Equilibrium Measurements of Cognate and Noncognate Interactions between Aminoacyl Transfer RNA Synthetases and Transfer RNA†

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ABSTRACT: The interaction of *Escherichia coli* isoleucyl-tRNA synthetase with its cognate and five noncognate tRNAs, and of yeast valyl-tRNA synthetase with its cognate and four noncognate tRNAs, has been measured directly by fluorescence quenching. The cognate associations are strongest (association constant of 10^8 M^{-1} or more at pH 5.5, 17°). A wide variation is found in the strengths of the noncognate interactions; these have association constants smaller than that of the cognate association by a factor of less than 10 to over 10^4, depending on the enzyme-tRNA pair. A more detailed study of the cognate isoleucyl-tRNA synthetase-$tRNA^{Ile}$ association suggests that the strength of the interaction is markedly sensitive to a pH-dependent transition in the enzyme centered at pH 6. On the other hand, Mg^{2+}-induced structural changes in $tRNA^{Ile}$ at 17° in low salt do not greatly affect the availability of the nucleic acid's receptor sites for enzyme. The temperature dependence of the *E. coli* isoleucyl-tRNA synthetase interaction with *E. coli* $tRNA^{Ile}$ and with yeast $tRNA^{Val}$ gives $\Delta H°$ = 0 kcal mol^{-1} and $\Delta S°$ = 34 cal deg^{-1} mol^{-1} for the cognate interaction and $\Delta H°$ = 8 kcal mol^{-1} and $\Delta S°$ = 54 cal deg^{-1} mol^{-1} for the noncognate one, at pH 6.5, 5 mM Mg^{2+}. Thus, both associations are entropically driven. Since the binding is also very salt sensitive, both in the presence and absence of Mg^{2+}, the large entropy changes may reflect a strong electrostatic component in the association. The difference in $\Delta H°$ for the two interactions decreases the discrimination between cognate and noncognate tRNA as temperature is raised.

The interaction of tRNAs with aminoacyl-tRNA synthetases has been subjected to numerous investigations which have aimed at characterizing the phenomenon and at uncovering the molecular basis for the strength and specificity of the association (for reviews see Chambers, 1971; Zachau, 1972; Cramer and Gauss, 1972; Schimmel, 1973; Söll and Schimmel, 1974). The most extensive studies characterizing cognate and noncognate associations have used the aminoacylation assay as a probe for the interactions (e.g., Dudock et al., 1971; Giegé et al., 1971; Ebel et al., 1973; Roe et al., 1973; Yarus and Mertes, 1973). The nitrocellulose filter technique has also provided useful insights, but it is hazardous for making quantitative measurements and it is not generally useful much above pH 5.5 (Yarus and Berg, 1967, 1970). Some important studies of enzyme-tRNA interactions measured directly by fluorescence quenching have been reported (Hélène et al., 1969, 1971; Bruton and Hartley, 1970; Rigler et al., 1970; Farelly et al., 1971; Engel et al., 1972; Lapointe and Söll, 1972; Blanquet et al., 1973; Parfait, 1973; Pachmann et al., 1973; Pingoud et al., 1973; Maelicke et al., 1974), although each of these has been limited in scope. For example, measurements of the pH, ion, and temperature dependence of cognate and/or noncognate interactions by direct methods such as fluorescence are sparse or nonexistent. In fact, the only thermodynamic data come from measurements at pH 5.5 of the temperature dependence of the association measured by the nitrocellulose filter assay (Yarus and Berg, 1967).

This situation provides the motivation and rationale for the present study where we have used fluorescence quenching to explore the cognate system of *E. coli* IleRS[1] and $tRNA^{Ile}_{E.coli}$ in some detail, as well as a variety of noncognate interactions of *E. coli* IleRS and of yeast ValRS. These enzymes are single chain polypeptides with molecular weights in the range of about 110,000–125,000 (Baldwin and Berg, 1966a,b; Ardnt and Berg, 1970; Rymo et al., 1972). The results obtained suggest that the association of $tRNA^{Ile}_{E.coli}$ with IleRS is markedly affected by a pH dependent alteration in enzyme structure centered around pH 6, that Mg^{2+}-induced structural changes in tRNA at 17° in low salt solutions do not greatly alter the availability of the nucleic acid's receptor sites for enzyme, and that cognate and noncognate interactions are both entropically driven. The thermodynamic results and supporting data suggest electrostatic interactions play a significant role in complex stabilization.

Materials and Methods

IleRS was purified from *E. coli* B to homogeneity (on polyacrylamide gels) by the method of Eldred and Schimmel (1972). ValRS was purified from Baker's yeast to homogeneity by the procedure of Kern (1972). The $tRNA^{Ile}$ was prepared from *E. coli* B unfractionated tRNA according to Gillam et al. (1968). The purified $tRNA^{Ile}$ accepted about 1320 pmol of Ile/A_{260} unit (in 0.1 N NaOH). Purified $tRNA^{Glu}_{E.coli}$, $tRNA^{Phe}_{E.coli}$, $tRNA^{Phe}_{yeast}$, and $tRNA^{Val}_{E.coli}$ were purchased from Boehringer Mannheim

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[1] Abbreviations used are: IleRS, isoleucyl-tRNA synthetase; ValRS, valine tRNA synthetase; $tRNA^{X}_{Y}$, the tRNA specific for amino acid X obtained from organism Y; $tRNA^{Phe(-Y)}_{yeast}$, yeast $tRNA^{Phe}$ from which the Y base has been excised; Pipes, piperazine-*N*,*N*′-bis(2-ethanesulfonic acid).

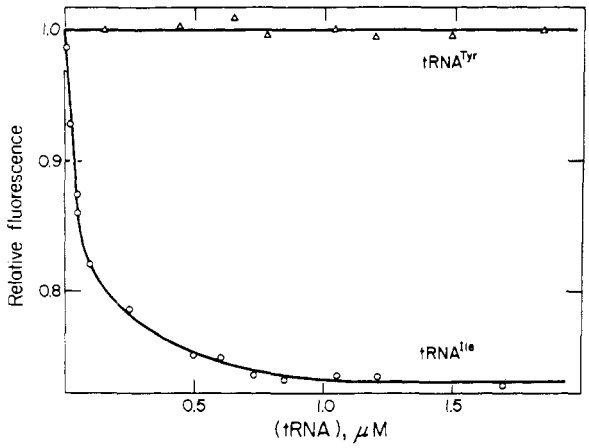

FIGURE 1: Relative fluorescence at 350 nm vs. total $tRNA^{Ile}$ or $tRNA^{Tyr}$ concentration in 67 m*M* sodium phosphate–1 m*M* $MgCl_2$, at pH 6.0, 17°. Enzyme concentration was about 20 n*M*. Excitation wavelength was 290 nm.

Gmbh. The amino acid acceptances were determined by us to be 1280, 1070, 1350, and 1240 pmol/A_{260} (in 0.1 *N* NaOH), respectively. The $tRNA^{Val}_{yeast}$ was a gift from Dr. R. Giegé (Strasbourg); $tRNA^{Tyr}_{E.coli}$ was isolated by E. Jekowsky of this laboratory and was at least 85% pure.[2] In order to prevent the fluorescence of the Y base of $tRNA^{Phe}_{yeast}$ from interfering with the protein fluorescence, this base was excised by acid treatment (Thiebe and Zachau, 1968) to give $tRNA^{Phe(-Y)}_{yeast}$.

All tRNA solutions were treated to remove endogenous Mg^{2+} and other heavy metals, according to the procedure of Schreier and Schimmel (1974). The treated tRNA samples were collected in polyethylene snap-cap vials (Eppendorf) since glass test tubes have heavy metals absorbed to the walls. Water used in various buffers was distilled, deionized on a mixed bed resin, and distilled again in an all glass still (Corning AG-3). In experiments using low Mg^{2+} concentrations, EDTA was used as a Mg^{2+} buffer (see Lynch and Schimmel, 1974a). Although phosphate buffers were used in some experiments, Pipes was used at those pH values where binding of Mg^{2+} to PO_4^{2-} seriously depletes the concentration of Mg^{2+}. The solution pH values were determined with a thermostated Radiometer pH meter.

Fluorescence measurements were carried out with a Farrand Optical Company Mark I spectrofluorometer equipped with a 200-W mercury–xenon arc lamp (Hanovia), using slits with a 5-nm band pass. The cell block was thermostated to ±0.2° with a Brinkmann/Lauda K2R regulated and circulating water bath. Fluctuations in lamp intensity were corrected by frequent comparisons of the sample to a tryptophan reference solution. Observed emission intensities were corrected for dilution of enzyme and for the inner filter effect. The correction factor used for the inner filter effect is $d(1 - 10^{-d_0})/d_0(1 - 10^{-d})$ where d_0 is the absorbance of enzyme alone at the exciting wavelength (290 nm) and d is the total absorbance at this wavelength when tRNA is added (Hélène et al., 1971). The validity of this correction was demonstrated by using tryptophan, instead of protein, to which successive known amounts of ATP were added.

Results

IleRS contains 24 tryptophan and 33 tyrosine residues (Baldwin and Berg, 1966b). When excited at 290 nm, the emission spectrum resembles that of tryptophan, showing a maximum around 336 nm. When the exciting wavelength is varied over the range of 260–295 nm, there is no significant change in the shape of the emission spectrum. Therefore, the emission is almost entirely due to the tryptophans.

Addition of $tRNA^{Ile}$ (*E. coli*) results in quenching of the enzyme's fluorescence. This is shown in Figure 1, which gives a plot of relative fluorescence at 350 nm *vs.* the concentration of $tRNA^{Ile}$ at pH 6.0, 17°. The emission drops progressively as $tRNA^{Ile}$ is added until a plateau is reached at which the fluorescence is about 75% of its original value. Addition of $tRNA^{Tyr}$ (*E. coli*), a noncognate tRNA, gives no change in emission over the same range of nucleic acid concentration.

Because of the large amplitude of the emission change, the data may be used to calculate the association constant K for the reaction

$$E + tRNA \rightleftharpoons E \cdot tRNA \quad (1)$$

where

$$K = (E \cdot tRNA)/(E)(tRNA) \quad (2)$$

$$= \frac{2}{2[tRNA]_{0,m} - [E]_0} \quad (3)$$

and E = enzyme, $[E]_0$ is the total enzyme concentration, and $(tRNA)_{0,m}$ is the value of the total tRNA concentration at the midpoint of the fluorescence titration curve. The entire fluorescence quenching curve was calculated for the value of K given by eq 3 and for slightly different values until the best fit with the experimental data was obtained, as determined by a least-squares analysis (see Appendix). For this purpose, the number of tRNA binding sites per enzyme molecule was determined by extrapolation of the initial slope of the quenching curve to the fluorescence base line obtained at high tRNA concentration, under conditions where the enzyme concentration is substantially greater than K^{-1}. In this situation, the ratio of the tRNA/enzyme concentration at the intersection of the two lines gives the stoichiometry (see Velick et al., 1960). For the titrations with cognate enzyme–tRNA pairs, a value of 1 ± 0.2 tRNA molecules/enzyme molecule was obtained for the stoichiometry at saturation. The K values are estimated to be accurate to ±30%.

Table I: Association Constants at Various pH Values for the *E. coli* IleRS–$tRNA^{Ile}$ Interaction at 17°.[a]

pH	K (M^{-1}), $-Mg^{2+}$	K (M^{-1}), + 5 m*M* Mg^{2+}
5.5	$\simeq 10^8$	$>10^8$
5.8	3.0×10^7	7.0×10^7
6.0	2.1×10^7	5.8×10^7
6.5	9.0×10^6	2.5×10^7
7.0	1.2×10^6	3.4×10^6

[a] Enzyme concentration was *ca.* 80 n*M*. Measurements in the absence of Mg^{2+} were done in 67 m*M* sodium phosphate buffer. Measurements in the presence of 5 m*M* total Mg^{2+} were done in 67 m*M* sodium phosphate at pH 5.5, 5.8, 6.0, and in 20 m*M* NaPipes at pH 6.5 and pH 7.0; the association constant in 67 m*M* sodium phosphate at pH 6.5 was the same as that in 20 m*M* NaPipes.

Association constants obtained in this way for the IleRS–$tRNA^{Ile}$ interaction are tabulated in Table I. This table gives values of K at 17° as a function of pH, in the presence

[2] Some of the tRNAs listed were specific isoacceptor species.

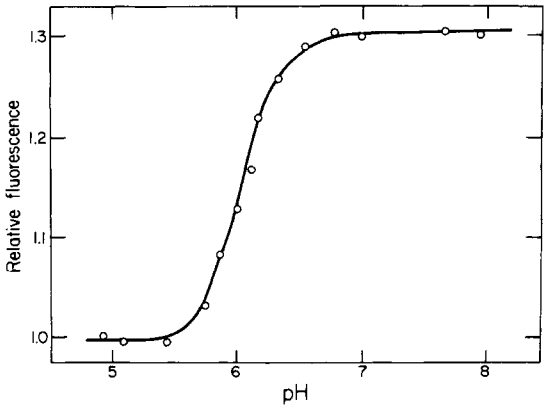

FIGURE 2: Relative fluorescence emission at 350 nm of about 50 n*M* IleRS vs. pH in a mixed buffer (5 m*M* Tris–5 m*M* KH_2PO_4–5 m*M* NaOAc) which was adjusted between pH 5.0 and pH 8.0 with 0.5 *M* HCl. Excitation was at 290 nm.

and absence of 5 m*M* Mg^{2+}. The association is about two orders of magnitude stronger at pH 5.5 than at pH 7.0, and at each pH the presence of magnesium enhances the binding by a factor of two- to threefold. The strong binding at pH 5.5 in the presence of Mg^{2+} is consistent with the result given by the membrane filter assay of Yarus and Berg (1967).

The effect of pH on the interaction must be due to pH-dependent changes in the tRNA and/or protein which in turn affect the protein–tRNA association. In the absence of Mg^{2+} or sufficient salt, tRNA structural changes accompany the ionization of abnormal base p*K*'s which ionize in the range of pH 5–6.5 (Lynch and Schimmel, 1974b; Bina-Stein and Crothers, 1974). However, one or more of these p*K*'s are depressed to more typical values (p*K* < 5) in the presence of Mg^{2+} (Lynch and Schimmel, 1974b). Since the association reaction is strongly pH dependent in the presence and absence of Mg^{2+} (Table I), changes in the enzyme must be responsible for the weaker binding at higher pH values.

In support of this conclusion, a fluorescence pH titration revealed a strong pH dependence of the fluorescence of the free enzyme. This is shown in Figure 2 which gives a plot of relative fluorescence at 350 nm vs. pH. The fluorescence increases by about 30% from a low pH plateau to a high pH limit, following a simple titration curve centered around pH 6.[3] This suggests that some of the enzyme's tryptophans are changing their environment in response to the ionization of a histidine residue(s). One possibility among others that can be envisioned is charge transfer complex formation between a protonated histidine(s) with a tryptophan(s). This kind of interaction is known to cause quenching of the tryptophan emission (Shinitzky and Goldman, 1967).

It might be argued that the changes in the enzyme reflected by the pH dependent emission changes are not necessarily the same as the changes responsible for the stronger binding of the tRNA at acid pH values. The latter changes, for example, might be associated with a component of the fluorescence which is pH independent. Although this possibility cannot be completely excluded, it was found that the percent quenching accompanying saturation of the enzyme with tRNA is also pH dependent, varying from approximately 30% at pH 5.5 to about 10% at pH 7.0. This further suggests that events responsible for fluorescence changes in the free enzyme are linked to the stability of the enzyme–tRNA complex.

[3] The titration in Figure 2 was done by sequential additions of HCl to an initially basic solution. When NaOH additions were made to an initially acidic solution, a somewhat different curve was observed. This probably is due to some denaturation caused by the base. When a constant amount of enzyme was placed in each of several solutions adjusted to various pH values (using a combination of Tris, phosphate, and acetate), a fluorescence titration closely similar to Figure 2 was obtained.

Table II: Effect of Mg^{2+} on *E. coli* IleRS–$tRNA^{Ile}$ Association at pH 6.5, 17°.[a]

$[Mg^{2+}]$ (m*M*)	K $(\mu M)^{-1}$	$[Mg^{2+}]$ (m*M*)	K $(\mu M)^{-1}$
0.001	6.0	5	25
1	13	10	6.5
2	37		

[a] Enzyme concentration was about 80 n*M*. All measurements done in 20 m*M* NaPipes buffer. The concentration of free Mg^{2+} was buffered with 10^{-4} *M* EDTA for the lowest free Mg^{2+} concentration given (see text). For other values of $[Mg^{2+}]$, the free and total concentrations are assumed to be identical.

The effect of Mg^{2+} on the association was examined more closely at pH 6.5, 17°. The results are tabulated in Table II, which gives association constants over a 10^4-fold range of Mg^{2+} concentration in the presence of about 20 m*M* Na^+. Over this wide range of (Mg^{2+}), the free energy of interaction varies by only 1 or so kcal mol^{-1}. Using different conditions and the nitrocellulose filter assay, Yarus (1972a) also found a relatively small effect of Mg^{2+} in this concentration range. Since tRNA conformation varies with Mg^{2+} concentration (Henley et al., 1966; Tao et al., 1970; Cole et al., 1972; Lynch and Schimmel, 1974a,b), the data suggest that the receptor sites on the tRNA for the enzyme are not greatly altered by these Mg^{2+}-induced changes. This is consistent with the fact that although Mg^{2+} is required for the formation of enzyme bound aminoacyl adenylate (Cole and Schimmel, 1970), transfer of the aminoacyl group from the adenylate to tRNA occurs in the presence of Mg^{2+} (Yarus and Rashbaum, 1972). In addition, the weak Mg^{2+} dependence of the synthetase–tRNA interaction implies there is little effect of the divalent ion on the enzyme conformation. The lack of a clearly significant change in the enzyme's quantum yield upon addition of Mg^{2+} further supports this conclusion.

There are few studies with other synthetases against which the present results may be compared, in order to check on the generality of the conclusions obtained. Hélène et al. (1969, 1971) have conducted fluorescence pH titrations and tRNA binding studies with *E. coli* ValRS. In those investigations, it was also found that the enzyme's fluorescence follows a simple titration centered around pH 6 and that $tRNA^{Val}$ binding is stronger at pH 5.5 than at pH 7.0. Although the pH dependence of the enzyme's emission was not reported, Pachmann et al. (1973) found that the yeast SerRS–$tRNA^{Ser}_{yeast}$ interaction is stronger and the magnitude of quenching is greater on the acid side of neutrality. These limited examples together with the present work suggest that some common threads underlie the molecular mechanism of various synthetase–tRNA interactions.

Noncognate Interactions. It is well known that a given synthetase can bind to and mis-acylate certain tRNAs (e.g., Dudock et al., 1971; Giegé et al., 1971; Yarus, 1972b; Ebel et al., 1973; Roe et al., 1973; Yarus and Mertes, 1973). Es-

Table III: Association Constants for Cognate and Noncognate Enzyme–tRNA Complexes at pH 5.5, 17°.[a]

Enzyme	tRNA	K (M^{-1})
Yeast ValRS	Val (yeast)	$\simeq 10^8$
	Val (*E. coli*)	3.2×10^7
	Ile (*E. coli*)	9.1×10^6
	Phe (*E. coli*)	2.8×10^6
	Glu (*E. coli*)	$<10^4$
E. coli IleRS	Ile (*E. coli*)	$\simeq 10^8$
	Val (yeast)	2.0×10^7
	Phe (yeast, −Y)	2.5×10^6
	Phe (*E. coli*)	1.1×10^5
	Tyr (*E. coli*)	$<10^4$
	Glu (*E. coli*)	$<10^4$

[a] All measurements done in 67 mM sodium phosphate.

Table IV: Thermodynamic Parameters for Cognate and Noncognate Interactions at 17°, pH 6.5.[a]

Enzyme–tRNA Complex	$\Delta H°$ (kcal mol^{-1})	$\Delta S°$ (cal deg^{-1} mol^{-1})
E. coli IleRS–$tRNA^{Ile}_{E.coli}$	0.0	34
E. coli IleRS–$tRNA^{Val}_{yeast}$	8.0	54

[a] Parameters are calculated from Figure 3.

timates of the strengths of noncognate interactions have been obtained from K_m determinations (Yarus, 1972b; Ebel et al., 1973; Roe et al., 1973), although some limited direct determinations have been made by using fluorescence quenching as well (Rigler et al., 1970; Pachmann et al., 1973). In the present study, a more extensive examination of a variety of noncognate interactions was undertaken, with the fluorescence approach outlined above.

The interaction of *E. coli* IleRS with six tRNA species ($tRNA^{Ile}_{E.coli}$, $tRNA^{Phe}_{E.coli}$, $tRNA^{Tyr}_{E.coli}$, $tRNA^{Glu}_{E.coli}$, $tRNA^{Val}_{yeast}$), and $tRNA^{Phe(-Y)}_{yeast}$ and of yeast ValRS with five species ($tRNA^{Val}_{yeast}$, $tRNA^{Val}_{E.coli}$, $tRNA^{Ile}_{E.coli}$, $tRNA^{Phe}_{E.coli}$, $tRNA^{Glu}_{E.coli}$) was studied at pH 5.5, 17°. The results are summarized in Table III. It is clear that the strength of the interaction with either synthetase is quite sensitive to the tRNA species. Interestingly enough, no binding of $tRNA^{Glu}_{E.coli}$ to yeast ValRS or of $tRNA^{Tyr}_{E.coli}$ and $tRNA^{Glu}_{E.coli}$ to *E. coli* IleRS could be detected. The lack of interaction of $tRNA^{Tyr}_{E.coli}$ with *E. coli* IleRS has been confirmed by a different method (Dickson and Schimmel, 1975). The variation in the association constants between the remaining tRNAs is considerable. For example, in the case of IleRS the association constant spans three orders of magnitude in going from $tRNA^{Phe}_{E.coli}$ to $tRNA^{Ile}_{E.coli}$. In the case of yeast ValRS, all tRNAs which are found to bind can be aminoacylated to some degree by this enzyme, without the addition of an organic solvent (Kern et al., 1972). Complete data are not available for IleRS, although some results suggest it can acylate $tRNA^{Phe}_{E.coli}$ and $tRNA^{Tyr}_{E.coli}$, but not $tRNA^{Glu}_{E.coli}$ (Yarus, 1972b; Yarus and Mertes, 1973).

The structural reasons for the wide variation in the strength of association are not clear. Photochemical cross-linking studies suggest that cognate and noncognate complexes are similar in their morphological features; that is, a given enzyme orients on a tRNA in the same fashion in both kinds of complexes (Budzik et al., 1975; Schoemaker et al., 1975). The precise bonding within this supposedly common orientation determines the strength of the interaction. Although more definitive structural interpretations cannot be made at this stage, it is of interest to note that the tRNAs which have detectable interactions with IleRS or ValRS each have an A in the fourth position from the 3′-terminus. The base in this position has been proposed as a "discriminator" which limits the interaction of a given tRNA to those enzymes which are within the same general class (Crothers et al., 1972). On the other hand, while $tRNA^{Glu}_{E.coli}$ contains a G in this position (Ohashi et al., 1972) and does not significantly bind to IleRS, $tRNA^{Tyr}_{E.coli}$ contains an A (Goodman et al., 1968) and interacts with neither enzyme. Thus, the occurrence of an A in the fourth position from the 3′-terminus in all tRNAs which bind to the two synthetases may not be of particular significance.

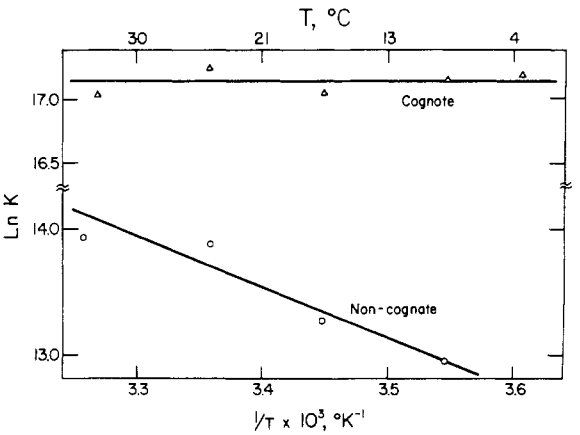

FIGURE 3: Ln K vs. $1/T$ for the association of IleRS with $tRNA^{Ile}_{E.coli}$ and $tRNA^{Val}_{yeast}$ in 20 mM NaPipes–5 mM $MgCl_2$ (pH 6.5).

Thermodynamic Parameters. Very little is known concerning the thermodynamics of synthetase–tRNA interactions. To obtain information on this issue, the temperature dependence of the interaction of *E. coli* IleRS with $tRNA^{Ile}_{E.coli}$ and with $tRNA^{Val}_{yeast}$ was studied at pH 6.5 in the presence of 5 mM Mg^{2+}. The results are shown in Figure 3 which gives plots of ln K vs. $1/T$ for both interactions. The association constant for the cognate complex shows no significant variation with temperature over the range of 4–32°. This agrees with the result of Yarus and Berg (1967) who found by the nitrocellulose filter assay that the association at pH 5.5 does not greatly vary between 0 and 17°. The noncognate interaction, on the other hand, is quite temperature sensitive and becomes stronger as the temperature is raised, gradually approaching that of the cognate interaction.

Thermodynamic parameters are given in Table IV. The reaction is entropically driven in both cases. The large positive $\Delta H°$ for the noncognate case is offset by a $\Delta S°$ of 54 cal deg^{-1} mol^{-1}, which is 20 cal deg^{-1} mol^{-1} greater than the $\Delta S°$ of the cognate case. These results suggest electrostatic interactions play a prime role in stabilizing the complexes, with the liberation of solvating water molecules upon complex formation accounting for the large $\Delta S°$ values (Kauzmann, 1959). Further support for this conclusion comes from the observation that additions of Na^+, in the presence or absence of Mg^{2+}, cause a sharp decrease in the stability of the IleRS–$tRNA^{Ile}$ complex. For example, at pH 5.5 (17°) with no Mg^{2+}, K is $\simeq 10^8$, 2.2×10^6, 5.0×10^5 M^{-1} in the presence of 0.07, 0.3, and 1.1 M Na^+, re-

spectively. In the presence of 10 mM Mg^{2+} under the same conditions the corresponding K values are $>10^8$, 9.5×10^6, and 1.6×10^6 M^{-1}, respectively. Yarus (1972a) has also noted the sensitivity to monovalent cation concentration of the IleRS-$tRNA^{Ile}$ association at pH 5.5, 10 mM Mg^{2+}, using the nitrocellulose filter assay.

Discussion

The data given in Table III show a wide variation in the stabilities of enzyme-tRNA complexes. The cognate interactions are strongest, although noncognate associations in some instances are also substantial. For some cases the difference in binding between the cognate and noncognate tRNA is sufficiently high to be a major source of specificity. An additional source of specificity lies in the maximal velocity, however (Ebel et al., 1973; Roe et al., 1973).

The data in Table III on noncognate enzyme-tRNA complexes were obtained at pH 5.5, where we expect the interactions to be easiest to detect. It is of interest to compare these association constants with those obtained from K_m determinations in aminoacylation reactions carried out at other pH values, but without the addition of special additives to the reaction mixture (e.g., organic solvents). Roe et al. (1973) have made K_m determinations for the mis-aminoacylation of a variety of *E. coli* tRNAs by yeast phenylalanine tRNA synthetase at pH 6.0 and at pH 8.2, 30°. At both pH values the association constants for the noncognate interactions fall in the range of 10^5–10^6 M^{-1} and are almost as strong as the homologous cognate interaction. Ebel et al. (1973) have made K_m determinations for misacylations in a yeast system. Association constants at pH 7.5 in the approximate range of 10^5–10^6 M^{-1} have been obtained for the interaction of valyl-tRNA synthetase with $tRNA^{Phe}$ and $tRNA^{Ala}$ and for the interaction of arginyl-tRNA synthetase with $tRNA^{Asp}$. These association constants are about 10- to 100-fold less than the cognate ones (Ebel et al., 1973). Finally, Yarus (1972b) has estimated from a K_m measurement an association constant of less than 10^4 M^{-1} for the interaction of *E. coli* $tRNA^{Phe}$ with the homologous isoleucyl-tRNA synthetase at pH 7, 37°. Therefore, the range of association constants for noncognate interactions measured by fluorescence methods (Table III) agrees well with those found for other systems in which measurements were done by aminoacylation assays under different sets of conditions.

The data in Table IV show that entropic changes provide the driving force for enzyme-tRNA association. For other specific protein-nucleic acid systems, data are sparse. In one study, Riggs et al. (1970) measured the temperature dependence of the *Lac* repressor-operator interaction. For the association, they obtained $\Delta H = +8.5$ kcal mol^{-1} and $\Delta S = +90$ cal deg^{-1} mol^{-1}. Like us, they suspect that solvation changes associated with charged sites account for the thermodynamics. These two examples, for entirely different systems, suggest that positive ΔS values may be the pattern for specific protein-nucleic acid associations.

Figure 3 shows that discrimination between the cognate and noncognate tRNA is diminished as temperature is raised. This arises from a difference in the apparent enthalpy of the cognate and noncognate associations (Table IV). It is possible that tRNA structure relaxes as temperature increases and that the enzyme binds preferentially to the more relaxed form (cf. Yarus, 1972a). It is well known that misacylations occur readily when organic solvents are added to an aqueous aminoacylation reaction mixture (Giegé et al., 1971, 1972, 1974; Yarus, 1972b; Kern et al., 1972; Mertes et al., 1972; Ebel et al., 1973; Yarus and Mertes, 1973). Yarus (1972a) has suggested that such solvents permit a relaxation of tRNA structure. Therefore, the effects of the organic solvents and those of temperature (Figure 3) may have a common basis.

When the many experiments on synthetase-tRNA interactions are considered in the light of the three-dimensional tRNA structure (Kim et al., 1975; Robertus et al., 1974), a consistent picture is suggested (A. Rich and P. R. Schimmel, in preparation). It appears that some major enzyme-tRNA contact points lie along and around the inside of the L-shaped tRNA structure (Budzik et al., 1975; Schoemaker et al., 1975; A. Rich and P. R. Schimmel, in preparation). This part of the tRNA structure is believed to be similar for all tRNAs with regard to the spatial arrangement of the phosphate backbone (Kim et al., 1975). Variations in the lengths of specific tRNAs occur in the extra loop and in the dihydrouridine loop, which both lie on the opposite side of the structure (Kim et al., 1975). In view of data cited above a common system of enzyme-tRNA interactions may include electrostatic bonds with certain phosphate groups lining the inside of the L (see A. Rich and P. R. Schimmel, in preparation). Some binding and aminoacylation specificity might be achieved by favorable, and unfavorable, interactions with a few particular bases.

Appendix

For the equilibrium of eq 1 the degree of association α is

$$\alpha = \frac{[E]}{[E] + [E \cdot tRNA]} \qquad (I\text{-}1)$$

$$= \frac{\Delta F}{\Delta F_\infty} \qquad (I\text{-}2)$$

where ΔF is the fluorescence decrease induced by total tRNA concentration $[tRNA]_0$ and ΔF_∞ is the maximal decrease. Making use of the two mass conservation relationships

$$[E]_0 = [E] + [E \cdot tRNA] \qquad (I\text{-}3)$$

$$[tRNA]_0 = [tRNA] + [E \cdot tRNA] \qquad (I\text{-}4)$$

and the equilibrium constant expression (eq 2 of text), we obtain

$$\alpha = \frac{1 + K[tRNA]_0 + K[E]_0}{2K[E]_0} - \sqrt{\left[\frac{1 + K[tRNA]_0 + K[E]_0}{2K[E]_0}\right]^2 - \frac{[tRNA]_0}{[E]_0}} \qquad (I\text{-}5)$$

Theoretical fluorescence titration curves (as a function of $[tRNA]_0$) were constructed for an initial K value (see text), which was then varied in order to construct new curves. The best K value was chosen as the one in which the sum of the squares of the deviations of the observed ΔF values from the calculated ones is a minimum.

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