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On the Hydration of DNA. I. Preferential Hydration and Stability of DNA in Concentrated Trifluoroacetate Solution

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Synopsis

The hydration of DNA is an important factor in the stability of its secondary structure. Methods for measuring the hydration of DNA in solution and the results of various techniques are compared and discussed critically. The buoyant density of native and denatured T-7 bacteriophage DNA in potassium trifluoroacetate (KTFA) solution has been measured as a function of temperature between 5 and 50°C. The buoyant density of native DNA increased linearly with temperature, with a dependence of $(2.3 \pm 0.5) \times 10^{-4}$ g/cc-°C. DNA which has been heat denatured and quenched at 0°C in the salt solution shows a similar dependence of buoyant density on temperature at temperatures far below the T_m and above the T_m. However, there is an inflection region in the buoyant density versus T curve over a wide range of temperatures below the T_m. Optical density versus temperature studies showed that this is due to the inhibition by KTFA of recovery of secondary structure on quenching. If the partial specific volume is assumed to be the same for native and denatured DNA, the loss of water of hydration on denaturation is calculated to be about 20% in KTFA at a water activity of 0.7 at 25°C. By treating the denaturation of DNA as a phase transition, an equation has been derived relating the destabilizing effect of trifluoroacetate to the loss of hydration on denaturation. The hydration of native DNA is abnormally high in the presence of this anion, and the loss of hydration on denaturation is greater than in CsCl. In addition, trifluoroacetate appears to decrease the ΔH of denaturation.

INTRODUCTION

The nature of the interaction of DNA with water is central to the study of the structure of DNA since, as is well known, water is an important factor in preserving the helical form and in fact is probably necessary to this structure. X-Ray diffraction and polarized infrared and ultraviolet spectroscopy of oriented fibers of DNA show that the B form of NaDNA is stable only over a limited range of relative humidity (above 75–80%), and that below 55% RH there appears to be a general loss of ordered structure.[1,2] Falk et al.[2] have shown that under these conditions DNA fibers bind definite quantities of water at specific sites. The humidity range below which water molecules begin to be removed from the grooves of the helix, i.e., from contact with the bases, appears to be the same

range below which helical structure disappears by the x-ray diffraction and spectroscopic evidence.

Hydration of DNA in solution, however, and its influence on structure is less well defined. As the relative humidity becomes very high, the macroscopic orientation of the fibers is lost, and clear diffraction patterns cannot be obtained. Above about 80% RH, water is apparently no longer bound to definite sites on the DNA molecule but is adsorbed on the water layer already present on the surface of the molecule. It is commonly assumed that the structure in solution is close to the B form observed in the fibers, and there is no reason to believe that any profound changes in structure occur.[3,4] There is considerable evidence that the binding of water molecules to the helix is necessary to the structure in solution as well. The native helical form is stable only in aqueous solvents (with a few exceptions[5,6]). Although every method of measuring bound water in solution seems to give a different value from all others, all methods indicate that there is at least a small amount of water bound firmly by the DNA molecule. It seems that the DNA must influence a considerable amount of the water in its neighborhood, the influence decreasing with the distance from the DNA molecule. Since each method of measuring hydration is sensitive to different factors in this region of affected water, that measurement will tend to show the cut-off in the hydration layer at a different point.

There is some evidence that DNA in solution may undergo some changes in structure, perhaps analogous to the changes in the fibers as a result of varying the water content of the fibers. High concentrations of some salts, and the mixtures of nonaqueous and aqueous solvents can produce changes in some properties—optical density, optical rotation, viscosity—which do not imply denaturation, but may be associated with subtler changes in structure.[7–12] The hypothesis has been made that these structural changes may be a result of changes in solvent structure; it is not unreasonable to think that they may be a direct result of changes in the structure or size of the hydration "layer" around the DNA.

Types of Hydration Studies

Different types of experiments give different values for water of hydration. This is not surprising when one realizes the wide variety of parameters being measured. The x-ray diffraction experiments indicate that no water molecules are bound firmly and regularly enough to the helix to contribute to the crystal structure of DNA,[13] but there are several other ways by which water may be associated with the molecule.

Nuclear magnetic resonance and infrared spectroscopy look at the average properties of all the water molecules. Most of the early NMR experiments were apparently invalid due to artifacts and errors in interpretation of data. Of the more recent work, neither NMR nor infrared techniques have been able to detect large changes in the water as a result of the addition of the DNA to the solution. However, all one can conclude from these results is that there is not a large amount of water whose properties

experience a large change due to the presence of DNA. If the hydration "shell" is small, and/or the properties of the bound water are not greatly different from those of free water, water of hydration might not have a large enough effect on the average water properties to be detectable by these means.

Lubas and Wilczok[14] measured proton magnetic relaxation times in DNA solutions by a spin-echo technique and obtained a value of 8–10% by weight of water, or only about 2 molecules of water per base, bound nonrotationally to the DNA. This may amount to only a small percentage of the water associated with the DNA, and is of the same order of magnitude as that measured by Falk et al.[2] in films at near 0% RH. The latter authors attributed this most tightly bound water to binding by the phosphate groups.

Several authors have observed a broadening of the water proton resonance peak in the presence of DNA and have attributed it to various factors. Discussions by Kavanau[15] and by Gordon et al.[16] indicate that this broadening may be due to protons in a small hydration shell with a shorter transverse relaxation time than free water molecules. These observations are not inconsistent with the hydration values obtained by Lubas and Wilczok[14] or with larger values measured by other investigators.

Infrared studies by Depireux and Williams[17] on DNA in solution did not reveal any changes in the properties of water due to the presence of DNA. However, it is not certain that they would be expected to do so.[15] Infrared spectroscopy has been very helpful in studying the properties of the water of hydration of DNA in solid films,[2] but under the latter conditions these properties are not masked by the properties of the large amounts of free water in solution.

The above methods are apparently able to detect only the most tightly bound water. Hydrodynamic measurements, on the other hand, yield an average amount of water which migrates with the DNA through the solution. This water may exchange very rapidly with the bulk water and thus may be indistinguishable from the bulk by the above techniques.

Wang's measurements[18] of the self-diffusion coefficient of H_2O in DNA solutions appear to give the most reliable values for the average hydration. He obtained a value of 35% by weight hydration for NaDNA, or about 6.5 molecules water per base, which is about enough to fill the grooves.

Hearst and co-workers[19,20] calculated an effective hydrodynamic diameter for the DNA molecule, using data on the variation of sedimentation coefficient with molecular weight. They obtained a value of 26–27 Å for the hydrated DNA molecule, which corresponds to an extra layer of water molecules around the helix. However, this is not a precise method for determining hydration, and the approximations used to obtain this value are such that this is in reasonable agreement with Wang's value.

Electrical properties of DNA solutions have been extensively interpreted in terms of large shells of immobilized water around the DNA. However,

much of the electrical behavior can be adequately accounted for in terms of double-layer polarization and mobility of counterions. Thus these experiments are not very useful in studying hydrations.[15,21]

Another class of hydration measurements determines the amount of water preferentially associated with the DNA, in thermodynamic equilibrium with water at a given chemical potential, and yields curves of the form seen in Figure 1. Density gradient ultracentrifugation and isopiestic measurements of hydration both give this kind of information for DNA in a three component solution.[22,23] So do Cohen and Eisenberg's measurements of density increments at constant chemical potential of diffusible solutions.[24] The environment of the individual water molecules is irrelevant to this kind of measurement and may be indistinguishable on a microscopic scale from the bulk solution, since there may be a continuous decrease in the concentration of water with distance from the DNA molecule. Furthermore, this water cannot be necessarily considered to be bound to the DNA molecule. At high water activities, the hydration attains values much larger than that amount of water bound in a hydrodynamic sense. This is to be expected, since by the nature of this definition of hydration, the value measured will go to infinity for a polyelectrolyte as one approaches pure water as the solvent.[23]

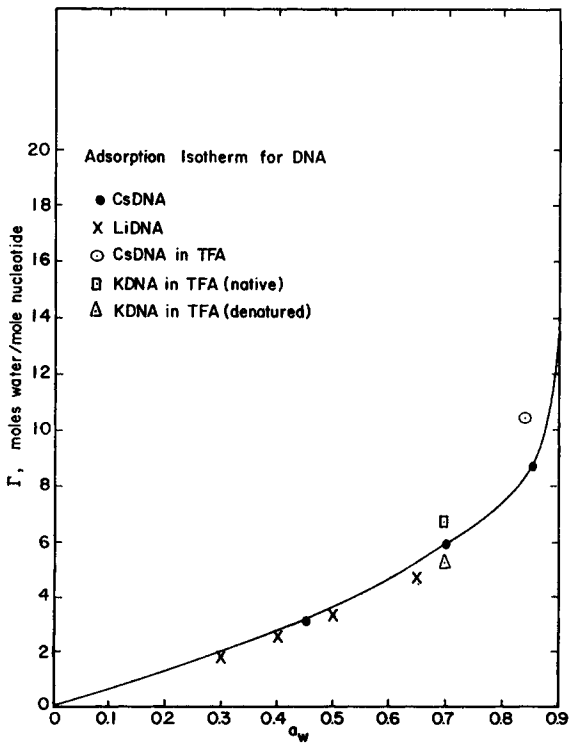

Fig. 1. Preferential hydration of DNA, determined from buoyant densities in the ultracentrifuge (TFA = trifluoroacetate).

Such a hydration of proteins and viruses observed by ultracentrifugation in a two-component solvent has widely been interpreted in terms of a steric exclusion of the more bulky component of the solvent from the neighborhood of the macromolecule.[25,26] This does not appear to be a significant factor in values of hydration measured for DNA. As previously discussed,[22] the assumptions on which this model is based are poor ones in the case of the ionic solutions in which DNA is centrifuged. The dependence of the thermodynamic hydration primarily on the chemical potential of water is well established by isopiestic and density measurements in solvents where there is no variation in size of solvent components and a steric exclusion mechanism could not account for the observed variation in hydration. Even in the ultracentrifuge, the hydration does not follow the trends predicted by this model, since decreasing values of hydration are measured as the size of the anion increases in the series chloride, formate, acetate.

Small-angle x-ray scattering experiments are also sensitive to a thermodynamic hydration. Luzzati et al.[4] interpreted a linear dependence of their experimental parameter $(A/c_e)^{1/2}$ on ρ_0 (electron density of solvent) to be due to a constant hydration of about 67% by weight. However, this is not the only valid interpretation of such a curve. Bruner and Vinograd,[27] in studying the dependence of the sedimentation coefficient of DNA on the solution density, have an experimental parameter which depends on hydration in an identical manner to that of Luzzati. They also observed a linear dependence on density. However, they showed that this data is reasonably consistent with the hydration values measured by Hearst and Vinograd.[22] They incorporated the data for the preferential solvation at the appropriate water activity into their equation:

$$s^0\eta_r f_s = M_3[1 + \Gamma' - \rho(\bar{v}_3 + \Gamma'\bar{v}_1)] \quad (1)$$

where s^0 is the observed sedimentation coefficient; η_r is the relative viscosity of the solvent; f_s is the frictional coefficient of the hydrated DNA; M_3 is the anhydrous molecular weight of DNA; $\bar{v}_3$ and $\bar{v}_1$ are the partial specific volumes of the DNA and water of hydration, respectively; and Γ' is the preferential hydration in grams water per gram DNA. They then plotted $s^0\eta_r f_s/M_3$ versus density of the CsCl solution. They found the plot to be linear and fit the data very well at densities between 1.3 and 1.9 (Fig. 2).

The appropriate equation for small-angle x-ray scattering is:

$$(A/c_e)^{1/2} = \mu^{1/2}[1 + \alpha - \rho_0(\psi + \alpha\psi_{H_2O})] \quad (2)$$

where A is determined experimentally; c_e is the concentration of DNA, measured as the ratio between the number of electrons of the DNA and the number of electrons of the solution; μ is the linear mass of anhydrous DNA, measured by the number of electrons in a segment of unit length; α is the preferential hydration, measured as the ratio between the number of electrons of bound water and the number of electrons of DNA; and ψ and ψ_{H_2O} are the partial specific volumes (per electron) of DNA and water,

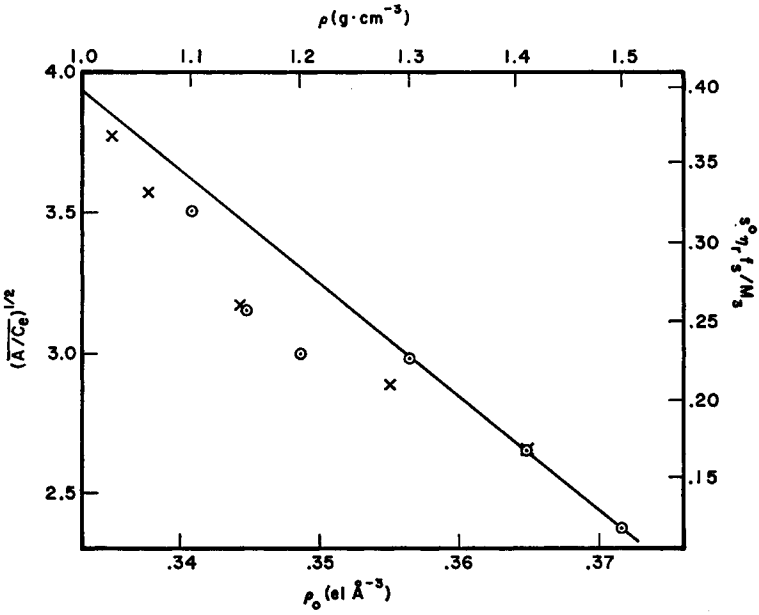

Fig. 2. Comparison of x-ray scattering[4] and sedimentation[27] data on DNA as a function of salt concentration: (——) linear behavior of experimental data; (×) values of $(A/C_s)^{1/2}$ in NaCl calculated by using eq. (2) and preferential solvation data of Hearst and Vinograd;[22] (⊙) similar treatment of sedimentation data in CsCl by Bruner and Vinograd.[27] Calculated values fit linear plot for densities between 1.3 and 1.9 g/cc.

respectively. If one substitutes the data of Hearst and Vinograd[22] into this equation and solves for $(A/c_s)^{1/2}$ as a function of the electron density of the solvent, the results are very similar to those obtained by Bruner and Vinograd[27] (Fig. 2). Unfortunately, the x-ray-scattering data exist only for salt concentrations up to 3M NaCl, which corresponds to a water activity of 0.88. This in turn corresponds to a CsCl density of about 1.41 which is near the low-density end of the linear portion of $s^0\eta_r f_s/M_3$ versus ρ curve. The negative deviations from linearity are of the same order of magnitude, and in the same range of water activity as those obtained by Bruner and Vinograd. There is every reason to believe that the agreement with the data of Hearst and Vinograd would be much better at lower water activities. The experimental parameters are very sensitive to the partial specific volume at high water activities. Cohen and Eisenberg[24] have observed a decrease in $\bar{v}$ of NaDNA and CsDNA at low salt concentrations. Although the values they measured for NaDNA do not fit Luzzati's data, such a variation in $\bar{v}$ might contribute to deviations from linearity at low a_w.

Luzzati[4] observed that the radius of gyration of the DNA decreased from 8.3 Å at 0.5M NaCl to 7.6 Å at 3.00M NaCl. Calculations of radii of gyration for simple models of hydration show that this is exactly the kind of change one would expect if the hydration depends on water activity

in the manner shown in Figure 1. Thus there appears to be no inconsistency between the x-ray-scattering data and other equilibrium measurements of hydration.

Pouyet et al.[28] have measured the sedimentation coefficient of DNA in solutions of various salt concentrations, and have attributed all variations of s to changes in the $(1 - \bar{v}\rho)$ factor. They thus claim to be measuring a thermodynamic hydration, although the values they obtained are very different from those measured by equilibrium methods. Their assumption that the friction factor is constant is probably a very poor one. Phosphate repulsion is likely to be still significant enough to contribute to increasing the friction factor at the lower ionic strengths they use. Furthermore, the sedimentation coefficient becomes extremely insensitive to the hydration term as the solution density gets closer to unity, and this is where their results are the most anomalous. An assumption that the friction factor decreases by 25% or less as the salt concentration is increased from their low-salt limit ($\leq 0.1M$) to concentrations greater than $1M$ brings most of their data into very close agreement with hydration values measured by Hearst and Vinograd.[22] Certainly, an equilibrium method is more reliable for the measurement of a thermodynamic hydration.

Measurements on DNA films and fibers in equilibrium with a humid atmosphere give very similar curves of hydration versus relative humidity to those obtained by density gradient and isopiestic methods as a function of water activity. Again in this case, a thermodynamic hydration is being measured by an equilibrium method. Falk et al.[2] have obtained some data on the form of the water by infrared spectroscopy. They conclude that all hydration sites are filled at 80% RH where about 10 moles water per mole nucleotide are bound. At above 92% RH, the infrared bands are characteristic of those of liquid water. Below 80%, down to 55% RH, 4–5 water molecules per base are removed, mainly from the grooves. These authors conclude that the phosphates are the strongest binding sites, and bind approximately 2 molecules water per phosphate even at very low humidity. This information cannot necessarily be applied to DNA in solution, since the relative forces involved are different, but it gives some insight as to how the water might be bound.

Recent measurements of hydration by calorimetric means by Privalov and Mrevlishvili[29] are also interesting in this regard. They measured heat capacity versus temperature in the neighborhood of 0°C for DNA in the presence of varying amounts of water. They observed that about nine to ten molecules water per nucleotide can be added to the DNA without observing a phase transition in the water as the temperature is changed. They conclude that this water is bound in an ordered state. Above this amount, one begins to observe a transition with temperature, but it is still broad and the water shows the influence of the DNA. Two or three times this amount of water must be added before one observes heat capacity behavior typical of liquid water.

We might tentatively compare these data for solid DNA and DNA in solution and reach some general conclusions on the form of the water of hydration of DNA in dilute aqueous solution. There appears to be a very small amount of water, one or two moles per base, which is bound very firmly to the DNA. Then there is a layer of water up to one molecule thick, which is bound firmly enough to be carried along with the DNA molecule as a hydrodynamic unit, but which is exchanged very rapidly with the bulk water. Beyond this there is an expanse of water which cannot be considered bound to the DNA, but which contains a defficiency of salt as compared to the bulk solution and thus is influenced by the presence of the macromolecule.

The data on the hydration of denatured DNA is much less extensive than for the native form, but most evidence indicates that the former is slightly less hydrated. Centrifugation data would imply that the thermodynamic hydration decreases on denaturation, since there is an increase in density. Falk[30] observed a similar decrease for solid DNA by gravimetric measurements. Using the NMR technique, Lubas and Wilczok[14] observed a decrease in nonrotationally bound water by a factor of 1.45, but Gordon et al.[16] did not observe any change in hydration on denaturation. According to the calorimetric data of Privalov and Mrevlishvili,[29] the amount of immobilized water on denatured DNA is slightly higher than for native. It is not clear whether this observation conflicts with the other evidence, since again, the factors being measured are different.

Mechanisms of Hydration

There are several ways in which water molecules can be bound to or affected by the DNA molecule. There is a long-range electrostatic effect due to the charged phosphates and their counterions. One would expect that the most strongly bound water would be that bound to these ionic groups. Falk[30] has estimated that in films one phosphate group might strongly bind two molecules of water, while infrared studies indicate that at higher relative humidity there are about six water molecules hydrating the phosphate group.[2] The charged phosphates also lead to a Donnan-type exclusion of salt from the vicinity of the polyelectrolyte. Hearst[23] has calculated the hydration of DNA at various water activities expected on the basis of electrostatic long-range interactions, depending on an estimate of effective charge on the DNA molecule. The Donnan effect was found to account for only a part of the hydration measured by ultracentrifugal and isopiestic techniques, and a significant percentage of the hydration must be due to short-range interactions. These calculations show the electrostatic contribution to be between 30 and 75% at $a_w = 0.95$ in NaCl, the smaller value being more probable. The relative contribution of long-range interactions would be greater at high water activity and smaller at low water activity, and the values calculated are independent of the specific anion used. On denaturation, the charge density on the surface of the molecule decreases,[31,32] which might be expected to result in a decrease in this part of the hydration.

Short-range electrostatic interactions and hydrogen bonds should make an important contribution to hydration. One would expect dipole–dipole interactions between the neutralized phosphates and water molecules. Hydrogen bonding is probably quite extensive, since there are several hydrogen-bonding groups on the bases and sugar which are exposed to the solvent even in the helical conformation, and on denaturation the specific interbase bonding sites also become available to water. The dipole moments of the bases[33] are probably pretty well shielded in the native structure, but for denatured DNA there may be considerable dipole–dipole and charge–dipole interaction with the solvent. In fact, one explanation for the effect of some anions in facilitating the denaturation of DNA involves the direct interaction of these anions with the bases, perhaps by a partially hydrophobic mechanism.[34,35] This would influence the value of the preferential hydration measured.

The extent to which "hydrophobic hydration" would contribute to measured values of hydration is uncertain. Since there are so many electrostatic groups associated with the DNA molecule, and the bases themselves are so polar, it is uncertain whether hydrophobic bonding is really a factor in the DNA stability, in Franks'[36] sense of increased structuring of water due to the presence of a nonpolar solute. Thermodynamic and hydrodynamic measurements do not give direct evidence as to the structure of the water, and NMR has indicated only that there is a lack of extensive ordering. The infrared studies of Depireux and Williams[17] also gave negative evidence. The most extensive data in this regard are those of Falk et al.[2] on the structure of water adsorbed on DNA films. They conclude that since the infrared spectrum of this water is very similar to that of films of pure liquid water, there is no evidence for the existence of "icebergs." However, they also found that the OH-stretching frequency of water in DNA increases with increasing relative humidity, indicating that the average strength of hydrogen bonding decreases as subsequent molecules absorb. Even at 92% RH, the water band at about 4.7 μ, which is the one Depireux and Williams[17] looked at in solution, is shifted to slightly but significantly lower wavelength. Thus one cannot rule out the possibility of a slight increase in icelike structure due to hydrophobic hydration.

The nature of hydrophobic interactions is salt solutions is especially obscure. Since the structure of water would be totally disrupted at appreciable salt concentrations, a nonpolar group might not be able to induce structure in the presence of salt, even though it did so in pure water. However, if one considers the surface tension factors discussed by Sinanoglu and Abdulnur,[37] one might expect to measure a net hydration under these conditions, since for many salt solutions interfacial surfaces are deficient in ions.[38]

Denatured DNA might be expected to induce more structure in the water than would native DNA by a hydrophobic mechanism, since the bases have considerable hydrophobic character and would afford relatively

few specific sites for interaction if they extend into the solvent. Sinanoglu and Abdulnur[37] have estimated that one base would be surrounded by about 50 water molecules, and since it might have only five to ten possible hydrogen-bonding sites, there might be a tendency to increase the water structure. This would be particularly true for thymine and adenine, whose dipole moments are much smaller than those of guanine and cytosine.[33]

The discussion of the hydration of denatured DNA is especially difficult, since its structure is so uncertain, and is quite variable with environment. For instance, there is probably considerable reformation of nativelike structure on cooling DNA below its T_m after denaturation. Under most conditions, the "coil" form in the helix–coil transition is a purely hypothetical state.

Thus the species involved in the helix–coil transition:

$$\text{DNA(native)} \cdot (H_2O)_x + \text{bulk solvent} \rightarrow \text{DNA (denatured)} \cdot (H_2O)_y + (x - y)H_2O + \text{bulk solvent}$$

are very poorly defined. Since the solvent is so critical for the stabilization of the helical structure, an adequate understanding of its stability requires a knowledge not only of the intrahelical interactions, but the DNA–solvent interactions both for native and denatured DNA. Furthermore, as discussed by Sinanoglu and Abdulnur,[37] one must also consider the difference of energy of the solvent in the presence of the two forms of DNA.

In this work, the buoyant density of native and denatured DNA in potassium trifluoroacetate solution has been measured as a function of temperature in an attempt to estimate the change in hydration of DNA on denaturation. It was of special interest to observe the buoyant density of denatured DNA above the transition temperature in order to avoid the effects of re-formation of structure on cooling. In the following paper,[39] the dependence of the net hydration of DNA on its base composition was measured by density gradient ultracentrifugation. Although a large amount of the water of hydration is probably due to binding by the phosphate groups and counterions, any water bound specifically by the bases might be expected to vary with the kind of base. Since the buoyant density of DNA depends on the guanine–cytosine content, it seemed worth attempting to determine if this is due to differences in hydration of the two base pairs. Any quantitative information of this sort should be useful in determining the nature of the interactions of water with the bases.

EXPERIMENTAL

Denatured DNA at room temperature has a higher buoyant density than native DNA, in both CsCl and Cs_2SO_4. This could be due to two factors: either the partial specific volume of denatured DNA is smaller than of native DNA, or the denatured form is less hydrated in solution. We have considerable reason to believe that the major part of the effect

is due to the latter cause. We therefore studied the buoyant density of native and denatured DNA as a function of temperature in a potassium trifluoroacetate (KTFA) density gradient in order to determine the change in hydration on denaturation and obtain estimates of the enthalpy of hydration of native and denatured DNA. KTFA was used because the temperature of denaturation is much lower in this salt than in the standard salts used for centrifugation experiments, and we wanted particularly to measure the density of denatured DNA above the transition temperature (T_m) in order to be sure we were looking at the fully denatured form.

The most convincing evidence we have that the partial specific volume of native and denatured DNA are the same comes from the recent experiments of Chapman and Sturtevant,[40] who measured the apparent specific volume of calf thymus DNA in dilute buffer as a function of temperature through the transition region and found no sign of an apparent specific volume versus temperature transition at neutral pH. This is not surprising, since for proteins the partial specific volume is quite insensitive to secondary or tertiary structure. The $\bar{v}$ of proteins of many different structures can be predicted to within 1% from their amino acid composition.[41] To account for the density differences observed in CsCl, denatured DNA would have to have a partial specific volume 1.3% smaller than native; in Cs_2SO_4, 4.0% smaller; and from the present experiments, in KTFA, 6.4% smaller. These differences are much larger than would be expected for different secondary structures of the same molecule, especially in the presence of high concentrations of salt, where any different effects on the water structure would be expected to be small.

Materials and Methods

Bacteriophage T-7 DNA was used in all experiments in this section. The DNA was extracted from phage (gift of Horace B. Gray) according to the phenol procedure of Mandel and Hershey[42] and stored in a phosphate buffer (0.1*M* NaCl, 0.1*M* Na_2HPO_4 + NaH_2PO_4, 0.001*M* EDTA).

The KTFA stock solution was prepared by titrating distilled trifluoroacetic acid with a filtered saturated solution of K_2CO_3 to a pH of 7.5–8.0. The titration was done at about 50–60°C with vigorous stirring to drive off CO_2 as it was liberated. The thermal expansion of the KTFA solution was measured by using a 1-ml Gay-Lussac specific gravity bottle calibrated with mercury. The pycnometer containing the solution was immersed in a water bath at a given temperature and allowed to come to thermal equilibrium. The volume of the solution was adjusted to the calibrated volume, and the pycnometer was then weighed. The value obtained for the thermal expansion coefficient was $6.4 \times 10^{-4}°C^{-1}$ for a solution whose density at room temperature was about 1.50 g/cc (Fig. 3). Water activity as a function of solution density was determined isopiestically as previously described,[22] and the results are shown in Figure 4. The exact concentrations of KTFA in solutions of various densities were not determined. In a solution of density 1.48 g/cc the KTFA concentration is about 5.0*M*.

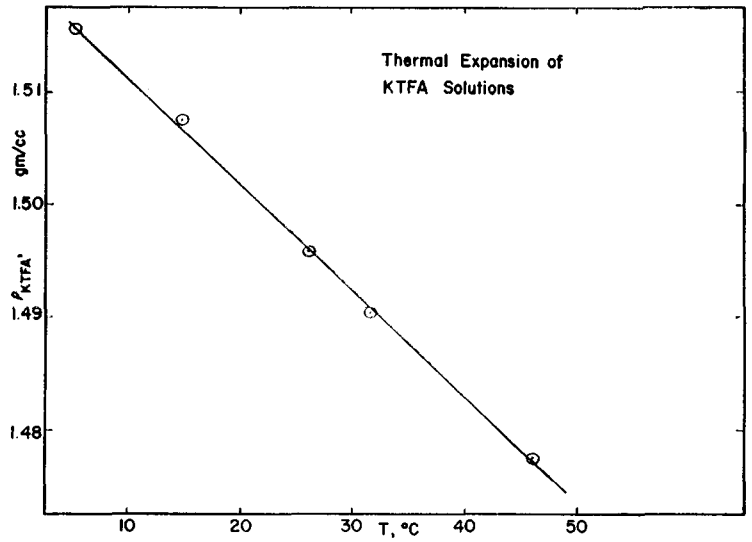

Fig. 3. Thermal expansion of KTFA solutions, measured pycnometrically.

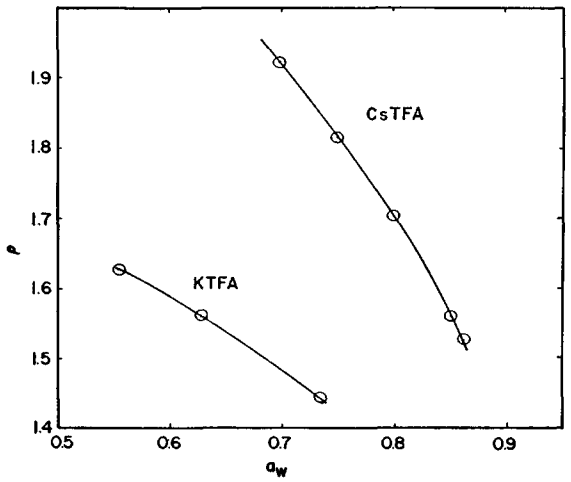

Fig. 4. Water activities of trifluoroacetate solutions, measured isopiestically.

The denaturation of the DNA was followed by watching the OD_{260} versus temperature in a Gilford multiple sample absorbance recorder, mounted on a Beckman DU spectrophotometer. Hyperchromicity at 260 mμ was 35–40% in concentrated KTFA solutions, and the temperature of the midpoint of the transition decreased with increasing salt concentration in the manner observed by Hamaguchi and Geiduschek.[7] (See Fig. 5.)

Solutions were prepared for centrifugation by dilution of the KTFA stock solution with distilled water and addition of DNA stock to a final DNA concentration of about 0.1 OD. Denatured DNA solution samples were prepared by heating the DNA in the salt solution of desired density

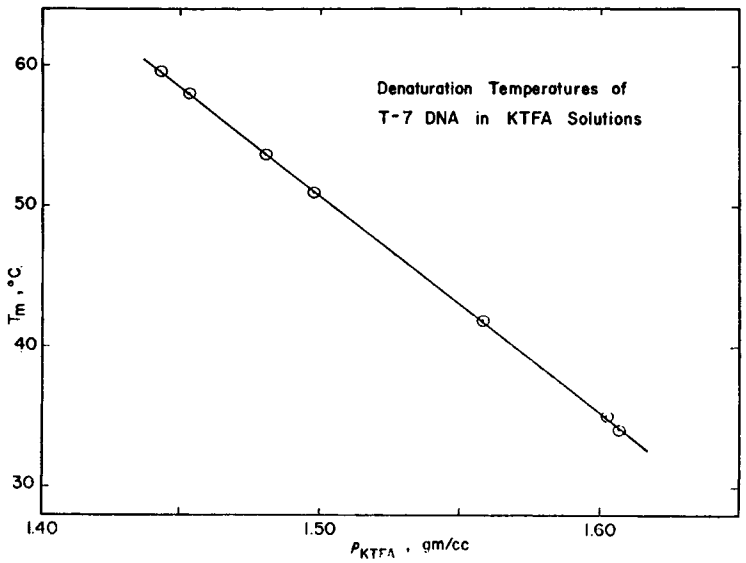

Fig. 5. Effect of increasing concentrations of trifluoroacetate in lowering T_m of DNA.

for 10 min in a water bath at a temperature 6–8°C above T_m and then quenching at 0°C. Solutions were maintained in the refrigerator until they were run in the centrifuge at the desired temperature, generally within one or two hours.

The densities of the solutions were determined by weighing in 100-μl pipets calibrated with mercury. Samples were run in a Beckman Model E analytical ultracentrifuge at 52640 rpm until the position of the center of the band did not change for 6 hr. Temperatures were controlled to within ± 0.1°C by the standard RTIC temperature unit. Ultraviolet optics were employed, and films were analyzed on a Joyce-Loebl double-beam recording microdensitometer. In some experiments the Beckman photoelectric scanning attachment was used. The buoyant densities were determined by banding the DNA in two cells in solutions of slightly different densities, as previously described.[22] Densities are estimated to be accurate to $\pm 0.2\%$.

There was a tendency for the denatured DNA to disappear at the highest salt concentrations. This was observed both by a gradual loss of optical density above T_m and a gradual decrease in area under the peak in the centrifuge. This problem was serious enough to limit the maximum temperature at which we could measure the density of denatured DNA, since higher and higher salt concentrations are needed as the buoyant density increases at higher temperatures. There is some evidence that high salt concentrations promote aggregation[8] and absorption of denatured DNA onto dust particles and walls of containers.[43] No hypersharpening of bands was observed in the density gradient, so if any aggregates did form, they must have been absorbed out of solution.

RESULTS

Figure 6 shows the buoyant density data for native and denatured DNA as a function of temperature. The solid lines are the "apparent" buoyant densities, which are the densities at 25°C of the KTFA solutions buoyant for the DNA at temperature T. The dotted lines give the actual buoyant density of DNA at temperature T, the densities at 25°C of the solutions being corrected to temperature T on the basis of the pycnometrically determined dependence of density on temperature (Fig. 3).

The buoyant density versus temperature curve for native DNA is similar to that eabontd biy Vinograd et al.[44] for three DNA's in CsCl, and the positive slope is a measure of the loss of hydration with increasing temperature. However, the slope of the curve is significantly smaller: $(2.3 \pm 0.5) \times 10^{-4}$ g/cc-°C, as compared to 4.2×10^{-4} in CsCl. The corresponding curve for denatured DNA is similar to that for the native form in the high- and low-temperature regions, where the plot is a straight line, displaced to higher densities. However, there is an inflection region over a wide range of temperatures below the T_m's in these solutions, where the change of density with temperature is much greater.

The effect of KTFA on denatured DNA is clarified by observing the behavior of the optical density of denatured DNA as a function of temperature in the presence of varying concentrations of the salt. In three of the experiments shown in Figure 7, the DNA was denatured as previously described, quenched at 0°C, and then OD_{260} was observed in the Gilford recording spectrophotometer as the temperature was raised. It

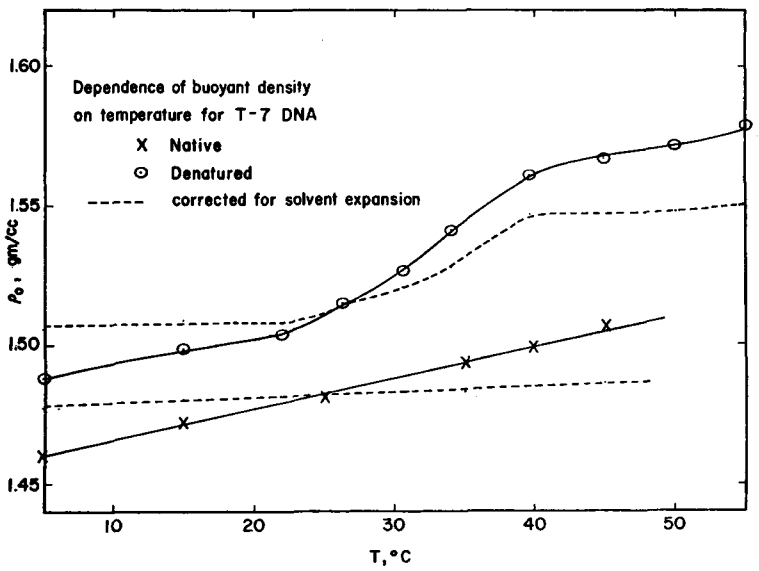

Fig. 6. Buoyant density of DNA in KTFA density gradients in the ultracentrifuge as a function of temperature.

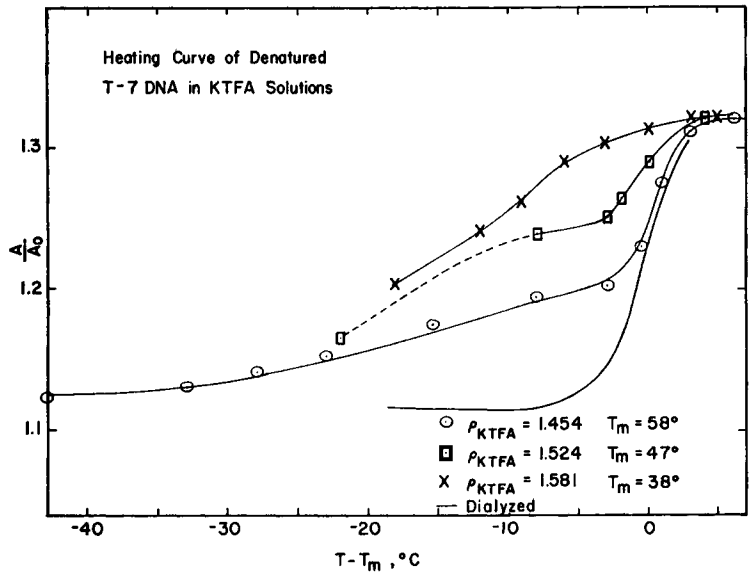

Fig. 7. Optical density at 260 mμ of denatured DNA as a function of temperature in solutions of various KTFA concentrations (A_0 = optical density of native DNA)

appears that under the conditions of denaturation in these experiments (10 min at T_m + 6–8°C) the strands do not separate completely, although the DNA is totally denatured by OD criteria. At the lower solution densities, there is considerable re-formation of structure on cooling, much of it similar enough to the native to melt out at the T_m. This corresponds to the low-temperature linear portion of the OD versus T curve for denatured DNA. At higher KTFA concentrations, where the melting temperature is lower, there is also considerable recovery of hypochromicity at very low temperatures. However, less of the structure is stable, and more of it melts out over a wide range below the T_m. In the high-density region, the hypochromicity which is observed at low temperatures all melts out below the T_m. However, the absence of formation of stable secondary structure under these conditions is due only to the presence of KTFA rather than to more complete strand separation. If DNA is melted in the presence of the highest concentration of salt and the salt is then diluted before cooling to 0°C, the melting curve is identical to that of DNA melted originally at the lower salt concentration. On the other hand, the structure which is reformed at the lower salt concentrations is stable in the presence of higher concentrations of KTFA. If DNA is melted in a KTFA solution of density about 1.45 g/cc, quenched at 0°C, and KTFA is added to bring the density up to 1.58, a sharp transition is observed at 38°C.

Thus KTFA appears to inhibit the re-formation of structure. On removal of the trifluoroacetate, a considerable amount of stable nativelike structure may be recovered rapidly. The higher the KTFA concentration,

the less long-range order can be formed on cooling, although local stacking may occur at temperatures relatively far below the T_m.

From the buoyant density shift of denatured DNA in the low-temperature region compared with that extrapolated from the high-temperature region, one can estimate that the maximum amount of recovery of structure is about 60% in a solution of density about 1.48–1.51 g/cc. This is very close to the value obtained by OD at $\rho = 1.45$. Figure 7 shows the melting curve of DNA denatured in a KTFA solution of density 1.58 g/cc and then dialyzed against dilute buffer. Under these conditions approximately 60% of the structure is formed, all of which is stable below the T_m This figure of 60% appears to be the maximum of native structure it is possible to recover. In the presence of KTFA no renaturation over longer periods of time was observed, either in the OD or centrifuge experiments.

Given the assumption that the partial specific volume of DNA does not change on denaturation, one can easily calculate the change of hydration on denaturation. In addition, the change in hydration with temperature and the heat of hydration can be calculated for native and denatured DNA, according to the method of Vinograd et al.[44] Table I gives these values for native and denatured DNA in KTFA. A value of 0.562 cc/g for the partial specific volume of KDNA was used in calculating hydrations.[45] "Denatured DNA" in these calculations means the fully denatured form, densities being extrapolated to 25°C from above the T_m. The hydration of the intermediate forms appear to be approximately proportional to the degree of structure measured by OD_{260}. The possible absence of strand separation above T_m in the presence of KTFA should not make any difference in the calculations for denatured DNA. Above the T_m the density and OD appear to be that of fully denatured DNA. No further changes in the buoyant density occur even at long periods of time at $T_m + 15$°C, except for small effects due to thermal expansion, and the OD remains constant even up to 40 or 50°C above the T_m. In order to eliminate the effect of hydration dependence on water activity, all buoyant densities used for these calculations have been adjusted to a water activity of 0.70. It was assumed that ρ_0 depends linearly on a_w, as is the case for LiDNA and CsDNA in this region of water activity. The value used for the thermal expansion coefficient of dry DNA was 7.0×10^{-4}°C^{-1}

TABLE I

Results of Temperature-Dependent Buoyant Density Studies on Native and Denatured DNA in KTFA

DNA	$(d\rho_0/dT)$, g/ml-°C	ΔH, cal/mole H_2O	$(\partial\Gamma/\partial T)a_w$, mole H_2O/ mole nucleotide, °C	Γ, mole H_2O/ mole nucleotide	
				25°C	54.5°C
Native	11.8×10^{-4}	397	−0.024	6.9	6.2
Denatured	10.0×10^{-4}	341	−0.015	5.3	4.9
3 DNA's in CsCl[a]		409	−0.028	6.6–7.4	

[a] Data of Vinograd et al.[44]

(obtained by Chapman and Sturtevant[40] for NaDNA at 25°C) which leads to a value of $5.3 \times 10^{-4}°C^{-1}$ for hydrated DNA.

Values obtained by Vinograd et al.[44] for three DNA's (native) in CsCl are included in Table I for comparison. The hydration depends on the kind of DNA, but is approximately the same at $a_w = 0.76$ in CsCl as it is in KTFA at $a_w = 0.70$. Values of ΔH of hydration and $(d\Gamma/dT)$ have been corrected for the thermal expansion of the DNA. Values for both ΔH of hydration and $(d\Gamma/dT)$ are the same within experimental error for DNA in both salts at this hydration. The values for denatured DNA are somewhat less.

DISCUSSION

The denaturation of DNA can be considered as a phase transition.[46] The coexistence of native and denatured states in the same molecule has been shown to exist in the transition region due to intramolecular heterogeneity of base composition.[47] It is reasonable to assume a reversible equilibrium between helix and coil in one region of the molecule in a small neighborhood of the T_m. Since this is the midpoint of the transition, one might then assume this region to represent the average properties of the molecule such as it would have if there were a sharp phase transition in the whole molecule. It is possible then to apply a kind of Clapeyron equation to calculate an enthalpy of denaturation of DNA in trifluoroacetate solution.

At constant pressure, the chemical potential of the DNA is a function of the temperature and salt concentration of the solution, which can also be expressed in terms of the chemical potential μ or activity a of water:

$$d\mu^{n}_{DNA} = \frac{S^{n}}{N^{n}_{DNA}} dT + \frac{N^{n}_{w}}{N^{n}_{DNA}} d\mu_{w}$$

$$d\mu^{d}_{DNA} = \frac{S^{d}}{N^{d}_{DNA}} dT + \frac{N^{d}_{w}}{N^{d}_{DNA}} d\mu_{w}$$

where N is the number of moles, S is entropy, and T temperature; the subscripts refer to the substance (DNA, w = water), and the superscripts n and d to the native and denatured phases, respectively.

For a small change in the conditions of temperature and water activity, such that we are still at the midpoint of the melting curve:

$$d\mu^{n}_{DNA} = d\mu^{d}_{DNA}$$

Then

$$\left(\frac{S^{d}}{N^{d}_{DNA}} - \frac{S^{n}}{N^{n}_{DNA}}\right) dT_m + \left(\frac{N^{d}_{w}}{N^{d}_{DNA}} - \frac{N^{n}_{w}}{N^{n}_{DNA}}\right) d\mu_{w} = 0$$

If the hydration of the DNA is different for native and denatured form, the coefficient of $d\mu_w$ is just equal to the loss of hydration of the DNA on denaturation. Thus we have:

$$\frac{d\mu_{w}}{dT_m} = -\frac{\Delta S}{\Delta\Gamma} = -\frac{\Delta H}{T_m \Delta\Gamma}$$

Expressing μ_w in terms of water activity, we have:

$$\Delta H = -\frac{RT_m^2}{a_w}\Delta\Gamma\frac{da_w}{dT_m} - RT_m\Delta\Gamma \ln a_w$$

We can calculate a ΔH of denaturation of 4.2 kcal/mole base pairs at $a_w = 0.7$ in KTFA (about $5M$) where $T_m = 54.5°$ C. At 25°C, where the change in hydration is perhaps slightly larger, the ΔH is approximately the same. This is considerably lower than the values in the vicinity of 8 kcal usually measured[48] but is certainly reasonable considering the widely different conditions.

Thus we have related the tendency of trifluoroacetate to destabilize the DNA helix to the loss of hydration of denaturation. If this relationship is even qualitatively correct, these phenomena should accompany each other in other salts as well. There is enough data available for CsCl solutions to test this hypothesis. Since the buoyant density in a CsCl gradient is greater for denatured than native DNA, there must be a loss of hydration on denaturation in this salt. The exact change is uncertain, because the buoyant density change on denaturation is known only at room temperature, far below the T_m. Optical density evidence indicates that there may be some re-formation of structure and recovery of hydration such as is observed in KTFA.

The effect of CsCl on the transition temperature has been published by Schildkraut and Lifson.[49] Since the effect of chloride is so much smaller than that of trifluoroacetate, the denaturing action of CsCl at high concentrations is obscured by the stabilizing effect of the salt by diminishing electrostatic interactions. However, a rough calculation can be done by separating the free-energy change on denaturation into an electrostatic and a nonelectrostatic part, and substracting off the electrostatic effect. Then if it is assumed that, as in KTFA, the change in hydration observed at room temperature is about 40% what it would be if the DNA were totally denatured, the ΔH calculated at 25°C is 10 kcal/mole base pairs, assuming reversibility at 25°C. Again, this is quite reasonable, considering the amount of approximation in the calculation.

Thus we would predict that in any salt which tends to destabilize the native form of DNA at high salt concentrations, the hydration of the denatured form would be lower than that of the native. (If a salt could be found which had a stabilizing effect on the helical structure, as has been found for some proteins, one would predict that the hydration in this salt would be higher for the denatured form.) Conversely, any salt in which the hydration of the denatured form is lower than that of the native would be expected to destabilize the helix, the extent of destabilization being proportional to the change in hydration. However, the denaturing ability of the salt may be due to much more than simply its influence on hydration, which is merely an effect on stoichiometry. The large effect of KTFA compared to CsCl is much more a result of the lowering of the ΔH of denaturation than of the larger $\Delta\Gamma$. The latter, however, might be

influenced by the same factors that influence the ΔH. For instance, if there is a specific interaction between the TFA^- and the bases in denatured DNA, which would lower ΔH, the measured hydration of the denatured DNA would be abnormally low and the $\Delta\Gamma$ would be greater. The tendency of KTFA to inhibit the recovery of a stable secondary structure below the T_m is consistent with such an interaction. However, in trifluoroacetate, the larger $\Delta\Gamma$ seems to be due to an abnormally high hydration of the native DNA (Fig. 1); the hydration of the denatured form is not unusually low. On the other hand in $NaClO_4$, whose destabilizing effect is also very large, native DNA does not appear to have an unusually high hydration, at least at water activities above about 0.92 where a significant lowering of the T_m is already observable.[7,23]

Thus the relationship between T_m, hydration, and salt concentration is complex, and the relative importances of various factors may be different in each specific case. Hamaguchi and Geiduschek[7] have attributed the destabilizing effect of the large anions to effects on the structure of water, while Robinson and co-workers[34,35] have interpreted the effect as due to binding of the large anions to the denatured form. From the data of the latter authors, both perchlorate and trifluoroacetate might be expected to bind to the DNA bases. Their effects on water structure are probably in opposite directions, however, ClO_4^- being a structure breaker and TFA^- a structure former (since both acetate and trichloroacetate are structure formers[15,34]). Perhaps the effect on water structure contributes to a higher hydration for both native and denatured DNA in TFA^-, while the binding of the anion brings the hydration back to normal for the denatured form, leading to the large $\Delta\Gamma$ on denaturation. If this were the case, one would expect to measure abnormally low hydrations for denatured DNA in other destabilizing salts, such as ClO_4^- and SCN^-, which are structure breaking.

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