Changes in the Solution Structure of Yeast Phenylalanine Transfer Ribonucleic Acid Associated with Aminoacylation and Magnesium Binding†

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ABSTRACT: The effect of aminoacylation on the structure of yeast phenylalanine tRNA was evaluated by laser light scattering. In these experiments, the translational diffusion coefficient ($D_{20,w}$) of phenylalanyl-tRNA was monitored continuously during spontaneous deacylation in a variety of solution conditions. The results reveal that significant changes can occur in the hydrodynamic volume and electric charge as a consequence of aminoacylation but that the effects are magnesium dependent. At neutral pH, 20 °C, and 0.1 M salt, the $D_{20,w}$ value increased by 18% when deacylation occurred in 2–10 mM Mg^{2+} concentrations while no change in diffusivity was observed for tRNA deacylating in 0.5–1.0 mM Mg^{2+}. The Mg^{2+} concentration dependence of the $D_{20,w}$ changes behaves in a highly cooperative manner. The electric charges of aminoacyl-tRNA and nonacylated tRNA in 1 and 10 mM Mg^{2+} were estimated from the diffusive virial coefficients. In the higher Mg^{2+} condition, aminoacyl-tRNA has a charge of $15 \pm 2e^-$ while that of the nonacylated form is $10 \pm 2e^-$; both acylated and nonacylated tRNA have a charge of $11 \pm 4e^-$ in 1 mM Mg^{2+}. Taken together, the results indicate that aminoacylation permits the binding of additional Mg^{2+}, resulting, in turn, in the formation of a more extended conformer of lower diffusivity and greater negative charge. The results also provide a possible explanation for several contradictory results in the literature.

Considerable effort is being devoted to the analysis of the structure of the transfer ribonucleic acids (tRNA) with a view toward the complete elucidation of the solution structure and identification of important structure–function relationships. Perhaps the most important and by far widely studied of these relationships is that involving the aminoacylation of tRNA. It has often been suggested that structural changes in tRNA associated with aminoacylation provide the basis for discrimination between aminoacyl-tRNA and nonacylated tRNA in a number of biological processes. Unfortunately, much of the experimental evidence available on this important point is in apparent disagreement.

The basis (bases) for the lack of agreement in the two dozen or so reports available is (are) not clear but is (are) likely related to differences in solution conditions, individual tRNAs, and physical properties being evaluated. A broad assortment of biophysical and biochemical techniques have been employed to study a variety of tRNAs under a variety of solution conditions. The techniques include the following: circular dichroism (CD) (Hashizume & Imahori, 1967; Bernardi & Cantoni, 1969; Adler & Fasman, 1970; Melcher et al., 1971; Watanabe & Imahori, 1971; Wickstrom, 1971) and optical rotatory dispersion (ORD) (Sarin & Zamecnik, 1965); nuclear magnetic resonance (NMR) (Cohn et al., 1969; Wong et al., 1973; Davenloo et al., 1979) and electron spin resonance (ESR) (Caron et al., 1976); Raman (Thomas et al., 1973) and fluorescence (Beres & Lucas-Lenard, 1973) spectroscopy; X-ray scattering (Ninio et al., 1972); ethidium bromide (Tritton & Mohr, 1973), steroid (Danchin & Grunberg-Manago, 1970), and polynucleotide (Chin & Kidson, 1971; Dvorak et al., 1976; Pongs et al., 1976) binding; ^3H-exchange kinetics (Gantt et al., 1969; Chatterjee & Kaji, 1970; Englander et al., 1972); sedimentation (Kaji & Tanaka, 1967; Chatterjee & Kaji, 1970); nuclease susceptibility (Hänngi & Zachau, 1971); column chromatography (Stern et al., 1969); kinetics of transformylation (Schofield, 1970); tRNA–ribosome binding (Phillips, 1970; Kirillov & Odinzov, 1978). The results from these varied experiments exhibit little consensus, with somewhat more than half showing some change in tRNA structure upon aminoacylation, while no significant change was evident in the remainder.

Among the early experiments, Kaji and co-workers (Kaji & Tanaka, 1967; Chatterjee & Kaji, 1970) showed that some, but not all, *Escherichia coli* aa-tRNAs have lower sedimentation rates in 10 mM Mg^{2+} at pH 7.0 than the corresponding nonacylated form, thus suggesting an "extended" conformer. On the other hand, no significant differences were detected in either the CD spectra (Hashizume & Imahori, 1967; Adler & Fasman, 1970; Wickstrom, 1971) or the kinetics of ^3H exchange (Gantt et al., 1969; Chatterjee & Kaji, 1970; Englander et al., 1972) for a variety of tRNAs under nearly identical solution conditions, indicating that within the limits of detection and other confines of the technique, no changes occur in tRNA structure upon aminoacylation. Taken together, these results suggest that at least some individual tRNAs undergo structural changes upon aminoacylation, although the changes may be so small or of a type to be beyond the detection capabilities of some techniques.

Subsequent experiments, using techniques more sensitive to tRNA substructure, have similarly produced conflicting results. Analysis of aminoacylated tRNA by low-field NMR, a technique sensitive to secondary and tertiary hydrogen bonding, has shown no differences for aminoacylated and nonacylated yeast $tRNA^{Phe}$ in a pH 5 solution condition (Wong et al., 1973); a second study performed at pH 6.6 by another laboratory showed changes occurring in the region around ribothymidine-54 in the Ψ loop (Davanloo et al., 1979). No changes with aminoacylation were detected in the Y-base fluorescence spectra of yeast $tRNA^{Phe}$ at neutral pH and in

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the presence of Mg^{2+}, strongly limiting the occurrence of changes in the anticodon region of this tRNA (Beres & Lucas-Lenard, 1973). Cohn et al. (1969) have detected differences in the water proton NMR spectra for samples of aminoacylated and nonacylated *E. coli* tRNA in the presence of manganese ions at near neutral pH. These particular results indicate that there are changes in both conformation and Mn^{2+} binding properties upon aminoacylation. Under solution conditions similar to those used in this last study, differences were observed in the intensity of X-ray scattering with the aminoacylation of *E. coli* $tRNA_1^{Val}$, suggesting that changes occurred in Mg^{2+} ion binding and in the distribution of associated counterions (Ninio et al., 1972).

Taken together, the results from these and some of the other investigations cited above suggest that tRNA conformational changes can occur with aminoacylation but may be dependent upon solution conditions. Of particular importance are the effects of Mg^{2+} ions, which have been shown to have a profound influence on the crystal structure [see the review by Rich & RajBhandary (1976)] and tertiary structural transitions of nonacylated tRNAs (Olson et al., 1976; K. W. Rhee, R. O. Potts, C. C. Wang, M. J. Fournier, and N. C. Ford, unpublished experiments; Ehrenberg et al., 1979). To date, however, there has been no comprehensive investigation of the influence of solution conditions and, particularly, Mg^{2+} ion effects on conformational transitions associated with aminoacylation.

We present here the results of a laser light scattering investigation of the effects of aminoacylation on the structure of yeast $tRNA^{Phe}$ at a variety of solution conditions. The technique of laser light scattering [reviewed earlier by us (Olson et al., 1976 and references cited therein)] is sensitive to the overall shape and electrostatic charge of a biopolymer plus the surrounding ion–water atmosphere. With this technique, we have been able to demonstrate a Mg^{2+}-dependent, tertiary structural change for several nonacylated tRNAs (Olson et al., 1976; K. W. Rhee, R. O. Potts, C. C. Wang, M. J. Fournier, and N. C. Ford, unpublished experiments). The results presented here show that as phenylalanine-$tRNA^{Phe}$ is allowed to spontaneously deacylate at neutral pH, an increase in the translational diffusion coefficient ($D_{20,w}$) is observed at high Mg^{2+} concentrations (2–10 mM), while no change can be detected at low Mg^{2+} concentrations (0.5–1.0 mM). These findings indicate the occurrence of a more "extended" conformer for aa-tRNA in the high magnesium condition. tRNA concentration dependent changes in $D_{20,w}$ indicate that electrostatic charge differences are also obtained at high Mg^{2+} concentrations with the aminoacylated form having a greater negative charge. Furthermore, the Mg^{2+} ion concentration dependence of the $D_{20,w}$ changes behaves in a highly cooperative manner, consistent with a two-state transition involving the binding of additional Mg^{2+} ions to the aminoacylated form.

These findings provide the first clear-cut demonstration of the dependence of aa-tRNA structure upon solution conditions and offer a plausible explanation for a number of earlier, apparently contradictory results. Preliminary and abbreviated accounts of this work have been presented previously (Potts et al., 1977, 1979).

Materials and Methods

Transfer RNA. Bulk yeast tRNA (Plenum Scientific, Ft. Lee, NJ) with a phenylalanine acceptor activity of 50–60 pmol/A_{260} was enriched for $tRNA^{Phe}$ by a two-step procedure. An initial enrichment of ~10-fold was achieved by chromatography on benzoylated DEAE-cellulose using conditions similar to those of Gillam et al. (1967). Further enrichment was effected by high-pressure, reversed-phase column chromatography on RPC-5 sorbent (Pearson et al., 1971). The resulting tRNA had a phenylalanine acceptor activity of 1100–1200 pmol/A_{260}.

Phenylalanyl-tRNA Synthetase. Synthetase was prepared from fresh baker's yeast using the fractionation procedures and assay conditions of Schmidt et al. (1971) with the omission of the gel filtration and final hydroxylapatite chromatography steps. The enzyme had a specific activity of 1.5 units/μg, where 1 unit corresponds to the amount of protein required for the formation of 1 pmol of phenylalanyl-tRNA/min at 37 °C. The aminoacylation reaction mixture contained, per milliliter 25 μmol of Tris-HCl, pH 7.2, 25 μmol of ATP, 12 μmol of $MgCl_2$, 1 nmol of $tRNA^{Phe}$, 5 nmol of [^{3}H]phenylalanine (200 mC/mmol; New England Nuclear Corp.), and 30 μg of synthetase.

Preparation of Aminoacyl-tRNA. The preparation of [^{3}H]Phe-$tRNA^{Phe}$ for light scattering has been described previously (Potts et al., 1977). In a typical experiment ~5 A_{260} of $tRNA^{Phe}$ with an acceptor activity of at least 1100 pmol/A_{260} was aminoacylated as described above. Just prior to light scattering, the aminoacyl-tRNA was solubilized in ~25 μL of 1 mM sodium acetate, pH 5.0, and 0.1 M NaCl and dialyzed vs. the same buffer with Mg^{2+} at the appropriate concentration. Spontaneous deacylation was in effect initiated by raising the pH to 7.2 by the addition of 0.2 volume of 5 times concentrated stock of the appropriate buffer. Eight microliters of sample was then filtered (Flath-Lundin filter, Hamilton Syringe Co.) in a 2 × 2 × 40 mm cuvette for light scattering; the remainder was filtered into a test tube containing the same buffer and thermostatically maintained at the same temperature (20 °C). Portions of the larger sample were withdrawn with time and acidified with trichloroacetic acid, and the acid-insoluble radioactivity was trapped by filtration through glass-fiber filters (Whatman type AP from Reeve-Angel). The extent of aminoacylation was measured by scintillation spectrometry.

Data Collection and Analysis. These techniques have been described previously (Olson et al., 1976, and references cited therein).

Results

Effect of Aminoacylation on the Translational Diffusion Coefficient. Figure 1 shows results from two separate experiments in which changes in $D_{20,w}$ were monitored as phenylalanyl-$tRNA^{Phe}$ deacylated spontaneously in solutions containing respectively 1 and 10 mM magnesium; all samples were previously dialyzed against solutions containing magnesium at the appropriate concentration. The data in panel A show that a linear increase in $D_{20,w}$ occurs for tRNA deacylated in 10 mM Mg^{2+} over a range of about 900 to nearly 0 pmol/A_{260}. On the other hand, no change was detected for aminoacyl-tRNA deacylated in 1 mM Mg^{2+} (Figure 1B), all other conditions remaining the same. These results are virtually identical with those reported earlier by us for $tRNA^{Phe}$ which was only acylated to the extent of ~600 pmol/A_{260} (Potts et al., 1977).

When linearly extrapolated by a least-squares technique to a starting point of pure, fully aminoacylated tRNA (1800 pmol/A_{260}), the data obtained for the 10 mM Mg^{2+} condition show an 18 ± 3% increase in $D_{20,w}$, while the data for 1 mM Mg^{2+} show no change (0.4 ± 0.6%). In each case, no significant changes in scattered intensity were detected over the course of the experiment, and the final $D_{20,w}$ value obtained upon complete deacylation was nearly identical with that observed for nonacylated tRNA under the same conditions.

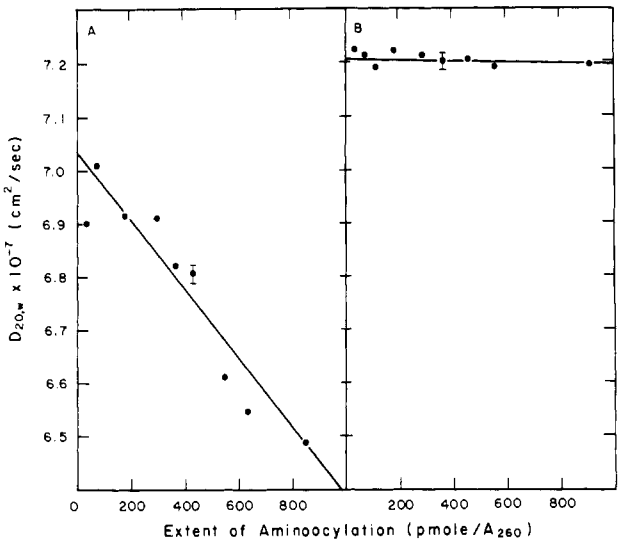

FIGURE 1: Translational diffusion constant ($D_{20,w}$) of yeast tRNA^{Phe} as a function of the extent of aminoacylation. Values of $D_{20,w}$ and the extent of acylation were monitored as [^{3}H]Phe-tRNA^{Phe} deacylated spontaneously at 20 °C in 0.1 M Tris-HCl, pH 7.2, 0.1 M NaCl, and 10 or 1 mM $MgCl_2$. Each point represents the average of at least six determinations of $D_{20,w}$ made within a 15-min period. The error bars represent ±1 standard deviation about the point. The data were fit to straight lines by a least-squares method. The Mg^{2+} and tRNA concentrations and half-time of deacylation were (A) 10 mM, 1.2 mg/mL, and 75 min, respectively, or (B) 1 mM, 1.5 mg/mL, and 55 min. Full details are provided under Materials and Methods.

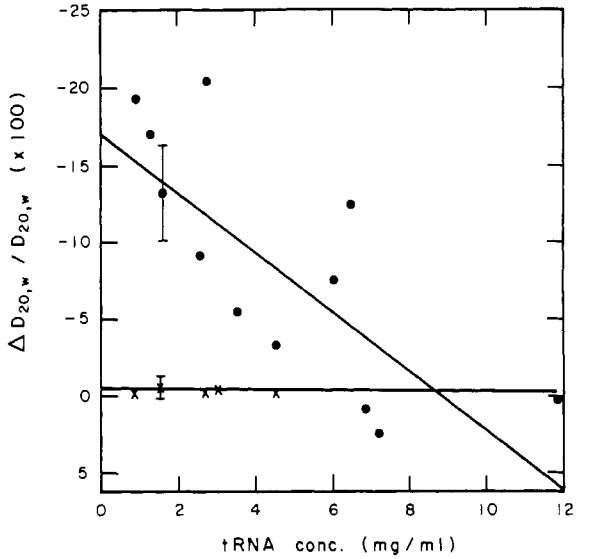

FIGURE 2: Relative change in diffusion constant ($\Delta D_{20,w}/D_{20,w}$) upon aminoacylation as a function of tRNA concentration. Experiments similar to those described in Figure 1 were repeated at a variety of tRNA concentrations, and the relative change in $D_{20,w}$ was determined by a linear extrapolation to a starting point of pure, fully aminoacylated tRNA^{Phe}. The error bars signify ±1 standard deviation determined from the linear extrapolation of data from $D_{20,w}$ vs. the extent of aminoacylation at each tRNA concentration. The data at each Mg^{2+} concentration were fit to a straight line by a least-squares technique. The experimental conditions are those of Figure 1 with either 10 mM Mg^{2+} (●) or 1 mM Mg^{2+} (×).

Further, tRNA which had been subjected to a mock aminoacylation, i.e., incubation with synthetase in a reaction mixture lacking amino acid and recovered in the same way as aminoacyl-tRNA, behaved like untreated, nonacylated tRNA (results not shown). In another control experiment it was determined that the change in diffusivity did not occur because of the shift in pH from 5.0 to 7.2. Here, aminoacylated tRNA was maintained at pH 7.2 during recovery from the acylation step. Although the sample had deacylated to the extent of ~350 pmol/A_{260} when light scattering measurements were initiated, the diffusion coefficient changed in the same manner as seen in Figure 1, with the calculated changes in $D_{20,w}$ in good agreement (results not shown).

Light scattering theory suggests several alternative explanations of the results shown in Figure 1. One hypothesis is that the lower $D_{20,w}$ value for aminoacyl-tRNA in the 10 mM Mg^{2+} condition reflects conformational and/or electrostatic changes. Other hypotheses are that the $D_{20,w}$ change results from (1) the added mass and volume of phenylalanine or (2) aggregation of aminoacylated tRNA.

In the absence of $D_{20,w}$ changes with deacylation in 1 mM Mg^{2+}, it seems clear that the changes observed are not due simply to the additional mass and volume of the amino acid. Changes in $D_{20,w}$ due to aggregation can also be evaluated from measurements made of the intensity of the scattered light taken over the course of the experiment. Theoretical considerations and results from light scattering experiments performed in our laboratories on the formation of tRNA dimers show the scattered intensity to increase markedly upon aggregate formation (Wang et al., 1980). Furthermore, according to theory, the smallest possible increase in scattered intensity accompanying a given change in $D_{20,w}$ results from the formation of a few, very large aggregates. In such a case, the relative change in the magnitude of the scattered intensity should be approximately half the relative change in the diffusion constant. The relative increase in scattered intensity for the experiment of Figure 1A is 3 ± 3% (data not shown)—two standard deviations less than the minimal value predicted by theory (corresponding to half of the observed 18% change in $D_{20,w}$). We conclude that the observed changes in $D_{20,w}$ are related to conformational and charge changes and not to increased aggregation of aa-tRNA.

Effect of tRNA Concentration on tRNA Diffusivity. Light scattering theory predicts that changes in the diffusion constant with the concentration of scatterers can be related to the shape and electrostatic charge of the scatterers through the equation [see Olson et al. (1976) for the derivation]

$$D_{20,w} = D^0_{20,w}\left[1 + \left(\frac{10^3\bar{\nu}_1\bar{Z}^2}{2\mu M} + V_e\bar{\nu}_2\right)c\right] \quad (1)$$

where $D_{20,w}$ is the diffusion constant in water at 20 °C, $D^0_{20,w}$ is the value of $D_{20,w}$ extrapolated to zero concentration of scatterers, $\bar{\nu}_1$ and $\bar{\nu}_2$ are the solvent and solute partial excluded volumes, respectively, $\bar{Z}$ is the average electrostatic charge of the scatterer plus associated ions, M is the molecular mass of the scatterer, μ is the ionic strength, V_e is an excluded volume factor dependent upon the molecular shape, and c is the concentration of scatterers. Calculations show that the excluded volume effects for a charged molecule like tRNA are negligible when compared to the electrostatic effects [$V_e\bar{\nu}_2 \ll 10^3\bar{\nu}_1\bar{Z}^2/(2\mu M)$]. Thus, eq 1 predicts that a plot of $D_{20,w}$ vs. c at constant ionic strength will yield a straight line of intercept $D^0_{20,w}$ and slope proportional to the square of the average electrostatic charge.

For determination of the relative contribution of conformational and electrostatic effects to the changes in $D_{20,w}$, the tRNA concentration dependence of the effect was determined. Here, the basic experiment described in Figure 1 was repeated over a broad range of tRNA concentrations. When plotted as relative change in $D_{20,w}$ (extrapolated to 1800 pmol/A_{260}) vs. tRNA concentration, the results shown in Figure 2 are obtained. The data for tRNA in 1 mM Mg^{2+} exhibit no tRNA concentration dependence, indicating that neither

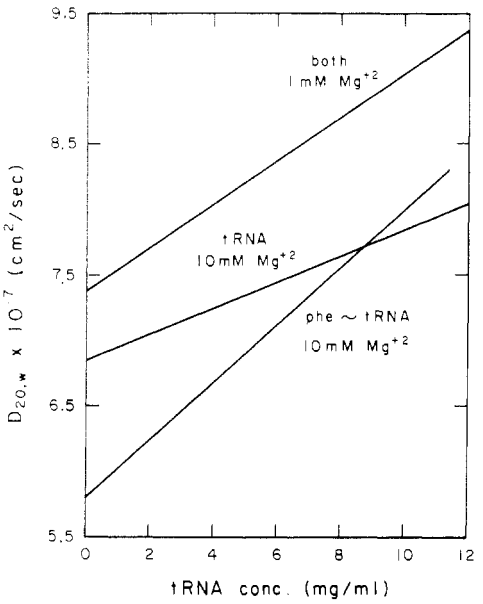

FIGURE 3: Diffusion constants of nonacylated and aminoacylated $tRNA^{Phe}$ as a function of tRNA concentration. The data shown are from Figure 2, replotted to show the effects of RNA concentration on diffusivity.

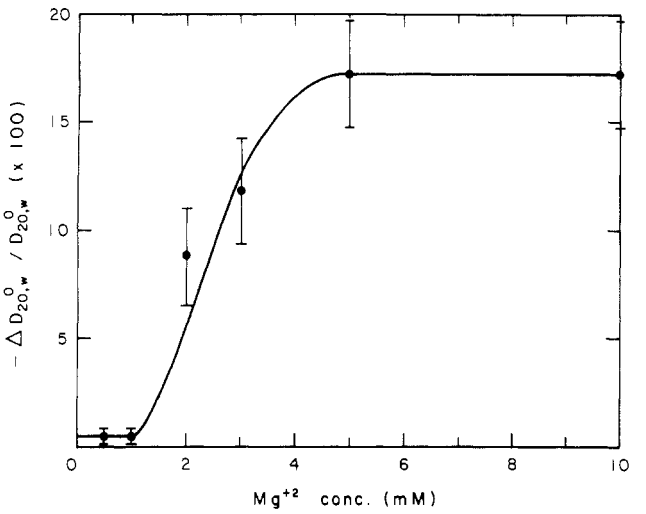

FIGURE 4: Relative change in diffusion constant ($\Delta D^0_{20,w}/D^0_{20,w}$) upon aminoacylation of $tRNA^{Phe}$ as a function of Mg^{2+} concentration. Each point represents the linear extrapolation to zero tRNA concentration of data similar to that in Figure 2, at various Mg^{2+} concentrations. The number of data points, n, for each concentration were 3 for 0.5 mM Mg^{2+}, 5 for 1.0 mM Mg^{2+}, 3 for 2 mM Mg^{2+}, 4 for 3 mM Mg^{2+}, 6 for 5 mM Mg^{2+}, and 12 for 10 mM Mg^{2+}. The error bars signify ±1 standard deviation obtained by the least-squares technique. The experimental conditions are those of Figure 2 with the exception that Mg^{2+} concentrations were varied from 0.5 to 10 mM.

conformational nor electrostatic changes occur under this solution condition. The data obtained for aminoacylated tRNA in the 10 mM Mg^{2+} condition, however, show that the relative change in $D_{20,w}$ is linearly dependent on tRNA concentration. When extrapolated to zero tRNA concentration, the increase in $D_{20,w}$ upon deacylation is 17 ± 2.7%. As before, the $D_{20,w}$ values obtained after complete deacylation were essentially identical with those obtained for nonacylated tRNA under the same condition.

It seems most likely that the tRNA concentration dependence of the $D_{20,w}$ increase upon deacylation in 10 mM Mg^{2+} reflects electrostatic charge differences upon aminoacylation. However, it is also possible that these changes result from association of aminoacylated and/or nonacylated tRNAs. The possibility of aggregation was considered unlikely as the diffusion constant and scattered intensity values monitored over the course of the experiment were consistent with those expected for a monodisperse, monomeric sample. A plot of the relative change in scattered intensity (extrapolated to 1800 pmol/A_{260}) as a function of tRNA concentration, for experiments done in 10 mM Mg^{2+}, yields values for the slope and intercept more than three standard deviations less than the smallest effect of aggregation predicted by theory (data not shown). From these measurements it seems clear that the relative changes in $D_{20,w}$ with tRNA concentration cannot be attributed to an association phenomenon but rather reflect electrostatic differences associated with aminoacylation.

Contributions of Conformation and Charge to the Change in $D^0_{20,w}$. For quantitation of conformational and electrostatic changes, the data of Figure 2 are replotted to show the tRNA concentration dependence of the absolute values of $D_{20,w}$ for both aminoacyl-tRNA and nonacylated tRNA. As indicated above (eq 1), the slope of such a plot is proportional to the square of the average charge, while the intercept, $D^0_{20,w}$ is related to the molecular conformation. The diffusion coefficients for completely aminoacylated and deacylated tRNA are plotted vs. tRNA concentration for 1 and 10 mM Mg^{2+} in Figure 3. In 1 mM Mg^{2+} both aminoacylated and nonacylated tRNAs exhibit the same $D^0_{20,w}$ [(7.39 ± 0.08) × 10^{-7} cm^2/s] and slope, (0.14 ± 0.05) × 10^{-7} (cm^2/s)/(mg/mL)]. By use of the value of the slope and eq 1, an average charge of 11 ± 4e^- is calculated for both, in good agreement with the value of 10 e^- previously determined by us for bulk *E. coli* tRNA and yeast $tRNA^{Phe}$ under the same conditions (Olson et al., 1976).

In contrast, however, the data obtained with the aminoacylated and nonacylated species generate different slopes and intercepts in 10 mM Mg^{2+}. Aminoacylation results in a decrease in $D^0_{20,w}$ from (6.84 ± 0.10) × 10^{-7} to (5.8 ± 0.2) × 10^{-7} cm^2/s and an increase in the slope from (0.10 ± 0.02) × 10^{-7} to (0.22 ± 0.06) × 10^{-7} (cm^2/s)/(mg/mL). From the slope values, the average charge of nonacylated $tRNA^{Phe}$ is estimated to be 10 ± 2e^-, while the average charge of the aminoacylated form is 15 ± 2e^-, corresponding to an increase in negative charge of 5 ± 2e^-. Thus, while aminoacylation of $tRNA^{Phe}$ results in no changes in 1 mM Mg^{2+}, an increase in negative charge and decrease in $D^0_{20,w}$ are observed with the 10 mM Mg^{2+} condition.

Role of Magnesium in the Transition. Insight into the role of Mg^{2+} ions in the conformational/electrostatic changes accompanying aminoacylation was obtained from the tRNA concentration dependence of $D_{20,w}$ changes at several Mg^{2+} concentrations, all other conditions remaining the same. Data for each Mg^{2+} concentration were fit to a straight line, and the final results, shown in Figure 4, plotted as the relative change in $D^0_{20,w}$ vs. Mg^{2+} concentration. The data for 0.5 and 1.0 mM Mg^{2+} yield the same line, with no increase in $D^0_{20,w}$ upon deacylation. Measurements made at 2 and 3 mM Mg^{2+} show increases in $D^0_{20,w}$ upon deacylation of 8.8 ± 2.3% and 11.7 ± 2.7%, respectively. The data for 5 and 10 mM Mg^{2+} yield the same line, with an increase in $D^0_{20,w}$ of 17 ± 2.7%. The tRNA concentration dependence of the scattered intensity changes observed with deacylation indicate that association is not responsible for $D^0_{20,w}$ changes at any of the Mg^{2+} concentrations investigated. Taken together, the data in Figure 4 suggest a cooperative effect involving the binding of Mg^{2+} ions and are consistent with a two-state transition.

In summary, changes observed in the diffusion constant ($D^0_{20,w}$) and average charge of yeast $tRNA^{Phe}$ upon deacylation are not related to association/dissociation of the molecules.

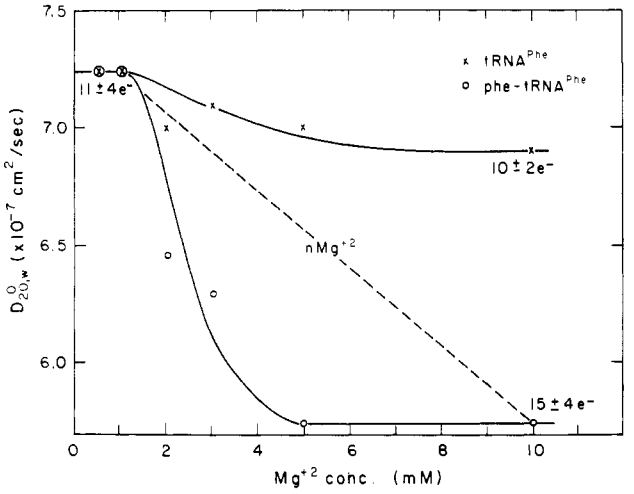

FIGURE 5: Summary of the effects of Mg^{2+} on the changes in yeast tRNA^{Phe} conformation and charge associated with aminoacylation. The data are taken from Figures 2–4. (×) Nonacylated tRNA^{Phe}; (○) Phe-tRNA^{Phe}.

The conformational and electrostatic changes observed are dependent upon the Mg^{2+} concentration in a manner summarized in Figure 5. At low Mg^{2+} concentrations (0.5 and 1.0 mM), no differences in charge or conformation are detected between the aminoacylated and nonacylated forms. At higher Mg^{2+} concentrations, however, the aminoacylated form has a substantially smaller diffusion constant and greater negative charge. Furthermore, while the conformation and charge of nonacylated tRNA^{Phe} are insensitive to the different Mg^{2+} levels evaluted, both of these properties are affected in a highly cooperative, Mg^{2+}-dependent fashion when the tRNA is aminoacylated. The dramatic change observed with aminoacyl-tRNA is consistent with the occurrence of a two-state transition involving the binding of additional Mg^{2+} ions.

Discussion

The results presented show a Mg^{2+}-dependent transition to a slower diffusing conformer of greater negative charge upon aminoacylation of yeast tRNA^{Phe}. The number of Mg^{2+} ions involved in a highly cooperative transition of this sort is often estimated from a Hill analysis: for this case a plot of log $[Mg^{2+}]$ vs. log ([B]/[A]) where A and B correspond to the conformers of lower and higher diffusivity, respectively, the resulting slope corresponds to the number of ligands bound. An analysis of the results from Figure 4 indicates that two to four additional Mg^{2+} ions are bound. This estimate is imprecise, however, and possibly low, owing to errors in determination of the relative change in $D_{20,w}$ and inaccuracies inherent in the estimate of the ratio of the two conformers and because of a possible deficiency in the Hill-type method of analysis for cooperative transitions involving the binding of several ligands. In this last regard, the estimate of the number of ligands involved can be much lower than the actual number (Kirschner, 1971). Thus, we only conclude that the Mg^{2+}-dependent change from higher to lower diffusivity and less to greater negative charge for aminoacylated tRNA involves the binding of additional magnesium.

The transition of tRNA^{Phe} to a conformer of lower diffusivity upon aminoacylation is in agreement with the results of sedimentation (Kaji & Tanaka, 1967), ESR (Caron et al., 1976), and NMR (Cohn et al., 1969) experiments carried out under nearly identical solution conditions, which show the existence of a more "extended" structure upon aminoacylation. However, upon first inspection it might appear that the large-magnitude changes observed in this study are contradictory to results from CD (Hashizume & Imahori, 1967; Bernardi & Cantoni, 1969; Adler & Fasman, 1970; Melcher et al., 1971; Watanabe & Imahori, 1971; Wickstrom, 1971) and ORD (Sarin & Zamecnik, 1965) studies which indicate that only small changes occur in the secondary structure upon aminoacylation and results of ESR (Caron et al., 1976), fluorescence (Beres & Lucas-Lenard, 1973), ligand binding (Danchin & Grunberg-Manago, 1970; Chin & Kidson, 1971; Dvorak et al., 1976), and an NMR study (Davanloo et al., 1979) which suggest that changes are localized to tRNA substructure. It must be recalled, however, that laser light scattering yields information about the hydrodynamic shape and electrostatic charge of the macromolecule *plus* the associated ion–hydration atmosphere. Thus, these surrounding ion and hydration atmospheres must also be considered when interpreting effective charge and diffusion constant measurements.

The tRNA molecule, due to its polyanionic character, will have associated with it a hydrogen atmosphere which includes various counterions. In addition to these loosely associated cations, some Mg^{2+} ions are likely to bind to the tRNA in a site-specific manner (Kim, 1978 and references cited therein). The effective charge then includes the intrinsic macromolecular charge plus the charge of the associated Mg^{2+} and other counterions. Furthermore, the entire complex is in a state of flux, in which these ions exchange with those in the surrounding solution. Because of the complexity of the polyanion and its surrounding ionic medium, it is not possible to give a strict interpretation to the "average" charge as measured by the light scattering method. However, we can discuss the division of many of the charges between consideration as part of the polyion's charge and consideration as part of the ionic background. Clearly any ions bound firmly to the tRNA constitute part of the polyanion's charge. Similarly, ions outside of the layer of hydration belong to the ionic background. The distribution of ions within the hydration layer are less easily assigned. It seems likely that those ions that remain for relatively long times within the hydration layer should be thought of as belonging to the polyion charge while those that move rapidly from within to without the hydration layer are part of the ionic background. Here, rapid or slow refers to the time required for the molecule to diffuse a distance $\lambda/(4\pi n)\sin(\theta/2)$ and is ~ 20 μs in the current study (Ford, 1972). NMR investigations of Eu^{3+} (Jones & Kearns, 1974) and Mn^{2+} (Chao & Kearns, 1977) binding to tRNA have shown exchange times ranging from 1 μs to 15 ms. Since these ions have tRNA binding properties similar to Mg^{2+}, it seems likely that both the diffusion constant and electrostatic charge values reflect the influence of Mg^{2+} ion binding.

We suggest that as a consequence of aminoacylation tRNA undergoes a conformational change—possibly quite small—and alterations in the ion–hydration atmosphere and Mg^{2+} binding. While it appears possible that the effects on $D^0_{20,w}$ and electrostatic charge observed could occur without an intrinsic molecular change, that possibility seems quite remote. Quite likely, there is a substantial change in the ion–hydration atmosphere—an hypothesis supported by the results of two other investigations. Cohn et al. (1969), monitoring changes in the NMR relaxation constants of water protons surrounding tRNA, found that aminoacylation of *E. coli* bulk tRNA and, separately, tRNA^{Phe} resulted in increased Mn^{2+} binding and a "looser" tRNA structure in the vicinity of binding. Ninio et al. (1972) found no intensity changes in small-angle X-ray scattering upon aminoacylation of *E. coli* tRNA^{Val}, indicating that large-scale structural changes do not occur; however,

significant intensity differences were observed at larger angles. These changes were presumed to result from alterations in Mg^{2+} binding and changes in the distribution of counterions associated with the tRNA. Consistent with our interpretation is a new finding by low-field NMR spectral analysis that shows the spectrum of aminoacylated yeast $tRNA^{Phe}$ at pH 6.6 and 10 mM Mg^{2+} to differ from that of nonacylated tRNA only in the region corresponding to ribothymidine-54 (Davanloo et al., 1979).

Should it turn out that the L-shaped tRNA molecule is actually hinged and flexible, say, at the nonhelical apex, yet another, different interpretation of these results can be advanced. A consequence of aminoacylation could be a change in the rate of flexing of the acceptor-TΨC arm of the L. The reduction in breathing rate could be caused or accompanied by alterations in solvation and ion shielding which allow additional Mg^{2+} to be bound. The major effect of any or all of these events could be on a flex rate constant. It could follow then that the change in diffusivity is due largely to a change in the flex constant. A faster rate of flexing could be manifest as apparent higher diffusivity. Conversely, a reduction in flexing would lead to a lower rate of diffusion. This view is consistent with a theoretical prediction of the diffusion behavior of hinged molecules described recently (Harvey, 1979a,b).

Of major importance in the findings presented by us is the role of solution conditions—in particular the dependences of the observed conformational and electrostatic changes on magnesium. One important methodological feature of our investigation is the use of exhaustive dialysis of the tRNA just prior to light scattering, thus ensuring a known equilibrium Mg^{2+} concentration during the experiment. The results presented here provide at least a partial explanation for some of the apparent inconsistencies in other results. It seems probable that many of the negative results reported, that is, lack of structural differences upon aminoacylation of tRNA, are related to the solution conditions used during the investigation. Solution conditions alone, however, cannot adquately explain many of the negative results. It seems equally likely that the techniques used in a number of other investigations are simply not sensitive to the changes which do occur. This suggestion is consistent with the results of several CD studies done at neutral pH and high Mg^{2+} concentrations which indicate that no changes occur in secondary structure upon aminoacylation within the limit of one to two base-pair interactions (Hashizume & Imahori, 1967; Melcher et al., 1971; Wickstrom, 1971). Similarly, the results of two ^3H-exchange experiments, done under solution conditions similar to those used here, indicate little if any change in the extent of hydrogen bonding upon aminoacylation (Gantt et al., 1969; Englander et al., 1972). In light of the results presented here, we would suggest that neither of these techniques is sensitive to perturbations of the counterion distribution and electrostatic charge which could accompany relatively small changes in conformation.

We have demonstrated Mg^{2+}-dependent conformational and electrostatic transitions for tRNA at near-physiological solution conditions in response to aminoacylation. It is tempting to speculate that these transitions are relevant in regulating tRNA function. This hypothesis appears to be supported by recent experimental evidence. Kirillov and Odinzov (1978) have measured the poly(U)-directed binding of *E. coli* phe-$tRNA^{Phe}$ to *E. coli* 70S ribosomes using a nitrocellulose filter assay. Their results show a reversible, Mg^{2+}-dependent, cooperative transition between two "isomers" of the phe-$tRNA^{Phe}$, in which the high Mg^{2+} conformer has a binding constant [tRNA·70S·poly(U)] orders of magnitude larger than that of the low Mg^{2+} conformer. These authors speculate that such differences could provide the basis for ribosomal translocation. The large tRNA–ribosome binding differences described by these authors serve to illustrate the possible biochemical implications of the Mg^{2+}-dependent conformational/electrostatic changes identified in this study. Structural changes of this sort could provide the molecular basis for the specificity in assorted tRNA recognition/binding processes.

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Purification and Characterization of a Transformation-Dependent Protein Secreted by Cultured Murine Fibroblasts†

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ABSTRACT: The major excreted protein (MEP) of transformed mouse fibroblasts has been purified, and monospecific antisera against it have been prepared. Synthesis and secretion of this protein have previously been shown to be stimulated by transformation or treatment with tumor-promoting phorbol esters, but its function is still not known [Gottesman, M. M. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 2767–2771; Gottesman, M. M., & Sobel, M. E. (1980) *Cell (Cambridge, Mass.) 19*, 449–455]. The purified protein shows charge heterogeneity by two-dimensional gel electrophoresis; the major intracellular and extracellular species have a molecular weight of 35 000 and a p*I* of 6.8–7.3. The purified secreted protein contains approximately 5–10% neutral sugar by weight and binds specifically to a concanavalin A–Sepharose affinity column. Translation of messenger ribonucleic acid (mRNA) from cells actively synthesizing MEP in cell-free reticulocyte or wheat germ systems, which are reported to be unable to glycosylate translated proteins, results in a product of M_r 33 000 which is presumably devoid of neutral sugar. However, on two-dimensional electrophoresis, the MEP mRNA translation products continue to show charge heterogeneity similar to that seen in intact cells, suggesting that there may be multiple coordinately controlled mRNAs for MEP or a single mRNA species which can be translated in a variety of ways.

Malignant tumor cells are capable of local invasion and distant metastasis and, in many cases, also have effects on their host's blood supply, immune system, nervous system, and ability to assimilate nutrients. Some of these phenomena are presumed to be mediated by biologically active compounds secreted by the tumor cells. Ectopic secretion of a number of bioactive hormones by tumors has been described, and transformed cells in culture have been shown to release a number of factors affecting phenomena such as cell growth (Dulak & Temin, 1973; De Larco & Todaro, 1978), cell migration (Hammond et al., 1974; Burk, 1973), tumor angiogenesis (Klagsbrun et al., 1976), proteolysis (Unkeless et al., 1973; Chen & Buchanan, 1975), and protein phosphorylation (Senger et al., 1979).

Assuming that some of these putative biologically active compounds will be proteins, we have recently investigated the effects of transformation on protein secretion by cultured mouse 3T3 fibroblasts and found that transformation by various RNA viruses, a DNA virus, or chemical carcinogens dramatically stimulates (up to 100-fold) the secretion of a polypeptide of M_r 35 000 which we have called MEP[1] (Gottesman, 1978). Its rate of synthesis and secretion appears to be regulated at a pretranslational level since transformation controls levels of translatable mRNA for MEP (Gottesman & Sobel, 1980). Tumor-promoting phorbol esters also stimulate synthesis and secretion of MEP by regulating translatable mRNA levels (Gottesman & Sobel, 1980). Taken together, these data suggest that MEP may serve as a useful marker of both transformation and tumor promotion and that an

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[1] Abbreviations used: MEP, major excreted protein of transformed mouse fibroblasts; NIH, NIH Swiss 3T3 cells; KNIH, Kirsten virus transformed NIH cells; BSA, bovine serum albumin; $NaDodSO_4$, sodium dodecyl sulfate; Cl_3AcOH, trichloroacetic acid; phosphate-buffered saline, Dulbecco's phosphate-buffered saline without Ca^{2+} or Mg^{2+}.