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Structure of Phenylalanine-Accepting Transfer Ribonucleic Acid and of Its Environment in Aqueous Solvents with Different Salts

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ABSTRACT: Yeast $tRNA^{Phe}$ was studied in different salt-containing solvents by UV absorbance and small-angle neutron scattering (SANS). This extends results obtained previously in NaCl and KCl solutions [Li, Z.-Q., Giegé, R., Jacrot, B., Oberthür, R., Thierry, J. C., & Zaccai, G. (1983) *Biochemistry 22*, 4380-4388]. As expected, at low concentrations of all salts studied, the tRNA molecule is unfolded. The importance of specific counterion interactions and the flexibility of the macromolecule are emphasized by the observation that it cannot take up its folded structure in $N(CH_3)_4Cl$ solvents, even when that salt concentration is increased to 1 M, in the absence of Mg ions. In CsCl solvents, on the other hand, the folded conformation is obtained in salt concentrations above about 0.2 M, similar to NaCl or KCl. By a comparison of SANS results in CsCl H_2O and CsCl ^2H_2O solvents with the data from NaCl and KCl solvents, thermodynamic and structural parameters were derived for the solvated macromolecule. All the data are accounted for, quantitatively, by a model for the particle in NaCl, KCl, or CsCl solution made up of $tRNA^{76-}$, closely associated with 76 positive hydrated counterions, surrounded by an aqueous solvent layer that excludes salt (and, therefore, of density different from that of bulk solvent). The mass of water in that layer depends on salt concentration, and the values found are consistent with those predicted by the Donnan effect.

Specific effects of salts on nucleic acid interactions have been known and studied for several decades [e.g., Chargaff et al. (1953), Latt and Sober (1967), review by Von Hippel and Schleich (1969), and Li et al. (1983)]. They remain incompletely understood, however, even though their importance is firmly established. Latt and Sober (1967a,b) studied the binding of synthetic oligopeptides to synthetic double-stranded polynucleotides as a function of salt and showed that there were specific effects associated with different cations. They interpreted their results in terms of competition between cation and oligopeptide for nucleic acid binding. Von Hippel and Schleich (1969) reviewed studies of salts and macromolecules

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and concluded that salt effects on DNA are of two types: electrostatic and lyotropic. The first are dominant at low salt concentrations where the native DNA conformation is stabilized by cations neutralizing the negatively charged phosphate groups. At high salt concentrations, electrostatic effects are saturated and the helix-destabilizing properties of different salts follow the lyotropic (or Hofmeister) series that ranks salts in aqueous solution, phenomenologically, according to their ability to increase the solubility of nonpolar chemical groups. More recently, hydration and cation binding in nucleic acids were explored by high-resolution crystal studies of oligodeoxynucleotides (Dickerson et al., 1982), and different hydration networks were proposed for different structural forms.

Transfer RNAs are good models for the study of solvent effects on nucleic acid structure. They are small, well-defined macromolecules that participate in many important biological processes [review by Schimmel and Redfield (1980)], and, as expected from their polyionic character, their biological interactions are strongly affected by salt (Fritzinger & Fournier, 1980; Dessen et al., 1979; Giegé et al., 1982). A few tRNA crystal structures have now been solved, and they all show L-shaped molecules with small structural differences. The most recent study is of yeast $tRNA^{Asp}$ (Moras et al., 1980). Structural information from high-resolution crystallography is the most detailed available, but crystallizing a macromolecule in different environments and solving its structure and that of the solvent molecules interacting with it are not easy tasks. Small-angle neutron scattering (SANS) from solution provides limited structural information but has the advantage that any solvent condition can be examined, and through contrast variation with the solvent, complex structures can be resolved into their different components (Zaccai & Jacrot, 1983). It is an approach that provides information at an intermediate resolution between biochemical binding studies, on the one hand, and crystallography, on the other. Yeast $tRNA^{Phe}$ was studied by SANS in NaCl and KCl solutions (Li et al., 1983). The quantitative results emphasized that the "particle" in solution is different for each condition. The particle is not just the macromolecular polynucleotide chain but includes also its interactions with counterions and solvent molecules, i.e., that volume of solution, associated with the macromolecule, different from bulk solvent. It is the particle that participates in biological and scattering processes and not the polynucleotide chain alone. The present work is an extension of the Li et al. (1983) study to other salts, in particular CsCl and $N(CH_3)_4Cl$ (TMA-Cl). These are not physiological conditions, but, because of their extreme natures, they are relevant to the understanding of the NaCl and KCl solutions. Cs^+ is the largest ion of the alkali-metal family but has a similar ranking to Na^+ in the lyotropic series. TMA^+ is a very large ion that, in the lyotropic series, decreases the solubility of nonpolar groups when compared to Na^+ or K^+; on this basis it would be expected to stabilize a folded conformation.

Materials and Methods

Transfer RNA. Preparation of Solutions. Pure brewer's yeast $tRNA^{Phe}$ was acquired from Boehringer (Mannheim, Germany). A weighed amount of the powder was dissolved in 1 mL of buffer A [10 mM sodium cacodylate, pH 7.0, 0.1 mM ethylenediaminetetraacetic acid (EDTA)] and dialyzed against buffer A with added 1 M NaCl and 5 mM EDTA in order to eliminate divalent cations and establish well-defined salt conditions. It was dialyzed, subsequently, against buffer A with no additions. This sample was named tRNA stock A. Concentration was determined by UV absorption; samples were diluted in buffer A plus 10 mM $MgCl_2$, and the concentrations was calculated by taking 1.86 nmol/A_{260} unit (Guéron & Leroy, 1978). Other salt solutions were prepared with the appropriate concentration in buffer A; for example, "0.2 M TMA-Cl" is a solvent of buffer A with 0.2 M TMA-Cl. In all such salt solutions, the pH was adjusted to 7.0 after the addition of salt to the buffer. All volume measurements were done with high-precision Petersen pipets.

UV Absorption Experiments. Stock A was made with a tRNA concentration close to 1 mg/mL. Four hundred microliters of each salt solution was measured into Eppendorf tubes. Four tubes were prepared for each salt condition, and to each tube was added 5 μL of stock A. The tubes were capped, put on a vortex mixer, and centrifuged briefly in an Eppendorf centrifuge to homogenize the solution and collect any drops trapped on the sides or below the cap. The solutions were transferred to spectrophotometer quartz cuvettes, and the UV absorption was measured in a double-beam spectrophotometer against the solvent alone. For each salt condition, the average of the measuremnts on the four identical solutions was taken. Concentration dependence of the UV absorption was measured by diluting samples with the appropriate solvents in Eppendorf tubes followed by vortex mixing and centrifugation.

Thermal melting curves were measured on a Beckman Model 25 spectrophotometer with an in-house modification for temperature scans.

Neutron Scattering. SANS experiments were performed on the D11 camera at the Institut Laue-Langevin in Grenoble (Ibel, 1976). Samples were contained in quartz spectrophotometer cuvettes of 0.100-cm path length for H_2O solvents and of 0.200-cm path length for ^2H_2O solvents. Scattering data were collected in the scattering vector range $0.01 < q < 0.1$ Å^{-1} with an 8% wavelength bandwidth and exposures of about 0.5 h. Data were put on an absolute scale by normalizing to the scattering of 0.100 cm of H_2O (Jacrot & Zaccai, 1981). Scattered intensities were analyzed in the Guinier approximation (Guinier & Fournet, 1955)

$$\ln I(q) = \ln I(0) - \tfrac{1}{3}R_g^2 q^2$$

from which are derived $I(0)$ and the radius of gyration of contrast in the particle, R_g. The scattering parameter $q = (4\pi \sin\theta)/\lambda$ (θ is half the scattering angle and λ the neutron wavelength). The forward scattered intensity $I(0)$ can be expressed in terms of thermodynamic variables of the solution (Eisenberg, 1981)

$$I(0) = (c_2 M_2/N_A)(\partial\rho_N/\partial c_2)_{\mu\neq\mu_2} \quad (1)$$

where subscripts refer to the solution component (1 = water, 2 = macromolecule, 3 = salt), c_2 is mass concentration, M_2 is molar mass, N_A is Avogadro's number, and $(\partial\rho_N/\partial c_2)_{\mu\neq\mu_2}$ is the neutron-scattering density increment at constant chemical potentials μ_1 and μ_3 (i.e., for a sample of concentration c_2 in dialysis equilibrium with a bath of μ_1, μ_3) given by

$$(\partial\rho_N/\partial c_2)_{\mu\neq\mu_2} = b_2 + \xi_3 b_3 - \rho_N^\circ(\bar{v}_2 + \xi_3\bar{v}_3) \quad (2)$$

where b_i is the scattering length per unit mass of component i, $\bar{v}_i$ is the partial specific volume of i, ρ_N° is the neutron-scattering density of the solvent, and ξ_3 is an interaction parameter (mass of salt per mass of macromolecule) (Eisenberg, 1981). If the macromolecule were added to a salt solution, it would perturb the salt–water ratio through preferential interactions; ξ_3 is the mass of salt required per mass of macromolecule to reestablish chemical potential equilibrium of water and salt when the macromolecule solution is dialyzed against the initial salt solution. The interaction can be expressed equivalently in terms of a parameter ξ_1 for water, with

Table I: Experimental Scattering Density Increments and Parameters Required for Their Interpretation

condition	$\lvert\partial\rho_N/\partial c_2\rvert^a$ (10^9 cm/g)	$\rho_N{}^{0b}$ (10^9 cm^{-2})	$b_2{}^b$ (10^9 cm/g)	$b_3{}^b$ (10^9 cm/g)	$\xi_3{}^c$ (g/g)	$\bar{v}_3{}^d$ (cm^3/g)	$\bar{v}_2{}^e$ (cm^3/g)
0.3 M CsCl in 95% ^2H_2O	7.5 ± 0.2	60.4	18.3	5.41	−0.098	0.24	0.442 ± 0.003
0.9 M CsCl in 95% ^2H_2O	7.8 ± 0.2	60.0	18.3	5.41	−0.148	0.24	0.458 ± 0.003

[a] Obtained from $I'(0)/c_2Tt = 10^{-3} f(\lambda)\, 4\pi/(1 - T_{H_2O})\,(M_2/N_A)\,(\partial\rho_N/\partial c_2)^2$, where $I'(0)$ is the observed forward-scattered intensity divided by the incoherent scattering of 0.100 cm of water (H_2O) in the same conditions, T is the transmission of the sample solution, t is the path length (0.100 cm), $f(\lambda)$ is a wavelength-dependent normalization factor, 0.79 for $\lambda = 7$ Å in this case, T_{H_2O} is the transmission of 0.100 cm of H_2O (0.52 in this case). [b] Calculated from the chemical composition. [c] ξ_3 was estimated from interaction parameters found for DNA by Cohen and Eisenberg (1968). [d] Calculated from the mass density of CsCl solutions (Cohen & Eisenberg, 1968). [e] Calculated from the data in the table and eq 2.

$\xi_1 = -\xi_3/w_3$, where w_3 (g of salt/g of water) is the mass molality of the solvent (Eisenberg, 1981).

Results

Yeast tRNA^{Phe} was studied in the following solvents: 10 mM sodium cacodylate, pH 7.0, plus a given salt [LiCl, NaCl, CsCl, TMA-Cl, $(NH_4)_2SO_4$] at a given concentration (0–1 M). All conditions were characterized by UV absorbance measurements. Thermal denaturation curves and SANS experiments were performed in selected cases. The molar absorbance of tRNA at 260 nm is sensitive to its conformation, and the value for a solvent with 10 mM $MgCl_2$ was taken for the native state (Guéron & Leroy, 1978). Thermodynamic [$I(0)$] and structural (R_g) parameters were derived from the SANS experiments.

Ultraviolet Absorbance Measurements. The folded-state molar absorbance value at 260 nm is defined as that obtained in 10 mM $MgCl_2$. It was found also for 0.2 M LiCl and for the other monovalent cations at concentrations higher than 0.2 M. The salts NaCl and CsCl at 0.2 M and $(NH_4)_2SO_4$ at 0.1 M gave absorbance values that were higher by 4%. The buffer (10 mM Na^+) in the absence of added salt gave a value 12% higher, and the highest absorbance, 17% higher than for 10 mM $MgCl_2$, was observed for TMA-Cl solvents (0.2–1.0 M). This high absorbance is similar to the value for tRNA in distilled water in which the molecule is presumed to have an extended conformation (Cole et al., 1972; Guéron & Leroy, 1978).

Thermal melting curves were measured for 0.2 M TMA-Cl, 0.2 M NaCl, and 10 mM $MgCl_2$. A fairly sharp transition at 79 °C was observed for 10 mM $MgCl_2$. The transition in TMA-Cl is very broad and almost covers the entire temperature range scanned. It is slightly narrower in 0.2 M NaCl with $T_m \approx 60$ °C. All the scans were performed with increasing temperature. The transitions were not perfectly reversible.

The absorbance measurements showed that tRNA cannot take up its folded conformation and stability in TMA-Cl solvents, even at very high concentrations of the salt. To explore this effect further, the absorbance was measured in mixtures of TMA-Cl with NaCl and $MgCl_2$. Experiments were performed with tRNA concentrations of $\approx 10^{-6}$ M. There is competition between the two salts; NaCl tends to establish the native state of lower absorbance, while TMA-Cl inhibits its formation. In about equal concentrations of the two salts the native state is complete, suggesting a marginally higher affinity for Na^+ as a counterion. The experiment was repeated in 0.1 and 1.0 M TMA-Cl with similar results. In the competition experiment with Mg^{2+}, the starting solvent contained 0.2 M TMA-Cl. The affinity of the divalent cation to fold tRNA is so high that is was not possible to observe a titration curve. The minimum absorbance was reached at 0.1 mM, the minimum amount of $MgCl_2$ added.

Neutron-Scattering Experiments. The Guinier approximation scattering plots of tRNA in 1 M NaCl and 1 M TMA-Cl are shown together in Figure 1. The macromolecule has an extended conformation in TMA-Cl, with a radius of gyration of 35 Å. In 1 M NaCl, its radius of gyration is 23 Å, which corresponds to the folded-state value (Li et al., 1983).

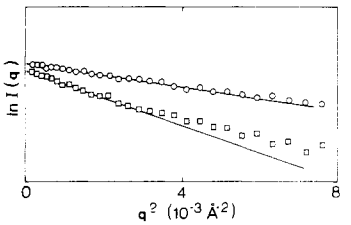

FIGURE 1: Neutron-scattering curve plotted as ln $I(q)$ versus q^2 of tRNA in 10 mM sodium cacodylate, pH 7.0, and 0.1 mM EDTA with added 1 M NaCl (○) or 1 M TMA-Cl (□). The concentration of tRNA was the same in both experiments, but the curves are displaced slightly for clarity.

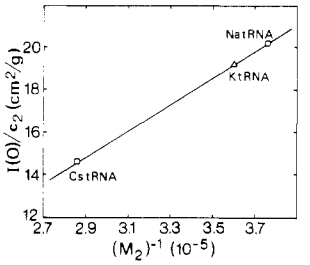

FIGURE 2: Forward-scattered intensity divided by concentration plotted against the reciprocal of molecular weight for tRNA in different salt solvents in H_2O buffer, 10 mM sodium cacodylate, pH 7.0, and 0.1 mM EDTA. Data for Na-tRNA and K-tRNA are from Li et al. (1983).

Neutron data were collected for different CsCl solvent conditions. Data in 0.3 M salt showed no concentration dependence between 4 and 12 mg/mL Cs-tRNA. Other data were collected with Cs-tRNA concentrations close to 5 mg/mL. Scattered intensity was put on an absolute scale and interpreted according to eq 1 and 2. In H_2O solvents it is a good approximation to write $\partial\rho_N/\partial c_2 \propto 1/M_2$ for Na-tRNA, K-tRNA, and Cs-tRNA. Then, from eq 1 $I(0)/c_2 \propto 1/M_2$. Data from Li et al. (1983) for Na-tRNA and K-tRNA are replotted as $I(0)/c_2$ versus $1/M_2$ for comparison with the present Cs-tRNA data in Figure 2. The straight-line relationship shows the precision and reproducibility of the neutron data, even when taken from different experiments more than 1 year apart.

The forward scattering per mole, $[I(0)/c_2]M_2$, in ^2H_2O solvents is shown in Figure 3. It is not constant for the different species (as it is in H_2O solvents, Figure 2) and increases with salt concentration in the solvent. In ^2H_2O solvents $\rho_N{}^0$ is large and positive and the $\bar{v}_i$ terms dominate $\partial\rho_N/\partial c_2$ (eq 2). The partial specific volume, $\bar{v}_2$, of Cs-tRNA was derived as a function of solvent salt from the scattering data from ^2H_2O by using eq 2 with values for the other parameters from Table I. The calculated partial molar volumes ($M_2\bar{v}_2$) are plotted in Figure 4.

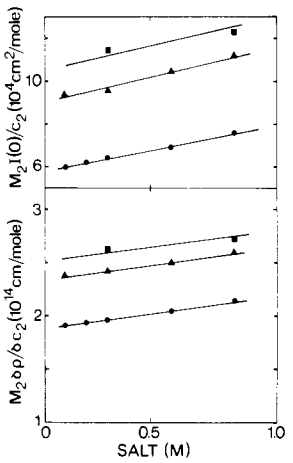

FIGURE 3: Forward-scattered intensity per mole (top panel) and scattering density increment per mole (bottom panel) of tRNA in different salt solvents in ^2H_2O buffer: 10 mM sodium cacodylate, pH 7.0, and 0.1 mM EDTA, as a function of salt concentration. Data for Na-tRNA and K-tRNA are from Li et al. (1983).

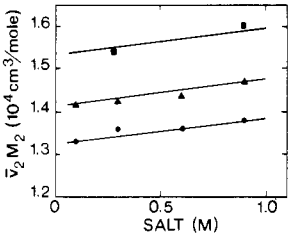

FIGURE 4: Molar volume of tRNA in different salt conditions calculated from the neutron-scattering data.

The radii of gyration found for the CsCl conditions are 23.5 and 20 Å for H_2O and ^2H_2O, respectively, independent of salt concentration.

Discussion

Type of Counterion and tRNA Conformation. The UV absorbance and SANS data from tRNA in TMA-Cl solvents show that the macromolecule has an open conformation in these conditions, even at the highest salt concentrations examined. Thus, tRNA conformation does not depend on ionic strength but on salt type and concentration. The competition experiments (TMA^+, Na^+ and TMA^+, Mg^{2+}) and the ranking of TMA^+ in the lyotropic series as an ion that reduces the solubility of nonpolar groups and would therefore stabilize the folded macromolecule suggest that tRNA does not take up its folded conformation in TMA-Cl solvents because of unfavorable specific counterion interactions between TMA^+ and the nucleic acid phosphates rather than unspecific solvents effects. Latt and Sober (1967a,b) interpreted the inhibition of binding between oligopeptides and nucleic acids in TMA-Cl in terms of competition between the cation and the oligopeptide. This view is not incompatible with our results. The sensitive dependence of tRNA structure on the nature of the counterion is an indication of its flexibility, even at high ionic strength, and suggests that large conformational changes might occur when it interacts with aminoacyl tRNA synthetases, elongation factors or ribosomes. The effect of TMA-Cl on tRNA structure is shown schematically in Figure 5a.

Volume of tRNA. The partial molar volumes found for Na-tRNA, K-tRNA, and Cs-tRNA are shown in Figure 3. The differences in volume are 1100 ± 200 cm^3/mol between Cs-tRNA and K-tRNA and 850 ± 200 cm^3/mol between K-tRNA and Na-tRNA. Ionic volumes at infinite dilution are −6, 3, and 15 cm^3/mol for Na^+, K^+, and Cs^+, respectively (Millero, 1972). There are 76 negative charges on $tRNA^{Phe}$

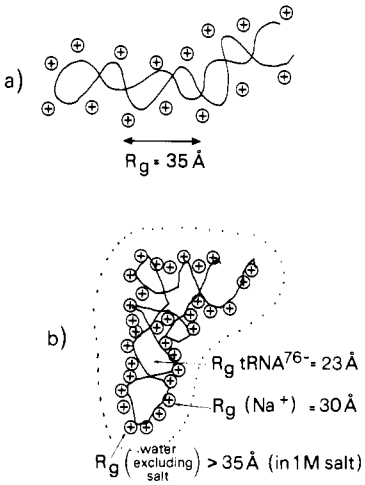

FIGURE 5: Schematic diagrams of tRNA structure in (a) TMA-Cl solvents and (b) NaCl, KCl, or CsCl.

at pH 7.0, with which are associated 76 positive counterions. The volume differences calculated are 912 cm^3/mol between 76 Cs^+ and 76 K^+ and 684 cm^3/mol between 76 K^+ and 76 Na^+. Ionic volumes increase with salt concentration, but the differences between them remain approximately constant. The data are consistent, therefore, with a molar volume for tRNA made up of the sum of the volume of $tRNA^{76-}$ and the volume of 76 counterions. In this hypothesis, the volume of $tRNA^{76-}$ alone in 0.2 M salt is calculated to be 14 000 cm^3/mol or 23 000 Å^3 for the molecule. Similar to DNA (Cohen & Eisenberg, 1968), the partial volumes of Na-tRNA, K-tRNA, and Cs-tRNA are observed to increase with salt concentration (Figure 3); this is probably because of the counterion contribution increasing following less electrostriction of the water relative to the bulk solvent, the molar volume of $tRNA^{76-}$ remaining constant. It is interesting to note that the partial specific volumes calculated from SANS ^2H_2O data for Cs-tRNA in this paper and for Na-tRNA by Li et al. (1983) are virtually identical with the respective values for Cs-DNA and Na-DNA found by Cohen and Eisenberg (1968) by conventional means. This is quite remarkable considering that the only input from the DNA data are values of ξ_3, which have a small effect on the absolute value for $\bar{v}_2$.

Solvent Environment of tRNA. The solvent environment of polyions in salt-containing solution is expected to be different from that of bulk solvent for a number of reasons: salt is excluded due to the macromolecule charges and its counterions (the Donnan effect); there is electrostriction around counterions; and there are hydration networks linking counterions and charges on the marcomolecule. In general, this associated solvent layer will contribute to the observed radius of gyration in a SANS experiment if there is contrast between it and bulk solvent, i.e., if its neutron-scattering density is significantly different from that of bulk solvent [review by Zaccai and Jacrot (1983)].

In the previous paper, the radii of gyration observed for Na-tRNA and K-tRNA were interpreted in terms of a "denser than bulk" solvent layer surrounding the macromolecule (Li et al., 1983), control experiments having shown that the lower value of radius of gyration observed in ^2H_2O solvents was not due to a conformational change of the macromolecule but to a contrast variation effect. The particle was analyzed as having two components: the macromolecular chain and its associated solvent layer. In H_2O solvents the contrast between this solvent layer and the bulk solvent is negligible, so that the R_g observed is that of the macromolecular chain. In ^2H_2O solvents, a denser than bulk solvent layer has positive contrast, while the

Table II

condition	contrast of water associated with ξ_1 (10^{-12} cm/molecule of tRNA)	contrast of 76 counterions (10^{-12} cm/molecule of tRNA)
1 M NaCl	14.5×10^{-12} cm	75×10^{-12} cm
1 M KCl	43.7×10^{-12} cm	-4.3×10^{-12} cm
1 M CsCl	72.7×10^{-12} cm	-91.2×10^{-12} cm

macromolecular chain has negative contrast; this leads to an R_g of the particle which is smaller than that of the macromolecule alone. Because it was expected to be small, the Donnan effect contribution, i.e., the difference in density between the water (excluding salt) associated with tRNA and the bulk salt solvent, was neglected in the interpretation of Li et al. (1983). This difference, however, is emphasized in CsCl solvents, and we show here that it had significant influence on the SANS data in NaCl and KCl as well.

We shall consider the R_g of the tRNA to be made up of contributions from the nucleic acid chain itself, from the water excluding salt, and from the hydrated counterions. The last two, the contributions of the associated solvent, will be considered separately.

(1) The water associated with tRNA in a given salt solvent is the sum of the water of hydration and the exclusion of salt by the Donnan effect (Eisenberg, 1981). The former is a constant, but the latter is strongly solvent dependent; to exclude a constant amount of salt, a larger volume of water is required at lower salt concentrations than at higher salt concentratons. Thus, in 1 M NaCl, the interaction parameter for water, ξ_1 = 1.1 g/g of tRNA, and in 0.2 M NaCl this increases to 4.8 g/g or 1600 and 7000 mol of water/mol of Na-tRNA, respectively. In ^2H_2O solvents, this associated water is pure ^2H_2O and the bulk solvent is the appropriate salt solution in ^2H_2O. The neutron-scattering length densities of ^2H_2O salt solutions are smaller than that of pure ^2H_2O. The water associated with tRNA through the Donnan effect, therefore, has higher neutron-scattering density than the bulk solvent, independent of electrostriction. We calculated the excess neutron-scattering length over bulk solvent of the salt excluding water associated to one molecule of tRNA for different solvents (Table II): $\xi_1(b_1 - \rho_N{}^\circ v_1)(M_2/N_A)$. Note that in lower salt concentration the difference in neutron-scattering density between bulk solvent and water is smaller, but the number of water molecules associated with macromolecules is larger.

(2) The counterion contribution to the scattering of one tRNA molecule is $76(b_c - \rho_N{}^\circ v_c)(M_c/N_A)$, where M_c, b_c, and v_c are the counterion molar mass, specific scattering length, and partial specific volume, respectively. These values are also given for 1 M salt in ^2H_2O solvents in Table II. In NaCl, both water and counterion contributions are positive. In KCl, the counterion contribution is small, and the balance is positive. In CsCl, the balance is negative, the counterion having a larger volume. The R_g values measured in CsCl, however, are similar to the values in NaCl and KCl. If the Cs counterions were distributed homogeneously in the associated solvent layer, it would have a negative contrast in ^2H_2O similar to that of the macromolecular chain, leading to a larger R_g than in H_2O. The observed R_g, however, is smaller in ^2H_2O. This means that the Cs^+ counterions are not distributed homogeneously in the water surrounding tRNA but that they are close to the molecule, the "denser" water extending to larger radii, its positive contrast contributing to the smaller R_g value.

In light of these considerations, the Li et al. (1983) results should be reinterpreted. The "observed" solvent perturbation was calculated for the NaCl and KCl conditions by Li et al. (1983), and excess scattering lengths of $146 \pm 10 \times 10^{-12}$ and $42 \pm 10 \times 10^{-12}$ cm were found at 0.9 M NaCl and KCl, respectively. The values calculated from the associated water and counterion contributions (Table II) are 90×10^{-12} and 39×10^{-12} cm, respectively, in fair agreement with the Li et al. (1983) values. The radius of gyration of the perturbation was found to be smaller for NaCl ($\simeq$30 Å) than for KCl ($\geq$35 Å) (Li et al., 1983). Since in KCl the counterion contribution is small compared to that of the associated water and vice versa for NaCl, we can conclude that the R_g of the perturbation in KCl is essentially that of the water, and, that in NaCl it is essentially that of the Na^+ counterions. The tRNA solution environment is solvent dependent, and it is shown schematically in Figure 5b for 1 M NaCl. Na^+ counterions are distributed with an R_g of 30 Å; the macromolecular chain has an R_g of 23 Å, which is the value calculated from the crystal structure and observed in H_2O solvents. At lower salt, the water layer extends further out, but the difference in density between it and bulk solvent is smaller than at 1 M salt.

Registry No. TMA-Cl, 75-57-0; NaCl, 7647-14-5; KCl, 7447-40-7; CsCl, 7647-17-8.

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