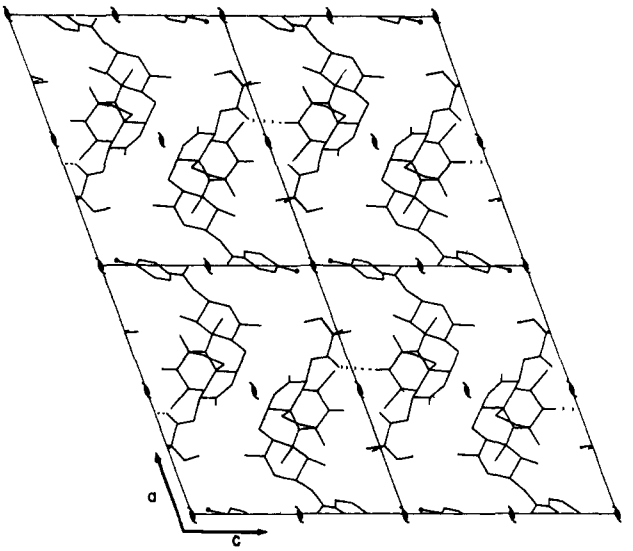

Fig. 4.—Packing diagram of the molecule. The hydrogen-bonded molecules form spirals parallel to the *b* axis; the spirals are held together by van der Waals' attractions.

sufficiently, we have listed in Table V all the oxygen atoms near enough to one another to permit internal hydrogen bonds.

TABLE V
CLOSE OXYGEN–OXYGEN DISTANCES AND PERTINENT ANGLES

	Units, Å.
O_2–O_3	2.83
O_3–O_4	2.60
O_4–O_5	2.86
O_{10}–O_{11}	2.70
	Units, deg.
C_1–O_3...O_2	57.3
C_1–O_3...O_4	110.4
C_2–O_2...O_3	57.4
C_{11}–O_4...O_3	100.3
C_{11}–O_4...O_5	55.0
C_{12}–O_5...O_4	54.5
C_{20}–O_{10}...O_{11}	64.4
C_{21}–O_{11}...O_{10}	62.5

Acknowledgment.—We wish to express our thanks to Drs. D. Harker, J. Bello, and H. H. Mills for many stimulating discussions, Dr. D. R. Harris for the use of some of his IBM 1620 crystallographic programs, and Drs. H. J. Schaeffer and K. K. Kaistha for giving us the crystalline sample. We also express thanks to Mrs. C. Vincent for aiding in the collection of the intensity data and acknowledge the use of the ERBR1 and ER-FR2 least-squares and Fourier summation programs for the IBM 7090, by Van den Hende, Sly, and Shoemaker.

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On the Interaction of Magnesium with Deoxyribonucleic Acid[1a]

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Counterion activity coefficients of the pure sodium and magnesium salts of DNA (NaDNA and MgDNA, respectively) were measured by means of ion-exchange membrane electrodes. Viscosity measurements and determination of the molar extinction coefficients at 258 mμ were carried out on the same material. The following conclusions have been reached: (1) The magnesium activity coefficient of thermally denatured Mg-DNA is slightly lower than that of the native salt, whereas the sodium activity coefficient of thermally denatured NaDNA is substantially higher than that of the corresponding native salt. (2) A correlation is made between the magnitude and the concentration dependence of the activity coefficient and the thermal stability of the native structure. (3) Evidence is provided which suggests that the modes of binding of sodium and magnesium ions by DNA are quite similar, being diffuse electrostatic attractions typical of similar salts of a wide variety of synthetic polyelectrolytes. Evidence for some site binding is discussed in relation to the behavior of MgDNA when heated in solutions of simple magnesium salts.

Introduction

The importance of magnesium in many biological processes is well known. To cite several well-documented examples, there is a magnesium requirement both in the enzymatic synthesis of deoxyribonucleic acid (DNA)[2] and in the function of ATP-ase.[3] It has also been suggested that magnesium plays a significant role in holding ribosomal nucleoprotein together.[4] However, the nature of the interaction of magnesium with nucleic acids has been unclear with regard to molecular interpretation and, indeed, with regard to phenomenological description of the interaction. Shack and co-workers have reported that native DNA binds magnesium more strongly than does denatured DNA on the basis of conductivity data[5] and titration with eriochrome black T.[6] Zubay and Doty have concluded precisely the opposite based on conductivity data.[7] The latter attributed the stronger binding by denatured DNA to the availability of purine and pyrimidine bases as possible binding sites. However, recent magnetic resonance studies of 0.1–0.2 *M* adenosine triphosphate[8a] and deoxyadenosine and cytidine[8b] have shown that there is no detectable interaction be-

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tween Mg ion and the adenine portion of these molecules at neutral pH and at 1:1 mole ratio of Mg to nucleotide, or indeed, even up to 8:1 mole ratio in deoxyadenosine and cytidine.[8b]

The most recent study of the conductance of polynucleotides and "denatured" DNA[9] has shown that there is a definite interaction up to an equivalent Mg/P ratio of 1.0 and thus clarifies some of the earlier work.[5,7] Careful dialysis studies have shown there is no significant difference between the affinities of native or denatured DNA for magnesium.[10] Clearly, the results of some of the magnesium binding studies referred to above are mutually incompatible.

That magnesium interacts quite dramatically with DNA has been demonstrated by the interesting experiments of Dove and Davidson[11] in which the effect of magnesium on the melting temperature, T_m, was studied. They showed that, at a concentration of DNA phosphorus of 10^{-4} M and an equivalent ratio of magnesium to phosphorus of 1.0, T_m first decreases and then rises with decreasing sodium concentration. It is clear from this work that the interaction of magnesium with DNA, under the above conditions, is such as to eliminate the usual ionic strength effects on T_m. Another indication of the stabilization of the native structure of DNA by magnesium was reported by Eichhorn.[12]

Since all of the experiments on magnesium binding by DNA have been done in the presence of usually large amounts of sodium, it seemed to us that the study of the pure magnesium salt of DNA and solutions of the latter containing only magnesium cations would lead to a great simplification of the interpretation of any experiments concerning magnesium binding. Indeed, we will show that such studies shed considerable light on the ostensibly incompatible earlier work alluded to above.

It should be pointed out that the term *ion binding* has been used to describe binding phenomena including physical or chemical adsorption, specific ion pairing, chemical complex formation, and diffuse electrostatic interaction between counterion and polyion.[13] As is usually the case in measuring the extent of ion binding, the experimental manifestation of the binding is insufficient in itself to elucidate the nature of the binding in molecular terms. In what follows, we refer to the extent of binding as it is manifested in the magnitude of the thermodynamic activity coefficient of counterions (Na^+ or Mg^{2+}) in the sense that the lower the magnitude of the activity coefficient, the greater is the extent of binding. This operational definition of ion binding is made for reasons of clarity; clearly, the definition in no way distinguishes the microscopic details of the phenomenon. However, it will be shown that activity coefficient data when combined with viscosity and denaturation data lead to a description of the binding phenomenon in molecular terms.

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Experimental

Materials.—The sodium salt of DNA (NaDNA) was isolated from calf thymus tissue by the method of Kay, *et al.*[16] The magnesium salt (MgDNA) was prepared by exhaustive dialysis using 0.1 M $MgCl_2$ and sufficient $Mg(OAc)_2$ to maintain a pH of from 7 to 8. The ratio of outer-to-inner solution volumes was *ca.* 40:1. Four changes were sufficient to remove all sodium ion from the inner solution as determined by flame photometry; however, six changes of solution were used routinely. With shaking and stirring the outer solution was changed every 3 hr.; without agitation the solution was changed after holding overnight. When going from the magnesium form back to the sodium form, ten changes of the outer solution were made. Trace analysis for magnesium was done with a Jarrell–Ash emission spectrograph. All solutions were stored at 0–4°. Manipulations of DNA at low ionic strength were carried out at 0–4° also. MgDNA was isolated by alcohol precipitation followed by exhaustive washing with 3:1 alcohol–water. Analysis for Mg and P was done by EDTA titration[17] and colorimetric analysis,[18] respectively, on the residue from wet-ashing in H_2SO_4–HNO_3. The equivalent ratio was consistently found to be 1.0 ± 0.02. The molecular weight of the NaDNA was estimated from the intrinsic viscosity correlation of Eigner.[19] The DNA samples labeled II were carefully prepared in the same manner and in the same environment so as to give MgDNA and NaDNA of the same molecular weight; *e.g.*, while dialyzing half the material to the Mg form, the other half was dialized against sodium buffer in exactly the same manner.

All other chemicals were at least ACS reagent grade. The standard saline–citrate buffer (SSC) was 0.14 M NaCl, 0.014 M sodium citrate, ionic strength 0.2. The standard magnesium buffer was 0.062 M $MgCl_2$, 0.005 M $Mg(OAc)_2$, ionic strength 0.2. NaCl and $MgCl_2$ used in the ion-exchange membrane e.m.f. cell were recrystallized from solutions of reagent grade materials.

Denaturation.—Both Na- and MgDNA were denatured by heating, at 10^{-2} M in P, to 95° and holding for 10 min. This was followed by rapid quenching to room temperature. The presence or absence of buffer salts depended on the specific experiment.

Spectroscopy.—A Cary Model 14 spectrophotometer was used for all ultraviolet measurements. The 10-mm. cell required dilution of the DNA solutions to *ca.* 10^{-4} M in P. To obtain a melting curve, the sample was placed in a standard Cary heating chamber to which water of a given temperature was pumped *via* a Haake Model F thermostat. For the cooling section of the curve, a coil immersed in ice was inserted into the Haake pumping circuit. The temperature of the solution was recorded directly by a thermocouple in contact with the solution in the neck of the Cary cell. The cell was closed tightly enough so that several cycles of heating and cooling could be made without noticeable evaporation.

Activity Coefficient Measurements.—The basic e.m.f. cell design was that of Scatchard and Helfferich.[20] Stirring at the membrane surfaces was done by miniature d.c. motors. The lucite cell was not thermostated, since the laboratory temperature was essentially constant at 22°. The cation-exchange membrane was kindly supplied byAmerican Machine and Foundry, Springdale, Conn.—designated AMF C103C. It was converted to the Na or Mg form by exhaustive dialysis and washing. Contact with the cell solutions was made through agar–agar bridges saturated with KCl. The tips of these bridges were curved upward to minimize diffusion of the KCl. Several pairs of bridges were used so that duplicate readings could be taken and so that the tips could be regenerated in saturated KCl solution between readings. The bridges were connected to saturated calomel electrodes which were specially prepared and equilibrated.[21] The e.m.f. was detected by a Leeds and Northrup K-3 potentiometer and 2430 galvanometer. Calibration of the cell was carried out with chloride solutions of known Na or Mg concentration. The

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cation activity, a_+, of the reference soilutons was calculated as follows:

(1) Using reported values of the mean molal activity coefficients, $\gamma_\pm$, of KCl, NaCl, and $MgCl_2$, the ionic size parameters, $\mathring{a}$, appearing in the Debye–Hückel theory[22] were calculated. Utilizing the value of $\mathring{a}$ for each of the salts, the theory then allows one to compute the mean molal activity coefficient for any concentration or ionic strength.[22]

2. To calculate cation activity coefficients, γ_+, we make use of the assumption that the activity coefficient of the chloride ion is a function of ionic strength only and independent of the cationic species present, and also that the ionic activity coefficients of K^+ and Cl^- are equal to each other and equal also to the mean molal activity coefficient of KCl in solutions containing only KCl.[14]

3. On the basis of these assumptions, we have the simple relationships for the molal activity coefficients, γ_+, that

$$\gamma_{Na^+} = \frac{(\gamma_{\pm\ NaCl})^2}{(\gamma_{\pm\ KCl})} \text{ and } \gamma_{Mg^{2+}} = \frac{(\gamma_{\pm\ MgCl_2})^3}{(\gamma_{\pm\ KCl})^2}$$

where it is understood that the mean molal quantities in each equation correspond to the same ionic strength. The ionic activity is thus obtained from $a_+ = \gamma_+ m_+$.

Plots of e.m.f. *vs.* a_+ gave essentially straight lines with slopes of 0.058 and 0.030 for Na and Mg, respectively (*cf.* 0.0588 and 0.0294 from theory at 22°). To obtain a more nearly perfect fit, a quadratic in the e.m.f. was fitted by an IBM 704, and the resulting predicting equations were used to convert e.m.f. values on unknowns to cation activities. Experiments with the Mg electrodes showed that the e.m.f. was essentially independent of the anion; *e.g.*, Cl^-, NO_3^-, or SO_4^{2-}.

Viscosity.—A multibulb capillary viscometer was constructed using 454 cm. of polyethylene tubing (radius 7.5×10^{-2} cm.) wound on an aluminum drum and connected to an efflux chamber similar to that of a Ubbelohde viscometer. This device yields Newtonian shear rates, σ_N, for water at the capillary wall of 195, 122, and 48 sec.$^{-1}$. Correction for non-Newtonian behavior was not made, since it was found to be negligible at the concentrations of DNA studied; *i.e.*, d ln σ_N/d ln $S_w \approx 1.0$ in the Rabinowitsch equation for generalized capillary flow, where S_w is the unit shear stress at the wall.[23] The reciprocal of the reduced viscosity, η_{sp}/C, was plotted against σ_N to obtain the reduced viscosity at zero gradient. In general, as has been observed,[19] this method of extrapolation proved best. The resulting reduced viscosities, obtained at several concentrations, were extrapolated for native samples in the usual way to yield the intrinsic viscosity. For the denatured samples in pure water the reciprocal reduced viscosity was plotted against $\sqrt{C}$ to make the extrapolation (Fuoss-type plot).[24] The molecular weight of the DNA used in the viscosity work (DNA II) was found from the value of the intrinsic viscosity of the native NaDNA in SSC[19] to be 7×10^6.

Results

The characteristics of the various DNA samples are shown in Tables I and II and Fig. 1. Note that the experiments on MgDNA were done in the absence of any other kind of cation—an important distinction when comparing the data with other published results. Table I shows that MgDNA has a molar extinction coefficient, ϵ (P), not unlike that for NaDNA. The increase in ϵ (P) on heating NaDNA in SSC is in agreement with results reported by many other workers—in the range of 30–40%, depending on the ionic environment and DNA concentration. MgDNA in pure water exhibits, reproducibly, only about a 10% increase in ϵ (P) after heating and cooling. The rate of cooling has little effect on this result, as shown in Fig. 1. At elevated temperature the increase in ϵ (P) is the same as for NaDNA,[11,12] but about $^3/_4$ of the hypochromicity is recovered on slow cooling—just as was the case for the quenched samples in Table I. This heat-treated and cooled MgDNA then shows reversible behavior on successive heating and cooling cycles as is clearly shown in Fig. 1. The melting temperature, T_m, of MgDNA in pure water is 82°, about the same as for NaDNA in 0.1–0.2 *M* NaCl.[11,25] When MgDNA is heated at a concentration of DNA phosphorus of 10^{-2} *M* in a buffer of $MgCl_2$–$Mg(OAc)_2$ at an ionic strength of 0.2, the mixture precipitates at a temperature lower than 82°. A brief study of this phenomenon showed that at the low DNA concentrations used in the Cary instrument (*ca.* 10^{-4} *M* in P), there is no lowering of T_m over a range of concentrations of the buffer. However, at the highest buffer concentration (ionic strength 0.2), the melting curve became erratic at about the inflection point, and a precipitate was observed in the cell after the experiment was completed. In a series of visual observations at constant ionic strength of 0.2 (in the simple Mg salts), the temperature at which turbidity appeared was noted *vs.* DNA concentration. The result is that precipitation occurs at successively lower temperatures as the DNA concentration is increased. That the precipitate so formed was denatured MgDNA is strongly suggested by the fact that addition of $MgCl_2$–$Mg(OAc)_2$ buffer to samples of native and heat-denatured MgDNA at 5×10^{-3} *M* in P to a final ionic strength of simple salt of 0.2 precipitated only the denatured MgDNA. It should be pointed out that this result, though indicative, does not constitute a complete proof of the fact that the precipitate formed at elevated temperatures is denatured.

The viscosity data show that MgDNA is hydrodynamically smaller than NaDNA under the same con-

TABLE I

EXTINCTION COEFFICIENTS AT 258 mμ OF MAGNESIUM AND SODIUM DNA

Sample	ϵ (P)	$\Delta\epsilon$ (P)/ϵ (P)[a]
MgDNA (I) in H_2O	6253	0.10
NaDNA (I) in H_2O	9071[b]	..
MgDNA (II) in H_2O	6552	0.08, 0.11
MgDNA (II) in buffer[c]	6570	Ppt.[d]
NaDNA (II) in H_2O	8630[b]	..
NaDNA (II) in buffer[c]	6958	0.33

[a] Relative increase in ϵ (P) after heating 10 min. at 95° and quenching rapidly to room temperature. [b] Partially denatured by dilution. [c] Buffers are at 0.2 ionic strength (see Experimental). [d] See the text for details of this phenomenon.

TABLE II

INTRINSIC VISCOSITIES OF DNA AT ZERO GRADIENT

Solution	Intrinsic viscosity (dl./g.) NaDNA	MgDNA
Native in buffer[a]	54	32
Native in pure water	(530)[b]	165
Denatured in buffer[a]	(4)[c]	Ppt.
Denatured in pure water	204	69

[a] Buffered at pH 7–8; ionic strength 0.2. [b] Estimated from the data of T. Kurucsev, *Arch. Biochem. Biophys.*, **102**, 120 (1963). [c] Estimated from the data of S. A. Rice and P. Doty, *J. Am. Chem. Soc.*, **79**, 3937 (1957).

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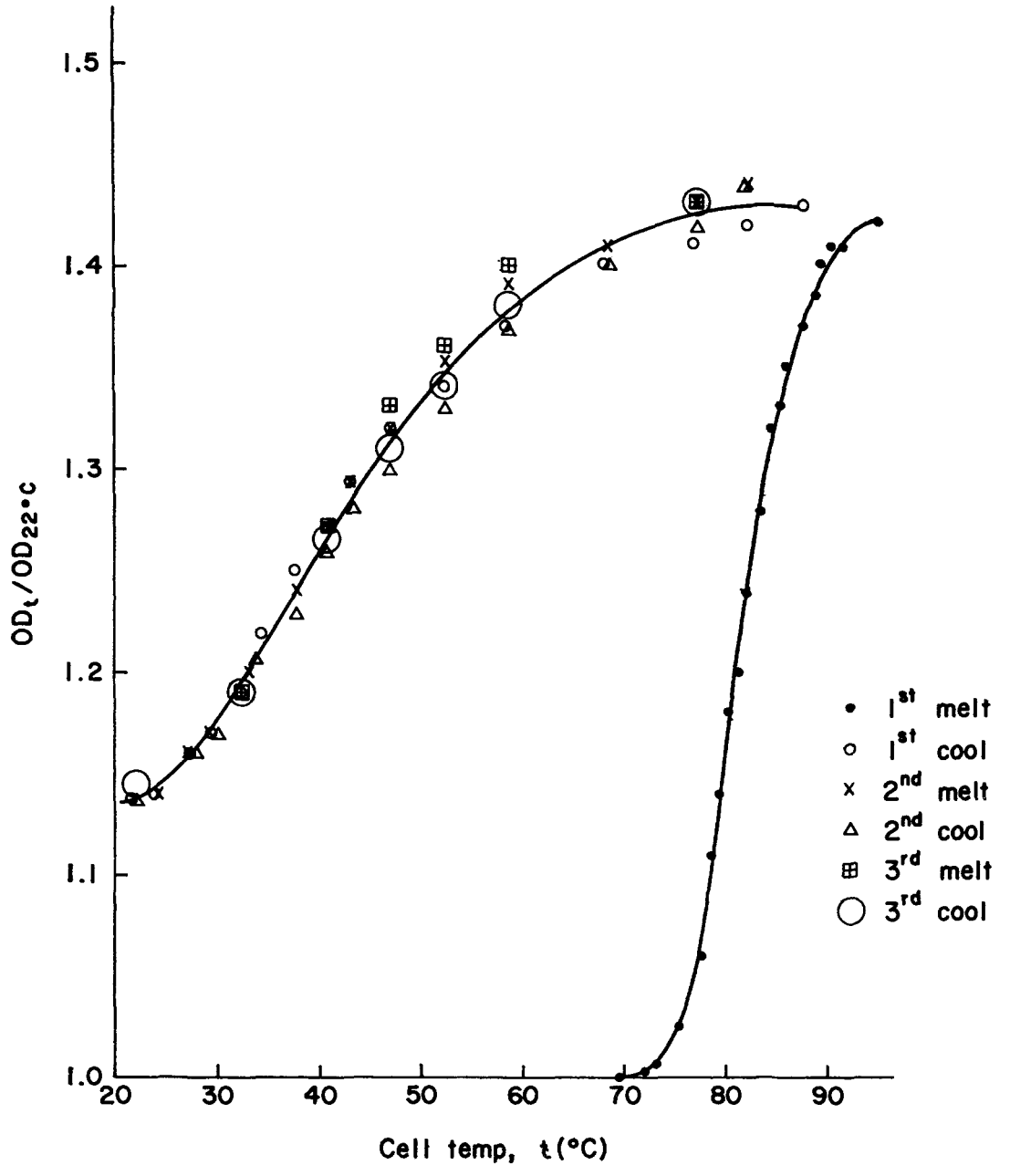

Fig. 1.—Dependence on temperature of the optical density at 258 mμ of MgDNA in pure water: heating rate, *ca.* 2½°/min.; cooling rate, *ca.* 3°/min.

ditions. The plot of η_{sp}/C *vs.* C for native DNA in pure water or in buffer is a straight line of positive slope at DNA concentrations of 5×10^{-3} g./dl. and lower. The plots for denatured DNA samples in pure water show upward curvature with decreasing concentration necessitating the use of the Fuoss-type plot to obtain the intrinsic viscosities. This upward curvature is characteristic of flexible polyelectrolytes in pure water, and the behavior was only observed on denatured samples in the absence of simple salt. That the differences in viscosity between the Mg and Na systems are real was demonstrated by dialyzing some of the native MgDNA back to the Na form and remeasuring the viscosity in SSC. Despite some 3 months elapsed time during which degradation might have occurred, the reduced viscosity, measured at a single concentration, returned to approximately the original value. This experiment also proved, by the absence of Mg in the final NaDNA solution as determined by emission spectroscopy, that the interaction between DNA and Mg is a reversible one with respect to replacement by Na.

Figure 2 shows that the activity coefficient for Mg ion in pure aqueous solutions of MgDNA is very low indeed. Denaturing causes a slight but definite further lowering of the value. Over the range studied, the activity coefficients decrease with decreasing polymer concentration. In the NaDNA system, the activity coefficient of native DNA is very much larger than for Mg in MgDNA, and the value increases markedly on denaturation in agreement with the results of Ascoli and co-workers.[26,27] As the native

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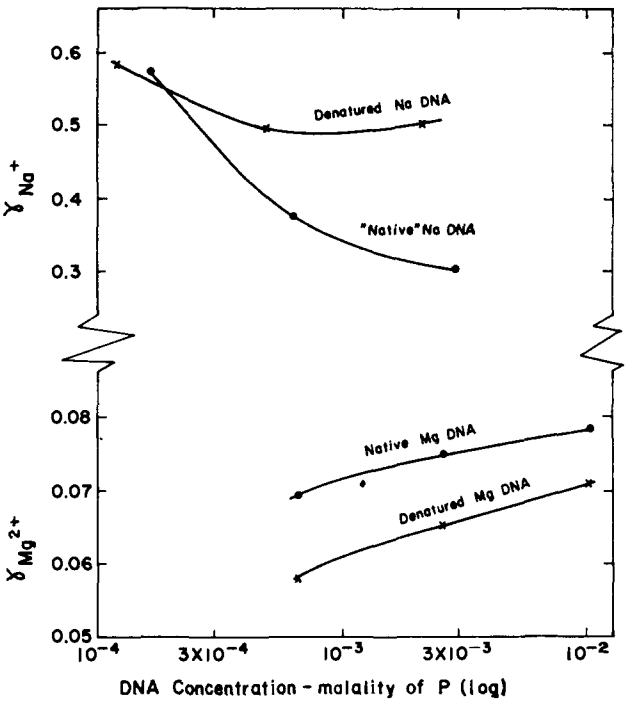

Fig. 2.—Counterion activity coefficients of salt-free DNA solutions at 22° and neutral pH.

NaDNA is diluted, the activity coefficient of the Na ion rises until it essentially coincides with the value for denatured NaDNA. The value for the denatured NaDNA begins to show an upturn at approximately 5×10^{-4} *m* in P.

Discussion

The physical properties of polymeric systems are intimately correlated with macromolecular configuration. In particular, one expects that the magnitude and concentration dependence of the counterion activity coefficient in DNA solutions ought to reflect significant alterations in the structure of the molecule. In the following section we consider the relationship of counterion activity to the thermal stability of the native DNA structure and, in the next section, the most probable mode of interaction of the counterions with the DNA.

Counterion Activity and Structural Stability.—A number of experiments have indicated a striking dependence of the melting temperature, T_m, of DNA on ionic strength.[11,25] T_m, which is a direct measure of the thermal stability of the DNA helix, is quite generally found to rise with increasing ionic strength in cases where sodium chloride is employed as the supporting electrolyte. The interpretation of these observations is simply that the added electrolyte serves to screen the electrostatic repulsion of charged phosphate groups on opposite strands.[28] We have, therefore, a rather simple criterion for relative stability of the helical structure; namely, systems in which the phosphate repulsion is somehow screened or reduced are more thermally stable. It must be emphasized that we are dealing with a question of relative stability in the sense that the forces tending to stabilize the DNA helix are certainly more complicated than the long-range electrostatic forces of the present discussion. We exclude from consideration those ions which are capable of affecting the secondary structure through direct and specific interactions with nucleotide bases.[12]

We turn now to the correlation between counterion binding and secondary structure. If one compares two DNA solutions in which the molecules have identical secondary structure and concentration and differ only in the nature of the counterion, it is a reasonable expectation that the magnitudes of the counterion activity coefficients will provide a measure of the relative stability of the helix according to our criterion. Clearly, the solution in which counterions are bound to a greater extent is expected to be more thermally stable towards denaturation. However, the magnitude of the counterion activity coefficient is not, by itself, sufficient to give any information concerning the secondary structure or stability, *a priori*, since it is well established that the extent of binding may change significantly with structure of the polyion. For example, it is known experimentally that NaDNA undergoes dilution denaturation at ordinary room temperature.[26,29] Figure 2 shows that the extent of binding for native NaDNA decreases rapidly with dilution. In this case the rapid increase of γ_{Na^+} with dilution is a reflection of the breakdown of secondary structure. Hence some caution must be exercised in any attempt to draw inferences concerning thermal stability from comparisons of the magnitudes of activity coefficients. Such comparisons are meaningful only when the secondary structures are reasonably identical. In the present instance, the values of the activity coefficients of Mg-DNA and NaDNA obtained at the highest concentrations possible are, in fact, indicators of relative thermal stability.

From Fig. 2 one sees that not only is the magnitude of $\gamma_{Mg^{2+}}$ considerably smaller than γ_{Na^+} for both native and denatured species, but, over the concentration range studied, the value of $\gamma_{Mg^{2+}}$ is slightly decreasing while an upturn is noted for both sodium salts The very low magnitude of $\gamma_{Mg^{2+}}$ is clearly consistent with the observed effect of magnesium on the thermal stability of the DNA helix and the fact that $\gamma_{Mg^{2+}}$ is so insensitive to dilution indicates that the secondary structure is essentially undisturbed by the process. Comparison of the concentration dependences of the denatured and native sodium salts shows that the extent of binding by the latter decreases rapidly with dilution. The concentration at the point of intersection of the two curves, 2×10^{-4} *m*, might possibly correspond to the concentration at which the native structure has broken down. Unfortunately, it was technically impossible to extend the measurements to lower concentrations in order to observe whether the two curves coalesce.

On the other hand, it should be pointed out that the precipitation of MgDNA at high ionic strength at temperatures lower than the T_m found for pure solutions of MgDNA may mean that the thermal stability of Mg-DNA has been reduced by the addition of excess $MgCl_2$. Although this observation is seemingly inconsistent with our discussion of the electrostatic criterion for

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stability, it will be shown to be understandable in the subsequent discussion of the ion-binding mechanism.[30] Although we have no evidence concerning the nature of the precipitate obtained by heating MgDNA in Mg buffer at high ionic strength, we re-emphasize that increasing the DNA concentration at fixed (0.2) ionic strength lowers the temperature at which the precipitate first appears. This suggests that under these conditions a destabilization of the helix may be occurring.

The Nature of the Ionic Interaction.—If the binding of a counterion by a polyion is strictly of a diffuse electrostatic nature (no specific ion pairing) one expects that the extent of binding will depend primarily upon the density of charge of the polyion.[14,15] The density of charge in the case of DNA must certainly be higher for the polyion in the native state than in the denatured state.[27] Hence, one anticipates that the native polyion should bind more strongly than the denatured if diffuse attraction is the binding mechanism. Figure 2 shows that this qualitative behavior is indeed followed by NaDNA. However, the reverse is true of MgDNA.

A comparison of the intrinsic viscosities of native and denatured MgDNA in pure water indicates the axial ratio of the latter is considerably lower than the former, and the observed upward curvature of the reduced viscosity of the denatured salt implies extensive coiling. The secondary structure of heat-denatured DNA is of paramount importance in interpreting the ion-binding results of the two denatured materials in pure water. Denatured NaDNA in dilute salt-free solutions has virtually no helical content as measured by the hypochromic effect and can be assumed to be in the form of single-stranded random coils. The viscosity data coupled with the ionic activity coefficients show that these coils are extended by charge repulsion. In contrast, denatured MgDNA is largely in a multistranded helical structure as shown by the recovery of three-fourths of the original hypochromicity. The viscosity results show that, despite its large amount of helical content, denatured MgDNA is more compact than the NaDNA coils. This is due to the low degree of charge repulsion (most of the phosphates are screened by magnesium ions) and to the flexibility arising from the disordered regions within the helices. The helical regions would be expected to bind magnesium to the same degree as in the native MgDNA. The nonhelical regions are therefore responsible for the slightly increased binding observed in the denatured MgDNA. We believe the nonhelical regions contain a higher charge density than the helical regions because (1) many of these regions contain two strands intertwined randomly and (2) the phosphates in the denatured structure can, if screened electrically, actually be closer together than in the native structure. Thus the magnesium ions would be held more tightly due simply to the higher charge density. The effect would be expected to be small because there is not much difference in the separation between phosphates in the two states and there is not much nonhelical content.

We now present arguments for assuming the mode of binding for MgDNA is essentially all of a diffuse electrostatic nature *at the concentrations studied in this work*. If there were extensive site binding (complexes, specific ion pairs), one would expect the binding equilibrium to be such that the activity coefficient would rise with increasing dilution. Such behavior is illustrated by the silver salt of carboxymethylcellulose,[31] a case where one expects site binding of a covalent character. On the other hand, a decrease of the activity coefficient with dilution is characteristic of polyelectrolyte solutions in which little site binding is expected to occur.[14] Figure 2 shows this latter type behavior for MgDNA and this is not surprising in view of the paucity of known stable complexes of magnesium with monodentate ligands in dilute aqueous solution. (Note that the spacing of nearest neighbor phosphate groups in DNA is about 7 Å.) Moreover, the relative magnitudes of $\gamma_{Mg^{++}}$ and $\gamma_{Na^{+}}$ may be satisfactorily accounted for in terms of a theoretical model which does not involve site binding of any sort.[15] We therefore conclude that the interaction between magnesium and DNA *in pure water* is largely of a diffuse electrostatic nature with site binding assuming a very secondary role. In the case of MgDNA with added $MgCl_2$, however, there is some evidence for site binding which evidence we shall present in a forthcoming paper along with discussion of the magnitudes of the activity coefficients.

We now offer some speculation about the precipitation of MgDNA at higher simple salt concentration. If we assume that the precipitate formed by heating native MgDNA in salt solution is denatured, then the explanation for the precipitation may be simply that the denatured DNA, having some exposed bases, is intrinsically less soluble than the native material and is therefore salted out. The observation that precipitation occurs at successively lower temperatures as the DNA concentration is increased at constant $MgCl_2$ strength implies that the helix is destabilized under these conditions. There are several possible explanations. A mass action effect, wherein one magnesium atom binds to one phosphorus atom thereby reversing the charge at that site, could occur despite the small stability constant for site binding. This would explain why the destabilizing effect is detectable only at high salt concentrations. If this mechanism is operative, the result is a positively charged polyelectrolyte which would behave like NaDNA at low levels of added salt. There is at this time no direct evidence to support this proposed mechanism. However, the magnetic resonance studies argue strongly against the concept of specific interaction with the bases as a destabilizing effect of the type demonstrated by Eichhorn.[12] The effect of large quantities of some simple salts can cause destabilization through an indirect influence on the structure of the water around and within the helix reducing the hydrophobic bonding between base pairs.[32] A clear explanation of the precipitation phenomenon cannot be advanced on the basis of the available data.

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(30) The fact that magnesium salts precipitate only denatured DNA at room temperature under the specified conditions is probably intimately related to similar effects of magnesium salts on soluble ribonucleic acid and synthetic polynucleotides; see, for example, W. E. Razzell, *J. Biol. Chem.*, **238**, 3053 (1963); J. Eisinger, F. Fawaz-Estrup, and R. G. Shulman, *Biochim. Biophys. Acta*, **72**, 120 (1963).

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Diffusion Studies of Bovine Plasma Albumin at 25° with the Help of Jamin Interference Optics

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Diffusion coefficients of bovine plasma albumin in acetate buffer (pH 4.55, ionic strength 0.2) were measured at 25° for different values of mean protein concentration $\bar{C}$ with the help of Jamin interference optics and using a microdiffusion cell. $D(\bar{C})$ was found to be linearly dependent on $\bar{C}$ with a small negative slope of 0.0837×10^{-7}, $\bar{C}$ being in g./100 ml. The extrapolated value of the diffusion coefficient was obtained as $D_{25}^{0,w} = 6.728 \times 10^{-7}$ cm.²/sec. The nature of the concentration dependence of the diffusion coefficient was in conformity with the theoretical deduction made by Creeth from a single diffusion experiment carried out at 25°. The probable theoretical basis of the present observations has been discussed.

The diffusion coefficient of bovine plasma albumin has been measured at a finite concentration by a number of workers[1-3] using either Gouy or Rayleigh interference optics. Unfortunately, experimental data on the nature of the concentration dependence of bovine plasma albumin have been lacking. Wagner and Scheraga[4] happen to be the only authors who carried out such studies at 1° and obtained a linear dependence with a small positive slope. On the other hand, Creeth[5] developed a theory with the help of which the nature of the concentration dependence could be deduced from a single diffusion experiment. From such an experiment at 25°, Creeth[6] observed that the straight line describing the concentration dependence of the diffusion coefficient of bovine plasma albumin (BPA) should have a negative slope, *i.e.*, just opposite in the sense to what was obtained by Wagner and Scheraga at 1°

The need for having experimental data on this aspect in the temperature region where many of the physicochemical studies on BPA are usually carried out is thus naturally felt. Accordingly, in the present investigation the diffusion coefficients of bovine plasma albumin have been measured at 25° over a range of concentrations.[7] The measurements have been carried out with the help of the Jamin interference optics and using a microdiffusion cell.

Experimental

Materials and Methods.—Crystalline bovine plasma albumin, fraction V, was obtained from L. Light & Co., Colnbrook, England. The sample was dissolved in acetate buffer (0.18 *M* NaCl + 0.02 *M* sodium acetate + 0.02 *M* acetic acid) of pH 4.55 and ionic strength 0.2. The solution was dialyzed against the said buffer for a total period of about 28 hr. In the course of dialysis, the solution outside the bag was constantly stirred with the help of a magnetic stirrer, changed twice (after 4 and 16 hr.) and then allowed to equilibrate for about 12 hr. The outer solution from the final equilibration was used, whenever necessary, for the dilution of the dialyzed protein solution. In all cases, the concentration of the final protein solution was measured by noting the absorbancy at 278 mμ ($E_{1\text{cm.}}^{1\%} = 6.67$)[8] with the help of a Zeiss PMQ II spectrophotometer. In all the experiments, the difference in concentration between the diffusing and the diffusate protein solution was kept small and of the order of 2×10^{-3} g./ml.

Diffusion experiments were carried out at 25 ± 0.01° with the help of Jamin interference optics and a microdiffusion cell provided by the Antweiler microelectrophoresis diffusion equipment.[9] The instrument was standardized with the help of Merck reagent grade sucrose and the details of the theory and methods used in the present technique have already been described.[10] In short, for an average protein concentration $\bar{C}$, a number of symmetrical curves were obtained, describing the solute concentration distribution within the cell at different times after the layering had taken place. From each of such curves, an average value D', corresponding to the particular time t, was obtained. The zero time correction was performed as usual. This gave the value of the diffusion coefficient $D(\bar{C})$ corresponding to the mean protein concentration $\bar{C}$. The heterogeneity of the sample was checked from a number of ultracentrifugal runs carried out with the help of Spinco Model E ultracentrifuge.

Results and Discussion

Ultracentrifugal analysis of the sample revealed the presence of 4–6% of material of higher molecular weight. Fig. 1 gives an illustration of the nature of the solute distribution in the cell occurring in the course of the diffusion process. As stated beforehand, each of the curves gave an average value, D', corresponding to the particular time t at which the curve was obtained. In these averaging procedures the maximum standard deviation in any value of D' was found as ±0.5%. The zero time correction plot corresponding to the curves of Fig. 1 is shown in Fig. 2. The values of the diffusion coefficients, $D(\bar{C})$, thus obtained corresponding to different values of the average solute concentration

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