Magnesium Ion Inner Sphere Complex in the Anticodon Loop of Phenylalanine Transfer Ribonucleic Acid[†]

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ABSTRACT: The binding of Ca^{2+} and Mg^{2+} to $tRNA^{Phe}$ is analyzed by equilibrium titrations and temperature-jump measurements using the Wye base fluorescence as a label. Titration experiments starting with the folded structure of the tRNA (high salt and low temperature) show that Ca^{2+} and Mg^{2+} binding detected by Wye base fluorescence changes is associated with equilibrium constants between 1×10^3 and 3×10^3 M^{-1}. The binding of Ca^{2+} leads to an increase of the relaxation time associated with a conformation change of the anticodon loop and to a decrease of the corresponding amplitude. These data are represented quantitatively by a two-step reaction scheme with a preferential binding of Ca^{2+} to one of the anticodon conformations. When Mg^{2+} is added, an extra relaxation process is observed with time constants around 1 ms. This process demonstrates the formation of a Mg^{2+} inner sphere complex. Relaxation time constants and amplitudes are represented quantitatively by a three-step reaction scheme. Mg^{2+} binds preferentially to one of the anticodon conformations. In the absence of Mg^{2+}, these conformations are populated almost equally with a transition rate constant around 5×10^3 s^{-1}. The Mg^{2+} inner sphere complex is formed with a relatively low rate constant of $(1-2) \times 10^3$ s^{-1}, indicating a conformational barrier. These data strongly suggest that the Mg^{2+} site analyzed in the present investigation corresponds to the anticodon site with a distorted octahedral coordination characterized by X-ray analysis. The results are discussed in terms of the anticodon function and also with respect to their implications upon Mg^{2+} binding to nucleic acids in general.

It has been known for a long time already that the translation of the messenger RNA at the ribosome requires relatively high Mg^{2+} concentrations (Szer & Ochoa, 1964; Gassen, 1980). Apparently the Mg^{2+} is required for various parts and reactions of the translation complex (Gassen, 1980). For example, Mg^{2+} serves to stabilize the tertiary structure of tRNA (Riesner et al., 1973; Stein & Crothers, 1976). Mg^{2+} may also be involved in the reading process itself, i.e., in the complex formation between codon and anticodon. For an understanding of the function of Mg^{2+}, information is required on both the structure and the dynamics of the Mg^{2+} complexes. Recently very detailed information on the structure of some Mg^{2+} binding sites in yeast $tRNA^{Phe}$ has been obtained by X-ray analysis (Jack et al., 1977; Holbrook et al., 1977; Quigley et al., 1978; Hingerty et al., 1978; Teeter et al., 1980). One of the Mg^{2+} binding sites, which has been characterized in detail, is located at the anticodon loop. In the present paper, we describe the dynamics of this complex and analyze the influence of Mg^{2+} binding on the conformation of the anticodon loop. Finally we discuss some general implications of the present results on Mg^{2+} binding to nuclei acids in general.

Materials and Methods

Yeast phenylalanine tRNA from yeast was obtained from Boehringer Mannheim. Its phenylalanine acceptor activity was 1.39 nmol/A_{260} unit as determined by the manufacturer. Mg-free $tRNA^{Phe}$ was prepared by dialysis against 0.4 M $NaClO_4$, and 0.02 M Na_3EDTA solution, followed by H_2O and finally buffer A (0.1 M $NaClO_4$ and 0.05 M Tris–cacodylate, pH 7.1) at 0 °C (Janssens de Varebeke et al., 1978). After such a procedure, there is less than 0.1 Mg^{2+} left per tRNA molecule as determined by atomic absorption spectrophotometry. Solutions of $tRNA^{Phe}$ were prepared from stock solution by appropriate dilution with buffer A containing the required concentrations of Mg^{2+} (c_{Mg}) or Ca^{2+} (c_{Ca}). So that contamination by divalent cations could be checked for, the kinetic measurements at c_{Mg} and $c_{Ca} = 0$ were performed for $tRNA^{Phe}$ in buffer A and in buffer A containing 0.5 mM EDTA; the results were identical. Experiments at pH 6.0 (in buffer B of the same composition as buffer A but the pH changed to 6.0) were done with the same $tRNA^{Phe}$ samples, only the pH was readjusted to pH 6.0 by appropriate amounts of perchloric acid. Prior to measurements, $tRNA^{Phe}$ solutions were annealed at 65 °C for 3 min as described by Grosjean et al. (1976), except that the samples at c_{Mg} and $c_{Ca} = 0$ were exposed to 45 °C for 5 min. The concentration of $tRNA^{Phe}$, c_{tRNA}, was 2.5 A_{260} units/mL if not stated otherwise.

Temperature-jump experiments were carried out essentially as described previously (Labuda & Pörschke, 1980), using the same equipment (Rigler et al., 1974). This time, however, we used a Hanovia 600 W Hg/Xe lamp as a light source and a single Schoeffel GM-250 monochromator for excitation of the sample at 313 nm. The relaxation data were stored in the computer: three to six measurements made on the same solution were averaged and analyzed by using a deconvolution program (B. Morgeneyer, unpublished results). By this program, the relaxation signals were corrected for the heating time, the rise time of the photomultiplier (a 5-μs rise time was used in all measurements), and the cutoff of the first part of the signal (about half of the amplitude corresponding to the unspecific fluorescence quench associated with the temperature change; cf. Labuda & Pörschke, 1980).

The equilibrium titrations were performed by using a temperature-jump instrument adapted for equilibrium measurements. The fluorescence was excited at 312 nm and the emitted light selected by a WG 385/3 cutoff filter from Schott Gen.

Results

Equilibrium Titrations. The binding of Mg^{2+} to $tRNA^{Phe}$ is characterized by measurements of the Wye base fluorescence (Robinson & Zimmerman, 1971; Labuda et al., 1977; Labuda & Augustyniak, 1977). Owing to the relatively high quantum

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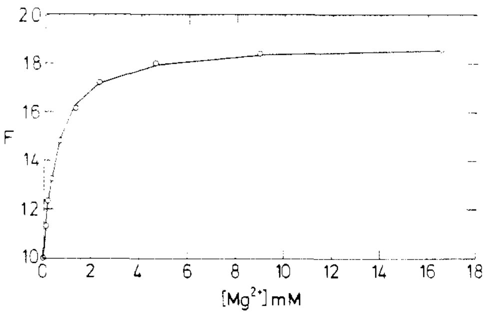

FIGURE 1: Relative fluorescence F of tRNA$^{\mathrm{Phe}}$ as a function of the Mg^{2+} concentration in buffer A. The line is a least-squares fit according to a simple one-step binding model: (tRNA$^{\mathrm{Phe}}$ 0.8 μM; 0 °C, pH 7.2) $K = 1.9 \times 10^3$ M^{-1}, limit $F \equiv 1.884 \equiv \Delta$.

yield of the Wye base fluorescence, the measurements can be carried out at tRNA concentrations below 1 μM. Under these conditions, the quantitative analysis is simplified by the fact that the reduction of the free Mg^{2+} concentration by Mg^{2+} uptake of the tRNA can be neglected. This is particularly important in the present case, since the tRNA has a relatively large number of binding sites (Römer & Hach, 1975; Schimmel & Redfield, 1980) and it would be difficult to describe the competition between the sites quantitatively. By using the Wye base as a label, we restrict our analysis to sites directly associated with the Wye base. Since the measurements are performed at a relatively high concentration of monovalent ions, the tertiary structure of the tRNA is essentially intact already before the addition of Mg^{2+}. Thus we do not expect any major contribution due to a Mg^{2+}-induced folding of the tertiary structure.

The change of tRNA$^{\mathrm{Phe}}$ fluorescence upon addition of Mg^{2+} is shown in Figure 1. Due to the rather large increase of fluorescence, the binding curve can be characterized with a relatively high accuracy. The data can be analyzed in terms of the simple model

$$\mathrm{L} + \mathrm{Mg} \rightleftarrows \mathrm{C}$$

with an equilibrium constant

$$K = c_{\mathrm{C}}/(c_{\mathrm{L}}c_{\mathrm{Mg}})$$

In the present case, the data were fitted with the aid of a least-squares procedure, which provided values for the stability constant K and the limit change of fluorescence Δ ($\Delta \equiv$ fluorescence of complex C relative to the fluorescence of free tRNA, L).

These titrations were performed at various temperatures, at two different pH values, and both for Ca^{2+} and Mg^{2+}. The stability constant measured for Ca^{2+} binding at 30 °C does not show a good linear correlation with the constants measured at 0, 10, and 20 °C. This is an indication for the onset of a thermal tRNA unfolding, which becomes more evident at higher temperatures. Because of this thermal unfolding, the measurements were restricted to the temperature range 0–20 °C. The resulting thermodynamic parameters are compiled in Table I.

Kinetic Measurements. (*A*) *Calcium.* All the kinetic data described below are obtained by temperature-jump experiments using the Wye base fluorescence as label. When tRNA$^{\mathrm{Phe}}$ in the absence of bivalent ions is subjected to temperature jumps (at a sufficiently low temperature to maintain the tertiary structure), two different relaxation processes are observed. One of them is associated with a very high amplitude and a time constant below the limit of time resolution. Such a process is found for all fluorophors and is assigned to thermal quenching. The second process is associated with a time constant around 100 μs. It has been described previously by Urbanke & Maass (1978) and is assigned to a transition between two conformations A and B of the anticodon loop. When Ca^{2+} is added, both the reciprocal relaxation time constant and the amplitude decrease (cf. Figure 2). This result suggests that Ca^{2+} is bound preferentially to one of the conformations of the anticodon loop according to the following mechanism:

Table I: Thermodynamic Parameters Obtained from Fluorescence Titrations[a]

	pH 6.0		pH 7.1	
parameter	Mg^{2+}	Ca^{2+}	Mg^{2+}	Ca^{2+}
ΔH (kcal/mol)	−2.5	−2.4	+0.7	−1.8
ΔS (eu)	4.9	6.3	17.6	9.6
K (3.4 °C)	1210	1820	1930	3520
Δ (3.4 °C)	2.75	2.17	1.874	1.392

[a] Estimated accuracy ±10%.

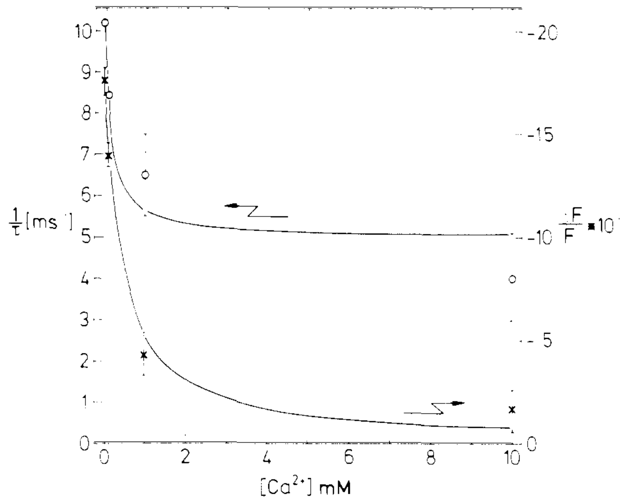

FIGURE 2: Reciprocal relaxation times $1/\tau$ and fluorescence amplitudes $\Delta F/F$ as a function of the Ca^{2+} concentration at 3.4 °C and pH 7.1. The lines represent least-squares fits with the parameters given in Table II together with $\alpha = 0.46$, $\beta = 0.99$, $\Delta H_{\mathrm{A}} = -4.1$ kcal/mol, and $\Delta H_{\mathrm{B}} = -0.04$ kcal/mol.

$$\mathrm{A} \underset{k_{\mathrm{A}}^-}{\overset{k_{\mathrm{A}}^+}{\rightleftarrows}} \mathrm{B} \tag{1a}$$

$$\mathrm{B} + \mathrm{Ca} \underset{k_{\mathrm{B}}^-}{\overset{k_{\mathrm{B}}^+}{\rightleftarrows}} \mathrm{C} \tag{1b}$$

The relaxation times and the amplitudes can be calculated according to the standard procedures (Eigen & deMaeyer, 1963; Bernasconi, 1976). In the present evaluation, we have used a least-squares routine to fit time constants and amplitudes simultaneously according to the above mechanism. Since we observe a single relaxation process and yet fit a two-step mechanism to the data, we have to use some additional information. It is known from various investigations that the rate of Ca^{2+} binding is very high (Eigen, 1963; Eigen & Maass, 1966). Thus the second step (eq 1b) is very fast compared to the first step and can be considered to be in equilibrium. Furthermore, we may use the information obtained from the equilibrium titrations: the overall equilibrium constant K

$$K = \frac{K_{\mathrm{A}}}{K_{\mathrm{A}} + 1}K_{\mathrm{B}} \qquad K_{\mathrm{A}} = \frac{k_{\mathrm{A}}^+}{k_{\mathrm{A}}^-} \text{ and } K_{\mathrm{B}} = \frac{k_{\mathrm{B}}^+}{k_{\mathrm{B}}^-}$$

the overall enthalpy change ΔH associated with Ca^{2+} binding

$$\Delta H = \left(\frac{1}{1 + K_{\mathrm{A}}}\right)\Delta H_{\mathrm{A}} + \Delta H_{\mathrm{B}}$$

Table II: Thermodynamic, Kinetic and Fluorescence Parameters Obtained for the Binding of Ca^{2+} to $tRNA^{Phe}$ from Relaxation Times and Amplitudes[a]

parameter	pH 7.1		pH 6.0	
	3.4 °C	7.2 °C	3.4 °C	7.2 °C
K (M^{-1})	3540	3350	1820	1720
K_A	0.9	1.0	1.0	0.9
K_B (M^{-1})	7300	6600	3600	3600
k_A^+ (s^{-1})	5.0×10^3	6.4×10^3	3.8×10^3	5.1×10^3

[a] Estimated accuracy ±10%; for K_A ±25%.

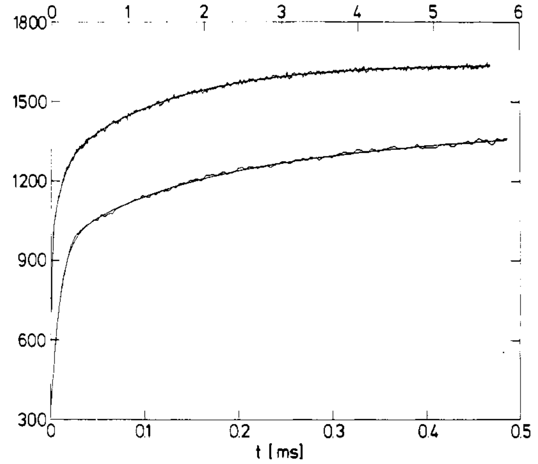

FIGURE 3: Temperature-jump relaxation of $tRNA^{Phe}$ in buffer B + 0.3 mM $Mg(ClO_4)_2$ at 3.4 °C shown at two different time scales. The lower and upper time scales are for the lower and upper curves, respectively. In addition to the fast thermal quenching, two relaxation processes are observed (τ_1 = 129 μs; τ_2 = 1.45 ms).

where ΔH_A and ΔH_B are the enthalpy changes associated with reactions 1a and 1b, respectively, and the reciprocal of the overall fluorescence factor

$$1/\Delta = \left(\frac{1}{1 + K_A}\right)\alpha + \left(\frac{K_A}{1 + K_A}\right)\beta$$

where α and β are the quantum yields of A and B relative to C, respectively.

An example for a fit is given in Figure 2. The parameters obtained for different conditions are compiled in Table II. We did not include the values found for α, β, ΔH_A, and ΔH_B, since the absolute values of these parameters cannot be determined with sufficient accuracy due to strong mutual coupling between the fluorescence and the enthalpy parameters. A sample set of these parameters is given in the legend to Figure 2.

(*B*) *Magnesium.* When Mg^{2+} is added to $tRNA^{Phe}$, another relaxation process in addition to the one found for tRNA alone is observed (cf. Figure 3). Since we do not see any similar process in the presence of Ca^{2+}, although the equilibrium parameters are similar for Ca^{2+} and Mg^{2+}, we have to conclude that the additional process is associated with the formation of an inner sphere complex by the Mg^{2+} ion. It is known from various investigations that in the case of Mg^{2+} the formation of inner sphere complexes is a relatively slow reaction whereas the corresponding reaction is very fast in the case of Ca^{2+} (Eigen, 1963; Eigen & Maass, 1966). This difference may be used for the characterization of Mg^{2+} inner sphere complexes (Pörschke, 1979).

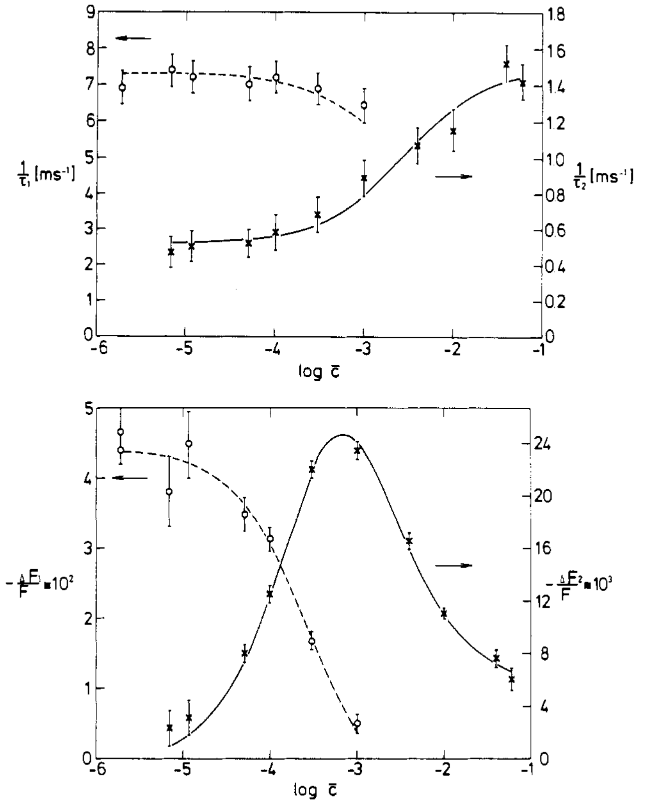

FIGURE 4: Reciprocal relaxation times $1/\tau_1$ and $1/\tau_2$ as well as fluorescence amplitudes $\Delta F_1/F$ and $\Delta F_2/F$ as a function of the free Mg^{2+} concentration (logarithmic scale) at 3.4 °C in buffer B. The lines represent least-squares fits with the parameters given in Table III together with α = 0.71, β = 0.95, γ = 3.0, ΔH_A = −28 kcal/mol, ΔH_B = 11 kcal/mol, and ΔH_C = −1.2 kcal/mol.

The concentration dependence of relaxation time constants and amplitudes is shown in Figure 4. The $1/\tau$ values associated with the slow relaxation process approach a limit at high Mg^{2+} concentrations. This also indicates that the slow relaxation process is associated with the formation of an inner sphere complex. Thus the reaction scheme has to be extended in the case of Mg^{2+} as

$$A \underset{k_A^-}{\overset{k_A^+}{\rightleftharpoons}} B \tag{2a}$$

$$B + Mg \underset{k_o^-}{\overset{k_o^+}{\rightleftharpoons}} C_o \underset{k_i^-}{\overset{k_i^+}{\rightleftharpoons}} C_i \tag{2b}$$

It should be emphasized, that Ca^{2+} will also form an inner sphere complex to some extent. The difference in the reaction schemes 1 and 2 comes from the fact that in the case of Ca^{2+} the second step is too fast to be detected by the temperature-jump method. As known from various investigations, the rate constant k_o^+ for the formation of the outer sphere complex is very high (10^{10} M^{-1} s^{-1}). Furthermore, we may use the information obtained from the equilibrium titrations. The overall binding constant K determined by titration experiments corresponds to

$$K = \frac{K_A}{K_A + 1} K_o(K_i + 1)$$

where $K_o = k_o^+/k_o^-$ and $K_i = k_i^+/k_i^{-1}$. The enthalpy change associated with the overall binding reaction is given by

$$\Delta H = \frac{1}{K_A + 1}\Delta H_A + \Delta H_o + \frac{K_i}{K_i + 1}\Delta H_i$$

where ΔH_A, ΔH_o, and ΔH_i are the enthalpy changes associated with the three consecutive steps defined by reaction scheme 2. The overall fluorescence factor corresponds to

$$\Delta = \left(\frac{1}{K_i + 1} + \gamma\frac{K_i}{K_i + 1}\right) \Bigg/ \left(\alpha\frac{1}{K_A + 1} + \beta\frac{K_A}{K_A + 1}\right)$$

where α, β, and γ are the quantum yields of A, B, and C_i

Table III: Thermodynamic and Kinetic Parameters Obtained for the Binding of Mg^{2+} to $tRNA^{Phe}$ from Relaxation Times and Amplitudes[a]

	pH 7.1		pH 6.0	
parameter	3.4 °C	7.2 °C	3.4 °C	7.2 °C
K (M^{-1})	2000	2000	1200	1140
K_A	0.6	0.9	1.2	0.4
K_o (M^{-1})	1460	1240	780	1190
K_i	3	2.5	1.9	2.4
k_A^+ (s^{-1})	3.4×10^3	5.9×10^3	4.0×10^3	3.0×10^3
k_i^+ (s^{-1})	1.4×10^3	1.9×10^3	1.0×10^3	1.9×10^3

[a] Estimated accuracy ±10%; for K_A and K_i ±25%.

relative to that of C_o, respectively.

As in the case of Ca^{2+}, the relaxation time constants and the amplitudes were fitted simultaneously by using a standard least-squares procedure. An example for a fit is given in Figure 4. The parameters obtained are compiled in Table III. Because of mutual coupling between the fluorescence and the enthalpy parameters (cf. previous section), the values α, β, γ, ΔH_A, ΔH_o, and ΔH_i could not be determined with sufficient accuracy and thus are not included in Table III. A sample set of these parameters is given in the legend to Figure 4.

Discussion

It has been shown by various authors that tRNA may bind a high number of Mg^{2+} ions [for a recent review, cf. Schimmel & Redfield (1980)]. Up to five of these ions bind with high equilibrium constants between 2×10^4 and 5×10^6 M^{-1} depending upon the conditions. Some of these strongly bound Mg^{2+} ions may form "inner sphere" complexes, as suggested by proton magnetic relaxation studies of Mn^{2+} binding to tRNA (Danchin & Gueron, 1970). Another "class" of about 20 ions binds with lower binding constants in the range from 5×10^2 to 10^4 M^{-1}. Some authors also define a third class of binding sites with a further reduced affinity. Obviously this classification is somewhat arbitrary. Nevertheless it may be useful to put some order into various phenomena. Recently Teeter et al. (1980) have extended the scheme by a classification of various binding sites on the basis of their molecular structure. According to Teeter et al., strong Mg^{2+} binding sites provide the coordination of two phosphate groups with the metal ion, whereas intermediate sites are characterized by contacts of the metal with base atoms and a single phosphate group.

According to the equilibrium constants, the site characterized in the present investigation belongs to the intermediate class. The relatively low values of the equilibrium constants indicate that this site is not located in an area with a particularly high electrostatic potential. Furthermore, the binding is associated with large changes of the Wye base fluorescence, and thus the site should be in the anticodon loop close to the Wye base. Finally the kinetic data demonstrate that an inner sphere complex is formed, i.e., the Mg^{2+} ion has lost at least one water molecule from its inner hydration sphere and makes at least one direct contact to the tRNA.

A binding site with all these special features has been characterized by X-ray analysis (Jack et al., 1977; Holbrook et al., 1977; Quigley et al., 1978). It is located in the anticodon loop with one direct coordination to a phosphate residue (P-37) and several indirect coordinations via water molecules to various base residues. Among these base residues is the Wye base, and thus we have an explanation for the large change of fluorescence intensity. Quigley et al. (1978) described the coordination as a "somewhat distorted octahedron". This distortion as well as the rather complicated coordination, which requires a special orientation of several nucleotide residues, may explain the relatively low rate found for the formation of the inner sphere complex.

Recently the mode of Mg^{2+} binding has been characterized for various oligonucleotides. From field jump–relaxation measurements, it was concluded (Pörschke, 1979) that oligo(A)'s do form inner sphere complexes but not oligo(U), oligo(C), oligo(I), or oligo(dA). These results demonstrated that special sites are required for inner sphere complexation. The present data together with the information from the X-ray analysis provide a basis for a more detailed interpretation. According to these data, the formation of inner sphere complexes by Mg^{2+} with nucleic acid chains in solution does not require a simultaneous contact of the ion with two phosphate residues in order to compensate its charge. However, one direct contact to a single phosphate diester residue is apparently not sufficient, and additional contacts to other residues seem to be required for stabilization. The X-ray data demonstrate that these additional contacts need not be arranged directly to the Mg^{2+} ion but can be mediated by water molecules of the inner hydration sphere. Apparently such contact sites are available in oligo(A) but not in various other oligonucleotides. It is remarkable that four of the contacts made by the Mg^{2+} in the anticodon site can be arranged in exactly the same manner with an oligo(A): two of them are to the phosphate residue, one to the N-1 of the Wye base, and another one to the amino group of A-38. The N-1 of the Wye base can be substituted by an N-7 of an adenine without any change of the conformation. The absence of corresponding sites in oligo(U), oligo(C), and partly oligo(I) may explain their low affinity for the formation of inner sphere complexes. In the case of oligo(dA), the formation of inner sphere complexes may be suppressed by a special conformation of these nucleotides, which has been well documented by various methods (Adler et al., 1969; Pörschke, 1973).

The binding of Mg^{2+} to the anticodon loop seems to be essential for the function of the tRNA. In some tRNAs, the binding affinity is reinforced by special modifications. For example, t^6A [*N*-[9-(β-D-ribofuranosyl)purin-6-ylcarbamoyl]threonine] is found in the anticodon loop of most tRNAs which read codons starting with an A. t^6A occurs in the same position as the Wye base and forms strong complexes with Mg^{2+} (Reddy et al., 1979). Another example of a modified base with an enhanced Mg^{2+} affinity (Thedford & Straus, 1974) is i^6A [N^6-(Δ^2-isopentenyl)adenosine], which is also found at the same position of the loop in many tRNAs recognizing codons starting with uridine. These observations suggest a general function of the Mg^{2+} ions bound to the loops of tRNAs.

The present relaxation data demonstrate that the Mg^{2+} ion stabilizes one of two loop conformations. In the absence of Mg^{2+}, these conformations are almost equally populated. According to various arguments (Labuda & Pörschke, 1980; Urbanke & Maass, 1978), the transition probably corresponds to the one described by Fuller & Hodgson (1967). From the X-ray analysis, it is apparent that the Mg^{2+} ion by its many contacts to various bases strongly favors the 3′-stacked conformation. This is in line with the present results. However, from the present results, we also know that the affinity of the Mg^{2+} ion is not very high, and thus the Mg^{2+} may be removed from its site or reoriented without too much difficulty. Furthermore, we learn from the present data that the conformations can be rearranged within a few milliseconds. Thus it is possible that the conformation of the anticodon loop is changed in the process of translation, for instance during

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translocation from the A to the P site of the ribosome (Woese, 1970).

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Codon-Induced Transfer Ribonucleic Acid Association: Quantitative Analysis by Sedimentation Equilibrium[†]

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ABSTRACT: It is shown by measurements of the sedimentation equilibrium that binding of the codon UUC to $\mathrm{tRNA^{Phe}}$ from yeast induces an association of tRNA molecules. Sedimentation measurements at different concentrations demonstrate that the tRNA–codon complexes form dimers. The sedimentation profiles are analyzed quantitatively in terms of a simple monomer–dimer model as well as a model which considers the sedimentation of four species (tRNA, tRNA·UUC, $\mathrm{(tRNA{\cdot}UUC)_2}$, and UUC) separately. The information resulting from the conservation of mass relation is used directly in the determination of equilibrium constants via integration of the sedimentation profiles. Using this procedure, we determine the equilibrium constants for dimerization of the $\mathrm{tRNA^{Phe}{\cdot}UUC}$ complex, $K_D = 8.6 \times 10^4\ \mathrm{M^{-1}}$, and for the binding of UUC to $\mathrm{tRNA^{Phe}}$, $K_L = 1800\ \mathrm{M^{-1}}$ (5 °C). The large free energy for dimerization of the tRNA–codon complex suggests that interactions between adjacent tRNAs are important for the ribosomal translation process.

During translation of the messenger RNA at the ribosome, tRNA molecules bind at adjacent trinucleotide codons and thus come very close to each other. Since tRNA molecules are rather large, it seems to be almost inevitable that they make direct contacts with each other at the ribosome binding sites. These contacts may be necessary for the peptidyl transfer reaction. If the contacts are favorable, it should be possible to provide evidence for them by experiments in free solution. Actually recent relaxation experiments (Labuda & Porschke, 1980) showed an association of $\mathrm{tRNA^{Phe}}$, which was induced by binding of the codon UUC. In the present contribution, this reaction is analyzed quantitatively by measurements of the sedimentation equilibrium in an analytical ultracentrifuge.

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