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Magnesium-Induced Conformational Change in Transfer Ribonucleic Acid as Measured by Circular Dichroism*

Gordon E. Willick and Cyril M. Kay†

ABSTRACT: The circular dichroism spectra of purified $tRNA^{fMet}$, $tRNA_2^{Phe}$, $tRNA^{Val}$, $tRNA^{Arg}$, and $tRNA^{Glu}$ from *Escherichia coli*, and also unfractionated tRNA from *E. coli*, have been examined in the region 200–380 nm. It was found that a minimum centered at about 210 nm showed an increase in magnitude of 20–28% on the addition of 10 mM magnesium chloride to a solution with an ionic strength of 0.2 M and a pH of 7.0. The 260-nm maximum showed an increase only 5–12% in magnitude. The region above 300 nm, primarily due to the presence of 4-thiouracil in many *E. coli* tRNAs, gave a variety of spectra, and the effect of adding Mg^{2+} was also very variable. However, all of the measured changes occurred in a concerted manner, supporting the idea that the binding of a small number of magnesium ions gives rise to a specific conformational change in the tRNA. The data, treated in terms of a two-state hypothesis, were consistent with the idea that the binding of three magnesium ions, with an average binding constant of 3.2×10^2 M^{-1} is required for the conformational charge. $tRNA^{Glu}$ was unusual in that it showed little absorption in the 4-thiouracil region, but had a strong negative circular dichroism in this same region. Also it did not have the 295-nm minimum found in all other tRNAs studied.

The affinity of magnesium ion for polynucleotides and its stabilization of their secondary structure is well known (Felsenfeld and Miles, 1967). Considerable work has also implicated Mg^{2+} as a necessary requirement for the correct secondary and tertiary structure of tRNA (Adams *et al.*, 1967; Ishida and Sueoka, 1967; Reeves *et al.*, 1970), and its reactivity in the aminoacylation reaction (Adams *et al.*, 1967). These latter authors have reported that metastable states of tRNA can be created under certain conditions. These states are inactive with respect to the aminoacylation reaction, which includes 5–10 mM Mg^{2+} in the assay system, but can be reactivated by heat treatment in the presence of Mg^{2+}. It has been shown that there are concomitant changes in the physical parameters of tRNA, and thus it has been suggested that Mg^{2+} is a necessary requirement for the biological activity of tRNA (Lindahl *et al.*, 1966; Adams *et al.*, 1967; Ishida and Sueoka, 1967).

On the other hand, experiments have been performed that suggest that Mg^{2+} (or any other divalent ion) is not a necessary requirement for the biological activity of tRNA, at least insofar as the aminoacylation reaction is concerned. Lagerkvist *et al.* (1966) have shown that $tRNA^{Val}$ can be charged by Val-AMP in the absence of Mg^{2+}. Also, a later communication by Ishida and Sueoka (1968) has indicated that an inactivated state of tRNA can be reactivated in the absence of Mg^{2+} by using high concentrations of monovalent salt.

Manganous ion substitutes for Mg^{2+} in reactions involving tRNA. The paramagnetic properties of Mn^{2+} have been useful in studying its binding to nucleic acids. Studies of the enhancement of the proton relaxation rate of water by Mn^{2+} (Cohn *et al.*, 1969) and of the free Mn^{2+} concentration as measured by electron spin resonance (Danchin and Guéron, 1970) suggested that there is a class of interacting binding sites in tRNA, which is not observed in synthetic polymers, such as poly(A). This cooperativity has been presumed to reflect a conformational change in the tRNA (Danchin and Guéron, 1970).

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In view of these results, and since a number of purified tRNAs are now available, we decided that it would be worthwhile to pursue further the effects of Mg^{2+} on the secondary and tertiary structure of tRNA, using a parameter sensitive to such changes. The optical activity has been shown to be a parameter which is highly sensitive to structural changes in nucleic acids. Thus, we have examined the effect of Mg^{2+} on the circular dichroism of the near- and far-ultraviolet region, including the spectrum attributed to the 4-TU region[1] in those tRNAs where this residue is found (Scott and Schofield, 1969). We decided to carry out our studies in a medium with an ionic strength and pH approximating that found in biological systems. We shall show that under these conditions, changes occur in the circular dichroism spectrum as Mg^{2+} is added to concentrations commonly used in biological assays and probably present in the intracellular fluid.

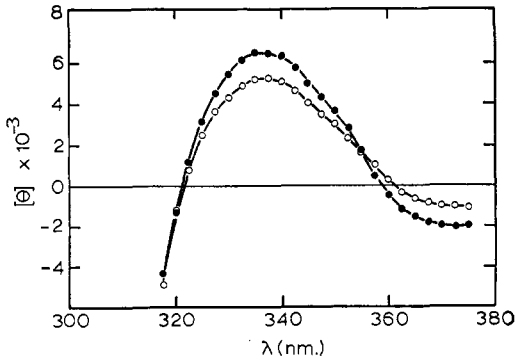

FIGURE 1: Circular dichroism spectrum of 4-TU region of unfractionated *E. coli* B tRNA in the standard solvent (○) and with 10 mM $MgCl_2$ (●).

Materials and Methods

Unfractionated yeast tRNA was purchased from Calbiochem. The unfractionated *Escherichia coli* B tRNA was purchased from Schwarz BioResearch, Inc. Purified samples of $tRNA^{fMet}$ from *E. coli* B, and $tRNA^{Val}$, $tRNA_2^{Phe}$, $tRNA^{Arg}$, and $tRNA^{Glu}$ from *E. coli* K12M07 were donated by the Oak Ridge National Laboratory. Based on amino acid acceptor activity with respect to terminal adenosine, the purities of the tRNAs were 88, 82, 78, 70, and 78%, respectively.

The standard solvent used in all these studies, unless stated otherwise in the text, consisted of 0.01 M cacodylic acid, 0.19 M potassium chloride, and 0.5 mM ETDA adjusted to a pH of 7.0 with potassium hydroxide. The Mg^{2+} concentration of the solvent was 6×10^{-6} M as measured with a Unicam atomic absorption spectrophotometer. Magnesium chloride was added to give the required Mg^{2+} concentrations.

Unfractionated tRNAs were first dissolved in about one-tenth final volume of distilled water, then brought to volume with solvent. These solutions were found to contain approximately 1.5×10^{-4} M Mg^{2+}. The purified tRNAs were obtained in a solvent containing 10^{-2} M magnesium chloride. To remove this, they were dialyzed against 200 volumes of distilled water containing 2 mM EDTA for 4 hr, then against the standard solvent containing 4 mM EDTA for 18 hr, and finally against two changes of solvent for 36 hr. These samples were found to still have a residual Mg^{2+} concentration of about 3×10^{-4} M or less. For our purposes, we shall consider this zero Mg^{2+} concentration, even though Mg^{2+} effects occur at concentrations lower than these (Reeves *et al.*, 1970).

Circular dichroism spectra were measured with a Cary 60 spectropolarimeter, fitted with a Model 6001 circular dichroism attachment. All measurements were conducted at a temperature of 27°. Spectra and difference spectra were obtained with a Cary 15 spectrophotometer.

Ellipticities are reported in terms of (deg cm^2) per mean residue weight, where the mean residue weight in all cases is taken to be 320. In the case of the spectra taken in the 4-TU region, ellipticities are reported per decimole, where the average molecular weight of unfractionated tRNA was taken as 26,500. This value was also assumed for $tRNA^{Arg}$ and $tRNA^{Glu}$. Values of 24,990, 24,587, and 24,659 were calculated from the published sequences for $tRNA^{fMet}$ (Cory and Marcker, 1970), $tRNA^{Val}$ (Yaniv and Barrell, 1969), and $tRNA_2^{Phe}$ (Barrell and Sanger, 1969). The absorptions of all tRNAs were measured in a potassium phosphate buffer (pH 7.0) with an ionic strength of 0.1 M. A value for $E_{258}^{0.1\%} = 23.4$ (Adler and Fasman, 1970) was used for all tRNAs. Extinction coefficients of unfractionated yeast and *E. coli* tRNA were determined by phosphate assay (Ames and Dublin, 1960) and, within experimental error, the same value was found as that for the purified tRNAs measured by Adler and Fasman (1970).

Results

Effect of Mg^{2+} on Unfractionated tRNA. The effect of 10 mM Mg^{2+} on the circular dichroism spectrum between 300 and 370 nm of unfractionated *E. coli* B tRNA is shown in Figure 1. The absorption and circular dichroism spectrum of this region has been attributed to the presence of 4-TU in many *E. coli* tRNAs. The shape of the curve, and the changes occurring as Mg^{2+} is added, suggest that there are two overlapping transitions, one positive with a maximum near 335 nm, and the other negative, with an ill-defined minimum. The absolute magnitudes of both are increased on the addition of Mg^{2+}. As will be shown later, the circular dichroism spectra of the various purified tRNAs, and the effect of Mg^{2+} on them, vary greatly in this region. The unfractionated circular dichroism spectrum is therefore a composite of the apparently large number of tRNA species in *E. coli* containing 4-TU, and perhaps other residues which are weakly absorbing but with relatively large optical activity. Figure 2a shows the absorption spectrum in the presence of 10 mM Mg^{2+}. There is a maximum at 337 nm and a shoulder at about 355 nm. The difference spectrum, with the sample without Mg^{2+} in the reference compartment, is shown in Figure 2b. There is a minimum on the high-wavelength side at about 360 nm, and a crossover at 354 nm which corresponds closely to the shoulder seen in the absorption spectrum. The rest of the difference spectrum is difficult to interpret, because the difference spectra due to the principle 4-TU transition and that due to the high-wavelength transitions from the seventy other bases now overlap. However, the high-wavelength portion of the difference spectrum is consistent with the interpretation that a blue shift of a transition centered about 355 nm occurs when Mg^{2+} is added.

The region of the circular dichroism spectrum from 200 to 310 nm which consists of the contributions from the remaining bases of the tRNA was also checked for a similar Mg^{2+} effect. The results are shown in Figure 3. The principal maximum is at 264 nm, and the principal minimum at 209 nm both in the presence and absence of 15 mM Mg^{2+}. There is an

[1] Abbreviation used is: 4-TU, 4-thiouracil.

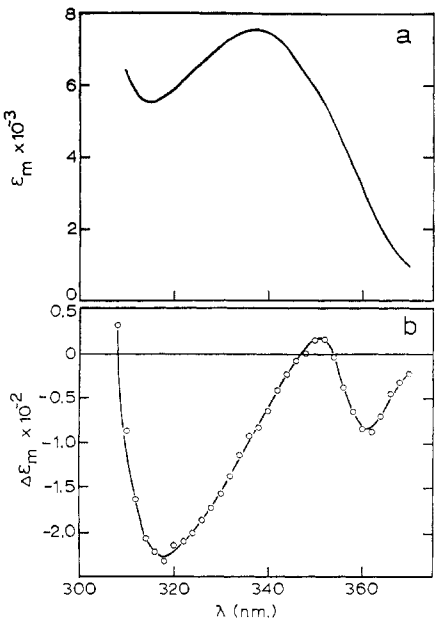

FIGURE 2: (a) Absorption spectrum of 4-TU region of *E. coli* B tRNA in standard solvent with 10 mM $MgCl_2$. (b) Difference spectrum of the sample with and without 10 mM magnesium chloride. The sample without Mg^{2+} is in the reference compartment.

increase in the magnitude of the 264-nm maximum of about 5%, and in the 209-nm minimum of about 20% after the Mg^{2+} is added. We shall see later that these results are qualitatively similar for all the purified tRNAs as well.

To show that these parameter changes are related, we have measured the change in the 4-TU maximum in *E. coli* B tRNA, and the change in the 209-nm minimum in both *E. coli* and yeast tRNA, as a function of Mg^{2+} concentration (Figure 4). Also included are data from two purified samples, $tRNA^{Phe}$ and $tRNA^{Glu}$. The results are plotted in terms of the function, f, defined as the change in ellipticity, $\delta\theta$, divided by the final observed change, $\Delta\theta$. It can be seen that the values all fall on the same curve, suggesting that they are all a measurement of the same change in the tRNA, as the concentration of Mg^{2+} varies from zero to 10 mM.

tRNA has been reported to undergo a concentration dependent self-aggregation, probably to dimers, in the presence

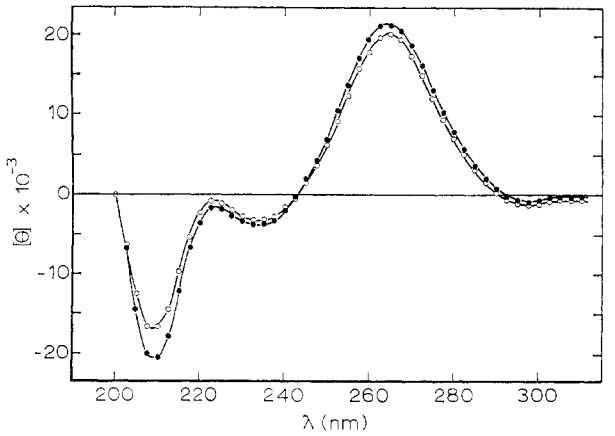

FIGURE 3: Circular dichroism spectrum of *E. coli* B unfractionated tRNA with (●) and without (○) 15 mM Mg^{2+}.

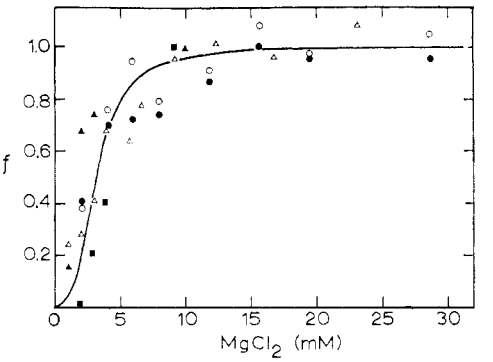

FIGURE 4: Fractional change, f, in the ellipticity of tRNA as a function of Mg^{2+} concentration. Unfractionated yeast tRNA (○) and *E. coli* tRNA (●) at 209 nm; unfractionated *E. coli* tRNA at 336 nm (△); $tRNA_2^{Phe}$ at 330 nm (▲); $tRNA^{Glu}$ at 310 nm (■).

of Mg^{2+} at the concentration we are using (Millar and Steiner, 1966). Therefore, the possibility exists that the changes in circular dichroism we have observed represent subtle changes in conformation, or simply changes resulting from proximity to new chromophores, as the tRNA molecules aggregate. The concentrations of tRNA normally used with 0.5-mm path-length cells is in the order of 1.5 mg/ml. In this case, at a Mg^{2+} concentration of 10 mM, the tRNA would be aggregated according to the data of Millar and Steiner (1966). Use of long-path-length cells is generally avoided since the absorption of the solvent makes the obtaining of data in the far-ultraviolet region more difficult. However, we obtained data down to 207 nm at a concentration of 0.04 mg/ml using a 1-cm path-length cell, and found that the spectrum and changes on the addition of Mg^{2+} were essentially the same as at the higher concentrations. At 0.04 mg/ml there is no appreciable aggregation of the tRNA (Millar and Steiner, 1966), so we conclude the Mg^{2+}-dependent changes we have observed represent an intramolecular rather than an intermolecular event.

Circular Dichroism of Purified tRNAs (310–380 nm). From the work on unfractionated tRNA, we have shown that the ellipticity changes we are observing are essentially complete when the Mg^{2+} concentration has reached 10 mM (Figure 4). Preliminary studies on the individual purified tRNAs indicates this assumption is valid, and we consequently here present the circular dichroism spectra of the individual tRNAs before and after the addition of 10 mM Mg^{2+}.

Figure 5 shows the absorption and circular dichroism spectrum of $tRNA_2^{Phe}$. The absorption spectrum has an extinction coefficient of about 12,000 M^{-1} at the maximum, as compared to about 7300 M^{-1} for the unfractionated *E. coli* tRNA. The shape of the two spectra is identical, and therefore suggests that the absorption spectrum of unfractionated tRNA in this region is essentially entirely due to 4-TU, but that some of the tRNAs in *E. coli* do not contain the 4-TU residue. The extinction coefficients for $tRNA^{Val}$ and $tRNA^{Arg}$ were also measured and were found to be about 12,000 M^{-1} also. However, $tRNA^{Glu}$ had an extinction of only about 3500 M^{-1} at 337 nm, with only a slight suggestion of a maximum at that wavelength. The extinction observed could be largely due to contaminating 4-TU-containing tRNAs, or alternatively the result of an intramolecular disulfide bond between two nearly 4-TU residues in $tRNA^{Glu}$, as has been observed for $tRNA^{Trp}$ (Lipsett, 1966). The disulfide bond results in a reduced extinction coefficient, and a shift into the blue so that the spectrum would be masked by the much higher intensity from the 260-

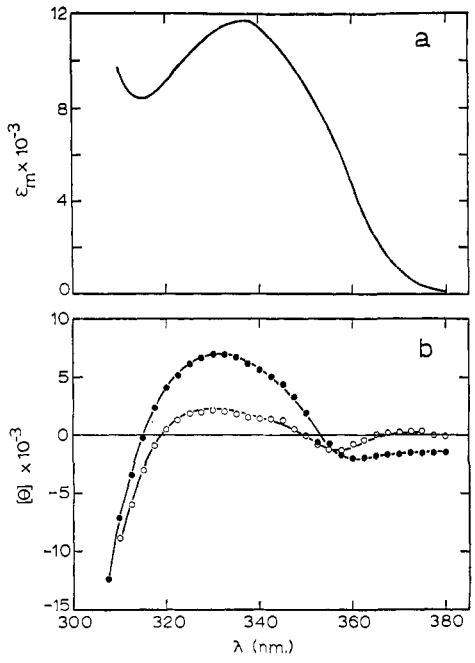

FIGURE 5: (a) Absorption spectrum of $tRNA_2^{Phe}$. (b) Circular dichroism spectrum of $tRNA_2^{Phe}$ in the region 300–380 nm with (●) and without (○) 10 mM Mg^{2+}.

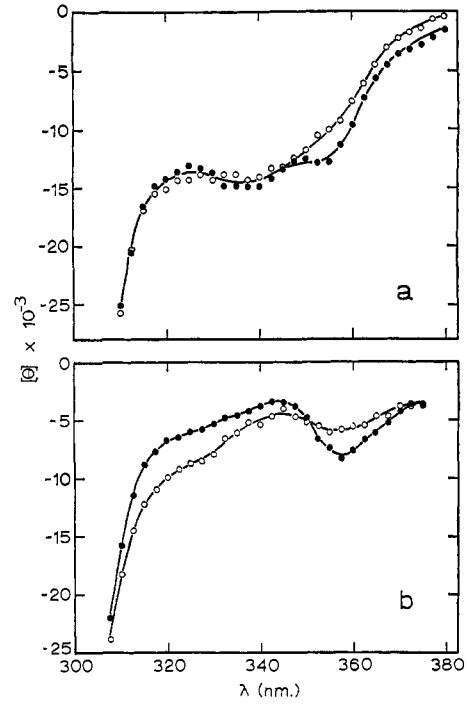

FIGURE 6: Circular dichroism of $tRNA^{fMet}$ (a) and $tRNA^{Arg}$ (b) in the 300- to 380-nm region. (○) Without added Mg^{2+}; (●) +10 mM Mg^{2+}.

nm absorption. However, treatment of the $tRNA^{Glu}$ with 50 mM sodium thiosulfate for 1 hr failed to produce any change in the absorption spectrum. These conditions were found by Lipsett (1966) to fully reduce the disulfide bond in $tRNA^{Trp}$. We therefore conclude that this *E. coli* $tRNA^{Glu}$ has no 4-TU residue.

The most striking aspect of the circular dichroism spectra in this region of these purified tRNAs (Figures 5–7) is their great diversity, and the widely varying effect of Mg^{2+} on them. $tRNA_2^{Phe}$ (Figure 5b) and $tRNA^{Val}$ (Figure 7a) both have positive ellipticities near 335 nm, but the effect of 10 mM Mg^{2+} is to greatly increase that of $tRNA_2^{Phe}$, as contrasted to a slight decrease in the value of $tRNA^{Val}$. $tRNA^{Arg}$ (Figure 6b) has a weak negative ellipticity throughout this region, with two minima. These are changed only slightly on the addition of Mg^{2+}. $tRNA^{fMet}$ (Figure 6a) and $tRNA^{Glu}$ (Figure 7b) both have a very similar negative band with a minimum at about 335 nm, but the ellipticity at the minimum of −23,000 for $tRNA^{Glu}$ is almost twice that of $tRNA^{fMet}$, with an ellipticity of about −13,000. Neither is significantly changed on addition of 10 mM Mg^{2+}. Unlike the other tRNAs, however, $tRNA^{Glu}$ does not have the band with a minimum at 295 nm (see also Figure 8b). Consequently, it is possible to detect another minimum at about 305 nm, with an ellipticity of about −13,000 in the absence of Mg^{2+}, which increases to about −18,000 on the addition of 10 mM Mg^{2+}. The band could be present in the other tRNAs, but would be masked by the relatively much stronger band centered at 295 nm.

Circular Dichroism of Purified tRNAs (200–310 nm). In contrast to the circular dichroism spectra of the region above 310 nm, the spectrum of this region is similar in all of the purified tRNAs and shows similar effects on the addition of Mg^{2+} to a concentration of 10 mM. Two representative spectra, those of $tRNA_2^{Phe}$ and $tRNA^{Glu}$, are shown in Figure 8. A summary of the pertinent data for all of the *E. coli* tRNAs studied is included in Table I. We would like to draw attention to the fact that shifts in the ellipticity extrema do not occur on the addition of Mg^{2+}, and also that the increases in magnitude are in general much less for the maximum at about 265 nm than for that located at about 210 nm. The spectrum for $tRNA^{Glu}$ (Figure 8b) shows no evidence of a 295-nm minimum, even in the presence of 10 mM Mg^{2+}.

Discussion

A few comments should first be made concerning the region above 300 nm. Comparison of the absorption spectra of unfractionated tRNA and purified tRNAs known to contain 4-TU makes it clear that this residue is primarily responsible for the absorption spectrum. Yeast tRNA, in which no 4-TU residues have been found, not only has no absorption band in this region, but also shows no circular dichroism except for the residual of the 295-nm minimum (G. E. Willick and C. M. Kay, unpublished data). Setting aside for the moment the problem of $tRNA^{Glu}$, which apparently contains no 4-TU residue, the circular dichroism, absorption, and Mg^{2+}-dependent difference spectrum of the *E. coli* unfractionated tRNA all suggest that the band consists of at least two transitions. Presuming there are only two principal transitions of 4-TU greater than 300 nm, they appear to be centered at about 337 and 355 nm. Such an interpretation of the spectrum of 4-TU is consistent with the published circular dichroism spectra of free 4-thiouridine and 4-thiouridylic acid (Samejima *et al.*, 1969). In the region greater than 300 nm, free 4-thiouridine was found to have two positive maxima, at 320 and 350 nm, with decimolar ellipticities of 1100 and 700, respectively. This compares with decimolar ellipticities of about 4000 at the 335-nm maximum in $tRNA^{Val}$ and $tRNA^{Phe}$ in the absence of Mg^{2+}. However, in all the purified tRNAs the higher wavelength transition is slightly negative. Just why the high-wavelength transition should be negative when 4-TU is in the tRNA polynucleotide is not clear, but it does appear that this may prove to be gen-

TABLE I: Circular Dichroism Band Values for Purified tRNAs in the Presence and Absence of Mg^{2+}.

tRNA	λ_{max} (nm)	$[\theta]_{max} \times 10^{-3}$ − $MgCl_2$	% Increase $[\theta]_{max}$ + 10 mM $MgCl_2$	λ_{min} (nm)	$[\theta]_{min} \times 10^{-3}$ − $MgCl_2$	% Increase $[\theta]_{min}$ + 10 mM $MgCl_2$
E. coli unfractionated	265	+20.3	5.4	209	−17.0	20.0
E. coli Val	265	+20.4	0	210	−18.8	21
E. coli Phe	262.5	+21.6	7.5	210	−17.6	25
E. coli Arg	265	+21.6	4.6	210	−18.6	22
E. coli Glu	267.5	+23.6	12.0	211	−16.0	27.5

erally the case. In addition to $tRNA^{fMet}$ and $tRNA^{Val}$, Scott and Schofield (1969) examined $tRNA^{Lys}$ and the high-wavelength contribution was also negative in this case. The high-wavelength transition appears to be at the same position, about 350 nm, in both free 4-thiouridine and 4-TU in the tRNAs. However, the lower wavelength transition appears to be red shifted about 10–15 nm when in the tRNAs, and the extrema correspond more closely to the absorption maxima of 331 nm for 4-thiouridine (Kochetkov *et al.*, 1963) and 4-thiouridylic acid (Saneyoshi and Sawada, 1969), and 336 nm for unfractionated *E. coli* tRNA.

The origin of the rather high optical activity in $tRNA^{Glu}$ is not at all clear, although it seems reasonably certain it is not 4-TU. It is possible that it arises from a more highly asymmetric derivative of 4-TU with a much lower transition probability. It is also possible that it is an accidental *in vitro* derivative of 4-TU (Scott and Schofield, 1969). We have not pursued the matter further, since its origin is not essential to the theme of this paper. Clearly, the sequence, or at least nucleotide composition, of *E. coli* $tRNA^{Glu}$ would be most useful.

We will proceed on the assumption that 4-TU is the source of the optical activity in the other tRNAs, although there is a striking similarity between $tRNA^{fMet}$ and $tRNA^{Glu}$. However, aside from 4-TU, the published sequence of *E. coli* $tRNA^{fMet}$ (Cory and Marcker, 1970) contains no residues likely to have an optically active band at this high a wavelength. To a first approximation, it has been assumed that the optical activity of a residue in a polynucleotide arises primarily from interaction with its first nearest neighbors (Bradley *et al.*, 1963), although more recent theoretical considerations suggest that such an assumption may not be valid for RNA, and that second and third neighbors together contribute as much as the nearest neighbors (Johnson and Tinoco, 1969). However, this interaction is inversely proportional to the square of the transition moment frequencies (Tinoco, 1962). Thus, as discussed by Scott and Schofield (1969), both theoretical considerations and the experimental observations on NADH by Miles and Urry (1968) suggest that 4-TU interaction with the 260-nm transition of adjacent nucleotides would not be an important contributing factor. The only other reasonable candidate for such interaction is the 295-nm band found in most tRNAs, but on a per residue basis the transition is weak. Sequences are available for only three of the purified tRNAs we have examined. In $tRNA^{Val}$ and $tRNA^{Phe}$, in which the low-wavelength transition has a positive value, the sequences about 4-

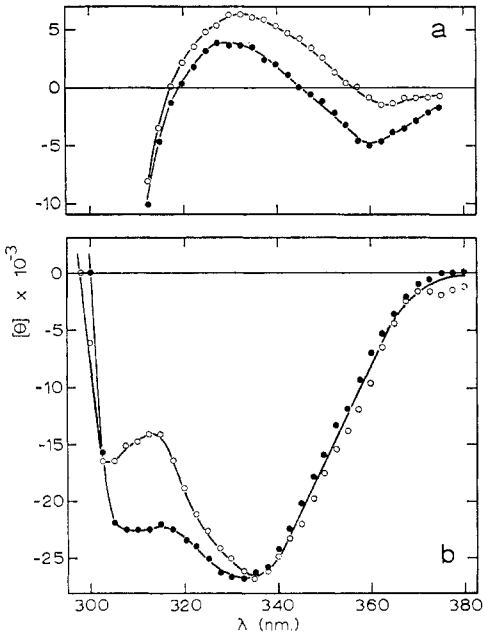

FIGURE 7: Circular dichroism of $tRNA^{Val}$ (a) and $tRNA^{Glu}$ (b) in the 290- to 380-nm region (○) without added Mg^{2+}; (●) +10 mM Mg^{2+}.

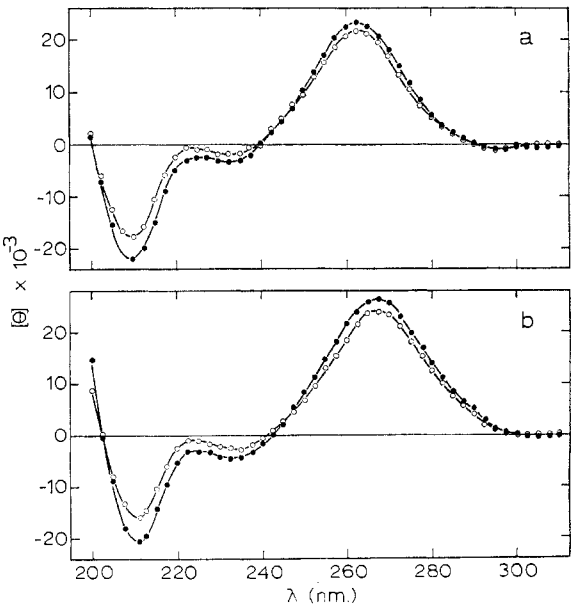

FIGURE 8: Circular dichroism of $tRNA^{Phe}$ (a) and $tRNA^{Glu}$ (b) in the 200- to 310-nm spectral region (○) without added Mg^{2+}; (●) +10 mM Mg^{2+}.

TU are U·4-TU·A (Yaniv and Barrell, 1969) and A·4-TU·A (Barrell and Sanger, 1969), respectively. $tRNA^{fMet}$, which has a negative ellipticity, has the sequence G·4-TU·G (Cory and Marcker, 1970). There is, from this limited data, no reason to believe there is a correlation between the sign of the 4-TU ellipticity and its nearest neighbors.

One other explanation for the variations in the circular dichroism spectra of the 4-TU in individual tRNAs may lie in the orientation of 4-TU with respect to the adjacent ribose. Miles *et al.* (1967) have pointed out that, for the purine nucleosides, interactions between the transition moments of the base and sugar appear to be responsible for at least 50% of the observed circular dichroism in these molecules. Scheit and Saenger (1969) have compared the nuclear magnetic resonance and optical rotatory dispersion of free 4-thiouridine and this residue in polynucleotides. Their results suggest the sign of the 4-TU Cotton effect changes as the base–ribose orientation goes from syn to anti. The 4-TU residue lies in a nonhydrogen-bonded region between the amino acid acceptor stem region and the stem of the dihydrouridine loop when tRNA is drawn in the cloverleaf configuration, and thus has fewer constraints imposed on its base–ribose orientation. There is a considerable body of evidence that tRNA is folded into a more compact tertiary structure (Cramer, 1969). It thus seems reasonable that the differences in the circular dichroism of the 4-TU region in the purified tRNAs examined may arise from the fact the different base–ribose orientations are imposed by the constraints of the different tRNA molecules.

As pointed out in the results, the Mg^{2+} dependency of the optical activity in the region of the 260-nm maximum is not the same as that reported by Reeves *et al.* (1970). The midpoint of the transition observed in unfractionated tRNA under our conditions is about 2.5 mM Mg^{2+}, and appears to be analogous to the Y base fluorescence Mg^{2+}-dependent transition reported for $tRNA^{Phe}$ (Beardsley *et al.*, 1970; Eisinger *et al.*, 1970). This suggests that the conformational change may involve a large part, if not all, of the tRNA molecules. If one assumes that the transition is, for all species involved, a two-state process involving the binding of Mg^{2+} ions, then one can obtain an estimate of the overall binding constant and the number of Mg^{2+} involved from the data for unfractionated tRNA shown in Figure 4. The reaction considered is

$$\text{tRNA} + i\text{Mg}^{2+} \rightleftharpoons \text{tRNA–Mg}_i^{2+} \qquad (1)$$

with

$$K_{app} = [\text{tRNA–Mg}_t^{2+}]/[\text{tRNA}][\text{Mg}^{2+}]^t$$

The fraction of tRNA molecules in the Mg^{2+}-bound state is then defined as f, where

$$f = \frac{\delta\theta}{\Delta\theta} = \frac{[\text{tRNA–Mg}_t^{2+}]}{[\text{tRNA}] + [\text{tRNA–Mg}_t^{2+}]} \qquad (2)$$

$$f = \frac{K_{app}[\text{Mg}^{2+}]^t}{1 + K_{app}[\text{Mg}^{2+}]^t} \qquad (3)$$

Equation 3 can be linearized by rearrangement and taking logarithms to give

$$\log (1/f - 1) = \log \frac{1}{K_{app}} - i \log [\text{Mg}^{2+}] \qquad (4)$$

Thus, an estimate of K_{app} and i can be obtained from a plot of $\log (1/f - 1)$ *vs.* $\log [Mg^{2+}]$. The fit to the data in Figure 4 was then refined further by using eq 3 and generating curves with slight variations in K_{app} and i using an IBM 360 time share computer. A value of $i = 3$ and $K_{app} = 3.2 \times 10^7$ M^{-3} gave the best fit as judged by eye. Defining $K_{Mg^{2+}}$ as the equilibrium constant for a single Mg^{2+}, we have $K_{Mg^{2+}} = K_{app}\sqrt{i}$. Curves with $i = 3 \pm 1$ and $K_{Mg^{2+}} = 3.2 \pm 1 \times 10^2$ will fit the data of Figure 4 within experimental error. It is possible that the individual tRNAs bind different amounts of Mg^{2+}, but, considering the inherent error of the measurements and the availability of the purified tRNAs it would be difficult to definitely prove this to be the case. Nonetheless, the data are consistent with some type of specific site binding of an average of about 3 Mg^{2+}, thus giving rise to a conformational change.

The idea that there is a specific site binding of Mg^{2+} has been suggested before (Lindahl *et al.*, 1966), although no estimate was made of the number of Mg^{2+} ions involved. However, recently, studies have been made of the binding of the paramagnetic Mn^{2+} to tRNA. Using the enhancement of the relaxation rate of the water protons as the Mn^{2+} is bound, Cohn *et al.* (1969) found that there is a class of about six interacting strong binding sites with an apparent binding constant of 2–4 $\times 10^4$ M^{-1}, in the range of ionic strengths of 0.01–0.1 M and a pH of 8.0. Danchin and Guéron (1970), using electron spin resonance to monitor the free Mn^{2+} concentration, estimated that the first four Mn^{2+} ions bind cooperatively, with an apparent binding constant of 1.8×10^5 M^{-1}. This was at a pH of 7.8 and an ionic strength of about 0.01 M. Danchin and Guéron suggested the cooperativity of the Mn^{2+} binding reflected a conformational change of the tRNA. Our results support this idea, namely, that the binding of a small number of Mg^{2+} or Mn^{2+} ions gives rise to a conformational change in the tRNA. In our case, a parameter measuring a conformational change was used to monitor the reaction. The value of 3 Mg^{2+} ions bound is, within experimental error, in agreement with the estimate of Danchin and Guéron (1970), although our estimated binding constant of 3.2×10^2 M^{-1} is considerably lower than theirs or that of Cohn *et al.* (1969). Since our conditions of ionic strength are higher, and the pH of 7.0 is lower, one would expect the binding constant to be reduced somewhat. For example, the mid point of the transition is shifted to about 25 mM Mg^{2+} when the total ionic strength is increased to 1 M by addition of potassium chloride (G. E. Willick and C. M. Kay, unpublished data). In addition, it is possible that Mn^{2+} binds more strongly to tRNA than does Mg^{2+}.

Comparison of our results with other circular dichroism results on the effect of Mg^{2+} on tRNA is difficult because the solvent conditions used were usually much different. Reeves *et al.* (1970) noted a pronounced blue shift in the 260-nm maximum when adding Mg^{2+} to $tRNA^{Ala}$ in 0.001 M Tris-HCl at pH 7.5. We suggest this result arose primarily because of the very low supporting electrolyte concentration. In one comparable case to our work, Beardsley *et al.* (1970) have studied the effect of adding 10 mM Mg^{2+} to yeast $tRNA^{Phe}$ in 0.1 M sodium chloride on the circular dichroism spectrum in the 220–320-nm region. Their results were very similar to ours in this case.

Although we have suggested that the conformational change seems to be reflected through much of the tRNA molecule, the question still remains as to the magnitude of this change. Firstly, when the changes occur they simply seem to be changes in the absolute magnitude of ellipticities, with no significant shifts in the maxima or minima. Secondly, the magnitudes of the changes, observed in the 200–310-nm region, summarized in Table I, show no relation to the magnitudes of the changes observed in the 310–380-nm regions of the purified tRNAs,

containing 4-TU. Thus, we conclude that although the Mg^{2+}-dependent transition always occurs in these tRNAs it is not always reflected in the same relative change in the 4-TU region. A blue shift in the 260-nm maximum of the circular dichroism spectrum, along with a slight increase in the magnitude of the ellipticity, has been reported both for tRNA (Reeves *et al.*, 1970) and wheat embryo RNA (Wolfe *et al.*, 1968) when the ionic strength is increased. Reeves *et al.* have interpreted their data on yeast $tRNA^{Ala}$ in terms of a conformational change involving a net increase of about 40% in the number of base pairs as they add Mg^{2+} to a low ionic strength medium. Their data do not extend below 220 nm. However, Wolfe *et al.* (1968) have studied the circular dichroism of wheat embryo RNA in the 200–300-nm region. It was found to be quite similar to that observed for tRNA. Increasing the ionic strength similar to that observed for tRNA. Increasing the ionic strength to about 0.1 M resulted in about a threefold in- in the magnitude of the 210-nm minimum, with no shift in the position of the minimum. They also interpreted their data in terms of the formation of additional base pairs. Since we see no shift in the 260-nm maximum, we cannot necessarily conclude that additional base pairing is responsible for the observed circular dichroism changes on the addition of Mg^{2+} when the ionic strength is 0.2 M. Certainly, the formation of one or two additional base pairs cannot be ruled out. Bush and Scheraga (1969), in a study of polyribadenylic and polydeoxyriboadenylic acids, have suggested that tilting of the bases might affect the 211- and 260-nm circular dichroism bands differently, since the polarization directions of these two transitions differ. We tentatively conclude, therefore, that the 20–30% increase in the magnitude of the 210-nm minimum as opposed to little or no increase in the magnitude of the 260-nm maximum suggests a subtle change in the base stacking of the tRNA molecule, perhaps involving the nonhydrogen-bonded regions, or the tilt of the bases in one or more of the base-paired regions.

Finally, a few comments on the biological implications of this study should be made. The study has been carried out under conditions of ionic strength and pH which approximate those found *in vivo* and are commonly used in *in vitro* studies of reactions involving tRNA. In addition to being recognized by its specific aminoacyl synthetase, the tRNA must recognize correctly its anticodon on the messenger RNA and is involved in a number of interactions with protein, and possibly nucleic acid, components of the ribosomes as protein biosynthesis proceeds. These have been summarized by Kim *et al.* (1969). The concentration of Mg^{2+} ion used in these systems is usually of the order of 5–15 mM. In few of these systems has any sort of systematic study been made on the effect of Mg^{2+} ion concentration. Even if such a study is made, however, it is difficult to sort out the precise effect of Mg^{2+} since the multicomponent systems present many possibilities where Mg^{2+} may be involved. However, these results support the concept that a unique tertiary structure of tRNA requires a minimal Mg^{2+} level, and that this may be at least part of the reason it is required in the protein biosynthesis steps.

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