$.[40] More mischarged tRNAs must be made and tested. Moreover, it is crucial that a variety of deacylation reaction conditions be investigated since there appears, for example, to be a strong sensitivity of the deacylation reaction to pH and Mg^{2+}.[30,37]

Another controversial and active area is concerned with the mechanism of aminoacylation. Although in the presence of Mg^{2+} the adenylate appears to be an intermediate in aminoacyl-tRNA formation, some question is raised when the multivalent cation is spermine, spermidine, or Nd^{3+}.[57-59] In the latter cases, aminoacyl-tRNA formation takes place, but barely any ATP-PP_i exchange is observable. This could be due to the inability of the enzyme to release PP_i in the presence of these ions. However, attempts to detect stimulation of adenylate complex formation, either by direct isolation or by trapping with hydroxylamine (to yield the aminoacyl hydroxamate), have proved abortive in the cases of spermine and spermidine.[59] One explanation is that a concerted mechanism of aminoacyl-tRNA formation is operative in which the adenylate is not formed.[29] Further studies are obviously required.

Fast kinetic studies of the Ile-tRNA synthetase are beginning to emerge.[60] These not only can give values for rate constants associated with the association and dissociation of small molecule substrates

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and tRNA but also can provide a means of elucidating transient intermediates and elementary steps in the pathway to aminoacyl-tRNA formation. Studies of this nature have been hampered thus far by the difficulties of obtaining substantial amounts of purified enzyme and of maintaining the enzyme activity.

Finally it will be of interest to identify groups on the enzyme which are involved in binding and catalysis. Although some work has been aimed at defining the role of the highly reactive sulfhydryl group,[61,62] little else has been done to unravel the chemical nature of the active site(s). This kind of work generally requires significant amounts of pure, stable enzyme, so it may yet be some time before definitive research in this area is carried out.

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Rapid Intramolecular Rearrangements in Pentacoordinate Transition Metal Compounds

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Pentacoordination is now found throughout the Periodic Table, but it occurs predominantly within two areas—in the compounds of group V (such as phosphorus(V)) and of the transition metals in a formal d^8 electronic configuration (*e.g.*, zerovalent iron).[1]

In group V, the ground-state geometry is almost exclusively trigonal bipyramidal (TBP) rather than square pyramidal (SP), and the relative energies of positional TBP isomers are determined by preference rules based on ligand electronegativity. Further, stereochemical nonrigidity is a characteristic and chemically important feature for which a general rearrangement mechanism has been established.[2]

In contrast, although many pentacoordinate transition metal complexes have been synthesized, and X-ray diffraction studies have established several solid-state structures, the factors determining the stability of geometric and positional isomers are not readily apparent. For d^8 complexes, most of the structures correspond closely to either TBP or, less commonly, SP geometry, although a few structures show quite large distortions from these idealized forms. In view of the close stability of SP and TBP forms in transition metal complexes, intramolecular rearrangement might be expected to occur readily. Indeed, at the outset of this work there were indications in the literature that this may well be so (*vide infra*), although no unambiguous example of such behavior had been reported.

In this Account we present the results of studies on a series of related complexes of d^8 electronic configuration, whose stereochemistries, dynamic behavior, and mechanism of rearrangement in solution have been investigated by nmr spectroscopy. From these studies some tentative generalizations have been drawn. However, pentacoordinate intermediates have also been invoked in ligand exchange reactions and catalytic processes involving d^8 metal complexes. Little consideration has been given to the detailed stereochemistry of such intermediates or to the implications of dynamic behavior occurring during their lifetime. Preliminary results involving such intermediates are also presented.

Background Observations.

Early ^{13}C nmr studies on iron pentacarbonyl found only one resonance line,[3] even though two signals (3:2 ratio) would be expected for the TBP ground-state geometry,[3] implying a rapid exchange of carbonyl ligands between nonequivalent sites. The first unambiguous evidence for a rapid intramolecular rearrangement in a d^8 pentacoordinate transition metal complex was reported in 1969 by Udovich and

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