Circular Dichroism Studies on the Conformation of Transfer Ribonucleic Acid in the Presence of Different Divalent Cations†

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ABSTRACT: The conformation of tRNA, as indicated by the circular dichroic spectra, was examined in the presence of each of the chloride salts of Mg^{2+}, Mn^{2+}, Ca^{2+}, and Ni^{2+}. The circular dichroic spectra of unfractionated tRNA from yeast and *Escherichia coli*, and two purified tRNAs from *E. coli*, $tRNA^{fMet}$ and $tRNA^{Glu}$, were studied in the region from 200 to 380 nm. The final spectrum in the region between 200 and 280 nm was found to be relatively insensitive to the ion used, except in the case of Ni^{2+}, where the change in the envelope of the 260-nm band was slightly different from that of the other ions. The minimum found near 295 nm was found to increase greatly, and to approximately the same extent, on the addition of all ions except Ni^{2+}. In the case of Ni^{2+}, the increase was either much less or a decrease was observed. The region associated with the thiolated residues in *E. coli* tRNAs was also examined. In the cases of unfractionated tRNA and $tRNA^{fMet}$, where the spectrum can be associated with a 4-thiouracil residue, the final spectra were essentially the same for all ions except Mn^{2+}, where a consistent difference was observed. This was interpreted as being due to a local conformational change near this residue when Mn^{2+} is inserted into the tRNA molecule. A band with a minimum near 330 nm found in $tRNA^{Glu}$ was shown to be due to the thiolated residue found at the 5′ end of the anticodon of this tRNA species. This circular dichroic spectrum increased in the same manner on the addition of all ions except Ni^{2+}, where a decrease was observed.

The results indicated that only Ca^{2+} appears able to interchange with Mg^{2+} without any apparent difference, as indicated by the circular dichroic spectra, in the final conformation of tRNA. It therefore appears that it is the coordination property of the divalent ion, rather than the ionic radius, which is of principal importance in determining the final conformation of tRNA obtained in the presence of divalent cations.

The requirement of divalent ions, particularly Mg^{2+} or Mn^{2+}, for the correct tertiary structure of tRNAs has been implied by many studies. These include studies on the effect of Mg^{2+} on the physical and thermal melt properties, as correlated with the amino acyl acceptor activity (Fresco *et al.*, 1966), on the circular dichroic spectra, including the total ultraviolet, (uv) spectra (Reeves *et al.*, 1970; Willick and Kay, 1971) and the near-uv spectra of thiolated residues (Willick and Kay, 1971), and on the fluorescence of the modified anticodon base of yeast $tRNA^{Phe}$ (Robison and Zimmerman, 1971). In addition, a study of Mn^{2+} binding to tRNA using either proton magnetic relaxation of bound water molecules (Cohn *et al.*, 1969) or the electron spin resonance of Mn^{2+} (Danchin and Guéron, 1970) has indicated the presence of two classes of binding sites, with the smaller class having a much larger binding constant than the larger class.

On the other hand, there have been reports that Mg^{2+} is not required for the recognition of some tRNAs by their respective synthetases (Yarus and Rashbaum, 1972; Lagerkvist *et al.*, 1966). Also, a thermodynamic study of the binding of Mg^{2+} to yeast $tRNA^{Phe}$ indicated that the stabilization of the tertiary structure resulted from a preferential binding of Mg^{2+} to the folded rather than the unfolded form of the molecule (Rialdo *et al.*, 1972), and there was no thermodynamically significant conformational change on Mg^{2+} binding.

One approach to the study of the conformation of tRNA is to utilize the fact that many tRNAs from *E. coli* contain thiolated bases, which have a spectra shifted about 70 nm to the red from the usual bases of tRNA. The circular dichroic spectra of these thiolated bases serve as sensitive markers of conformational changes in these tRNAs (Scott and Schofield, 1969; Willick and Kay, 1971). Using this approach, it has been shown that tRNAs demonstrate a conformational change on the addition of Mg^{2+} to solutions of physiological ionic strength and pH (Willick and Kay, 1971).

It has been assumed that the class of tight binding sites corresponds to a set of uniquely coordinated sites formed by the folded molecule of tRNA, as opposed to the rather nonspecific binding to the phosphate groups alone. One way in which it should be possible to test this hypothesis is to bind different divalent ions to tRNA and examine the final conformation obtained. We have chosen Mg^{2+}, Ca^{2+}, Mn^{2+}, and Ni^{2+} as divalent cations for use in this study. The conformation of unfractionated tRNAs from yeast and *Escherichia coli* and two purified tRNAs from *E. coli*, $tRNA^{fMet}$ and $tRNA^{Glu}$, have then been studied with a detailed analysis of their circular dichroic spectra.

Materials and Methods

Unfractionated *E. coli* B tRNA was donated by Dr. S. Igarashi, and was a stock lot from Schwarz BioResearch, Inc., which was found to be free of ribosomal RNA. Unfractionated yeast tRNA was obtained from Calbiochem. Purified samples of $tRNA^{fMet}$ and $tRNA^{Glu}$ from *E. coli* K12M07 were donated by Dr. A. D. Kelmers of the Oak Ridge National Laboratory. Based on amino acid acceptor activity with respect to terminal adenosine, the purities of

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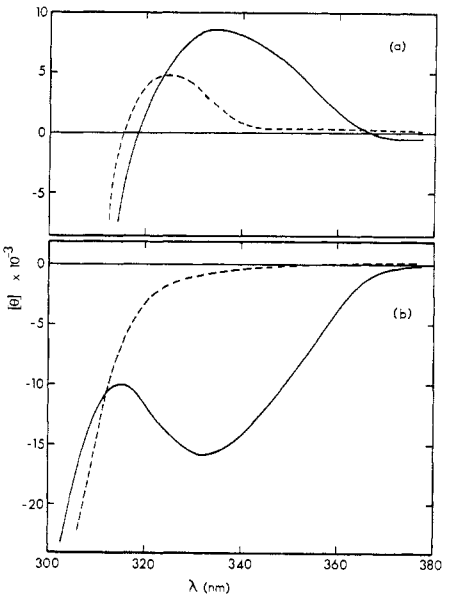

FIGURE 1: Circular dichroic spectra of *E. coli* unfractionated tRNA (a) and $tRNA^{Glu}$ (b) in buffer B before (——) and after (– – –) modification by cyanogen bromide.

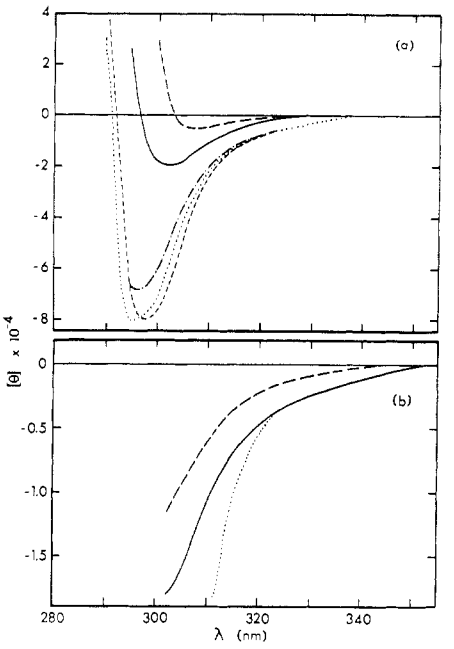

FIGURE 2: Circular dichroic spectra of unfractionated yeast tRNA in buffer A (——) and after the addition of Mg^{2+} (· · ·), Mn^{2+} (– – –), Ca^{2+} (— · —), and Ni^{2+} (— —). The concentration of tRNA is 0.25 mg/ml in a and 1.7 mg/ml in b. Final concentrations of added ions were 1 mM in a and 4 mM in b.

these tRNAs were 100 and 78%, respectively. All other chemicals were of the highest purity commercially available.

The following buffers were used: A, 0.5 mM triethanolamine–0.5 mM potassium chloride, pH 7.8; B, potassium dihydrogen phosphate–potassium monohydrogen phosphate, pH 7.0, total ionic strength of 0.2 M.

For experiments in which the initial solvent was the low ionic strength buffer A, divalent ions were removed by the procedure of Danchin and Guéron (1970). The tRNAs were dialyzed against three changes of 100 vol of a suspension of Chelex 100 in 0.1 M tetramethylammonium chloride, and then dialyzed against three changes of 100 vol of buffer A. Divalent ions were removed from buffer A by passage through a Chelex 100 column.

The thiolated residues in unfractionated tRNA and the s^4U[1] residue in $tRNA^{Glu}$ were converted to their corresponding thiocyanate derivatives by the procedure of Saneyoshi and Nishimura (1970). The tRNA was dissolved in 0.1 M sodium carbonate, pH 8.9, and a 40× excess of cyanogen bromide in 10% ethanol—0.1 M sodium carbonate was added with vigorous stirring. After 10 min at 20°, a solution of 2.5 M NaCl–0.5 M NaAc, pH 5.0, was added until the solution was approximately neutral. After precipitation with 2 vol of 95% ethanol at 4°, the tRNA was dissolved in 1 M NaCl, reprecipitated with ethanol, and finally dissolved in buffer B.

Circular dichroic spectra were measured with a Cary 60 spectropolarimeter, fitted with a Model 6001 circular dichroism attachment. All measurements were carried out at 27°. Spectra measured in the 200–300-nm region utilized cells with 0.5- or 1-mm path lengths. For spectra in the 300–375-nm region of the tRNAs with thiolated bases and the 295-nm bulk band of the tRNAs, cells with a 1-cm path length were used.

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 spectra taken in the 300–375-nm region and the 295-nm bulk tRNA band, ellipticities are reported per decimole, where the average molecular weight of unfractionated tRNA was taken as 26,500. Values of 24,990 and 24,500 were calculated from the published sequences for $tRNA^{fMet}$ (Cory and Marcker, 1970) and $tRNA^{Glu}$ (Ohashi *et al.*, 1972). 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 *E. coli* tRNAs. For the unfractionated yeast tRNA, a value of $E_{258}^{0.1\%} = 20.9$ was used (Adams *et al.*, 1967).

Results

Modification of E. coli tRNAs with Cyanogen Bromide. Saneyoshi and Nishmura (1970) have published the absorption spectra of s^4U and *E. coli* unfractionated tRNA before and after treatment with CNBr. The modification of s^4U results in a shift of the long wavelength absorption band from about 330 nm to about 305 nm, the latter band having about one-half the intensity of the former. This is reflected in the circular dichroic spectra of *E. coli* unfractionated tRNA shown in Figure 1a. After modification, there is essentially no spectrum above 340 nm, and this implies that above this wavelength the spectrum is entirely due to the thiolated residues, most commonly s^4U at position 8 from the 5′ end, found in *E. coli* tRNAs. Below 340 nm, the spectra due to the thiolated bases are increasingly overlapped by a relatively strong minimum centered at 295 nm, and associated with other residues in tRNA.

$tRNA^{Glu}$ does not contain s^4U, but does contain a 2-thiolated base mnm^5s^2U, located at the 5′ end of the anticodon. The reaction conditions used here have been used to specifically modify this base in $tRNA^{Glu}$ (Saneyoshi and Nishimura, 1971). The spectra of $tRNA^{Glu}$ in the region above

[1] Abbreviations used are: s^4U, 4-thiouridine; mnm^5s^2U, 5-methylaminomethyl-2-thiouridine.

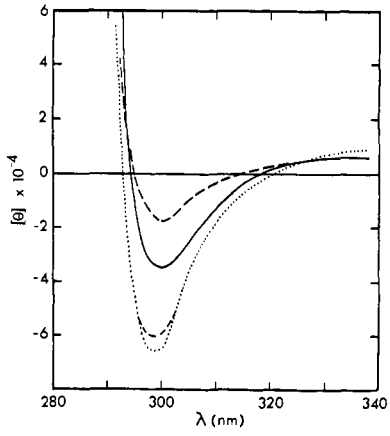

FIGURE 3: Circular dichroic spectra of unfractionated *E. coli* tRNA. The concentrations of tRNA and added ions are the same as in Figure 2, as are the symbols. The Ca^{2+} curve superimposes on the Mg^{2+} curve.

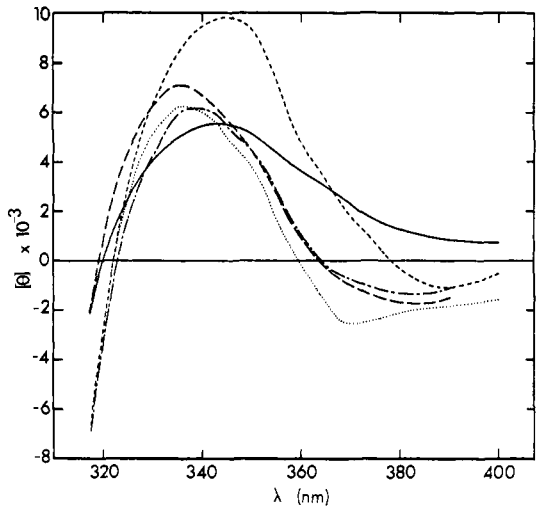

FIGURE 4: Circular dichroic spectra of unfractionated *E. coli* tRNA. The notation is as in Figure 2. The concentration of tRNA is 1.7 mg/ml and final concentrations of added divalent ions are 3.7 mM.

300 nm are shown in Figure 1b. The results indicate that the band is either eliminated or partially eliminated and blue shifted after CNBr modification.

Circular Dichroic Spectra of Unfractionated tRNA above 290 nm. In order to help in the analysis of the *E. coli* spectra to be considered later, the spectra of yeast tRNA above 300 nm have been examined in buffer A, and with the addition of the various divalent cations (Figure 2a). The addition of Mg^{2+}, Ca^{2+}, or Mn^{2+} gives an increase in ellipticity from about -2000 to about -8000, with the Mn^{2+} minimum at 298 nm rather than at 296 nm with Mg^{2+} or Ca^{2+}. In the presence of Ni^{2+}, however, the minimum is at 308 nm with a decrease in magnitude to about -500. The spectra of *E. coli* tRNA in the same region are qualitatively the same (Figure 3), except for a larger value of ellipticity in the absence of divalent ions. In this case, addition of Ni^{2+} decreases the value to about one-half, but the minimum remains at 300 nm.

Figure 2b gives a more detailed examination of the spectra above 305 nm under similar conditions of concentration and path length to those which are used in the examination of the *E. coli* tRNA spectra in this region. It should be noted that there is no indication of induced optical activity due to Mn^{2+} or Ni^{2+} transitions above 340 nm.

Circular Dichroic Spectra of Unfractionated E. coli tRNA above 300 nm. Above 320 nm, the circular dichroic spectra of unfractionated *E. coli* tRNA are largely due to the thiolated bases. Between 320 and 300 nm, the spectra become increasingly overlapped by the comparatively larger minimum associated with the bulk of the tRNA residues. Although there are other thiolated residues found in some *E. coli* tRNAs, about 80% of the sulfur in known sequences is found in a s^4U residue commonly found in position 8 from the 5′ end. Consequently, study of the behavior of the spectra of the unfractionated tRNA provides us with some clues as to the localized conformation in this region. Figure 4 presents the circular dichroic spectra of unfractionated tRNA in buffer A and in the presence of saturating amounts of the chloride salts of Mg^{2+}, Ca^{2+}, Mn^{2+}, and Ni^{2+}. In the absence of divalent ions, the spectrum has a broad maximum, centered at about 345 nm, and a shoulder at about 365 nm. Upon the addition of Mg^{2+}, the maximum shifts to 335 nm, with a shoulder at 350 nm and a broad minimum at about 370 nm. In the presence of Ni^{2+} or Ca^{2+}, the maximum is at about 335 and 340 nm, respectively. Both have a shoulder at 350 nm and a broad minimum at 370–380 nm. The crossover at 315 nm of the Ni^{2+} spectrum, as opposed to about 322 nm for the cases of the other divalent cations, is in accord with the results shown in Figures 2 and 3. In the case of the addition of Mn^{2+}, however, the maximum is shifted to 345 nm and the ellipticity is about 2000 greater than the spectra of the other added cations to tRNA. The shoulder is located at about 365 nm, and there is a weak minimum at 390 nm.

Circular Dichroic Spectra of $tRNA^{fMet}$ above 300 nm. $tRNA^{fMet}$ has only one thiolated residue, a s^4U residue located at position 8 from the 5′ end. Consequently, if any variations in the behavior of the spectra of the unfractionated tRNA spectra are largely due to some effect of Mn^{2+} ion, as opposed to other ions, on the local conformation near position 8, then the same general phenomenon should be observed with $tRNA^{fMet}$. The corresponding spectra are shown in Figure 5. In buffer A alone, the circular dichroic spectrum has a minimum at about 335 nm of about -6000, a shoulder at 355 nm, and a weak maximum at about 370 nm of about 800. The addition of Mg^{2+}, Ca^{2+}, or Ni^{2+} results in a minimum at 338 nm, with increased ellipticities to $-16{,}000$ for Mg^{2+} and about $-13{,}000$ for Ni^{2+} and Ca^{2+}. In addition, the shoulder at 355 nm increases to $-11{,}000$ for Mg^{2+} and about -9000 for Ni^{2+} or Ca^{2+}. There is no evidence for an additional band at higher wavelengths in the case of these added divalent cations. On the addition of Mn^{2+}, however, the low wavelength minimum is at about 334 nm and has a value of $-16{,}000$. There is a shoulder at about 355 nm with an ellipticity of about -8000, and now a very well-defined maxima at about 370 nm with an ellipticity of about 1600. There is no evidence that Mn^{2+} is any different than Mg^{2+} above about 315 nm with respect to its effect on the bulk tRNA minimum (Figure 2b). The data are consistent with the idea that Mn^{2+} effects a different conformational change in the s^4U region of $tRNA^{fMet}$ than Mg^{2+}, Ca^{2+}, or Ni^{2+}, for the relative rotational strengths of the bands are changed, whereas the only differences in the case of the other ions are some differences in the absolute strengths of the bands.

Circular Dichroic Spectra of E. coli $tRNA^{Glu}$ above 300 nm. The circular dichroic spectra of $tRNA^{Glu}$ shown in Figure 6 show two minima in each case, the long wavelength one being

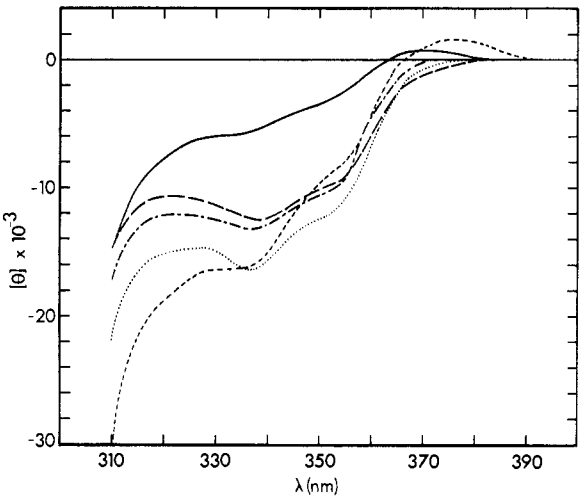

FIGURE 5: Circular dichroic spectra of *E. coli* $tRNA^{fMet}$. The notation is as in Figure 2. The concentration of $tRNA^{fMet}$ was 1.2 mg/ml and the final concentration of added divalent ions is 3.7 mM.

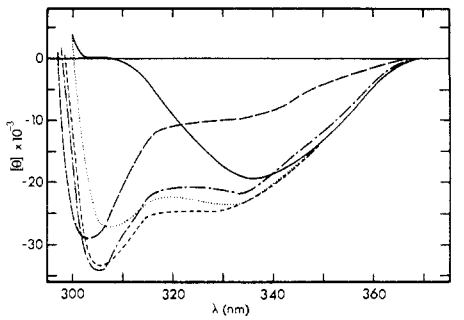

FIGURE 6: Circular dichroic spectra of *E. coli* $tRNA^{Glu}$. The notation is as in Figure 2. The concentration of $tRNA^{Glu}$ was 1.2 mg/ml and the final concentration of added divalent ions is 3.7 mM.

associated with the mnm^5s^2U residue, and the low wavelength one apparently being associated with the bulk of the tRNA. Focusing initially on the long wavelength minimum, we see that in buffer A there is a minimum at 337 nm with an ellipticity of −20,000. On the addition of Mg^{2+}, Ca^{2+}, or Mn^{2+} the minimum is shifted to 332 nm, with an increase in ellipticity of about −6000 at this wavelength. Applying an approximate correction from Figure 2b, only an increase of −2000 would be expected. Therefore, either $tRNA^{Glu}$ has a stronger bulk ellipticity in this region or, alternatively, another transition of mnm^5s^2U also contributes to the spectrum. In the case of the addition of Ni^{2+}, however, it is quite clear that the final local conformation of mnm^5s^2U is markedly different, since there is a decrease in the ellipticity at 337 nm from about −20,000 to about −10,000. Since $tRNA^{Glu}$ has a circular dichroic maximum at 267.5 nm (Willick and Kay, 1971) which is red-shifted about 2.5 nm from the value found for unfractionated *E. coli* tRNA, the bulk minima near 295 nm are largely obscured. The data suggest that the situation is qualitatively similar to the unfractionated tRNA as far as additions of the various ions are concerned. However, while the value of −3500 at 305 nm is about what would be expected from Figure 3 for the addition of Mg^{2+}, Mn^{2+}, or Ca^{2+}, the value of −2800 for Ni^{2+} at the same wavelength is about −1800 larger than expected. In addition, there is zero ellipticity at this wavelength in buffer A alone, whereas about −2500 would be expected from Figure 3. An obvious complication in the interpretation of these data by comparison to the unfractionated tRNA is the unknown contribution of the mnm^5s^2U residue in this region, which could be large.

Circular Dichroic Spectra of Unfractionated E. coli tRNA in the 200–300-nm Region. In buffer A alone, the long wavelength maximum has an ellipticity of 19,000 at 267 nm, and this is shifted to 265 nm on the addition of divalent ions (Figure 7). The ellipticity increases by about 10% to 20,800 on the addition of all ions except Ni^{2+}, where there is no change. The principal minimum, at 209 nm in buffer A, is shifted to 210 nm on addition of the divalent ions, and the ellipticity increases from −14,000 to −22,000. This region shows no variation with the ion added. The region between 220 and 240 nm does show some variations, but has not been explored in sufficient detail.

Yeast-unfractionated tRNA was also examined, and qualitatively the results were the same. Addition of Ni^{2+} actually resulted in a slight decrease in the ellipticity of the high wavelength maximum compared to that of buffer A alone.

Discussion

The original intent of this work was to attempt to establish what, if any, effect the replacement of Mg^{2+} by other divalent cations would have on the conformation of tRNA. The results make it clear that a similar divalent ion cannot necessarily replace Mg^{2+}. Of the three other ions tested, only Ca^{2+} appears to substitute with no conformational change.

The results of the studies on the circular dichroic spectra associated with the s^4U region suggest than Mn^{2+} alters the local conformation in this region. In order to aid in the interpretation of the data, it is necessary to establish the probable position of the transitions associated with s^4U. There appear to be three overlapping transitions visible outside of the bulk tRNA spectra. *E. coli* tRNA in a high ionic strength, Mg^{2+}-containing solvent, can be assigned a band at 355 nm (Willick and Kay, 1971; Scott and Schofield, 1969) agreeing with a free nucleoside band position (Samejima *et al.*, 1969). In addition, examination of the spectra of $tRNA^{fMet}$ suggests that there is a transition at 335 nm, since a distinct minimum occurs at this point (Figure 5). The positions of neither the transition at 335 nm, nor that at 355 nm, are sensitive to dimethyl sulfoxide (Scott and Schofield, 1969) and this implies that the positions of the bands should not be affected by changes in the hydrophobicity of the environment as a result of tRNA conformational changes. Finally, the results shown in Figure 5 suggest a weak band at 380–400 nm. The indication of this band in the absence of any divalent ion is important, since it argues against the increase in the presence of Mn^{2+} being due to an induced activity of a band associated with the Mn^{2+} ion itself. The position of this band could be sensitive to environmental changes.

The interpretation of the circular dichroic spectra of the unfractionated *E. coli* tRNA in terms of band positions is not possible because they represent the superposition of spectra from many individual tRNAs, whose signs and relative rotational strengths differ greatly (Scott and Schofield, 1969; Willick and Kay, 1971). However, it is clear that the addition of Mg^{2+}, Ca^{2+}, or Ni^{2+} gives final spectra where the positions of the extrema and shoulder are the same, and the rotational strengths at these positions are essentially the same, with the possible exception of the minimum at 370 nm on Mg^{2+} addition. This in turn implies that most of the individual tRNAs

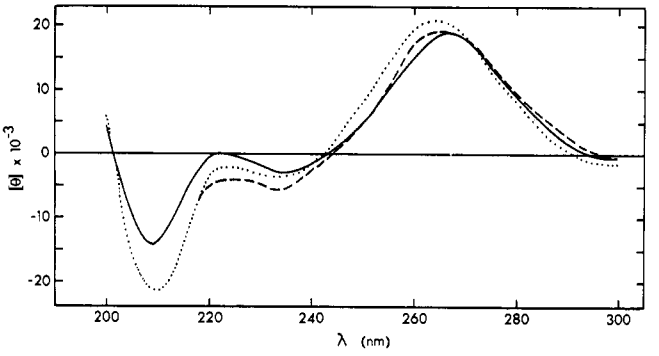

FIGURE 7: Uv circular dichroic spectra of *E. coli* unfractionated tRNA. The notation is the same as in Figure 2. The concentration of tRNA was 0.2 mg/ml and divalent ions were added to a final concentration of 0.8 mM. The Mn^{2+} and Ca^{2+} curves superimpose on the Mg^{2+} curve.

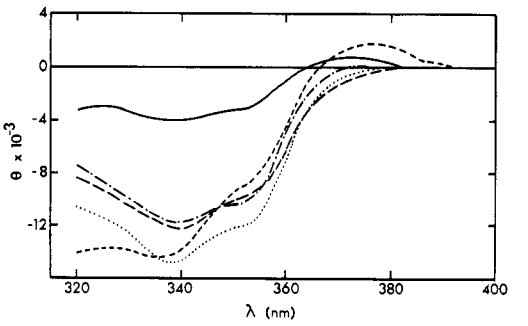

FIGURE 8: Circular dichroic spectra of $tRNA^{fMet}$ (Figure 5) adjusted with the data from Figure 2b. See text for details.

must be behaving similarly to each other on the addition of these ions. The addition of Mn^{2+} differs from the rest of the ions since the position of the maximum remains at 345 nm, and the ellipticity increases by about 80% compared to only 20–30% for the other ions.

The spectra of $tRNA^{fMet}$ are much simpler to interpret since they can be associated with the single s^4U residue located at position 8 from the 5′ end. The distinctive difference among the ions tested is that the addition of Mn^{2+} results in a significant band centered at about 376 nm (Figure 5). Figure 8 represents the $tRNA^{fMet}$ data of Figure 5 corrected by the yeast tRNA data of Figure 2b. The correction must be considered very approximate, but in the case of added ions is never greater than 30%. The purpose is to show that even with these corrections, the initial interpretation remains the same, and only Mn^{2+} appears to effect a conformational change in the s^4U region different from Mg^{2+}, Ca^{2+}, and Ni^{2+}. Since Mn^{2+} does not affect the final helical conformation (Figure 7), it appears this result reflects a change in the region about a binding site for divalent ions which either involves s^4U itself or a residue(s) very proximate to s^4U. It has been shown that Mn^{2+} interacts preferentially with guanine moieties (Anderson *et al.*, 1971) and this is a possible reason for this observed difference. It has also been observed that Mn^{2+}, as compared to group IIa ions, including Mg^{2+} and Ca^{2+}, gives rise to a different elution pattern of tRNAs during reversed phase chromatography (Kelmers, 1970). This might also be related to the present observation.

The interpretation of the ion effects on the minimum near 295 nm is difficult, since the origin of this band is not known for certain. It has been suggested that this is an $n \rightarrow \pi^*$ transition (Yang and Samejima, 1969). The presence of an $n \rightarrow \pi^*$ transition has been observed in adenosine (Miles *et al.*, 1969) and uridine (Rogers and Ulbricht, 1971) derivatives. The band is often visible only in organic solvents, since a pronounced blue shift of about 15 nm which occurs when the bases are placed in aqueous solution results in it being lost in the comparatively much stronger bands associated with the $\pi \rightarrow \pi^*$ transitions near 260 nm. The ellipticity of the 16 possible common dinucleotides is either slightly positive or zero at 300 nm (Warshaw and Cantor, 1970). No evidence for a negative band near 300 nm was found in poly(A), -(U), -(G), or -(C) (Wolfe *et al.*, 1969a) or in poly(A-U) or poly(G-C) (Wolfe *et al.*, 1969b). However, the minimum is observed in some naturally occurring RNAs in their native conformations such as ribosomal RNA (Wolfe *et al.*, 1968) and tRNA (Reeves *et al.*, 1970; Willick and Kay, 1971; Blum *et al.*, 1972). Recently, Blum *et al.* (1972) have published the uv circular dichroic spectra of nine species of tRNA. Six have the small negative band at 295 nm. Under the conditions of their experiments, any evidence of this band would be obscured in cases where the main envelope is near 268 nm. We originally reported this band was not present in $tRNA^{Glu}$ from *E. coli* (Willick and Kay, 1971), but it appears clear from the data of Figures 1b and 6 that the minimum at 305 nm in the presence of divalent ions at least partially corresponds to the band commonly giving rise to the minimum at 295 nm in other tRNAs. The basis set which Blum *et al.* (1972) use to calculate the best fit to these spectra consistently underestimates the value of this minimum by at least 50%. The reason would appear to be that their calculations ignore any effect of tertiary structure (and apparently demonstrate that tertiary structure has minimal effect on the circular dichroism band centered near 265 nm). However, it seems quite clear that the 295-nm band appearance is largely associated with the formation of the tertiary structure. This is suggested, since the work of both Reeves *et al.* (1970) and Blum *et al.* (1972) supports the idea that a very large portion of the maximum secondary structure is still present at low ionic strength and in the absence of divalent ions. The present work supports this view, since addition of the Mg^{2+} causes little change in the region of 260 nm. We suggest that the appearance or large increase of the band with a minimum near 295 nm results from the formation of a compact tertiary structure, and that this is likely due to a red shift caused by placing residues with an $n \rightarrow \pi^*$ transition into a hydrophobic, non-hydrogen-bonded environment when the RNA is folded. This would rationalize the effect of adding Mg^{2+}, Ca^{2+}, or Mn^{2+} (Figures 2a and 3). The situation with respect to Ni^{2+} is much more difficult to rationalize. Reference to Figure 7 shows that not only does addition of Ni^{2+} not give rise to the increase in ellipticity at 264 nm that the other ions give, but that the ellipticity from 280 to 290 nm is significantly greater. This might be rationalized in two ways; either the optical activity with respect to the postulated $n \rightarrow \pi^*$ transition changes sign or it is diminished. At the moment, it is not possible to draw a definite conclusion as to what this effect of Ni^{2+} represents.[2]

[2] It is highly unlikely that the effects noted here are caused by hydrolysis of RNA catalyzed by divalent ions, including Ni^{2+}, such as has been observed by Huff *et al.* (1964). Divalent ions are added immediately prior to a scan, and a rapid preliminary scan shows the effect is rapid (<5 min) compared to reported effects at 23°, which are at least in the order of hours. The longest scan time is about 30 min, and repetitive scans indicated no time-dependent phenomena.

The data of Figure 6 suggest that the conformation of the anticodon region is altered differently by Ni^{2+}, as compared to the other ions, in the case of $tRNA^{Glu}$. This conformational change might be restricted to the mnm^5s^2U residue itself, if it specifically chelates Ni^{2+}.

The blue shift and increase in magnitude of the band at about 260 nm have been implicated by Reeves *et al.* (1970) to be due to an increase in the number of base pairs. In addition, it has been suggested by Bush and Scheraga (1969) that a change in the ratio of the magnitudes of the 260-nm maximum to the 210-nm minimum is due to a change in the tilt of the bases with respect to the helical axis.[3] The difference noted in the region of 200–300 nm (Figure 7) when Ni^{2+} is added as compared to the other ions thus suggests that the final helical conformation of the tRNA is slightly different.

We thus conclude that while Ca^{2+} can replace Mg^{2+} with no detectable change in the final conformation of the tRNA, both Mn^{2+} and Ni^{2+} cannot. In the presence of either of the latter ions, at least some local conformational difference is observed. The fact that Mg^{2+} and Ca^{2+} behave similarly and have much different crystal ionic radii (0.65 *vs.* 0.99 Å (Pauling, 1960)), but similar coordination properties, suggests it is the coordination property rather than the size of the ion which is of primary importance. This in turn supports the presence of a set of specific coordination sites formed by the folded tRNA molecule, with a final conformation dependent on the ion occupying the sites.

No studies have been done on the functional integrity of the tRNAs in which different ions have been substituted, other than Mn^{2+}. Even in the latter case there is not sufficient evidence to indicate the tRNAs had fully exchanged Mg^{2+} with the Mn^{2+}. Such studies would be of interest, in view of the results reported here. For example, one might predict Ni^{2+}. tRNA would not have altered amino acid acceptor activity, but would have lost or altered coding properties in polypeptide synthesis from synthetic mRNAs. It is also of interest that the case of tRNA would appear to contrast with that of bacterial ribosomes, where a class of specific sites have been noted in which the ability of inorganic cations to maintain structural and functional integrity can be correlated with their crystalline ionic radii (Kimes *et al.*, 1971).

[3] We should reiterate that this change has been shown to be concentration independent (Willick and Kay, 1971) and is not due to any aggregation phenomena previously reported by Millar and Steiner (1966).

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