Exclusion of Alcohols from Spermidine-DNA Assemblies: Probing the Physical Basis of Preferential Hydration

Anne Hultgren[†] and Donald C. Rau*

Laboratory of Physical and Structural Biology, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20892

Received March 5, 2004; Revised Manuscript Received April 28, 2004

ABSTRACT: The interaction of the alcohols 2-methyl-2,4-pentanediol (MPD) and 2-propanol and of glycerol with condensed spermidine^{3+}-DNA arrays are investigated with direct force measurements using osmotic stress coupled with X-ray scattering. Thermodynamic forces between DNA helices are measured from the dependence of helical interaxial spacings on the osmotic pressure applied by poly(ethylene glycol) solutions in equilibrium with the DNA phase. The sensitivity of these forces to solute concentration can be transformed into a change in the number of excess or deficit solutes or waters in the DNA phase by applying the Gibbs−Duhem equation. The alcohols examined are excluded from the condensed DNA array and strongly affect the osmotic stress force curves. DNA is preferentially hydrated. MPD is significantly more excluded than 2-propanol. The exclusion of these alcohols, however, is not due to a steric repulsion since glycerol that is intermediate in size between MPD and 2-propanol does not observably affect DNA force curves. As the distance between DNA helices varies, the change in the number of excess waters is independent of alcohol concentration for each alcohol. These solutes are acting osmotically on the condensed array. The distance dependence of exclusion indicates that repulsive water structuring forces dominate the interaction of alcohols with the DNA surface. The exclusion measured for these condensed arrays can quantitatively account for the effect of these alcohols on the precipitation of DNA from dilute solution by spermidine^{3+}.

The assembly of highly charged polyelectrolytes as DNA using oligo- and multivalent cations into tightly packed arrays is the focus of much theoretical and experimental research (*1*−*3*). The reversible packaging of DNA has several potential applications; foremost among them is for use in gene delivery systems (*4*−*6*). The condensation of DNA by counterions such as cobalt hexammine ($Co(NH_3)_6^{3+}$), Mn^{2+}, oligolysines, protamines, and alkylamines as spermidine^{3+} ($NH_3^+-(CH_2)_3-NH_2^+-(CH_2)_4-NH_3^+$) and spermine^{4+} results in hexagonally packed, parallel arrays of helices. The center-to-center distance between helices varies between ∼27 and 32 Å, depending on the condensing ion and temperature for some ions as Mn^{2+}, corresponding to a relatively large distance between DNA surfaces of ∼7−12 Å (*7*−*10*). Assembly is commonly considered driven by an electrostatic attraction between phosphate groups on one DNA helix and bound cations on an apposing helix (*11*−*16*). Alternatively, an attraction between DNA phosphates and bound cations mediated by water structuring or hydration forces has also been proposed based on measured forces between DNA helices in condensed arrays (*8*, *9*, *17*).

The precipitation of DNA from dilute solution occurs reversibly over a narrow concentration range of these condensing ions (*18*−*21*). The critical precipitation concentration depends on the bulk NaCl concentration as well as the multivalent ion identity. Alcohols such as methanol, ethanol, and 2-propanol facilitate cation mediated precipitation of DNA (*22*). The critical concentration of condensing cations decreases with increasing alcohol concentration. There are two, essentially opposite, explanations for this action of alcohols. This effect has been interpreted as an increased attraction between DNA helices with bound multivalent ions due to a lowered dielectric constant (*22*−*24*). Increased attraction due to alcohol necessarily means that the alcohol concentration within the condensed DNA array would be higher than that around DNA in dilute solution (i.e., a preferential solvation of condensed DNA with alcohol).

An alternative explanation is that nonpolar alcohols are excluded from the vicinity of phosphate and bound cation charges on the DNA surface (i.e., DNA is "preferentially hydrated") (*25*, *26*). If the number of excess waters associated with DNA is greater for isolated helices than in the precipitated array, increased alcohol concentration will favor precipitation.

The direct measurement of force−distance curves between macromolecules, particularly DNA helices, in condensed arrays using the osmotic stress technique coupled with X-ray scattering has provided much detailed information about the interactions of molecules at close distance (*17*, *27*, *28*). Condensed DNA is equilibrated against a solution containing a polymer as poly(ethylene glycol) (PEG) that is excluded from the DNA phase. The osmotic pressure of the polymer acts as a force to push helices closer. The spacing between

* Corresponding author: Tel.: (301)-402-4698. Fax: (301)-402-9462. E-mail: raud@mail.nih.gov.

[†] Present address: Physics and Astronomy Department, Johns Hopkins University, Baltimore, MD 21205.

[1] Abbreviations: PEG, poly(ethylene glycol); MPD, 2-methyl-(2,4)-pentanediol; Spd, spermidine.

10.1021/bi049559s
Published on Web 06/04/2004

helices can be measured as a function of osmotic pressure using X-ray diffraction. Water, salts, and other small solutes are free to exchange between the polymer solution and condensed DNA phase and establish their own equilibrium. Thermodynamic force curves have been measured between helices spontaneously assembled by several different multivalent cations (*8*, *9*). The forces encountered in pushing helices closer than the equilibrium spacing are quite similar for the various condensing agents that range from metal ions as Mn^{2+} and cobalt hexammine to the alkyl chain oligoamines to small polypeptides as protamine, strongly suggesting that the equilibrium spacing is not determined by a hard ion bridging distance between phosphates, but by a balance of soft intermolecular forces.

An advantage of the osmotic stress experimental approach is its connection to rigorous thermodynamics. Changes in pressure−volume work, $\Pi\Delta V$, can be balanced by changes in temperature-entropy energies, $T\Delta S$, or by changes in the numbers of molecules-chemical potential work, $n\Delta\mu$, through the Gibbs−Duhem equation. We have previously (*9*) examined the sensitivity of DNA force curves with subcritical concentrations of $Co(NH_3)_6^{3+}$ to determine the number of extra ions bound in the assembly transition and to estimate the depth of the attractive energy well. We also used the temperature dependence of Mn^{2+}−DNA force curves to extract the change in entropy of the DNA phase as a function of interhelical spacing (*8*, *29*).

Here we measure the dependence of the forces between DNA helices condensed with spermidine (Spd^{3+}-DNA) on alcohol concentration. We extract, in particular, the change in the number of excess water molecules in the DNA phase inferred from the change in spacing between helices as the alcohol concentration is varied. We find that the alcohol-to-water ratio decreases in the DNA phase as helices move closer for both 2-propanol and MPD. By equilibrating dried Spd^{3+}-DNA arrays with small volumes of alcohol−water solutions and measuring the alcohol content remaining in the supernatant after solvation, we show that these alcohols are strongly excluded from the Spd^{3+}−DNA phase.

The change in the number of excess or preferentially included waters as the distance between helices varies is independent of alcohol concentration, but different for 2-propanol and MPD. The change in the number of preferentially included waters, $\Delta\Gamma_w$, increases approximately exponentially with distance between helices. The decay length of the exponential is 3−4 Å for both 2-propanol and MPD. This value is characteristic of a repulsive hydration force that has been now seen between many macromolecules at close separation and likely represents a water−water correlation length. These alcohols seem to interact with the DNA surface through water structuring forces. Virtually complete exclusion of MPD from the Spd^{3+}-DNA phase occurs at an interhelical spacing of ∼ 24 Å. Over the range of spacings examined (∼24−29 Å), MPD is about twice as excluded as 2-propanol and is also twice the size. The exclusion of these alcohols, however, is not simply a steric excluded volume effect since glycerol that is intermediate in size between 2-propanol and MPD is not excluded at all. Since size alone is not the determining factor, we postulate that the magnitude of the hydration repulsion is to a first-order approximation a sum of the contributions from the individual groups comprising the molecule.

The number of excess waters released as DNA helices are brought together from large distances to the spacings characteristic of DNA assembly can be calculated by integrating the distance dependence of $\Delta\Gamma_w$. The results are good agreement with the observed effect of MPD and 2-propanol on the critical concentration of spermidine necessary for precipitation of DNA from dilute solution.

The characteristics we observe for the exclusion of alcohols from DNA, the magnitude of $\Delta\Gamma_w$, its dependence on solute nature, but its insensitivity to concentration, are also commonly observed for the preferential hydration of proteins in the presence of excluded neutral solutes (*25*, *26*, *30*). Hydration forces seem likely to underlie the general exclusion of small osmolytes from macromolecular surfaces. This should not be surprising, given the dominant role of hydration forces in the close interaction between macromolecules that has been observed.

MATERIALS AND METHODS

Materials. High molecular weight chicken blood DNA was prepared as described previously (*9*). Poly(ethylene glycol) (MW 8,000), spermidine.3HCl, and 2-methyl-2,4-pentanediol were purchased from Fluka Chemical Company (micro select grade). Glycerol and 2-propanol ware purchased from JT Baker and Company (analytic grade).

Osmotic Stress. The method for direct force measurement by osmotic stress has been described in detail by Parsegian et al. (*27*). In brief, condensed macromolecular arrays are equilibrated against a bathing polymer solution, typically PEG of known osmotic pressure that is excluded from many condensed macromolecular arrays, DNA in particular. Water and small solutes are free to exchange between the PEG and condensed DNA phases. After equilibrium is achieved, the osmotic pressures in both the polymer and macromolecular phases are the same, as necessarily are the chemical potentials of all the permeating species. If the condensed macromolecular phase is sufficiently ordered, the intermolecular distance can be determined as a function of the applied PEG stress by Bragg scattering of X-rays.

Spermidine (Spd^{3+}) precipitated DNA was prepared by adding Spd^{3+} to a 1 mg/mL (∼3 mM DNA-phosphate) DNA solution to a final nominal concentration of ∼4 mM and mixing. Condensed Spd^{3+}-DNA samples (∼0.2−0.3 mg) are equilibrated against ∼1 mL PEG solution containing varying concentrations of $SpdCl_3$ and alcohols or glycerol in 10 mM TrisCl (pH 7.5) at room temperature for about 2−3 weeks with 2 changes of PEG solution with occasional mixing. Osmotic pressures of the PEG solutions were measured directly using a Vapro Vapor Pressure Osmometer (model 5520, Wescor Corp.). Osmotic pressures were additive over the range of PEG and solute concentrations to within ∼25% for glycerol and ∼10% for 2-methyl-2,4-pentanediol. The vapor pressure of 2-propanol itself is too high to allow direct osmotic pressure measurements. It was simply assumed that 2-propanol osmotic pressures are ideal in the concentration range used (as are glycerol and MPD to within ∼6%) and additive with PEG pressures.

X-ray Scattering. An Enraf-Nonius Service Corp. (Bohemia, NY) fixed copper anode Diffractis 601 X-ray generator (National Institutes of Health, MD) equipped with double focusing mirrors (Charles Supper Co.) was used for X-ray

scattering. DNA samples were sealed with a small amount of equilibrating solution in the sample cell and then mounted into a temperature-controlled holder at 20 °C. A helium filled Plexiglas cylinder with Mylar windows was between the sample cell and image plate, a distance of ~16 cm. Diffraction patterns were recorded by direct exposure of Fujifilm BAS image plates and digitized with a Fujifilm BAS 2500 scanner. The images were analyzed using the FIT2D (copyright A. P. Hammersley, ESRF) and SigmaPlot 8.0 (SPSS Inc.). The sample to image plate distance was calibrated using powered *p*-bromobenzoic acid. Mean pixel intensities between scattering radii $r - 0.05$ mm and $r + 0.05$ mm averaged over all angles of the powder pattern diffraction, $\langle I(r) \rangle$, were used to calculate integrated radial intensity profiles, $2\pi r\langle I(r) \rangle$. The sharp, intense ring corresponds to interaxial Bragg diffraction from DNA helices packed in a hexagonal array. X-ray scattering patterns were reproducible over at least several months of storage. No sample degradation was apparent.

The DNA samples showed complete reversibility. DNA pellets initially prepared by ethanol precipitation in Na acetate solution or Spd^{3+} precipitation in water or in water/alcohol solutions then equilibrated against PEG/Spd^{3+}/alcohol solutions gave the same X-ray spacings. Samples initially equilibrated against one set of PEG/Spd^{3+}/alcohol conditions can be equilibrated against another with complete reversibility in spacing. The measurements reflect equilibrium conditions.

Hydration of Dried Spd^{3+}-DNA. About 10 mg of Spd^{3+} precipitated DNA was washed three times with 10 mL 70% cold ethanol/water solution. The DNA pellets were lyophilized for ~ 2 days (Savant Speed Vac system VLP120, RVT4104, SC110A) and 40 μL of water/alcohol or water/glycerol solution added. Solvating samples were kept in screw-top Eppendorf tubes additionally sealed with Parafilm for ~1 month at room temperature with occasional mixing and centrifugation. The alcohol concentration of a 20-μL aliquot taken from the remaining supernatant was determined by measuring the refractive index using an Abbe C10 Refractometer. A control experiment solvating with water showed no change in the refractive index.

Critical Spd^{3+} Concentrations for DNA Precipitation. The critical concentration of Spd^{3+} necessary for the precipitation of DNA from dilute solution was determined as described basically by Pelta et al. (*31*). A series of chicken blood DNA samples were prepared with varying $SpCl_3$ concentrations and fixed solute concentration in 0.1 M NaCl, 10 mM TrisCl (pH 7.5). The DNA concentration was ~ 15 μM base pairs in 1 mL total volume. After incubating at room temperature for ~ 2 h, the solution was centrifuged at ~ 16000*g* for 10 min and the absorbance at 260 nm of the supernatant measured. Around the critical concentration, the Spd^{3+} concentration was varied in steps of 0.25 mM. We used the Spd^{3+} concentration at half loss of absorbance as the critical concentration.

Thermodynamics. A convenient thermodynamic scheme for considering the actions of the stressing polymer PEG and osmolyte is illustrated in Figure 1 (*29*, *32*). In practice, a pellet of condensed Spd^{3+}-DNA is equilibrated against a solution of PEG and solute. The PEG is sterically excluded from the Spd^{3+}-DNA phase, and the effect of its osmotic pressure is equivalent to a pressure, Π_{PEG}, acting through a

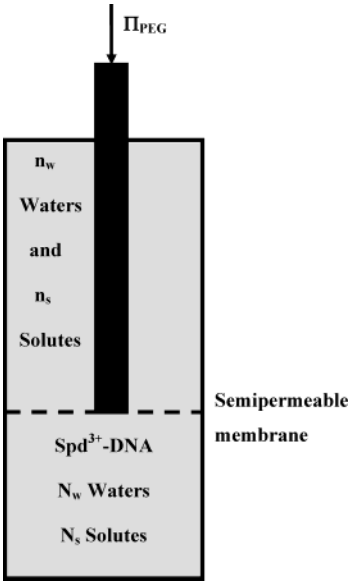

FIGURE 1: The thermodynamics of the effect of solutes on forces can be conveniently derived through a schematic representation of the osmotic stress experiment. Poly(ethylene glycol) is excluded from the DNA phase and applies an osmotic pressure on it. This stress can be modeled as hydrostatic pressure acting through a semipermeable membrane separating the polymer and DNA phases. Water and small solutes are free to exchange between the two phases. The reference phase contains n_w waters and n_s solutes and the DNA phase N_w waters and N_s solutes. If the solute affects the interaction between DNA helices, then n_s/n_w and N_s/N_w are necessarily different.

semipermeable membrane separating the Spd^{3+}-DNA phase from a reference solution. The membrane can pass water, salt, and solute, but not DNA. At constant temperature and hydrostatic pressure, the free energy of the Spd^{3+}-DNA can be related to the chemical potentials of the N_s solute and N_w water molecules included in this phase through a Gibbs−Duhem equation (*33*)

$$\mathrm{d}\, G_{Spd^{3+}-DNA} = -N_s\, \mathrm{d}\, \mu_s - N_w\, \mathrm{d}\, \mu_w \qquad (1)$$

There is another Gibbs−Duhem relation for the n_s and n_w solute and water molecules in the reference solution that connects the solute and water chemical potentials

$$n_s\, \mathrm{d}\, \mu_s^{ref} + n_w\, \mathrm{d}\, \mu_w^{ref} = 0 \text{ or } \mathrm{d}\, \mu_s^{ref} = -(n_w/n_s)\, \mathrm{d}\, \mu_w^{ref} \qquad (2)$$

In terms of the reference solution and PEG piston that are in equilibrium with the Spd^{3+}-DNA phase

$$\mathrm{d}\, \mu_s = \mathrm{d}\mu_s^{ref} \text{ and } \mathrm{d}\, \mu_w = \mathrm{d}\, \mu_w^{ref} - \bar{\nu}_w\, \mathrm{d}\, \Pi_{PEG} \qquad (3)$$

where $\bar{\nu}_w$ is the molecular volume of water. We have neglected the chemical potential of Spd^{3+} since DNA forces are insensitive to spermidine concentration over a broad range, ~ 0.5 to 20 mM (data not shown) as was observed for $Co(NH_3)_6^{3+}$ (*9*).

Combining these equations, we have either

$$\mathrm{d}\, G_{Spd^{3+}-DNA} = V_w\, \mathrm{d}\, \Pi_{PEG} - N_w\left(1 - \frac{(N_s/N_w)}{(n_s/n_w)}\right) \mathrm{d}\, \mu_w^{ref} = V_w\, \mathrm{d}\, \Pi_{PEG} + \bar{\nu}_w \Gamma_w\, \mathrm{d}\, \Pi_s \qquad (4a)$$

or

$$d\,G_{Spd^{3+}-DNA} = V_w\,d\,\Pi_{PEG} - N_s\left(1 - \frac{(n_s/n_w)}{(N_s/N_w)}\right) d\,\mu_s^{ref} = V_w\,d\,\Pi_{PEG} - \Gamma_s\,d\,\mu_s^{ref} \quad (4b)$$

where $V_w = \bar{v}_w N_w$, the incremental contribution to the osmotic pressure from the solute $d\,\Pi_s = -d\,\mu_w^{ref}/\bar{v}_w$, and Γ_w and Γ_s are the excess (or deficit) numbers of waters and solutes, respectively, associated with the Spd^{3+}-DNA phase, also termed preferential interaction coefficients. Solutes will only affect DNA forces if the ratio of solute to water is different in the Spd^{3+}-DNA and reference phases (i.e., only if $(N_s/N_w)/(n_s/n_w) \neq 1$). These two equivalent equations are a consequence of an intrinsic ambiguity in the Gibbs−Duhem equation, μ_w and μ_s cannot be varied independently. We focus here on Γ_w and Π_s (eq 4a) since, as will be seen, it is $\Delta\Gamma_w$ that remains constant as the solute concentration is varied.

Changes in the excess number of waters in the Spd^{3+}-DNA phase as the spacing between helices (or volume) changes can be calculated through a Maxwell relation associated with eq 4a

$$\left.\frac{\partial\Gamma_w}{\partial\Pi_{PEG}}\right|_{\Pi_s} = \frac{\partial\Gamma_w}{\partial V_w}\left.\frac{\partial V_w}{\partial\Pi_{PEG}}\right|_{\Pi_s} = \left.\frac{\partial V_w}{\bar{v}_w\partial\Pi_s}\right|_{\Pi_{PEG}}$$

or

$$\frac{\partial\Gamma_w}{\partial V_w} = \frac{1}{\bar{v}_w}\frac{\{\partial V_w\}/\{\partial\Pi_s\}|_{\Pi_{PEG}}}{\{\partial V_w\}/\{\partial\Pi_{PEG}\}|_{\Pi_s}} = -\frac{1}{\bar{v}_w}\left.\frac{\partial\Pi_{PEG}}{\partial\Pi_s}\right|_{V_w} \quad (6)$$

where we have recognized that if $V_w = V_w(\Pi_{PEG},\Pi_s)$, then

$$d\,V_w = \left.\frac{\partial V_w}{\partial\Pi_{PEG}}\right|_{\Pi_s} d\,\Pi_{PEG} + \left.\frac{\partial V_w}{\partial\Pi_s}\right|_{\Pi_{PEG}} d\,\Pi_s \quad (7)$$

and therefore at constant V_w

$$\left.\frac{d\,\Pi_{PEG}}{d\,\Pi_s}\right|_{V_w} = -\frac{(\{\partial V_w\}/\{\partial\Pi_s)\}|_{\Pi_{PEG}}}{(\partial V_w/\partial\Pi_{PEG})|_{\Pi_s}} \quad (8)$$

Equation 6 can be integrated to give

$$\Delta\Gamma_w = -\frac{1}{\bar{v}_w}\int\left.\frac{\partial\Pi_{PEG}}{\partial\Pi_s}\right|_{V_w} dV_w \quad (9)$$

Note that if solute is completely excluded and acts identically as PEG, then

$$-\frac{\partial\Pi_{PEG}}{\partial\Pi_s} = 1 \text{ and } \Delta\Gamma_w = \frac{\Delta V_w}{\bar{v}_w} = \Delta N_w \quad (10)$$

All the water removed is water of preferential hydration.

RESULTS

The Effect of Alcohols and Glycerol on Spd^{3+}-DNA Forces. Thermodynamic forces between spermidine condensed DNA helices without added solute and with 2 m glycerol, 2-propanol, or 2-methylpentane-2,4-diol (MPD) are shown in Figure 2. MPD significantly decreases the spacing between helices at constant PEG osmotic pressure. The smaller

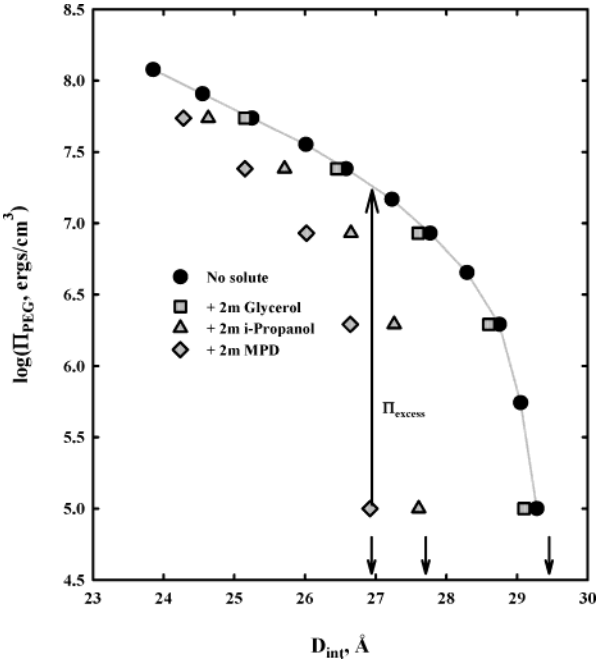

FIGURE 2: Both MPD and 2-propanol, but not glycerol, strongly affect the thermodynamic forces between Spd^{3+}-DNA helices. The interaxial spacing between helices was measured a function of the PEG osmotic pressure of the bathing solution for Spd^{3+}-DNA condensed arrays equilibrated against 2 mM $SpdCl_3$ and 10 mM TrisCl (pH 7.5) at 20 °C and either without added solute or with MPD, 2-propanol, or glycerol at a concentration of 2 molal. The arrows show the equilibrium spacing in the absence of PEG osmotic pressure for MPD, 2-propanol, and without added solute. Glycerol has very little effect on the force. Both MPD and 2-propanol further condense the array. Π_{excess} is the apparent osmotic pressure applied by the alcohols in addition to Π_{PEG} to account for the change in spacing.

2-propanol affects forces to a lesser extent. Glycerol that is intermediate in size has only a very slight effect on spacing at constant PEG pressure. Size alone is not the dominating factor. These force curves are not dependent on spermidine concentration between 0.5 and 20 mM (data not shown), as was seen previously for $Co(NH_3)_6^{3+}$ (*9*).

An effect of MPD and 2-propanol on DNA forces necessarily means a difference in the distribution of these alcohols between the Spd^{3+}-DNA phase and the bulk solution. This difference can be analyzed using the Gibbs−Duhem equation (eq 1−4) in terms of either an excess (or deficit) number of waters (a preferential hydration), Γ_w, or, equivalently, an excess (or deficit) number of solutes, Γ_s. A change in Γ_w as the spacing between helices changes can be calculated from eq 6, derived through a Maxwell relation of the Gibbs−Duhem equation. Figure 3 shows the variation in the interpolated PEG osmotic pressure necessary to maintain $D_{int} = 26$ Å (constant volume) as the MPD pressure changes. The linear relationship indicates that $\Delta\Gamma_w$ is independent of MPD concentration. The observed slope, $d\,\Pi_{PEG}/d\,\Pi_{MPD}$, at $D_{int} = 26$ Å is −0.55. The apparent, equivalent pressure applied by MPD at this spacing is slightly more than half the pressure if MPD were completely excluded from the Spd^{3+}-DNA phase.

Given a linear dependence, $d\,\Pi_{PEG}/d\,\Pi_{MPD}$ at constant volume can be calculated for each data point from the slope $-\Pi_{excess}/\Pi_s$, where Π_s is the contribution to the total osmotic pressure from solute MPD and Π_{excess} is the apparent pressure

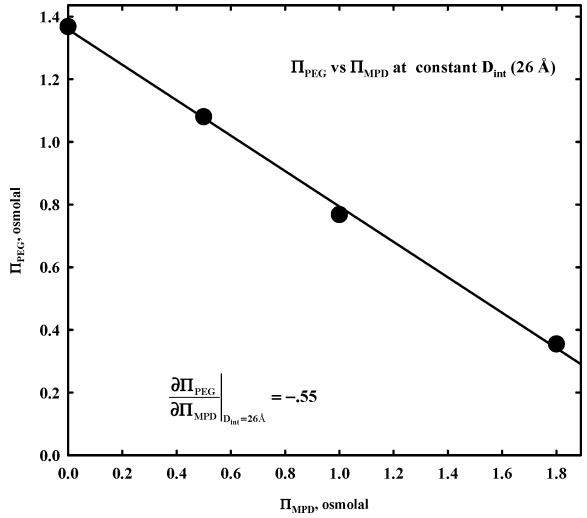

FIGURE 3: PEG and MPD osmotic pressures needed to maintain a constant spacing are linearly dependent. The interpolated PEG osmotic pressure at 26 Å from forces curves as shown in Figure 2 for various MPD concentrations is plotted against the MPD osmotic pressure. In terms of the osmolal concentration, $\Pi = kT$[osmolal]/(55.6 $\bar{v}_w$), where k is the Boltzmann constant, T is temperature, and $\bar{v}_w$ is the volume of a water molecule. The linear relationship indicates that the change in the excess number of waters in the Spd^{3+}-DNA phase is independent of MPD concentration at this spacing. For a simple exclusion, the slope of −0.55 indicates that about half the waters in the Spd^{3+}-DNA array are excess at this spacing.

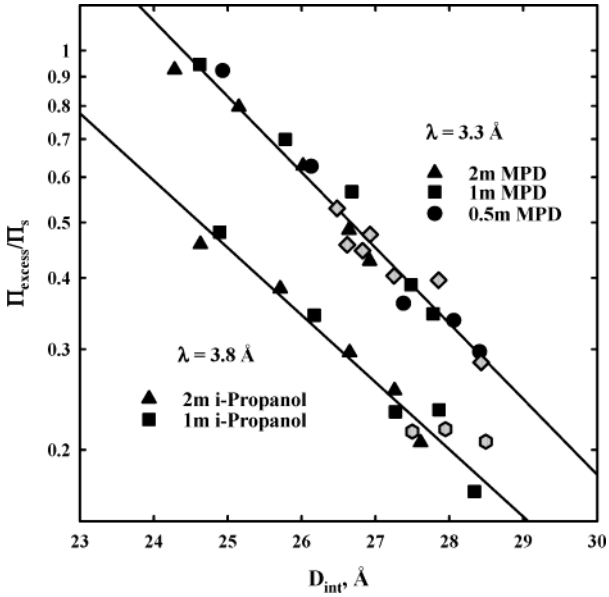

FIGURE 4: The apparent excess pressure applied by the alcohols MPD and 2-propanol is shown as a function of the spacing between DNA helices. For each data point in the force curves with added alcohol, an excess pressure was determined from the difference in PEG osmotic pressures at constant spacing, as schematically shown in Figure 2. The excess pressure is normalized by the alcohol osmotic pressure. For complete exclusion, the ratio $\Pi_{excess}/\Pi_s = 1$. The open symbols are for alcohols without added PEG, ranging between 1 and 3 molal for 2-propanol and 0.5 and 3 molal for MPD. The overlap of the different alcohol molal concentrations indicates that the excess water at any spacing is constant and independent of alcohol concentration. The excess pressure varies approximately exponentially over this range of spacings. MPD is about 2-fold more excluded than 2-propanol. The decay lengths of the exponentials are ∼3.8 and 3.3 Å for 2-propanol and MPD, respectively, characteristic of water structuring or hydration force repulsion.

applied by MPD on the DNA phase at a spacing D_{int}, (i.e., $\Pi_{excess}(MPD) = \Pi_{PEG}(D_{int}, [MPD] = 0) - \Pi_{PEG}(D_{int}, [MPD])$), as illustrated by the arrow in Figure 2. Figure 4 shows the dependence of Π_{excess}/Π_s on D_{int} for two concentrations of 2-propanol (0.5 and 1 molal) and three (0.5, 1, and 2 molal) for MPD. The overlap of the data for the different concentrations simply confirms that the slope $d\,\Pi_{PEG}/d\,\Pi_{alcohol}$ is indeed constant. The open symbols in the figure are for MPD or 2-propanol solutions without any added PEG. The insensitivity of Π_{excess}/Π_s to alcohol concentration indicates that changes in the excess water associated with the DNA phase, $\Delta\Gamma_w$, as the spacing between helices varies are also independent of alcohol concentration.

To a good first-order approximation Π_{excess}/Π_s, and therefore the changes in preferential hydration, $\Delta\Gamma_w$, vary exponentially with spacing between helices. The decay lengths are 3.3 (±0.2 Å) and 3.8 Å (± 0.5 Å) for MPD and 2-propanol, respectively. Within the range of measured spacings, Π_{excess}/Π_s for MPD is about twice as large as that for 2-propanol (a factor of 1.8 at 26 Å).

Solvation of Dry Spd^{3+}-DNA Shows Exclusion of Alcohols. Because we can only measure changes in excess water, $\Delta\Gamma_w$, we do not know the absolute sign of Γ_w (i.e., if there is more or less of these alcohols in the Spd^{3+}-DNA phase than in the bulk solution). We only know that the ratio of alcohol to water in the Spd^{3+}-DNA phase compared with the bulk, $(N_s/N_w)/(n_s/n_w)$ in eq 4a, decreases as the spacing between helices decreases. A rapid method for determining whether solutes are included or excluded from Spd^{3+} precipitated DNA is to solvate dried Spd^{3+} DNA pellets with a limited volume of alcohol−water solution. Initial and final concentrations of solute can be determined from refractive index measurements. Exclusion of solute from, or equivalently, preferential hydration of, the condensed Spd^{3+}-DNA phase necessarily results in a higher solute concentration in the bathing solution after equilibration than present initially. The results are summarized in Table 1. The alcohols are excluded. Because there is no exclusion of glycerol that is intermediate in size, this exclusion is not because these solutes are too large to enter the tightly packed pellet. Furthermore, inter-

Table 1: Hydration of Dried Spd^{3+}-DNA Assemblies Shows Alcohol Exclusion[a]

	C^0 (m)[b]	C_f (m)[c]	C_{DNA}/C_f [d]	D_{int} (Å)[e]	D_{int} (Å)[f]
	0	0		29.2	29.2
MPD	0.41	0.53	0.25	28.4	28.3
	0.85	1.09	0.34	27.8	27.75
	1.43	1.83	0.25	27.1	27.0
	1.51	1.96	0.29	27.0	26.9
	3.09	4.22	0.20	26.0	25.8
i-propanol	0.96	1.19	0.51	28.3	28.2
	2.12	2.56	0.52	27.5	27.4
glycerol	1.81	1.88	0.94	29.1	29.15

[a] Dried Spd^{3+} precipitated DNA (∼10 mg) was solvated with 40 μL of alcohol or glycerol solution, as described in Materials and Methods, and the concentration of alcohol or glycerol remaining in supernatant was measured. [b] Initial molal concentration of alcohol or glycerol. [c] Final molal concentration of solute in the supernatant determined from the change in refractive index. [d] The ratio of solute concentrations in the DNA phase and supernatant calculated using eq 13. [e] Interaxial spacings of the solvated DNA pellets. [f] Interaxial spacings of DNA precipitated with $SpdCl_3$ from solutions containing the calculated final concentration of solute in the supernatant.

helical spacings of solvated Spd^{3+}-DNA pellets are virtually identical to the spacings observed for DNA directly precipitated from alcohol−water solutions with added spermidine.

The ratio of alcohol concentrations in the Spd^{3+}-DNA pellet and in the bathing aqueous solution can be estimated, assuming that all base pairs in the sample are packed in an ideal hexagonal lattice. The dried sample has a weak interhelical reflection at ~ 20.4 Å. A total volume of solution taken up by the DNA phase, ΔV, can be estimated from the change in interaxial spacing D_{int} and from the weight, w, of the pellet, assuming a base pair molecular weight, M, of ~ 700 g/mole and a rise per base pair, L, of 3.4 Å.

$$\Delta V = \frac{\sqrt{3}}{2} L (D_{\text{int,final}}^2 - D_{\text{int,dry}}^2) \frac{wN_a}{M} \tag{11}$$

where N_a is Avogrado's number. If the volume of the solution of concentration C_i initially added to the pellet is V_0, then the ratio of the solute concentration in the DNA pellet, C_{DNA}, and in the bathing aqueous solution remaining after equilibration, C_f, is given by

$$\frac{C_{DNA}}{C_f} = 1 - \frac{V_0}{\Delta V}\left(1 - \frac{C_i}{C_f}\right) \tag{12}$$

These estimates are given in Table 1.

This ratio of concentrations can also be estimated from the data in Figure 4, assuming a simple exclusion of alcohols from the Spd^{3+}-DNA phase.

$$\frac{C_{DNA}}{C_0} \sim \frac{\int_{D_{\text{int,dry}}}^{D_{\text{int,final}}} \left(1 - \frac{\Pi_{\text{excess}}}{\Pi_s}\right) D \, \mathrm{d}D}{\int_{D_{\text{int,dry}}}^{D_{\text{int,final}}} D \, \mathrm{d}D} \tag{13}$$

We simply extrapolate the data in Figure 4 to $\Pi_{excess}/\Pi_s = 1$ (the limit for complete exclusion) and assume the alcohol concentration at closer spacings is 0. The calculated ratio of concentrations is ~0.22 for MPD at 27 Å and ~0.45 for 2-propanol at 28 Å. These values are somewhat smaller than those estimated in Table 1 (~0.27 and 0.52 for MPD and 2-propanol, respectively, at comparable spacings), but within reason given the crude assumption of a perfect DNA lattice made in calculating the ratio of concentrations from the solvation experiments.

Numbers of Preferentially Included Waters. The total number of preferentially included waters per base pair can be estimated in much the same manner

$$\Delta\Gamma_{w,\text{total}} = \int_{D_{\text{int,dry}}}^{\infty} \frac{\sqrt{3}}{\bar{v}_w} L \frac{\Pi_{\text{excess}}}{\Pi_s} D \, \mathrm{d}D \tag{14}$$

where L is the rise per base pair, assumed 3.4 Å, and $\bar{v}_w$ is the molecular volume of water, assumed 30 Å^3. Again, we simply extrapolate the data in Figure 4 and assume that Π_{excess}/Π_s can reach a maximum exclusion value of 1. The preferential hydration of Spd^{3+}-DNA in the presence of MPD is 36 waters/bp and 24 waters/bp with 2-propanol. Alternatively, an equivalent, two-state Gibbs dividing surface can be calculated (*33*). This is defined by the distance out from the DNA surface that would have to be swept entirely clear of solute to give the same number of included waters.

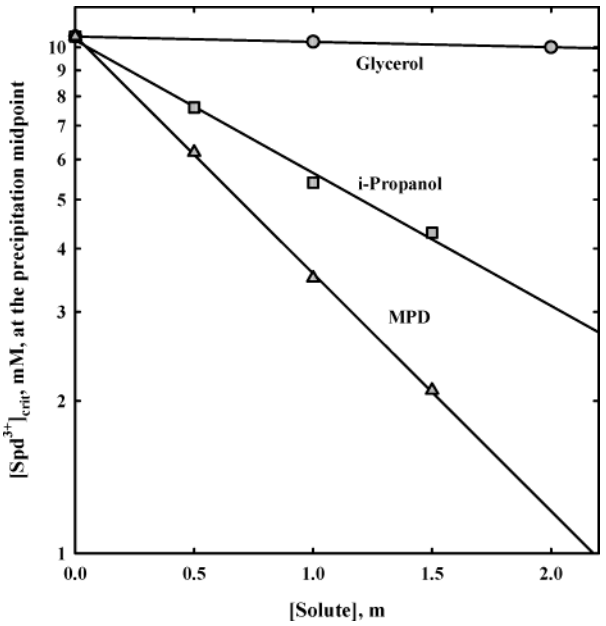

FIGURE 5: The critical concentration for Spd^{3+} precipitation of DNA from dilute solution varies with alcohol molal concentration. The extent of DNA precipitation by Spd^{3+} was monitored by centrifuging Spd^{3+}-DNA solutions and measuring the absorbance of DNA remaining in the supernatant, as described by Pelta et al. (*31*). The DNA concentration was ~15 μM in base pairs (A_{260} ~ 0.3 in a 1-cm cuvette). The salt concentration was 0.1 M NaCl in 10 mM TrisCl (pH 7.5). Samples were incubated at 20 °C for 2 h after addition of $SpdCl_3$. In agreement with the force measurements on condensed arrays, glycerol has almost no effect on the critical Spd^{3+} concentration, while both MPD and 2-propanol strongly decrease the $SpdCl_3$ concentration needed for precipitation. The linear dependence of $[Spd^{3+}]_{crit}$ on alcohol concentration is consistent with an osmotic action of these solutes. The slopes can be interpreted in terms of the number of extra Spd^{3+} ions that bind to DNA and the number of released water molecules as DNA goes through the transition from dilute solution to condensed phase.

Assuming cylindrical geometry and a 20-Å diameter, 36 waters corresponds to a distance of 4.1 Å from the Spd^{3+}-DNA surface and 24 waters to 2.9 Å. Essentially only the first hydration layer is affected.

Precipitation of DNA by Spd^{3+} from Dilute Solution. The precipitation of DNA from dilute solution provides a critical test of the extracted distance dependence of the excess water distribution function. Figure 5 shows the dependence of the critical Spd^{3+} concentration necessary for precipitation of DNA from dilute solution in 0.1 M NaCl on the molal concentration of MPD, 2-propanol, and glycerol. Not surprisingly, the efficacy of these osmolytes in facilitating precipitation is mirrored by their effect on the osmotic stress force curves seen in Figure 2. The slopes seen in Figure 5 are determined by the difference in numbers of Spd^{3+} ions and water molecules bound to DNA in the dilute solution and in the precipitated assembly at the critical Spd^{3+} concentration, $\Delta\Gamma_{Spd^{3+}}$ and $\Delta\Gamma_w$, respectively. For these solutes within this concentration range, osmotic pressure varies linearly with molal concentration m_s to a very good approximation, so that

$$\frac{\mathrm{d}\ln([Spd^{3+}]_{crit})}{\mathrm{d}\,m_s} \sim \frac{\Delta\Gamma_w/55.6}{\Delta\Gamma_{Spd^{3+}}} \tag{15}$$

Matulis et al. (*34*) has estimated $\Delta\Gamma_{Spd^{3+}}$ as ~0.1/bp. We can calculate $\Delta\Gamma_w$ by integrating Π_{excess}/Π_s from the distance

between helices at the precipitation transition, $D_{int} \sim 29$ Å, to infinite separation

$$\Delta\Gamma_w(/bp) \sim \int_{\infty}^{29\text{ Å}} \frac{\sqrt{3}}{\bar{v}_w} L \frac{\Pi_{excess}}{\Pi_s} D \, d\, D \qquad (16)$$

We further assume that the exponential form of Π_{excess}/Π_s seen in Figure 4 can simply be extrapolated to very large separations and that added solute does not affect $\Delta\Gamma_{Spd^{3+}}$ significantly. The estimated $\Delta\Gamma_w \sim -5.2$ waters with MPD results in a calculated slope of -0.95 m^{-1} compared with the measured -1.10 m^{-1}. With 2-propanol, $\Delta\Gamma_w \sim -3.7$ waters, corresponding to a slope of -0.65 m^{-1} compared with the experimental value of -0.60 m^{-1}. The predictions are in good agreement with experiment.

DISCUSSION

The action of solutes on DNA properties has commonly been considered from the standpoint of an effect on the solution dielectric constant on polyelectrolyte behavior. The electrostatic model for polyvalent cation assembly of DNA postulates an attractive screened electrostatic interaction between negatively charged phosphate groups on one DNA helix with positively charged polyvalent cations on an apposing helix. The effect of alcohol on decreasing the critical concentration of polyvalent ion necessary for precipitation has been rationalized as an increased attractive interaction between helices due to the lowered dielectric constant of alcohol-water solutions. Such a model would, of course, predict a problematic preferential inclusion of nonpolar alcohols within the high charge density Spd^{3+}-DNA phase. This framework ignores changes in self-energies or Born energies (*35*) resulting from the difference in solvation of the charged groups with water and with nonpolar alcohol. These energies are generally larger than the electrostatic interactions between charged groups. An alternative explanation is that alcohols are strongly excluded from the DNA surface (i.e., a preferential hydration) and that Spd^{3+} precipitation of DNA reduces the excess number of included waters (i.e., there is a release of excess water accompanying precipitation). A strong exclusion of MPD from the protein RNAse has been directly measured by densitometry (*25*, *36*) and rationalized as a strong repulsion from surface charge groups.

Indeed, we measure a strong exclusion of alcohols from Spd^{3+}-DNA arrays. Force curves of Spd^{3+}-DNA aggregates in alcohol-PEG solutions indicate that there is a very strong interaction of the alcohols MPD and 2-propanol with DNA. This is quite unlike glycerol, which shows no preferential interaction, inclusion or exclusion. The effect of alcohol is not through Spd^{3+} activity because force curves are insensitive to bathing solution Spd^{3+} concentrations between 0.5 and 20 mM. The PEG osmotic pressure necessary to maintain a constant interhelical spacing (or volume) is linearly dependent on the alcohol osmotic pressure (Figure 3). This indicates that the number of excess water molecules associated with the Spd^{3+}-DNA phase is constant, independent of alcohol concentration, at that spacing.

The great advantage of using the osmotic stress approach for measuring solute exclusion is that a spatial distribution of solute from the macromolecular surface can now be extracted or more precisely a change in the number of excess waters as a function of the distance between DNA helices. Figure 4 shows the variation in equivalent excess alcohol osmotic pressure normalized by total alcohol osmotic pressure with DNA helix spacing. The overlap of the various concentrations simply means that the excess water is independent of alcohol concentration at any particular spacing. The overlap of data with and without added PEG indicates we have properly accounted for the effect of PEG on alcohol activities. Over this limited range of spacings, the distance dependence of the excess pressure can be fit well by an exponential, with decay lengths 3.8 ± 0.5 and 3.3 ± 0.2 Å for 2-propanol and MPD, respectively. These decay lengths are characteristic of a repulsive hydration force (*17*, *28*). Forces have now been measured between many different kinds of macromolecules, many charged and zwitterionic lipid layers (*17*, *37*, *38*), highly charged DNA with various different salts (*39*, *40*), collagen triple helices (*41*, *42*), naturally occurring charged polysaccharides such as ι-carrageenan and xanthan (*43*), the naturally occurring, uncharged carbohydrate schizophyllan (*43*), and an uncharged, chemically modified polysaccharide, hydroxylpropyl cellulose (*32*). A striking common feature for many of these very disparate systems is the dominance of a 3−4 Å decay length exponentially varying force at close spacings, the last 10−15 Å of separation between surfaces. Force amplitudes may vary by several orders of magnitude. We have argued that these close interactions are due to water structuring energetics. Within this framework, these repulsive hydration forces result from the restructuring of water as hydrated surfaces come in close contact. The large forces are the aggregate sum of small perturbations of many water molecules. The 10−15 Å range of these forces corresponds to about two layers of water on each surface. The 3−4 Å decay length reflects a water−water correlation length that, for example, has been observed for density fluctuations in pure water by X-ray scattering (*44*). The amplitude of the force reflects the strength of the interaction between water and the macromolecular surface and the mutual water structure on apposing surfaces. The alcohols MPD and 2-propanol seem to interact with the DNA surface at close spacings through their effect on water structuring.

Only changes in preferential hydration, $\Delta\Gamma_w$, can be calculated from the data in Figure 4, not absolute numbers. The solvation of dry Spd^{3+}-DNA aggregates, however, shows that the alcohols are highly excluded from the DNA phase. The extent of exclusion is consistent with a simple exponential exclusion of alcohol, or

$$\frac{C(D)}{C_0} \sim 1 - Ae^{-D/\lambda}$$

where $C(D)$ is the average alcohol concentration in the DNA phase at a spacing D and C_0 is the bulk concentration. Complete exclusion ($C(D) = 0$ or $\Pi_{excess}/\Pi_s = 1$) of MPD occurs at a spacing of ∼24 Å and, by extrapolation, of 2-propanol at ∼ 22 Å. A total number of included waters can be calculated by extrapolating the exponentials in Figure 4 and integrating from $D = \infty$ to the "dry" diameter of 20.4 Å, assuming a maximal value for Π_{excess}/Π_s of 1. Assuming a 3 Å radius for a water molecule and approximating DNA as a smooth cylindrical surface, the total excess number of

waters associated with DNA for 2-propanol is roughly equivalent (Gibbs dividing surface) to the first hydration layer of DNA (24 waters/bp) and about 1.5 layers for MPD (36 waters/bp). These equivalent exclusion distances are on the same order as those calculated for the exclusion of several polar solutes from protein surfaces (*30*, *45*).

A demanding test of the preferential hydration distribution function is the comparison of theory and experiment for the effect of alcohols on the critical concentration of Spd^{3+} needed for precipitation of DNA from dilute solution (Figure 5). The calculation of water release associated with Spd^{3+} precipitation is dominated by an extrapolation of the data in Figure 4. The effect of alcohol on the binding competition between Na^+ and Spd^{3+} is neglected, and $\Delta\Gamma_{Sp^{d3+}}$ is assumed to be independent of alcohol concentration. Nonetheless, the observed and predicted slopes, $d \ln([Spd^{3+}]_{crit})/d\, m_{alcohol}$, agree quite well. It is possible that the potential complications due to addition of alcohol compensate.

There has long been a tension between interpreting solute, particularly alcohol, effects on DNA conformation and interactions in terms of dielectric constants and electrostatics or as an exclusion and preferential hydration. Given the results reported here, the primary effect of alcohols on Spd^{3+}-DNA assembly is through exclusion, not through the dielectric constant. Alcohol is acting osmotically on the excess water in the condensed array. There may be a secondary, dielectric-constant-based effect that leads to less exclusion than would occur in the absence of a possible attractive electrostatic interaction, but it would be difficult to establish this convincingly. It is well-established now through the work of Ivanov and co-workers (*46*) that alcohols produce the B−A transition of DNA also through their action on water activity. This, of course, implies an exclusion of alcohol from the hydrated DNA surface. A dehydrating effect of alcohols likely applies to other conformational transitions as well (*47*), as the B−Z transition. MPD is known to affect A-tract bending (*48*). Both electrostatics and dehydration effects have been invoked as explanations. The A-tract conformation associated with the bent DNA structure is characterized by a narrow minor groove and widened major groove. If the amount of preferentially bound water that excludes MPD is different for the bent and unbent A-tract conformations then an osmotic action of MPD is required.

Solute Size Effects. A striking correlation is that MPD, which is essentially two 2-propanol molecules joined methyl to hydroxyl carbon, is also about 2-fold more excluded. A simple steric argument cannot explain this because there is no apparent barrier for glycerol that is intermediate in size. A similar correlation was observed by the osmotic stress technique for the exclusion of glycerol and methyl glucoside from hydroxypropyl cellulose arrays (unpublished experiments). An approximate correlation between size and exclusion has been observed previously for homologous series of solutes, predominately polyols and sugars, both for the preferential hydration of proteins (*30*, *49*) and more strikingly for their effect on protein and DNA conformation changes (*50*, *51*). Given an apparent dominance of hydration interactions between the alcohols examined here and DNA, a simplistic formulation would be that the total repulsive force is a sum of the contributions from the component moieties, alkyl carbons and hydroxyl oxygens. MPD (2 hydroxyls, 6 alkyl carbons) and 2-propanol (1 hydroxyl, 3 alkyl carbons) effects would then simply scale with size as observed. The insensitivity of Spd^{3+}-DNA force to glycerol with its 1:1 ratio of hydroxyls/alkyl carbons rather than 1:3 suggests to a first order approximation that hydroxyls and alkyl carbons have oppositely signed interactions with the DNA surface.

Preferential Hydration. The exclusion of solutes from proteins has been widely measured by densitometry (reviewed by Timasheff (*25*, *26*)) and osmometry (*30*). The changes in protein free energy due to the presence of solute quite often vary linearly with solute concentration. To a good approximation, this dependence indicates that the number of included or excess waters associated with the protein is constant, independent of solute concentration. Thermodynamically, the energy change of the protein associated with varying the solute concentration is simply given by the product of the change in water chemical potential and the number of excess waters or, equivalently, of the change in osmotic pressure and the volume of the excess water. The measurements of preferential hydration can only determine a number of excess waters, not their distribution. The physical interactions underlying the exclusion of solutes have long been obscure. Only equations for hard sphere steric interactions have been developed (*52*, *53*). Here we can determine not only a number of preferentially bound waters but also a distribution function. A striking convergence of preferential hydration and hydration forces is seen; the distribution of excluded alcohols from the DNA surface is determined by water structuring interactions.

This is now the second system that the distance dependence of solute exclusion has been extracted. Exclusion of polar and charged solutes from condensed hydroxypropyl cellulose (HPC) arrays is almost a mirror image of the exclusion of nonpolar alcohols from Spd^{3+}-DNA (unpublished experiments). No exclusion of MPD is seen from this hydrophobically modified cellulose. HPC, however, does strongly exclude salts, the zwitterionic osmolyte betaine glycine, and neutral, but polar solutes such as glycerol and α-methyl glucoside. Remarkably, the same 3−4 Å decay length exponential is seen for these interactions. The magnitude of the exclusion for salts follows the anion Hofmeister series, which has long been thought to reflect differences in hydration structure and energies. These combined results present a unifying theme for the interaction of solutes with macromolecular surfaces. Preferential hydration is enforced through water structuring or hydration forces.

ACKNOWLEDGMENT

We gratefully acknowledge stimulating discussions with Dr. V. Adrian Parsegian.

REFERENCES

1. Bloomfield, V. A. (1996) DNA condensation, *Curr. Opin. Struct. Biol. 6*, 334−341.
2. Bloomfield, V. A. (1997) DNA condensation by multivalent cations, *Biopolymers 44*, 269−282.
3. Bloomfield, V. A., Crothers, D. M., and Tinoco, J., I (1999) *Nucleic Acids*: *Structures, Properties, and Functions*, University Science Books, Sausalito, CA.
4. Luo, D., and Saltzman, W. M. (2000) Synthetic DNA delivery systems, *Nature Biotech. 18*, 33−37.
5. Templeton, N. S., and Lasic, D. D. (2000) *Gene therapy*: *Therapeutic mechanisms and strategies*, Marcel Dekker, New York.

6. Huang, L., Hung, M.-C., and Wagner, E. (1999) *Nonviral Vectors for Gene Therapy*, Academic Press, San Diego.
7. Schellman, J. A., and Parthasarathy, N. (1984) X-ray diffraction studies in cation-collapsed DNA, *J. Mol. Biol. 175*, 313−329.
8. Rau, D. C., and Parsegian, V. A. (1992) Direct measurement of temperature-dependent solvation forces between DNA double helices, *Biophys. J. 61*, 260−271.
9. Rau, D. C., and Parsegian, V. A. (1992) Direct measurement of the intermolecular forces between counterion-condensed DNA double helices: Evidence for long-range attractive hydration forces, *Biophys. J. 61*, 246−259.
10. Pelta, J., Durand, D., Doucet, J., and Livolant, F. (1996) DNA mesophases induced by spermidine: Stuctural properties and biological implications, *Biophys. J. 71*, 48−63.
11. Rouzina, I., and Bloomfield, V. A. (1996) Macroion attraction due to electrostatic correlation between screening counterions. 1. Mobile surface-absorbed ions and diffuse ion cloud, *J. Phys. Chem. 100*, 9977−9989.
12. Ha, B.-Y., and Liu, A. J. (1997) Counterion-mediated attraction between two like-charged rods, *Phys. Rev. Lett. 79*, 1289−1292.
13. Lyubartsev, A. P., and Nordenskiold, L. (1997) Monte Carlo simulation study of DNA polyelectrolyte properties in the presence of multivalent polyamine ions, *J. Phys. Chem. B 101*, 4335−4342.
14. Raspaud, E., Cruz, O. d. l., Sikorav, J.-L., and Livolant, F. (1998) Precipitation of DNA by polyamines: A polyelectrolyte behavior, *Biophys. J. 74*, 381−393.
15. Shklovskii, B. I. (1999) Wigner crystal model of counterion induced bundle formation of rodlike polyelectrolytes, *Phys. Rev. Lett. 82*, 3298−3271.
16. Olvera de la Cruz, M., Belloni, L., Delsanti, M., Dalbiez, J. P., Spalla, O., and Drifford, M. (1995) Precipitation of highly charged polyelectrolyte solution in the presence of multivalent salts, *J. Chem. Phys. 103*, 5781−5791.
17. Leikin, S., Parsegian, V. A., Rau, D. C., and Rand, R. P. (1993) Hydration forces, *Annu. Rev. Phys. Chem. 44*, 369−395.
18. Gosule, L. C., and Schellman, J. A. (1978) DNA condensation with polyamines. I. Spectroscopic studies, *J. Mol. Biol. 121*, 311−326.
19. Wilson, R. W., and Bloomfield, V. A. (1979) Counterion-induced condensation of Deoxyribonucleic Acid. A light-scattering study, *Biochemistry 18*, 3192−2196.
20. Widom, J., and Baldwin, R. L. (1980) Cation-induced toroidal condensation of DNA: Studies with $Co^{3+}(NH_3)_6$, *J. Mol. Biol. 144*, 431−453.
21. Takahashi, M., Yoshikawa, K., Vasilevskaya, V. V., and Khokhlov, A. R. (1997) Discrete coil-globule transition of single duplex DNAs induced by polyamines, *J. Phys. Chem. B 101*, 9396−9401.
22. Arscott, P. G., Ma, C., Wenner, J. R., and Bloomfield, V. A. (1995) DNA condensation by cobalt hexaammine (III) in alcohol-water mixtures: Dielectric constant and other solvent effects, *Biopolymers 36*, 345−364.
23. Flock, S., Labarbe, R., and Houssier, C. (1996) Dielectric const and ionic strength effects on DNA precipitation, *Biophys. J. 70*, 1456−1465.
24. Mel′nikov, S. M., Khan, M. O., Lindman, B., and Jonsson, B. (1999) Phase behavior of single DNA in mixed solvents, *J. Am. Chem. Soc. 121*, 1130−1136.
25. Timasheff, S. N. (1993) The control of protein stability and association by weak interactions with water: How do solvents affect these processes? *Annu. Rev. Biophys. Biomol. Struct. 22*, 67−97.
26. Timasheff, S. N. (1998) Control of protein stability and reactions by weakly interacting cosolvents: The simplicity of the complicated, *Adv. Protein Chem. 51*, 355−432.
27. Parsegian, V. A., Rand, R. P., Fuller, N. L., and Rau, D. C. (1986) Osmotic stress for the direct measurement of intermolecular forces, *Methods Enzymol. 127*, 400−416.
28. Strey, H. H., R., P., Rau, D. C., and Parsegian, V. A. (1998) DNA−DNA interactions, *Curr. Opin. Struct. Biol. 8*, 309−313.
29. Leikin, S., Rau, D. C., and Parsegian, V. A. (1991) Measured entropy and enthalpy of hydration as a function of distance between DNA double helices, *Phys. Rev. A 44*, 5272−5278.
30. Courtenay, E. S., Capp, M. W., Anderson, C. F., and Record Jr., M. T. (2000) Vapor pressure osmometry of osmolyte-protein interactions: Implications for the action of osmoprotectants in vivo and for the interpretation of "osmotic stress" experiments in vitro, *Biochemistry 39*, 4455−4471.
31. Pelta, J., Livolant, F., and Sikorav, J.-L. (1996) DNA aggregation induced by polyamines and cobalthexamine, *J. Biol. Chem. 271*, 5656−5662.
32. Bonnet-Gonnet, C., Leikin, S., Chi, S., Rau, D. C., and Parsegian, V. A. (2001) Measurement of forces between hydroxypropylcellulose polymer: Temperature favored assembly and salt exclusion, *J. Phys. Chem. B 105*, 1877−1886.
33. Parsegian, V. A., Rand, R. P., and Rau, D. C. (2000) Osmotic stress, crowding, preferential hydration, and binding: A comparison of perspectives, *Proc. Natl. Acad. Sci. U.S.A. 97*, 3987−3992.
34. Matulis, D., Rouzina, I., and Bloomfield, V. A. (2000) Thermodynamics of DNA binding and condensation: Isothermal titration calorimetry and electrostatic mechanism, *J. Mol. Biol. 296*, 1053−1063.
35. Friedman, H. L., and Krishnan, C. V. (1973) Thermodynamics of Ionic Hydration, in *Water, A Comprehensive Treatment* (Franks, F., Ed.) pp 1−118, Plenum Press, New York.
36. Pittz, E. P., and Timasheff, S. N. (1978) Interaction of Ribonuclease A with aqueous 2-methyl-2,4-pentanediol at pH 5.8, *Biochemistry 17*, 615−623.
37. McIntosh, T. J. (2000) Short-range interactions between lipid bilayers measured by X-ray diffraction, *Curr. Opin. Struct. Biol. 10*, 481−485.
38. Petrache, H. I., Gouliaev, N., Tristam-Nagle, S., Zhang, R., Suter, R. M., and Nagle, J. F. (1998) Interbilayer interactions: High-resolution X-ray study, *Phys. Rev. E 57*, 7014−7024.
39. Podgornik, R., Rau, D. C., and Parsegian, V. A. (1994) Parametrization of direct and soft steric-undulatory forces between DNA double helical polyelectrolytes in solutions of several different anions and cations, *Biophys. J. 66*, 962−971.
40. Podgornik, R., Strey, H. H., Gawrisch, K., Rau, D. C., Rupprecht, A., and Parsegian, V. A. (1996) Bond orientational order, molecular motion, and free energy of high-density DNA mesophases, *Proc. Natl. Acad. Sci. U.S.A. 93*, 4261−4266.
41. Leikin, S., Rau, D. C., and Parsegian, V. A. (1994) Direct measurement of forces between self-assembled proteins: Temperature-dependent exponential forces between collagen triple helices, *Proc. Natl. Acad. Sci. U.S.A. 91*, 276−280.
42. Leikin, S., Rau, D. C., and Parsegian, V. A. (1995) Temperature-favoured assembly of collagen is driven by hydrophilic not hydrophobic interactions, *Nature Struct. Biol. 2*, 205−210.
43. Rau, D. C., and Parsegian, V. A. (1990) Direct Measurement of forces between linear polysaccharides xanthan and schizophyllan, *Science 249*, 1278−1281.
44. Xie, Y., Ludwig, J., K. F., Morales, G., Hare, D. E., and Sorensen, C. M. (1993) Noncritical behavior of density fluctuations in supercooled water, *Phys. Rev. Lett. 71*, 2050−2053.
45. Arakawa, T., and Timasheff, S. N. (1982) Stabilization of protein structure by sugars, *Biochem. 21*, 6536−6544.
46. Malenkov, G., Minchenkova, L., Minyat, E., Schyolkina, A., and Ivanov, V. (1975) The nature of the B−A transition of DNA in solution, *FEBS Lett. 51*, 38−42.
47. Saenger, W., Hunter, W. N., and Kennard, O. (1986) DNA conformation is determined by economics in the hydration of phosphate groups, *Nature 324*, 385−388.
48. Sprous, D., Zacharias, W., Wood, Z. A., and Harvey, S. C. (1995) Dehydrating agents sharply reduce curvature in DNAs containing A tracts, *Nucleic Acids Res. 23*, 1816−1821.
49. Ebel, C., Eisenberg, H., and Ghirlando, R. (2000) Probing protein-sugar interactions, *Biophys. J. 78*, 385−393.
50. Davis-Searles, P. R., Morar, A. S., Saunders, A. J., Erie, D. A., and Pielak, G. J. (1998) Sugar-induced molten-globule model, *Biochemistry 37*, 17048−17053.
51. Preisler, R. S., Chen, H. H., Colombo, M. F., Choe, Y., Short, J., B., J., and Rau, D. C. (1995) The B form to Z form transition of poly(dG-m^5dC) is sensitive to neutral solutes through an osmotic stress, *Biochemistry 34*, 14400−14407.
52. Minton, A. (1998) Molecular crowding: Analysis of high concentrations of inert cosolutes on biochemical equilibria and rates in terms of volume exclusion, *Methods Enzymol. 295*, 127−149.
53. Davis-Searles, P. R., Saunders, A. J., Erie, D. A., Winzor, D. J., and Pielak, G. J. (2001) Interpreting the effects of small uncharged solutes on protein-folding equilibria, *Annu. Rev. Biophys. Biomol. Struct. 30*, 271−306.

BI049559S