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Allosteric Interpretation of Mg^{2+} Binding to the Denaturable *Escherichia coli* $tRNA^{Glu}_2$†

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ABSTRACT: The Mg^{2+} binding properties of the denaturable $tRNA^{Glu}_2$ from *E. coli* in 0.1 M Na^+, pH 7, are characterized by equilibrium dialysis. At 34 °C, where the native and denatured conformers are in equilibrium, Mg^{2+} binding is cooperative. By trapping the tRNA completely in the native conformation at 4 °C it is shown that native $tRNA^{Glu}_2$ possesses one strong binding site, $K_1 = 7.5 \times 10^4$ M^{-1} and approximately 36 weak sites with $K_2 = 8.3 \times 10^2$ M^{-1}. A significantly lower affinity for the denatured conformer is indicated. We show that Mg^{2+} effects an allosteric transition from the low affinity denatured conformational state to the high affinity native state and develop the appropriate equations to fit the Mg^{2+} binding data with physically meaningful parameters. Our results also suggest the previously reported cooperative cation binding to tRNA arises from a cation induced conformational change to the native tRNA conformation and does not reflect the inherent Mg^{2+} binding properties of the native conformer.

It is now well known that there are macromolecules which undergo conformational changes in the course of their function and that allosteric effects may be important in the regulation of cellular activities (Monod et al., 1963). Evidence is accumulating which suggests that tRNA[1] may function in a similar fashion. In protein synthesis tRNA interacts with a number of different proteins. Although the generality of tRNA conformational changes accompaning interactions with various cellular components is not yet established, the results of Schwartz et al. (1974) and Erdman et al. (1973) indicate that the compact native structure of tRNA (Kim et al., 1973; Robertus et al., 1974; Ladner et al., 1975) is altered when tRNA is bound to the 30S ribosomal subunit. Possible roles for tRNAs in regulating cell differentiation are suggested by variations observed in chromatographic patterns of isoacceptor tRNAs in differentiating cells, different organs, and in response to hormones (Littauer and Inouye, 1973). The strong binding of tRNAs to some feedback-sensitive enzymes (Littauer and Inouye, 1973) and the high affinity of tRNA for the lysine-rich histones (Ilyin et al., 1971) provide further support for this idea. In addition, it has been suggested that tRNAs may be involved in regulating the biosynthetic pathways for their cognate amino acids (Goldberger and Kovach, 1972).

Mechanisms whereby tRNAs can function as regulatory molecules are therefore of interest. It is known that under certain conditions tRNAs bind divalent metal cations and polyamines cooperatively (Schreier and Schimmel, 1974, 1975). The reason for cooperative oxygen uptake in hemoglobin has been sought since the beginning of the century (Hill, 1910). Through extensive study over many years it has been shown that a conformational change between low and high affinity states is responsible for the cooperativity, in accordance with the ideas of Monod et al. (1965), and the triggering of the conformational transition can be explained in terms of chemical structure (Shulman et al., 1975). Most importantly, it is generally believed that the phenomenon exhibited by hemoglobin, a ligand-induced conformational change, may be a general feature of regulatory systems. Since tRNA may have regulatory functions, it could be of significance that ligands as diverse as divalent cations and polyamines interact with tRNA in a very similar fashion as evidenced by their binding properties and effect on tRNA conformation (Cohen, 1971; Schreier and Schimmel, 1975; Sakai et al., 1975).

In this paper we characterize a Mg^{2+} effected allosteric transition in a tRNA. *Escherichia coli* $tRNA^{Glu}_2$ was chosen because it is one of the "denaturable" tRNAs for which the native conformer exists in equilibrium with a nonnative, biologically inactive conformer at pH 7.0 over a range of Mg^{2+} concentrations in fairly high Na^+ (0.1 M), thus minimizing purely electrostatic effects. We show that Mg^{2+} binding to $tRNA^{Glu}_2$ is cooperative, and as for hemoglobin the cooperativity arises from a ligand affinity difference between two states. By kinetically trapping the tRNA in its native conformation for all degrees of ligation, it is shown that Mg^{2+} binds noncooperatively to this state with a high affinity, whereas a low affinity is indicated for the biologically inactive state. The relation between the degree of ligation and the extent of the conformational transition is expressed quantitatively using equations similar to those of Monod et al. (1965).

Experimental Section

Transfer RNA. Partially purified $tRNA^{Glu}_2$ from Oak Ridge National Laboratory was kindly provided by Drs. Z. Egan, A. Kelmers, and P. E. Cole. Further purification was

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[1] Abbreviations used: EDTA, ethylenediaminetetraacetic acid; tRNA, transfer ribonucleic acid; NMR, nuclear magnetic resonance.

achieved by RPC 5 column chromatography (Pearson et al., 1971). The final acceptor activity was 1450–1500 pmol/A_{260} unit.

Equilibrium Dialysis Measurements. tRNA samples (10–15 A_{260}/ml) were dialyzed for 4 to 7 days vs. 0.02 M sodium cacodylate, pH 7.0, 0.08 M sodium chloride, containing the desired magnesium chloride concentration. A thermostated box was used to maintain the temperature at 34 °C and a cold room provided temperature control at 4 °C. Mg^{2+} concentrations were measured using a Perkin-Elmer Model 305 B graphite furnace atomic absorption spectrophotometer as previously described (Stein and Crothers, 1976a). tRNA concentrations were measured by the absorbance at 260 nm after dilution with buffer containing 5 mM Mg^{2+}.

In one experiment it was necessary that equilibrium between the two conformers of $tRNA^{Glu_2}$ be attained for each Mg^{2+} concentration. Here, a temperature of 34 °C was chosen since Eisinger and Gross (1975) showed that at this temperature the complete conversion from one form to the other requires less than 1 h in 1 mM Mg^{2+}. A temperature of 4 °C was chosen to kinetically trap the tRNA completely in the native conformation for all Mg^{2+} concentrations in the second experiment (Eisinger and Gross, 1975). $tRNA^{Glu_2}$ was prepared in the native conformation by heating at 45 °C for 30 min in 5 mM Mg^{2+} and quenching in ice. Removal of Mg^{2+} was accomplished by extensive dialysis at 4 °C against buffer containing EDTA. In both experiments equilibrium with respect to Mg^{2+} binding was demonstrated by dialyzing Mg^{2+} both in and out in all regions of the binding isotherms. A 10% polyacrylamide gel was run on the tRNA samples for each point to demonstrate the absence of dimer formation (Eisinger and Gross, 1975).

Analysis of Data. Curves were fitted to the model equations by a simple iterative procedure using the M-Lab/PDP 10 interactive system at the National Institutes of Health designed and programmed by Knott, Reece, and Shrager (Knott and Reece, 1972; Knott and Shrager, 1972).

Results and Discussion

(a) There Are Only Two Resolvable Conformers of $tRNA^{Glu_2}$. $tRNA^{Glu_2}$ exists in two distinct conformational states in 0.1 M Na^+, pH 7.0; the denatured form (D) predominates in the absence of Mg^{2+} and the native form (N) predominates greatly with sufficient Mg^{2+} present (Eisinger and Gross, 1975; Bina-Stein et al., 1976). Eisinger and Gross (1975) demonstrated that two well-resolved peaks corresponding to the N and D conformers could be observed on polyacrylamide gels. They were able to follow the kinetics of renaturation or denaturation by the disappearance of one peak and concomitant appearance of the other as a function of time. The fraction of tRNA molecules converted from either state follows pseudo-first-order kinetics and is well represented by a single exponential. Similar results have been obtained with other denaturable tRNAs using column chromatography to separate two species at intermediate Mg^{2+} concentrations, and by following the renaturation kinetics by amino acid acceptor activity (Gartland and Sueoka, 1966; Ishida and Sueoka, 1968; Karpel et al., 1975). Also a single slow relaxation time is observed by following the absorbance change at 260 nm after a salt or Mg^{2+} jump from low salt solutions for various tRNAs (Cole et al., 1972; Bina-Stein, 1974). Therefore, we shall assume the existence of only two significantly populated states for $tRNA^{Glu_2}$: N and D.

(b) Mg^{2+} Binding to the Equilibrium Mixture of Denatured and Native Conformers. In Figure 1 the Scatchard plot

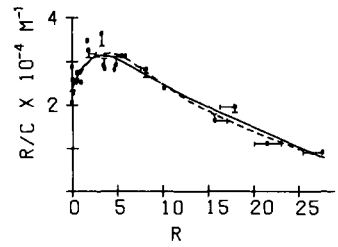

FIGURE 1: Scatchard plot of Mg^{2+} binding to $tRNA^{Glu_2}$ in 0.1 M Na^+, pH 7.0, 34 °C. The dashed curve is the best fit to eq 8, representing a class of strong cooperative sites, in addition to a weaker class of noninteracting sites. The solid curve given by eq 3 and 4 represents the allosteric model with parameters describing the N and D states as discussed in the text. *R* denotes *r*.

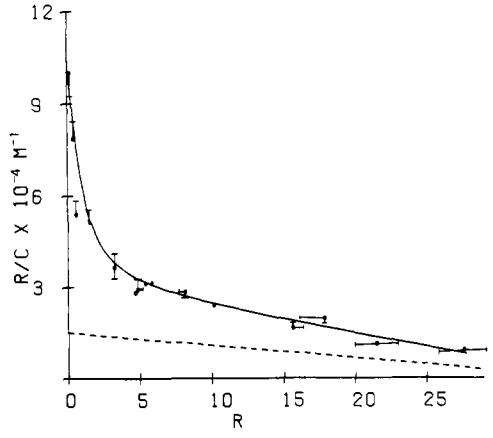

FIGURE 2: Scatchard plot of Mg^{2+} binding to native $tRNA^{Glu_2}$ in 0.1 M Na^+, pH 7, 4 °C. The solid curve is the best fit to eq 1. The dashed curve represents the Scatchard plot assumed for the denatured $tRNA^{Glu_2}$ conformer. *R* denotes *r*.

for Mg^{2+} binding to *E. coli* $tRNA^{Glu_2}$ in 0.1 M Na^+, pH 7.0, at 34 °C is presented. The positive slope at low *r* (Mg^{2+} ions bound per tRNA) values and maximum near $r = 3$ indicate cooperativity: the first Mg^{2+} ions bound exhibit lower apparent association constants than some of the subsequently bound ions. Error bars on representative points indicate the error propagated into *r* and *r/C*, where *C* is the "free" Mg^{2+} concentration, due to errors in measuring concentrations. At high Mg^{2+} concentrations ($r > 25$), the error in measuring *r* becomes unacceptably large due to persistent weak binding.

Our binding data at 34 °C (Figure 1) represent the interaction of Mg^{2+} with the *equilibrium* mixture of the N and D conformers of $tRNA^{Glu_2}$ since the D $\rightleftharpoons$ N conversion is fast on the time scale of the dialysis experiment. The position of this equilibrium is a function of the Mg^{2+} concentration (or *r* value); the Na^+ concentration is constant. Cooperativity suggests a Mg^{2+} affinity difference between the D and N states.

(c) Mg^{2+} Binding to the N Conformer Alone. In order to directly measure the affinity of the N state alone, $tRNA^{Glu_2}$ was kinetically trapped in the N conformation at 4 °C as described above. Ample evidence now exists indicating that Mg^{2+} binding to tRNA and other RNAs is not very temperature dependent; the enthalpy for Mg^{2+} binding is nearly zero in this temperature range (Krakauer, 1971; Rialdi et al., 1972; Privalov et al., 1975) and, therefore, any significant difference in Mg^{2+} affinity reflects the tRNA conformation. The Scatchard plot for $tRNA^{Glu_2}$ trapped in the native conformation is shown in Figure 2. It is significant that the curve does not indicate cooperativity and that the *r/C* intercept is 4.4 times

greater than in Figure 1 (note the change in the r/C scale). At r values near 4, the curve approaches the higher temperature curve within experimental error. Points for $r > 4$ are replotted from Figure 1. Figure 2 is quite similar in appearance to the Scatchard plot for *E. coli* $tRNA^{fMet}_1$, a nondenaturable tRNA in 0.17 M Na^+, pH 7.0 (Stein and Crothers, 1976a). The curve through the points represents the best fit to eq 1 for two classes of noninteracting sites:

$$r/C = K_1N_1/(1 + K_1C) + K_2N_2/(1 + K_2C) \quad (1)$$

where $K_1 = 7.5 \times 10^4\ M^{-1}$, $N_1 = 1$, $K_2 = 8.3 \times 10^2\ M^{-1}$, $N_2 = 36$. For $N_1 > 1$, a poorer fit is obtained. Our data are not precise enough to attempt to resolve more than two classes of sites, but this is clearly plausible.

(d) Mg^{2+} Binding to the D Conformer. Anticipating the results somewhat, it is shown below that the greatly increased affinity for Mg^{2+} of the N conformer (Figure 2) over that of the equilibrium mixture (Figure 1) requires that the D conformer must have a rather low affinity for Mg^{2+}. Ideally, it would be desirable to trap $tRNA^{Glu}_2$ completely in the D state in 0.1 M Na^+ and measure the Mg^{2+} binding. We could then calculate the shape of the higher temperature isotherm (Figure 1) without any adjustable parameters and compare the theoretical curve with the experimental points. However, the fraction of tRNA molecules existing in the N state is a function of the Na^+ concentration, and the extent of renaturation at 0.1 M Na^+ in the absence of Mg^{2+} is not independently known. In these measurements we chose to maintain 0.1 M Na^+ throughout in order to exclude the possibility of forming possible low salt structures (Cole et al., 1972) even though there is some evidence that the low salt and D conformations are the same (Bina-Stein, 1974). Furthermore, at low temperatures in the presence of Mg^{2+}, the D conformer readily forms dimers and higher aggregates (Eisinger and Gross, 1975). Therefore, as an approximation for the binding isotherm for the D conformer, we have chosen a single class of 36 noninteracting sites denoted by N_3 (equal to N_2) and the *highest* value of an association constant $K_3 = 4.3 \times 10^2\ M^{-1}$ which still permits a reasonably good fit to the 34 °C data. The dashed line in Figure 2 represents the Scatchard plot assumed for the D state. A lower affinity for the D form allows a better fit to the experimental data, but may be harder to justify.

A low affinity for the D form is likely since NMR and temperature-jump studies (Bina-Stein et al., 1976) have shown that the tertiary and D helix interactions are lacking in this species and, therefore, a relatively expanded structure possessing a considerably lower charge density is expected. Recent studies on the denaturable yeast $tRNA^{Leu}_3$ also indicate a very low cation affinity for the D conformer (Karpel et al., 1975). Moreover, by comparing the Mn^{2+} binding properties of yeast $tRNA^{Phe}$ with synthetic double helical polynucleotides, Schreier and Schimmel (1974) have assigned the predominant weak class of sites on tRNA to the cloverleaf helical sections. If this is true, then the affinity K_3N_3 we have chosen for the D state is quite reasonable and possibly high in view of the low Mg^{2+} affinity for synthetic double helical polynucleotides in 0.1 M Na^+ (Krakauer, 1971).

(e) Theoretical Description of Mg^{2+} Binding. We next derive an expression for the cooperative binding isotherm (Figure 1) in terms of the binding parameters describing the N and D states. Cooperativity arises due to a ligand affinity difference between two states in equilibrium, provided that the low affinity state predominates in the absence of ligand (Monod et al., 1965). The N state is characterized by K_1, K_2, N_1, and N_2 and the D state by K_3 and N_3. An allosteric constant L is defined for the reaction, $N \rightleftharpoons D$ as

$$L = [D]/[N] \quad (2)$$

in the absence of Mg^{2+}. The binding isotherm is most conveniently obtained using a statistical mechanical formalism (Crothers, 1968). We desire the partition function summed over all states, that is, both N and D for all degrees of binding. A statistical weighting factor $K_\alpha C$ arises for each Mg^{2+} bound where K_α is the intrinsic site association constant for a site in the class $\alpha = 1, 2$, or 3 and C is the "free" Mg^{2+} concentration. L is the weighting factor for forming the D state from the reference state, the N conformer with no Mg^{2+} bound. The summed partition function Q is given by:

$$Q = (1 + K_1C)^{N_1}(1 + K_2C)^{N_2} + L(1 + K_3C)^{N_3} \quad (3)$$

The average number of Mg^{2+} ions bound per tRNA, r, is obtained from the relation:

$$r = \partial \ln Q/\partial \ln C \quad (4)$$

which yields r (or r/C) as a function of C. For the simpler case where each conformational state possesses a single class of N noninteracting sites with an intrinsic site association constant K_α for the high affinity state and K_β for the low affinity state, the appropriate partition function is:

$$Q = (1 + K_\alpha C)^N + L(1 + K_\beta C)^N \quad (5)$$

and application of eq 4 results in the binding isotherm of Monod et al. (1965).

Equations 3 and 4 enable the calculation of the cooperative binding isotherm (Figure 1) from the known binding parameters describing the N and D states provided that L is known. Although we could not measure L independently, L can be calculated assuming the above model from the experimental r/C intercept in Figure 1. From eq 3 and 4

$$\lim_{r \to 0} r/C = (N_1K_1 + N_2K_2 + LN_3K_3)/(1 + L) \quad (6)$$

From Figure 1

$$\lim_{r \to 0} r/C = 2.35 \times 10^4\ M^{-1}$$

and using the above parameters for N_1K_1, N_2K_2, and N_3K_3, eq 6 yields $L = 10$. The Scatchard plot predicted by eq 3 and 4 is displayed as the solid curve in Figure 1 and is a satisfactory representation of the experimental points.

The fraction of tRNA molecules in the N conformation, F_N, as a function of the "free" Mg^{2+} concentration is easily calculated from the partition function as:

$$F_N = (1 + K_1C)^{N_1}(1 + K_2C)^{N_2}/Q \quad (7)$$

and is plotted in Figure 3. At $C = 0$, $F_N = 1/(1 + L)$ which is 0.091 indicating that 9.1% of the tRNA molecules are already renatured in 0.1 M Na^+ in the absence of Mg^{2+}. This is consistent with the higher than background levels of amino acid acceptor activity observed for $tRNA^{Glu}_2$ prepared under denaturing conditions and the measurable onset of the temperature-jump relaxation signal characteristic of the N conformer at about 0.2 M Na^+ without Mg^{2+} (Bina-Stein, unpublished results). In Figure 4, F_N is plotted vs. r, the average number of Mg^{2+} ions bound per tRNA molecule (both N and D forms). Approximately 92% renaturation is achieved by $r = 5$.

(f) Comparison with Other Work. Qualitatively similar cooperative binding of Mn^{2+} and polyamines to tRNA in solutions of low concentrations of monovalent metal ions has been

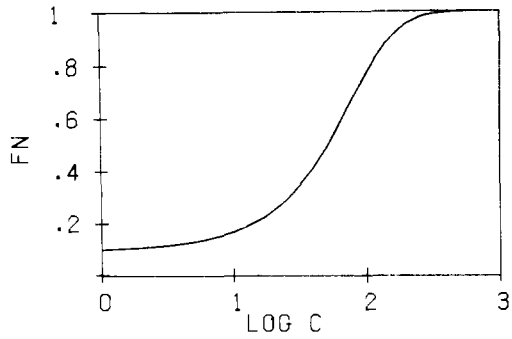

FIGURE 3: Theoretical curve of the fraction of $tRNA^{Glu_2}$ in the N state (F_N) as given by eq 7 for the two-state (allosteric) model vs. the logarithm of the free Mg^{2+} concentration (micromolar).

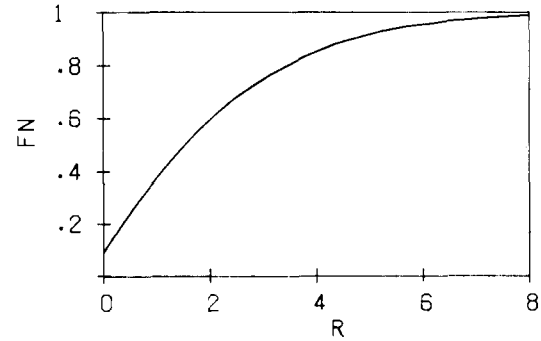

FIGURE 4: Theoretical curve of the fraction of $tRNA^{Glu_2}$ in the N state (F_N) vs. the average number of Mg^{2+} ions bound per tRNA molecule (both N and D forms). F_N as a function of r was obtained from eq 7, 3, and 4. R denotes r.

reported previously (Danchin and Gueron, 1970; Danchin, 1972; Schreier and Schimmel, 1974, 1975). These data have been represented in general by the Scatchard equation for a class of strong cooperative sites in addition to a weaker class of noninteracting sites

$$r = \frac{N_{1'}K_{1'}{}^{\alpha}C^{\alpha}}{1 + K_{1'}{}^{\alpha}C^{\alpha}} + \frac{N_{2'}K_{2'}C}{1 + K_{2'}C} \qquad (8)$$

where $N_{1'}$ and $K_{1'}$ are the number of sites and association constant, respectively, for the strong cooperative class, $N_{2'}$ and $K_{2'}$ are the corresponding parameters for the noninteracting class, and α is an empirical index of cooperativity or Hill coefficient. The dashed curve in Figure 1 is the best fit to eq 8, and the optimized parameters are: $K_{1'} = 6.66 \times 10^3\ M^{-1}$; $N_{1'} = 4$; $K_{2'} = 5.5 \times 10^2\ M^{-1}$; $N_{2'} = 36$, $\alpha = 1.8$. Since best fits were obtained in most cases for $N_{1'}$ approximately 5 (or 5 divalent equivalents in the case of polyamines), others have concluded that tRNAs possess a class of about five strong cooperative sites. Schreier and Schimmel (1974) have shown that various fragments of tRNA do not bind Mn^{2+} or polyamines cooperatively and argue that the cooperative sites arise from the tRNA tertiary structure.

There are several reasons why eq 8 is not a very satisfactory representation of tRNA binding data. First, eq 8 is empirical and gives little insight into the question of how cooperativity arises from the putative conformational change. Secondly, it is obvious that the five parameters of eq 8 can approximately accommodate any curve similar to the one shown in Figure 1, and therefore the values of these parameters may not be meaningful since a theoretical justification for eq 8 in terms of tRNA conformation is lacking. Finally, there is evidence that in the native conformation tRNA binds cations and polyamines noncooperatively (Jones and Kearns, 1974; Römer and Hach, 1975; Sakai et al., 1975; Stein and Crothers, 1976a) and some tRNAs at least do not possess a resolvable class of approximately five strong divalent cation binding sites corresponding to the cooperative phase of the Scatchard plot. In solutions of 0.17 M Na^+, $tRNA^{fMet_1}$, for example, possesses a single strong Mg^{2+} binding site in the neighborhood of the tertiary–D helix interactions in addition to a class of weaker sites (Stein and Crothers, 1976a). At low concentrations of monovalent ions, however, $tRNA^{fMet_1}$ binds Mg^{2+} cooperatively and effectively identically with most other tRNAs (Danchin, 1972).

In this study we similarly find that the native conformer of $tRNA^{Glu_2}$ is best represented by a single strong binding site and a large class of weaker sites. It is likely that this strong site is associated with the tertiary structure since the D conformer which lacks the native tertiary structure (Bina-Stein et al., 1976) has a very low affinity for Mg^{2+}. Equation 8, however, suggests that there is a class of four to five strong cooperative sites in addition to the weak sites. It is true that about five cations are required to effect the conformational change to the native state (Figure 4) but a resolvable class of four to five strong sites does not exist for native $tRNA^{Glu_2}$, nor need a distinguishable class of four to five sites exist on the D conformer.

The multivalent ion binding properties of nondenaturable tRNAs in solutions of low concentrations of monovalent ions can also be simply explained if tRNA undergoes a conformational change from a low salt structure to the native conformation as divalent ion binding increases. This explanation is supported by the existence of a distinct low salt region of the tRNA phase diagram (transition midpoint vs. logarithm of Na^+ concentration) and high activation energies upon conversion from the low salt to the high salt conformation (Cole et al., 1972). When Na^+ concentrations are greater than about 0.05 M, many, if not all, nondenaturable tRNAs are effectively in their native conformation and addition of Mg^{2+} to $tRNA^{fMet_1}$ and $tRNA^{Tyr_1}$ (both *E. coli*) causes no gross conformational rearrangement (Yang and Crothers, 1972; Stein and Crothers, 1976b). Denaturable tRNAs, such as *E. coli* $tRNA^{Glu_2}$, require substantially higher Na^+ concentrations to maintain their native structure in the absence of divalent ions (Ishida and Sueoka, 1968; Bina-Stein, unpublished results). It is not presently known if the ease of accessibility to nonnative conformations in denaturable tRNAs is biologically significant. However, analysis of primary structures indicates that all tRNAs possess potential alternative conformations which may be realized under nonphysiological conditions, i.e., no Mg^{2+}.

The two-state model also readily explains the effect of monovalent ions on the multivalent ion binding equilibrium. Simple considerations show that the effect of competition by Na^+, for example, is expected primarily to decrease the magnitude of the multivalent ion association constant while leaving the number of sites and character of the binding isotherm relatively unaffected. However, it is observed that with increasing Na^+ the nature of the Mg^{2+} binding for $tRNA^{fMet_1}$ changes from highly cooperative (Danchin, 1972) to noncooperative (Stein and Crothers, 1976a), and the number of strong sites changes from 5 to 1. This is not easily explained in terms of eq 8, whereas for the two-state model all that is required is that the allosteric constant L decreases rapidly with increasing Na^+.

In apparent disagreement with the two-state model, Lynch and Schimmel (1974) observe two relaxation times in a

Mg^{2+}-jump experiment with *E. coli* isoleucyl-$tRNA^{Ile}$ containing a fluorescent probe covalently attached to the isoleucyl amino moiety whose emission is sensitive to Mg^{2+}. They argue that for a two-state model one should observe a single relaxation time which increases with increasing Mg^{2+} in contrast to their observation of two slow relaxation times which decrease with increasing Mg^{2+}. The amplitude of the faster relaxation is small and only a single slow relaxation can be detected upon monitoring the absorbance change at 260 nm following the Mg^{2+} jump. It is not clear that a probe at the 3′ terminus of the tRNA can accurately monitor conformational changes in the neighborhood of the tertiary interactions. Furthermore, a kinetic mechanism consistent with the two-state model can easily be formulated for which the slow relaxation time decreases with increasing Mg^{2+}.

(*g*) *Possible Functional Significance.* The idea that a conformational change to the native conformation of tRNA from a nonnative conformation may be induced by Mg^{2+} or other divalent ions, also apparently by polyamines, and possibly by basic regions of proteins, is attractive and conducive to constructing models for tRNA functions. In solutions of physiological ionic strength in the presence of Mg^{2+}, the native conformation of tRNA is extremely stable and is required in order for aminoacylation to occur. However, on the ribosome, for example, an altered or nonnative conformation may exist since the TψC sequence in the TψC loop is exposed (Schwartz et al., 1974; Erdman et al., 1973; Ladner et al., 1975). The denatured conformation of $tRNA^{Glu}_2$ from *E. coli* could be roughly similar to the tRNA conformation on the ribosome. A more flexible molecule here lacking tertiary and D helix interactions with the acceptor stem intact could facilitate the peptidyl transferase reaction (Crothers, 1975). tRNA interaction with one or more molecules possessing a high affinity for the native state, perhaps a protein, could effect the allosteric transition to the N state accompanied by tRNA release from a ribosomal binding site. This step could be involved in the translocation mechanism.

Our ideas concerning tRNA as an allosteric macromolecule differ from the model recently proposed by Kurland et al. (1975) who attempt to account for the supposedly weak codon–anticodon interaction for native tRNA. Recent work on anticodon–anticodon interactions has shown that the codon–anticodon interaction may in fact be quite strong for certain possible messenger RNA conformations (Eisinger and Gross, 1975; Grosjean et al., 1976).

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Changes in Tertiary Structure Accompanying a Single Base Change in Transfer RNA. Proton Magnetic Resonance and Aminoacylation Studies of *Escherichia coli* $tRNA^{Met}_{f_1}$ and $tRNA^{Met}_{f_3}$ and Their Spin-Labeled (s^4U8) Derivatives†

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ABSTRACT: The properties of *Escherichia coli* $tRNA^{Met}_{f_1}$ and $tRNA^{Met}_{f_3}$ that differ by only one base change, m^7G to A at position 47, have been compared structurally by proton magnetic resonance and functionally by the aminoacylation reaction. The NMR spectra of the two tRNA species in the region between 0 and 4 ppm below 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS) (methyl and methylene region) were the same except for the absence of the lowest field peak at 3.8 ppm in $tRNA^{Met}_{f_3}$, thus unequivocally identifying this resonance as the methyl group of m^7G47 of $tRNA^{Met}_{f_1}$. The same resonance disappears in $tRNA^{Met}_{f_1}$ spin-labeled at s^4U8 and reappears in the diamagnetic reduced spin-labeled $tRNA^{Met}_{f_1}$ from which the average distance between the spin-label and the methyl protons of m^7G is estimated to be less than 15 Å. The proximity of m^7G47 but not T55 to s^4U8 in the structure of *E. coli* $tRNA^{Met}_{f_1}$ in solution is consistent with the crystallographic model for yeast $tRNA^{Phe}$. A spectral comparison of the hydrogen-bond regions (11–14 ppm below DSS) of $tRNA^{Met}_{f_1}$ and $tRNA^{Met}_{f_3}$ reveals major shifts of four resonances previously assigned to tertiary hydrogen bonds. Of the four, the one at lowest field (14.8 ppm) had been assigned by chemical modification to the tertiary (s^4U8–A14) hydrogen bond and the one at 13.3 ppm had been tentatively assigned to the tertiary hydrogen bond G23–m^7G47 of the 13–23–47 triple. A more positive assignment of the G23–m^7G47 at 13.3 ppm could be made from the additional evidence that this resonance, which was first observed in the difference spectrum between spin-labeled $tRNA^{Met}_{f_1}$ and its reduced form, is the only one missing in the analogous difference spectrum of $tRNA^{Met}_{f_3}$. At low ionic strength and in the absence of magnesium ions, the differences in the hydrogen-bonded region of the NMR spectra of $tRNA^{Met}_{f_1}$ and $tRNA^{Met}_{f_3}$ are much greater than in the presence of magnesium ions. The optimal magnesium concentration required for maximal initial velocities is also higher for $tRNA^{Met}_{f_3}$ than for $tRNA^{Met}_{f_1}$. The perturbation caused by the spin-label in destabilizing hydrogen bonds in the region between 13 and 14 ppm is greater for $tRNA^{Met}_{f_3}$ than $tRNA^{Met}_{f_1}$ but the distance relations for the hydrogen bonds in the region between 12 and 13 ppm (the major paramagnetic perturbations) are conserved in the two species. The disruption of one hydrogen bond relative to native $tRNA^{Met}_{f_1}$ either by spin-labeling (s^4U8–A14) or by substitution of m^7G by A in $tRNA^{Met}_{f_3}$ has little effect on the aminoacyl acceptor activity or the velocity of the aminoacylation reaction at optimal magnesium concentration, but the absence of both tertiary hydrogen bonds in the augmented D-helix region in the spin-labeled $tRNA^{Met}_{f_3}$ results in approximately 60% reduction both in acceptance activity and in initial velocity of the aminoacylation reaction.

Since it is clear that the tertiary structure of tRNA is crucial for its recognition by the appropriate aminoacyl tRNA synthetase, we are attempting to establish quantitative indicators of tRNA tertiary structure in solution. Because the effect of a paramagnetic species on the relaxation time of a nuclear spin is a function of distance between the two, initially, the effects of a nitroxide spin-label covalently bound to s^4U at position 8 of *E. coli* $tRNA^{Met}_{f_1}$ on the ^1H NMR spectrum of the methyl region and of the ring NH hydrogen bond region were investigated (Daniel and Cohn, 1975). Without assigning the resonances to particular hydrogens, one can, of course, follow changes in conformation as reflected in changes in the spectra of the spin-labeled derivatives, but interpretation in terms of detailed structural change is possible only if each proton resonance is assigned.

In the earlier report, the lowest field methyl peak in the region of 0–4 ppm from DSS[1] was tentatively assigned to m^7G at position 47[2] based on the assignments in yeast $tRNA^{Phe}$ (Kan et al., 1974). A comparison of the proton resonances of

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[1] Abbreviations used are: DSS, 4,4-dimethyl-4-silapentane-1-sulfonic acid; DEAE, diethylaminoethyl; Hepes, *N*-2-hydroxyethylpiperazine-*N*′-3-propanesulfonic acid; EDTA, (ethylenedinitrilo)tetraacetic acid; ATP, adenosine 5′-triphosphate; SL, spin labeled; ESR, electron spin resonance; NMR, nuclear magnetic resonance.