Ionic Strength Dependent Conformational Changes of Transfer Ribonucleic Acid Studied by Circular Polarization of Phosphorescence†

Nitza Steinberg, Ellen J. Wachtel, and Ari Gafni*

ABSTRACT: The circularly polarized phosphorescence emitted by the 4-thiouridine base was measured for bulk *Escherichia coli* tRNA, *E. coli* $tRNA_1^{Val}$, *E. coli* $tRNA_f^{Met}$, and *E. coli* $tRNA^{Phe}$ as a function of ionic strength in the presence of 1 mM Mg^{2+}. The emission anisotropy factor, g_{em}, was found to be dependent on the degree of local stacking in the vicinity of the chromophore. For bulk tRNA and $tRNA_f^{Met}$ a marked similarity was observed between the behavior of g_{em} and the translational diffusion coefficient $D^0_{20,w}$ [Potts, R. O., Wang, C. C., Fritzinger, D. C., Ford, N. C., & Fournier, M. J. (1979) in *Cold Spring Harbor Monograph Series* (Schimmel, P. R., Söll, D., & Abelson, J. N., Eds.) Part A, p 207, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY]. This was interpreted to mean that a relationship exists between the local stacking around the 4-thiouridine chromophore and the overall hydrodynamic shape of the tRNA molecule. Although a general similarity of behavior was observed, each species of tRNA examined exhibited a distinctive dependence of conformation on salt concentration. $tRNA^{Phe}$ was found to be a particular exception in the low-salt region (<50 mM). The value of the phosphorescence anisotropy factor was found to be remarkably larger (about 50-fold) than the value observed for the absorption anisotropy factor. Thus minor conformational changes in the tRNA molecule are more readily detected by circular polarization of phosphorescence than by circular dichroism.

Spectroscopic techniques have been used extensively to investigate conformational changes of macromolecules in solution. Among these techniques, those that measure optical activity relate directly to the molecular conformation, either in the ground state or in an electronically excited state. In particular, CD[1] (i.e., the preferential absorption of right- or left-handed circularly polarized light by an optically active chromophore) is related to the molecular conformation in the ground state, while circular polarization of luminescence (i.e., the preferential emission of circularly polarized light of one sense by a luminescent chiral chromophore) is related to the conformation in the emitting excited state (Emeis & Oosterhoff, 1967; Gafni & Steinberg, 1972; Steinberg, 1978a,b; Richardson & Riehl, 1977). Recently the scope of CPL measurements has been enlarged to include phosphorescent chromophores (Steinberg et al., 1981). Circular polarization of phosphorescence (CPP) is a manifestation of optical activity in triplet–singlet transitions and is thus related to the molecular asymmetry in the triplet state. Triplet–singlet transitions are forbidden by spin selection rules. The mechanism that renders this emission possible involves small perturbations of the pure spin states. It is therefore expected that the CPP will be particularly sensitive to minor changes in the vicinity of the chromophore.

4-Thiouridine, a constituent of many tRNA species obtained from *Escherichia coli*, is a highly phosphorescent nucleoside (Hélène et al., 1968; Hélène & Yaniv, 1970). It is located at position 8 from the 5′ end of the molecule, at the hinge of the AA stem and the D stem of the cloverleaf structure (Figure 1). Its long-wavelength absorption band is centered at about 335 nm, outside the main absorption band of the other nucleosides, thus enabling the excitation of this base exclusively. As pointed out by Shalitin & Feitelson (1976), the emission characteristics of s^4U are sensitive to conformational changes of the tRNA molecule. These properties render s^4U a natural probe for studying tRNA by CPP measurements.

The structure of tRNA in solution has been studied extensively (Crothers, 1979; Crothers & Cole, 1978). The weight of the evidence supports the conclusion that the solution behavior may be understood in light of the X-ray structure, if limited flexibility is permitted in the loops and the single-stranded 3′ end (Kim, 1978). Apparent exceptions to this are the laser light scattering experiments of Olson et al. (1976) and Potts et al. (1979), which revealed an anomalous increase of 11% in the translational diffusion coefficient of bulk *E. coli* tRNA upon decreasing the ionic strength from 0.2 to 0.1 M in the presence of 1 mM Mg^{2+}, pH 7.2 at 20 °C. These results prompted us to use CPP to explore the ionic strength dependent conformational changes that the tRNA molecule undergoes in the presence of 1 mM Mg^{2+}. We investigated bulk tRNA, $tRNA_1^{Val}$, $tRNA^{Phe}$, and $tRNA_f^{Met}$. The results of this study show that CPP is indeed a highly sensitive technique for monitoring local conformational changes in tRNA. We find that the local stacking around the s^4U nucleoside is intimately related to the overall hydrodynamic shape of the molecule, to the extent that the circularly polarized phosphorescence and the translational diffusion coefficient behave similarly with respect to changes in ionic strength.

Materials and Methods

Materials. Unfractionated tRNA, $tRNA_1^{Val}$, and $tRNA_f^{Met}$ from *E. coli* MRE 600 were purchased from Boehringer Mannheim. $tRNA^{Phe}$ from *E. coli* was obtained from Sigma. The suppliers' specifications regarding the amino acid acceptor activity of each tRNA were as follows (picomoles per OD_{260} unit): $tRNA_1^{Val}$, for valine 1085, isoleucine 6, and methionine 29; $tRNA_f^{Met}$, for methionine 1720, isoleucine 19, and lysine 4; and $tRNA^{Phe}$, for phenylalanine 1100, leucine 11, and serine 21. Ethylene glycol was a Fischer certified reagent. Aqueous

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[1] Abbreviations: CD, circular dichroism; CPL, circularly polarized luminescence; CPP, circularly polarized phosphorescence; AA stem, amino acid stem; D stem, dihydrouracil stem; s^4U, 4-thiouridine; NaOAc, sodium acetate; NMR, nuclear magnetic resonance.

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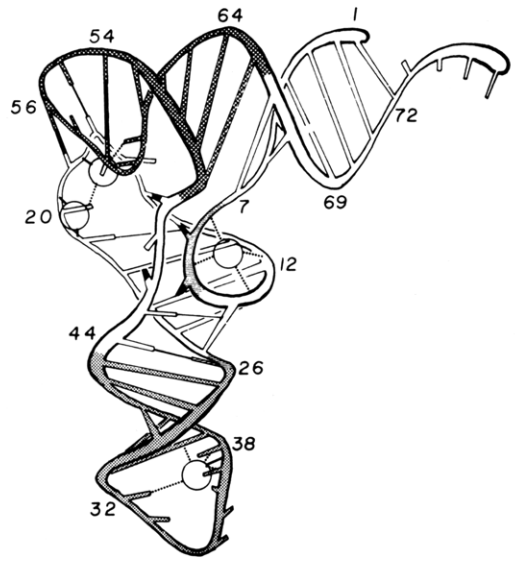

FIGURE 1: A schematic drawing of yeast $tRNA^{Phe}$ showing the ribose–phosphate backbone as a continuous tube, base pairs as long bars, and unpaired bases as short bars. Tertiary H bonds between the bases are indicated by solid dark rods. Adjacent arms are shaded differently. The four Mg^{2+} ions bound to the molecule are denoted by circles. The dotted lines represent hydrogen bonds connecting them to the backbone, and the short solid bars represent direct coordination bonds. Reprinted with permission from Holbrook et al. (1977). Copyright by Information Retrieval, Ltd.

solutions were prepared with doubly distilled water.

The tRNA samples, as purchased, contained small amounts of sodium and magnesium. An estimate of the amount of these cations in the lyophilizate was obtained by checking the atomic absorption of a sample of 0.6 mg/mL unfractionated tRNA dissolved in water. This sample was found to contain 1.3×10^{-5} M Mg^{2+} and 1.4×10^{-3} M Na^{+}. The tRNA concentration in the CPP measurements ranged from 0.5 to 1 mg/mL; i.e., the samples' inherent content of sodium and magnesium was at most 2.3×10^{-3} and 2.2×10^{-5} M, respectively. Subsequent measurements carried out on the samples used for the CPP measurements showed the magnesium content of the specimens to be as follows: unfractionated tRNA 1.06×10^{-3} M, $tRNA_1^{Val}$ 1.21×10^{-3} M, $tRNA_f^{Met}$ 0.99×10^{-3} M, and $tRNA^{Phe}$ 1.15×10^{-3} M. All concentrations mentioned hereafter refer to the concentration of the solutions in which the tRNA was dissolved at room temperature, neglecting the inherent salt content.

Methods. The instrument for CPL measurements was built in our laboratory (Steinberg & Gafni, 1972). The modifications introduced to enable CPP measurements have been described elsewhere (Steinberg et al., 1981). So that good phosphorescence yields can be obtained, it is necessary that the molecule be embedded in a rigid matrix at low temperature. A solvent mixture composed of ethylene glycol–water (2:1 v/v), which forms a strain-free clear glass at −120 °C, was therefore used.

Sample Preparation. Aqueous solutions containing appropriate concentrations of NaCl, $MgCl_2$, and sodium acetate, at neutral pH, were diluted 3-fold with ethylene glycol. The tRNA was dissolved in the solvent mixture, and the sample was centrifuged for 3 min at 3000 rpm to remove air bubbles.

The sample was cooled at a rate of 40 °C/min until a temperature of about −100 °C was reached, and the rate was then reduced to 7 °C/min. Care was taken to cool all samples at the same rate. Cells with an optical path of 2 mm were used in order to minimize effects of possible residual strain in the frozen glass. Such strain could interfere with the CPP measurement (Steinberg et al., 1981).

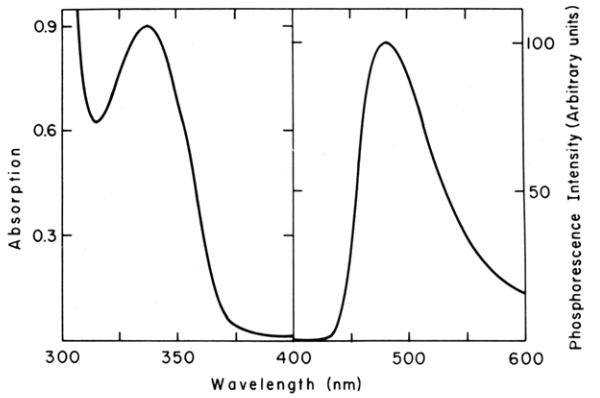

FIGURE 2: Absorption and emission spectra of s^4U as a constituent of unfractionated *E. coli* tRNA. On the left is the absorption spectrum of 1.6 mg/mL tRNA at room temperature, in an ethylene glycol–water (2:1 v/v) solvent mixture at neutral pH. The final concentration of salts in the mixture is 1 mM $MgCl_2$, 100 mM NaCl, and 5 mM NaOAc. The optical path is 2 cm. On the right is the phosphorescence spectrum of 1 mg/mL tRNA at −120 °C in an ethylene glycol–water (2:1 v/v) solvent mixture at neutral pH. The final concentration of salts in the mixture is 1 mM $MgCl_2$, 50 mM NaCl, and 3 mM NaOAc. The excitation wavelength is 335 nm.

The tendency of tRNA to dimerize in solution requires that precautionary measures be taken. Millar & Steiner (1966) reported that in aqueous solution, at room temperature and at a tRNA concentration of 1 mg/mL, dimerization becomes significant only when the magnesium concentration approaches 10 mM. The presence of ethylene glycol may be expected to disfavor dimerization whereas the low temperature favors it. Therefore the samples for the CPP measurements contained tRNA at a concentration of ca. 0.75 mg/mL and magnesium at a concentration of approximately 1 mM. Repeated CPP measurements of the same sample verified that no photoreaction occurred during the measurement.

The circular polarization of phosphorescence is expressed by the emission anisotropy factor, g_{em}, defined as $g_{em} = \Delta I/(I/2)$ where ΔI is the intensity of the circularly polarized component of the phosphorescence (defined as positive for left-handed circular polarization) and I is the total intensity of the phosphorescence. The absorption anisotropy factor g_{ab} is defined similarly as $g_{ab} = (\epsilon_l - \epsilon_r)/[(\epsilon_l + \epsilon_r)/2] = \Delta\epsilon/\epsilon$, where ϵ_l and ϵ_r are the molar extinction coefficients for left- and right-handed circularly polarized light, respectively, and ϵ is the average molar extinction coefficient.

Absorption spectra were measured on a Zeiss Model PMQ II spectrophotometer and on a Cary 14M-50 spectrophotometer. Circular dichroism spectra were recorded on a Cary 60 spectropolarimeter equipped with a 6002 CD accessory. Corrected phosphorescence spectra were obtained on a Hitachi Perkin-Elmer fluorometer, Model MPF-44a.

Results

The absorption spectrum of 4-thiouridine as a constituent of bulk *E. coli* tRNA, at room temperature, and its emission spectrum in a frozen glass at −120 °C are presented in Figure 2. The absorption peak lies at 336 nm and is shifted to the blue in low ionic strength. The phosphorescence is structureless and its maximum is at 480 nm.

The room temperature CD spectra of unfractionated tRNA were measured across the absorption band of s^4U, at several NaCl concentrations, in a mixture of ethylene glycol–water (2:1 v/v) containing 1 mM Mg^{2+} (Figure 3). The Cotton effect is positive across the absorption spectrum of the s^4U chromophore. At the blue edge of the spectrum, the absorption

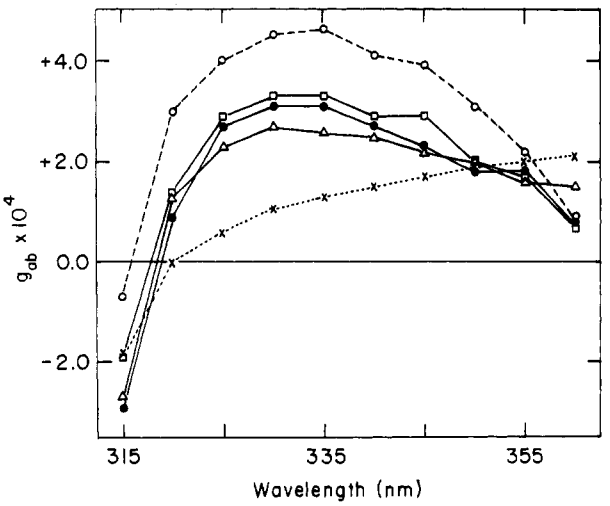

FIGURE 3: CD spectra of 1.5 mg/mL bulk *E. coli* tRNA at room temperature in aqueous solution (---) and in an ethylene glycol–water (2:1 v/v) solvent mixture (—), measured for various Na^+ and Mg^{2+} concentrations, at neutral pH: (○) 1 mM $MgCl_2$, 30 mM NaCl, and 5 mM NaOAc; (□) 1 mM $MgCl_2$ and 1 mM NaOAc; (●) 1 mM $MgCl_2$, 100 or 200 mM NaCl, and 5 mM NaOAc; (△) 1 mM $MgCl_2$, 300 mM NaCl, and 5 mM NaOAc; (×) 1 mM NaOAc. The spectra were recorded by using a cell with a path length of 2 cm, and the experimental error in g_{ab} is $\pm 5 \times 10^{-5}$.

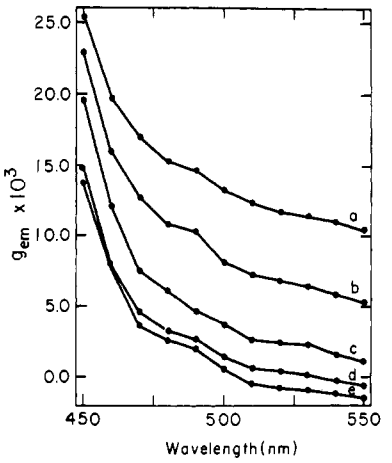

FIGURE 4: Representative CPP spectra of 0.75 mg/mL bulk *E. coli* tRNA at −120 °C, in an ethylene glycol–water (2:1 v/v) glass containing 1 mM $MgCl_2$ and various concentrations of Na^+, at neutral pH: (a) 1 mM NaOAc; (b) 30 mM NaCl and 5 mM NaOAc; (c) 100 mM NaCl and 5 mM NaOAc; (d) 400 mM NaCl and 5 mM NaOAc; (e) 200 mM NaCl and 5 mM NaOAc. The excitation wavelength in all cases is 335 nm. The experimental error in g_{em} is $\pm 10^{-4}$, and the spectral resolution is 16 nm.

of the normal bases of tRNA overlaps the s^4U absorption band, and the anisotropy factor changes sign. The weak dependence of g_{ab} on the NaCl concentration is conspicuous. The difference between the absorption anisotropy factors in a solution containing 100, 200, or 300 mM NaCl, in addition to 5 mM NaOAc, as opposed to a solution containing only 1 mM NaOAc, almost lies within the experimental error ($\Delta g_{ab} = \pm 5 \times 10^{-5}$). For determination of whether the conformation in ethylene glycol–water (2:1 v/v) is not affected by salt or whether it is the CD that is not sensitive enough to distinguish between various conformations that may exist at different salt concentrations, the CD spectra were also measured in aqueous solution at two ionic strengths. The first ionic strength (1 mM $MgCl_2$, 30 mM NaCl, 5 mM NaOAc) is appropriate for the formation of the native conformation of tRNA while the second ionic strength (1 mM NaOAc) is appropriate for the formation of the "extended" conformation (Cole et al., 1972). As seen from Figure 3 the difference between the CD spectra in the two cases is large, demonstrating that the CD of s^4U is capable of sensing local conformational changes if present. Apparently the salt effect on tRNA conformation in ethylene glycol–water at room temperature is very small. An explanation for this behavior will be offered under Discussion.

Representative CPP spectra of bulk *E. coli* tRNA in an ethylene glycol–water (2:1 v/v) glass at −120 °C, in the presence of 1 mM Mg^{2+} and various concentrations of Na^+, are given in Figure 4. Several points are worth noting. The anisotropy factor in the phosphorescence is very large and reaches a few percent while the maximum value attained for g_{ab} is only 4.6×10^{-4}. The CPP spectra are parallel to one another at the red edge and tend to converge at the blue edge, the dividing wavelengths being 470–490 nm. An examination of the g_{em} values measured at a given wavelength in the region where the spectra are parallel (530 nm for instance) reveals a clear trend in the emission anisotropy factor. As the NaCl concentration is increased to 200 mM, g_{em} decreases concomitantly. However, upon further increase of salt concentration this trend is reversed in a somewhat anomalous fashion, and g_{em} increases again.

The effect of magnesium on the conformation of bulk tRNA was checked by measurement of the CPP spectrum at the salt concentration of 200 mM where the emission anisotropy factor is most negative. So that possible dimerization could be avoided, the $MgCl_2$ concentration was not increased above 3 mM. The measurement showed that across the phosphorescence band, the emission anisotropy factor in the presence of 1 mM Mg^{2+} is more negative than in 3 mM Mg^{2+}.

CPP spectra were also obtained for $tRNA_1^{Val}$, $tRNA_f^{Met}$, and $tRNA^{Phe}$ at −120 °C in an ethylene glycol–water (2:1 v/v) glass containing 1 mM Mg^{2+} and various concentrations of Na^+ ranging from 1 to 500 mM. The general behavior observed for these specific tRNAs was similar to that exhibited by unfractionated tRNA (see Figure 4). However, significant differences were found among the details of the CPP spectra of the various species, indicating that each exhibits a distinctive dependence of conformation on salt concentration. $tRNA_1^{Val}$ and $tRNA_f^{Met}$ both show an anomaly; i.e., g_{em} decreases with an increase in NaCl concentration until a certain point is reached, after which it then increases. The peak of the anomaly for $tRNA_1^{Val}$ lies at 300 mM, with g_{em} values less positive than in the case of bulk tRNA, while for $tRNA_f^{Met}$ the anomaly peaks at 200 mM and the g_{em} values are more positive than those of bulk tRNA. Quite different behavior was observed for $tRNA^{Phe}$. It does not exhibit any anomaly within the salt range 50–400 mM. g_{em} decreases with increasing salt concentration until 400 mM, at which point it levels off. Furthermore, below 50 mM NaCl, g_{em} increases with increasing salt contrary to all other specimens examined. This exceptional behavior will be considered in more detail under Discussion.

Figure 5 presents the emission anisotropy factor, measured at 530 nm, as a function of NaCl concentration for bulk tRNA, $tRNA_1^{Val}$, $tRNA_f^{Met}$, and $tRNA^{Phe}$, allowing a comparison of the results obtained for the various tRNA species studied. Several points are worth noting: (1) The peak of the anomaly for each specific tRNA lies at a different NaCl concentration. (2) The magnitude of the variation of g_{em} at a given wavelength as a function of salt concentration differs from one specific tRNA to another. It is maximal for $tRNA_1^{Val}$ and minimal for $tRNA_f^{Met}$. (3) Bulk tRNA represents an average; each individual tRNA can be considerably different from this average. The significance of these obser-

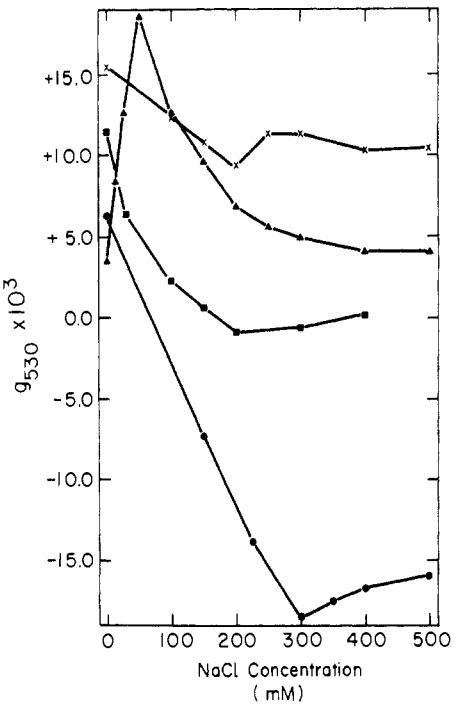

FIGURE 5: The salt (NaCl) dependence of the phosphorescence anisotropy factor measured at 530 nm exhibited by various species of *E. coli* tRNA: (×) $tRNA_f^{Met}$; (▲) $tRNA^{Phe}$; (■) bulk tRNA; (●) $tRNA_1^{Val}$. The excitation wavelength in all the cases is 335 nm, the experimental error in g_{em} is $\pm 10^{-4}$, and the spectral resolution is 16 nm.

vations will be discussed in the following section.

Discussion

We have used the circularly polarized phosphorescence of the naturally occurring s^4U nucleoside to probe conformational properties of several species of tRNA molecules as a function of NaCl concentration. For a study of this type, CPP has proven itself to be a much more sensitive spectroscopic technique than CD. This is due to the large difference in magnitude between the optical activities in absorption and phosphorescence. A possible explanation for this difference is that different electronic transitions are involved in the two processes, namely, singlet–singlet and triplet–singlet. Furthermore, the chirality of a molecule in the triplet state may differ from the chirality in the ground state, thus yielding different spectra.

The anisotropy factor for a single, symmetry-allowed electronic transition in a homogeneous population of chromophores is expected to be approximately constant across the emission band (Moscowitz, 1965). However, the situation for CPP may be more complex because triplet–singlet transitions are spin forbidden; hence, the phosphorescence usually involves multiple electronic transitions. The variation observed in the value of g_{em} across the s^4U phosphorescence band may be due either to properties inherent in the triplet–singlet optical activity or to sample heterogeneity. In the CPP of tRNA there are two possible sources of such heterogeneity. The first is heterogeneity of the tRNA conformers that are frozen during the cooling process, and the second is heterogeneity of the emission of the s^4U chromophore itself. As pointed out by Shalitin & Feitelson (1973, 1976) the phosphorescence spectrum of this chromophore is composed of two emission bands that are only partially resolved.

Since the CPP spectra are not constant, we have chosen to compare the g_{em} values for the different tRNA species at various salt concentrations, at one representative wavelength, 530 nm. This lies in a particularly advantageous region of the spectrum for such a comparison since the spectral dependence of g_{em} is very weak and changes in the relative contributions of the two emission bands to the overall emission spectrum are quite small. This is not the case at the blue edge of the spectra, near 450 nm where both the emission and anisotropy factor are extremely sensitive to spectral changes and the CPP data must consequently be interpreted with some caution.

A priori, it is very difficult to predict how the emission anisotropy factor will vary with changes in the local tRNA structure. However, it is interesting to examine the ionic strength dependence of g_{em} for unfractionated tRNA (Figure 4) in light of the salt jump experiments of Cole et al. (1972). These investigators measured the hyperchromicity at 335 nm, and according to their results for three species, increasing the salt concentration from 5 to 170 mM at room temperature results in a transition in the region of s^4U from a less stacked to a more stacked form. With a similar increase in salt concentration in our experiments, we find progressively less positive values for g_{em}, a trend that is independently borne out by each of the purified tRNAs examined—$tRNA_1^{Val}$, $tRNA_f^{Met}$, and $tRNA^{Phe}$ beyond 50 mM—at NaCl concentrations up to 200 mM. The only exception is the spectrum of $tRNA^{Phe}$ at very low ionic strength (<50 mM), which will be discussed in more detail below. On this basis we infer that a more negative g_{em} corresponds to a more highly stacked environment for the s^4U base. The valine-specific molecule, which shows the largest change in g_{em} in response to changes in ionic strength, therefore must undergo the most ordering in the vicinity of s^4U, while the $tRNA_f^{Met}$ correspondingly undergoes the least ordering.

The unique behavior of g_{em} of $tRNA^{Phe}$ in the range of salt concentration up to 50 mM, indicating decreasing local stacking with increasing ionic strength, may be understood by a comparison with the salt jump experiments of Cole et al. (1972) on $tRNA^{Phe}$ in aqueous solution. They observed an increase in absorbance at 335 nm when the concentration of Na^+ was raised from 5 to 170 mM. This indicates that, unique to $tRNA^{Phe}$, s^4U is in a more highly stacked environment at low salt than at near physiological salt concentration.

Beyond Na^+ concentration of 200 mM an anomaly is observed in the dependence of g_{em} on ionic strength for all tRNA species examined except $tRNA^{Phe}$. The environment of s^4U appears to reach a maximally stacked state, and then a relaxation sets in upon further addition of salt (Figure 5). Similar behavior has been observed for the hydrodynamic properties of tRNA. In laser light scattering experiments, Olson et al. (1976) and Potts et al. (1979) found that in 1 mM $MgCl_2$, the translational diffusion coefficient of bulk tRNA reaches a maximum at 0.1 M NaCl and decreases approximately 11% with a further increase in salt to 0.2 M NaCl. Furthermore, no anomaly was observed by them in 10 mM $MgCl_2$, and most significantly, the value of $D^0_{20,w}$ in 0.1 M NaCl and 10 mM $MgCl_2$ was found to be smaller than in 0.1 M NaCl and 1 mM $MgCl_2$. Our measurement of g_{em} at the Na^+ concentration corresponding to the peak of the anomaly for bulk tRNA but at 3 mM $MgCl_2$ is correspondingly less negative. It therefore appears that a correlation exists between the behavior of g_{em} and the behavior of $D^0_{20,w}$; i.e., a more negative g_{em} corresponds to a larger $D^0_{20,w}$. To allow for a more detailed comparison, we present our CPP data for $tRNA_f^{Met}$ and bulk tRNA along with the diffusion coefficient measurements of Olson et al. (1976) and Potts et al. (1979) for these species (Figure 6). The strong correspondence indicates that the local conformation of tRNA as probed by the CPP of the s^4U nucleoside is sensitive to the molecule's overall

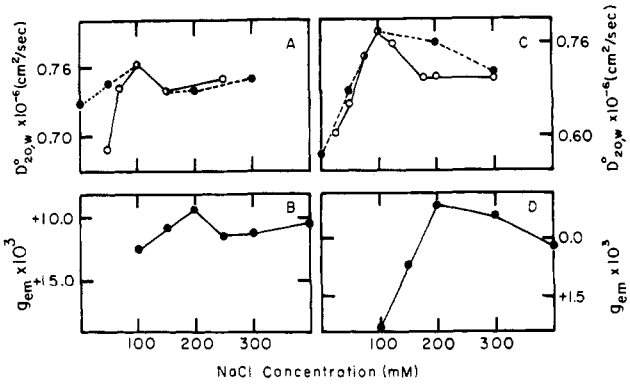

FIGURE 6: A comparison between the salt (NaCl) dependence exhibited by the phosphorescence anisotropy factor (●) and the salt dependence of the translational diffusion coefficient (○) drawn for $tRNA_f^{Met}$ (panels A and B) and for bulk tRNA (panels C and D). (●—●) The emission anisotropy factor at 530 nm as a function of NaCl concentration (data taken from Figure 5). (○—○) The translational diffusion coefficient as a function of NaCl concentration. The data points were taken from Olson et al. (1976) and from Potts et al. (1979), and the connecting line was redrawn. (●---●) In order to facilitate the comparison, we drew the CPP spectra shown in panels B and D with a 100 mM shift in NaCl concentration so that their peaks coincide with the corresponding peaks of the diffusion coefficient spectra.

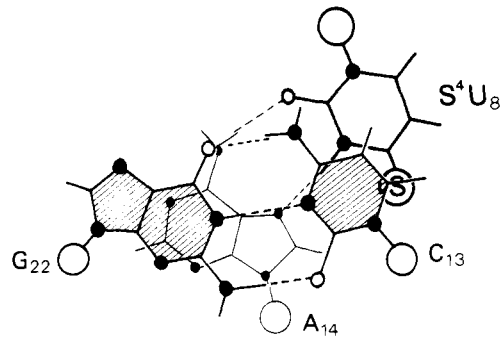

FIGURE 7: The reverse Hoogsteen hydrogen bonding pattern of the invariant residues s^4U_8 and A_{14}, and the position of s^4U_8 in relation to C_{13} (shaded). The large unfilled circles denote the $C_{1'}$ position of the ribose moiety.

hydrodynamic shape. When the overall shape of the molecule is more compact, the local environment of s^4U is more stacked. That the peaks of the anomalies, as observed by the two techniques, are shifted in salt concentration with respect to one another might indicate that the local stacking around s^4U lags the overall shape compaction. A more likely explanation, however, is that under our experimental conditions, i.e., an ethylene glycol–water (2:1 v/v) glass at low temperature, the ionic strength must be raised to higher values in order to compensate for a dielectric constant different from that of aqueous solution at room temperature. The reason that the anomaly in $tRNA_1^{Val}$ (for which $D^0_{20,w}$ has not been measured) occurs at still higher salt concentrations is unclear.

The influence of variation with temperature of the dielectric constant of the solvent may also be apparent in the CD results. All of our data indicate that the molecular configurations present in an ethylene glycol–water (2:1 v/v) mixture closely parallel those found in aqueous solution. Nevertheless, there is a puzzling lack of sensitivity to changes in ionic strength of the absorption anisotropy factor of bulk tRNA in ethylene glycol–water at room temperature (Figure 3). The low-temperature CPP data clearly reveal the presence of multiple conformational states, while the room temperature CD data, with similar variation in salt concentration, show the existence basically of only one conformation. This observation is also supported by thermal denaturation experiments in an ethylene glycol–water (2:1 v/v) solvent mixture, monitoring the hyperchromicity at 335 nm, which revealed an insensitivity of the melting curve to salt concentration between 30 and 200 mM (N. Steinberg, unpublished experiments). A study of solvent properties shows that the dielectric constant of the mixture is relatively low (ϵ = 59.7) at room temperature (Douzou, 1977) and intramolecular interactions are correspondingly modified with respect to aqueous solution. As the temperature is lowered, the dielectric constant increases, until between −30 and −40 °C it reaches a value that is approximately equal to that of water at room temperature (80.4). In this temperature range the cooling rate is slow enough and the viscosity of the solvent small enough that conformational changes can occur. Below this temperature range, the high solvent viscosity results in molecular conformations being essentially frozen. The absorption anisotropy factor measured in this solvent mixture at −120 °C should exhibit similar, although probably not identical, sensitivity to ionic strength as room temperature measurements in aqueous solution. Such low-temperature CD measurements are planned.

In summary, we have measured the variation of the emission anisotropy factor g_{em} of several tRNA species as a function of ionic strength. This variation has been correlated with corresponding changes in base stacking occurring in the local environment of the s^4U chromophore. For the cases of bulk tRNA and $tRNA_f^{Met}$, changes in g_{em} have been shown to behave in a similar way to ionic strength dependent changes in the translational diffusion coefficient $D^0_{20,w}$ as measured by Olson et al. (1976) and Potts et al. (1979). This rather surprising result implies that the local probe senses conformational changes that are intimately related to changes in the overall hydrodynamic shape of the molecule.

We may suggest a possible explanation for those cases in which parallel behavior of $D^0_{20,w}$ and g_{em} is observed. The explanation is based on the molecular model of yeast $tRNA^{Phe}$, and as yet undetermined species-related structural variations may explain its lack of complete generality, viz., *E. coli* $tRNA^{Phe}$ (Rhee et al., 1981). NMR data (Wong et al., 1975) and X-ray diffraction (Kim, 1978) indicate that in the native structure reverse Hoogsteen-type hydrogen bonds are formed between the invariant bases s^4U in position 8 and A_{14} (Figure 7). Consequently, the D loop is in close contact with the hinge region between the D stem and the AA stem. We suggest that the degree of overall compactness of the tRNA molecule, as regulated by ionic strength, determines the properties of these hydrogen bonds. Changes in these bonds, in turn, regulate the stacking interaction of bases s^4U_8 and C_{13}. It is to this base stacking, among other factors, that the emission anisotropy is sensitive. In addition, X-ray diffraction analysis of yeast $tRNA^{Phe}$ has revealed a Mg^{2+} ion interacting with the phosphate moiety of U_8 and stabilizing, via hydrogen bonds, the loop containing residues U_8 through U_{12} (Holbrook et al., 1977). Assuming that an analogous situation exists for *E. coli* tRNA, we cannot exclude the influence of salt-dependent changes in the binding of this Mg^{2+} ion on the CPP of s^4U_8.

We conclude that the spectral changes that we observe result from conformational changes at the level of tertiary structure. Such an effect has also been observed in melting experiments where a spin-label was placed at the s^4U site (Caron & Dugas, 1976). Independent of mechanism, our data clearly support the finding (Potts et al., 1979; Olson et al., 1976) of more than one conformation for tRNA under near physiological solution conditions. This is one of the few cases where the magnitude of the observed conformational change under these conditions is not readily explained by our current understanding of the

flexibility of the tRNA molecule, i.e., in the loops and the 3′ end.

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