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A Transition to a Compact Form of DNA in Polymer Solutions

(poly(vinyl pyrrolidinone)/poly(ethylene oxide)/T4 phage/centrifugation)

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ABSTRACT **In the presence of over-threshold concentrations of simple neutral polymers and salts, DNA undergoes a cooperative change in its solution structure. Sedimentation studies at low DNA concentrations show that phage DNA molecules collapse into particles approaching the compactness of the contents of phage heads. The interaction between DNA and polymers is thought to be nonspecifically repulsive.**

If the net interaction between the chains of two different polymers present in the same solvent is not attractive, their competition for the solvent space usually results in separation of the solution into two distinct phases (1). On the supposition that the space delimited by the random coil extension of a single high molecular weight DNA molecule is virtually large enough to be regarded as a phase, and that ordinary nonattractive contact effects would prevail in the presence of simple, neutral polymers that are soluble in water, I examined dilute phage DNA for structural alterations that might be equivalent to a virtual phase separation in solutions of poly(ethylene oxide) and poly(vinyl pyrrolidinone). If DNA were more flexible than the Watson–Crick helix implies, it might be surmised that the repulsions arising from contact with another polymer could be minimized by a collapse to a less extended configuration. The state of DNA in bacteriophage heads indicates the accessibility of a highly folded configuration, since the available volume is nearly spherical and only slightly greater than that of the DNA molecule alone.

Variation of the concentrations of the components of aqueous solutions containing DNA, polymer, and salt (including buffer) indicates that DNA indeed undergoes a cooperative structure transition, resulting in a compact form with interesting properties.

Studies on the transition by means of absorption and circular dichroism spectra, and the relation of the transition to virus structure will be presented in a separate communication.

METHODS AND MATERIALS

T4 and T7 DNA, with up to 13 ^{32}P atoms/phage, were prepared from purified virus by phenol extraction (2). Amounts and concentrations are expressed in terms of nucleotide equivalents (eq).

Poly(ethylene oxide) $(EO)_n$ was purchased from Union Carbide Corp. (Carbowax 6000). Poly(vinyl pyrrolidinone) $(VP)_n$, with nominal molecular weights of 10 and 40 $\times$ 10^3, was purchased from Sigma Chemical Co.

Abbreviations: $(EO)_n$, poly(ethylene oxide); $(VP)_n$, poly(vinyl pyrrolidinone); eq, nucleotide equivalents; RMS, root mean square.

For most of the sedimentation studies, 0.04 ml of DNA solution was layered over a 4.5 ml, linear, D_2O–H_2O gradient containing uniform concentrations of $(EO)_n$(100 mg/ml), buffer, and salt. The linear gradients were prepared by a digitally-controlled, positive-drive apparatus suitable for viscous solutions. A thin layer, 0.1 ml, of a solution at the same salt concentration but without polymer was usually interposed between the gradient and the DNA sample. Constant-volume fractions were deposited directly onto filter paper, dried, and counted by immersion in a standard liquid scintillator. Histograms were corrected by a weak statistical smoothing, consistent with counting error, and were converted to smooth, equal-area curves by means of a sliding fit to third degree polynomial segments (Venable, personal communication*). Sedimentation coefficients were calibrated, by means of a conventional calculation, including corrections for the variation of field, density, and viscosity with radius. Because of uncertainty in the starting level of DNA sedimentation and dilution of the polymer near the meniscus by the sample, there is a uniform error of at least $\pm$20 S in the abscissa calibration of each experiment. Viscosity and density measurements were made on only one batch of $(EO)_n$.

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TABLE 1. *Concentration independence of sedimentation rate*

DNA source	Duration of centrifugation (min)	Amount of DNA (peq)	Mean distance sedimented (cm)	Relative breadth of zone*	Distance sedimented at half peak† (cm)
T4	25	2.4	0.65	0.56	1.23
T4	25	4.7	0.73	0.54	1.24
T4	25	9.4	0.75	0.54	1.24
T4	45	2.4	1.23	0.55	2.29
T4	45	4.7	1.25	0.55	2.29
T4	45	9.4	1.19	0.58	2.22
T7	105	3.3	1.56	0.46	—
T7	105	16.5	1.65	0.45	—

All centrifugation was at 3 $\times$ 10^4 rotations/min in 100 mg/ml $(EO)_n$ and 1 M NaCl.

* Standard deviation of zone/mean distance sedimented.

† This is the distance from the meniscus to the layer in the front of the distribution that contains DNA at half the concentration found at the peak.

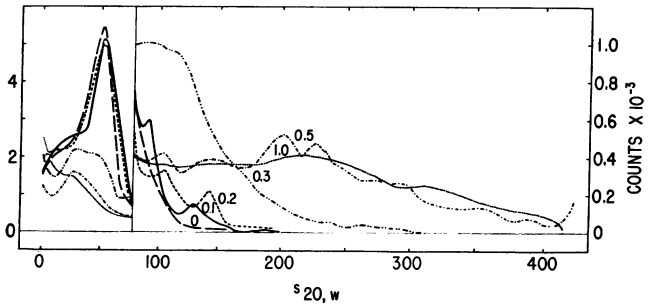

FIG. 1. Sedimentation of T7 DNA in $(EO)_n$ with increasing concentrations of salt. The gradients varied from 90% D_2O at the bottom to H_2O at the top and contained 10 mM phosphate (pH 6.8)–5 mM EDTA–100 mg/ml $(EO)_n$ 6000, and the molar concentration of NaCl shown adjacent to each curve. 16.5 peq DNA was layered on top. The tubes were centrifuged for 105 min at 30,000 rpm at 25°C in the SW 50 rotor. The ordinate calibration for data near the meniscus is at the left, while calibration for the data in the lower part of the tube is at the right. 35 fractions of 0.118 ml each were collected.

RESULTS

A family of curves showing the distribution of T7 DNA after relatively brief centrifugation in solutions containing 100 mg/ml of $(EO)_n$ and various concentrations of NaCl, together with 0.03 M sodium phosphate (pH 6.8), is shown in Fig 1. The corresponding data, plotted in a slightly different way, are shown in Fig. 2 for DNA extracted from T4. Where the sodium concentration is below 0.3 M, both types of DNA remain close to the meniscus in a compact peak. When the sodium concentration is 0.3 M or higher, there is an abrupt change in the sedimentation distribution, such that the DNA is found as a broad band extending to extremely high values of sedimentation coefficient. While the results with T4 DNA have been slightly variable, perhaps because of ^{32}P radiation damage, the peak is typically in the vicinity of 300 S, and the distributions extend to 500 or 600 S. With T7 DNA, the peaks lie in the vicinity of 200–250 S, and the distribution extends to about 400 S.

At the low DNA concentration used in these experiments, no dependence of the sedimentation coefficient on concentration is detected. Variation of concentration over a 20-fold range gives uniform results. Representative data are given in Table 1. Small amounts of DNA show no significant differences, but larger amounts sediment more rapidly, and, with much larger amounts, macroscopic aggregation occurs.

That the abrupt change in sedimentation properties between 0.2 and 0.3 M sodium is not attributable to a change in $(EO)_n$ between these salt concentrations is indicated by the absence of any anomaly in the viscosity of the polymer alone and the absence of a comparable effect on the sedimentation of whole phage. Sedimentation distributions of intact T7 phage in $(EO)_n$ are shown in Fig. 3 at three salt concentrations, one below and two above the critical value for the transition in DNA. The sedimentation coefficient of T7 changes by only about 5% and in a direction opposite to that of the effect on DNA. Our viscosity measurements show a small, gradual decline in $(EO)_n$ viscosity as a function of NaCl concentration, and similar results have been reported in terms of intrinsic viscosities of $(EO)_n$ in various salts (3).

A set of sedimentation patterns of T4 DNA in $(VP)_n$, molecular weight, 4×10^4, is shown in Fig. 4. Again, more-or-less

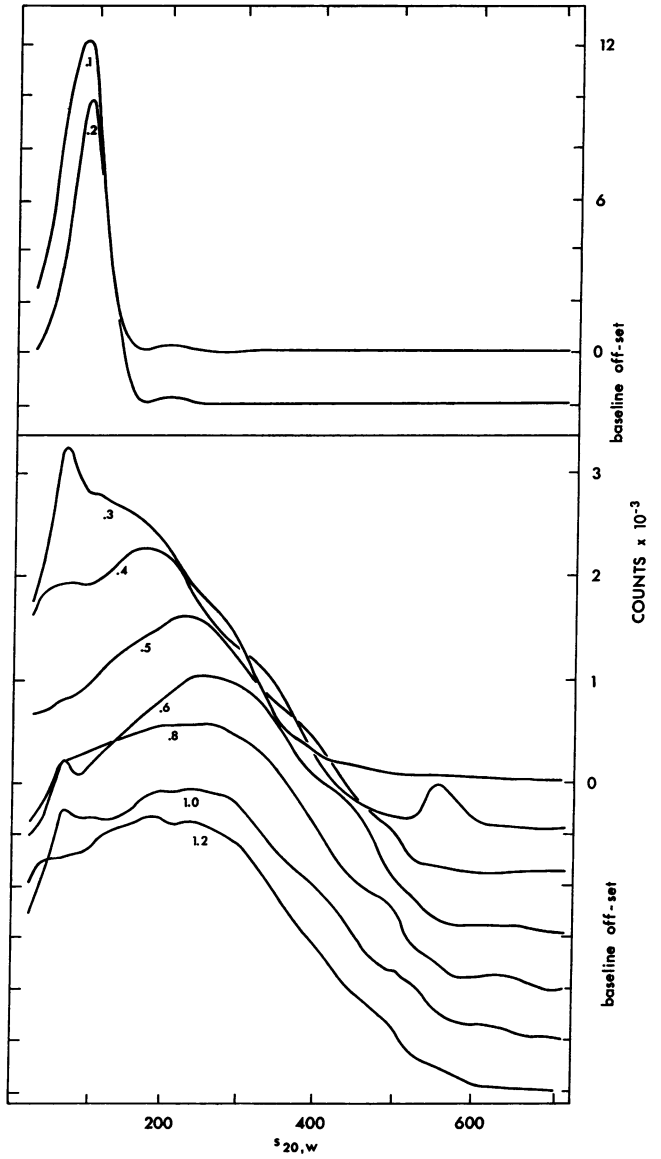

FIG. 2. Sedimentation of T4 DNA in $(EO)_n$ at different salt concentrations. The D_2O–H_2O gradients contained 0.03 M phosphate (pH 6.8)–0.01 M EDTA–100 mg/ml $(EO)_n$, and the molar concentration of NaCl specified. The tubes were centrifuged with 11.5 peq DNA at 30,000 rpm for 45 min, and provided 41 fractions of 0.118 ml. The ordinate calibration is expanded 4-fold for the curves representing 0.3 M NaCl or higher. The base line of successive curves has been displaced downward by a distance equal to 500 counts.

normal sedimentation is seen in low salt, changing to a rapid, broadly heterogeneous pattern in higher salt, similar to the $(EO)_n$ results, and indifferent to further increase in salt concentration. $(VP)_n$ of lower molecular weight, 1×10^4, gives similar patterns at 250 mg/ml in high concentrations of salt, but only slow, essentially normal, sedimentation at 100 mg/ml.

Rapid sedimentation is not seen in density gradients of uniform salt and polymer concentrations if the polymer is below threshold concentration, regardless of the salt concentration (within the range tested). A critical $(EO)_n$ concentration is also demonstrated in a different sedimentation scheme, shown in Fig. 5, in which the polymer concentration declines linearly from the top to the bottom of the tube, while the salt is

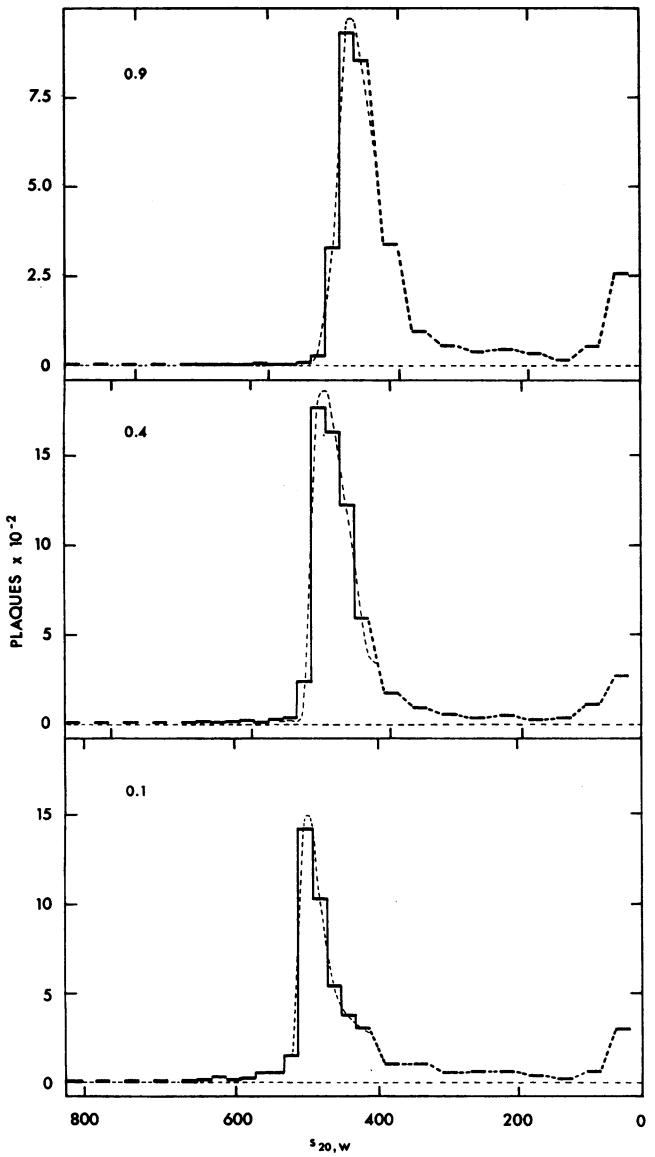

FIG. 3. Sedimentation of T7 phage in $(EO)_n$ and salt. 1–2 × 10^5 viable T7 were applied to the top of gradients of the same composition as in Fig. 2 and centrifuged for 30 min at 30,000 rpm. Fractions of 0.118 ml were diluted and assayed for viable count. The molar NaCl concentration is given in each panel. Both the histogram and calculated smoothed curves are shown. Close to 100% of the input phage was recovered.

uniform. Density stabilization is achieved by means of a combined D_2O–sucrose gradient. Since the sedimentation begins at high polymer concentration, the rate is initially rapid. As the DNA reaches a layer in the tube about 40% from the bottom, where the polymer concentration has declined to a value too low to maintain the rapidly sedimenting form, it appears to return quickly to the ordinary form. Very little further movement is detected during the period of the experiment, and DNA appears to accumulate at this layer in the tube. Because the rapid sedimentation rate is enhanced by aggregation at high DNA concentrations, the effect is more conspicuous in the upper panel of Fig. 5, but it is also plain in comparison of the 25- and 40- min distributions at the lower DNA concentration.

That the DNA is not denatured after suddenly returning from the rapidly sedimenting state is shown by isopycnic

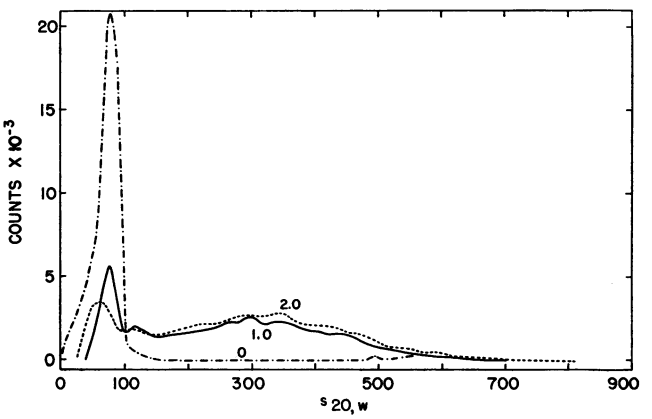

FIG. 4. Sedimentation of T4 DNA in $(VP)_n$ and salt. The composition of the gradients was as in Fig. 2, except that the solutions contained 100 mg/ml $(VP)_n$ of nominal MW 40,000, instead of $(EO)_n$. The tubes were centrifuged at 30,000 rpm for 85 min because of the greater viscosity of the $(VP)_n$ solutions. Molar NaCl concentration in each tube is indicated.

equilibrium in the presence of a reference DNA. A column of 100 mg/ml $(EO)_n$ and 1 M NaCl was floated over a column of CsCl solution. DNA labeled with ^3H or ^{32}P was applied to the top of the $(EO)_n$ layer, and reference DNA labeled with the other isotope, either native or denatured, was mixed with the CsCl. When both DNA samples were applied in the native state, the band that traversed the $(EO)_n$ was superposed on the reference band at equilibrium, but denatured reference DNA appeared denser than the $(EO)_n$-exposed sample by the usual increment.

DISCUSSION

The standard $(EO)_n$ concentration, 100 mg/ml, and the critical $(EO)_n$ concentration in 1 M salt, about 40 mg/ml, are many times lower than the volume fraction of low-molecular-weight organic solvents required to denature DNA at room temperature, and must have, therefore, much smaller effects on the activity of water in the solution. The requirement for a higher concentration of $(VP)_n$ than $(EO)_n$ of similar molecular weight is opposite to the relative hydrophobicity of these polymers (4); the insensitivity of hydrophobicity to molecular weight (4) also argues against that parameter as the predominating contribution to the interaction with DNA.

That the marked increase in sedimentation rates is not the result of aggregation of DNA molecules or a solvent anomaly has already been noted. If the $(EO)_n$ were merely wrapped around an otherwise unchanged double helix at a ratio of 1 or 2 ethylene oxide residues per nucleotide, the increase in sedimentation coefficient would be less than the molecular weight increment, that is, less than 6–12%. It would be hard to propose any plausible basis for the binding of a sufficiently large amount of $(EO)_n$ (that has a partial specific volume of 0.85 ml/g) to give an increase in sedimentation coefficient of 5- to 10-fold.

The possibility of complex formation between $(EO)_n$ and DNA is not supported by any obvious structural relationships and is electrostatically disadvantageous. We have made some observations on a similar system in which the expectation of complexing is much smaller. The circular dichroism spectra of DNA in the presence of $(EO)_n$ and salts show large cooperative changes in good correspondence with the changes

TABLE 2. *Calculated sedimentation coefficients for hypothetical configurations of a single molecule of T4 DNA*

Configuration	$S^0_{20,w}$	Scheme
T4 DNA, ordinary solutions, observed	62	(5)
Single strand, neutral, high salt, observed	208	(6)
Double-length single strand, as preceding (calculated)	282	(6)
Tight, narrow, double superhelical rod, 2.9 nm radius	95	*
Short, broad, tight, double superhelix, 25.5 nm radius	174	(7)†
100 arm star, ordinary chain configuration	265	(8)
Compact sphere	1035	‡
Prolate ellipsoid, axial ratio = 7:1	755	‡
Platelet, bundle of double helix segments, 16 nm long	704	§
Whole T4 phage, observed	890	(9)

* Minimum radius, approximated as the prolate ellipsoid equivalent to the cylinder enclosing two adjacent helices wound in a tight coil.

† We used the ratio of Gray's calculated S value for a polyoma DNA superhelix to the experimental value for the 2-ended polyoma DNA molecule.

‡ Same volume as the interior of the T4 head.

§ We assumed an hexagonal array, 2.77 nm between helices, close to the same volume as ‡, approximated as an oblate ellipsoid of axial ratio 10.2.

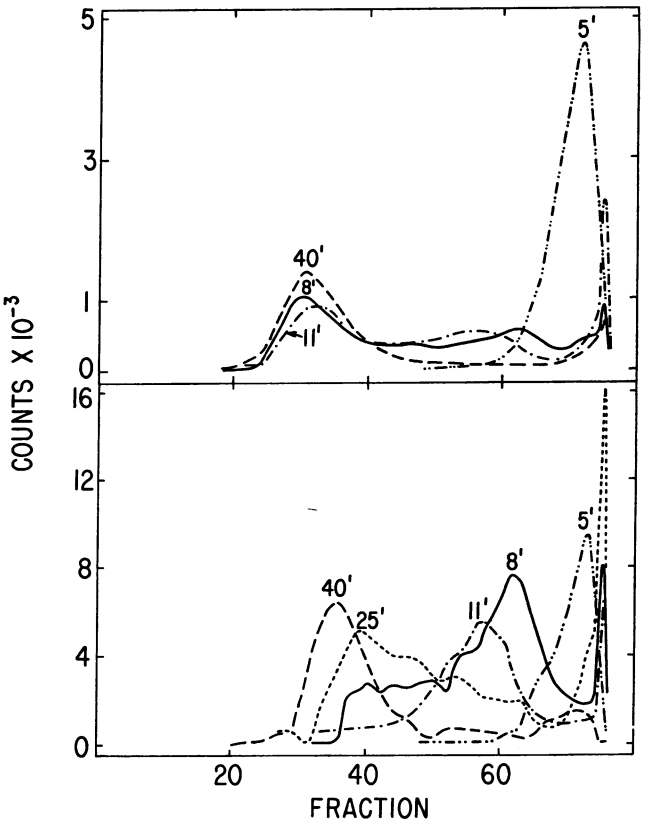

FIG. 5. Sedimentation of T4 DNA in a gradient of declining $(EO)_n$ concentration. The solutions contained 1 M NaCl–0.01 M EDTA–0.03 M phosphate (pH 6.8) throughout. Gradients were prepared as a linear mixture of a light solution, containing 100 mg/ml $(EO)_n$ and salts in H_2O, with a dense solution, containing 216 mg/ml sucrose and salts in 90% D_2O. Each tube in the upper panel was layered with 72 peq of T4 DNA and each tube in the lower panel with 7.2 peq. The numbers indicate the length of the time of centrifugation after the centrifuge reached full speed, 30,000 rpm.

in sedimentation; the same spectral changes are also observed when sodium polyacrylate is substituted for $(EO)_n$ (Jordan, Lerman, and Venable, in preparation). Since the polyacrylate is at least 80% negatively ionized under the conditions of the experiment, the possibility of its participation in a complex with DNA, as an association of two polyanions, seems remote.

On the other hand, the sedimentation data are easily compatible with the changes in frictional coefficient that would be expected from the conversion of the ordinary extended, stiff random configuration of double helical DNA into a more compact particle. It seems important that any calculated value be as compatible with the extremely high values that are attained by the fastest 10% of the particles as with the modal sedimentation coefficient. The fast DNA particles are presumably generated by the same interactions and show the same concentration independence; for T4 their rate corresponds to $s_{20,w}$ values in the vicinity of 500. Sedimentation coefficients calculated from the decreased frictional properties of various assumed configurations of the same molecular weight, that of T4 DNA, are presented in Table 2. A tightly twisted superhelix, approximated as an ellipsoid equivalent to a cylindrical rod, would sediment far too slowly, as would a denatured single strand of twice T4 molecular weight, an annular superhelix, and the 100-arm star. Each arm of the star is 1% of the length of T4, but retains the normal worm-like chain properties of the double helix in solution. Sufficiently large values of $s_{20,w}$ are attained only with models in which the filamentous character of DNA is replaced by a more compact shape. A sphere consisting of DNA and solvent, with the volume calculated for the inside of T4, according to the electron microscopic measurements of Eiserling *et al.* (10), provides an $s_{20,w}$ value appreciably larger than our experimental maximum. Deformation of the sphere into a prolate ellipsoid with an axial ratio of about 7, as seen by Lang (11) for alcohol-precipitated DNA, results in slightly slower sedimentation. If the DNA is folded into a platelet of about 16 nm thick, like the usual crystal of a linear polymer, according to an hexagonal net with a Bragg spacing of 2.4 nm (12), its sedimentation coefficient can be estimated as that of an oblate ellipsoid with an axial ratio of 10.2. The platelet would have a sedimentation coefficient of about 700, which approaches our experimental maximum.

Anticipation of phase instability in solutions containing both $(EO)_n$ and DNA is in accord with the approximate relations among activity coefficients in a ternary system derived by Ogston (13). In the absence of a critical experimental test of the applicability of the theory, a detailed discussion is not appropriate. However, the rough correspondence between the theoretical stability limit, if the collapse of a DNA molecule is regarded as a phase separation, and the present results is of some interest, and may not be entirely coincidental. The parameters of the theory are the dependence of the activity coefficients of each of two polymers on its own concentration and on that of the other. The mutual dependence is calculated on the assumption that the concentration of $(EO)_n$

within the region bounded by the root mean square (RMS) radius of the DNA coil is lower than that in the bulk solution because some of the interior space is blocked from occupancy by $(EO)_n$. The center of $(EO)_n$ random coil cannot come closer to the axis of the double helix than the sum of the radii of the helix and the $(EO)_n$ coil. If the space from which $(EO)_n$ is excluded is taken to be a caterpillar-like curving cylinder, coaxial with the DNA worm-like chain, and having a radius equal to the sum of the double helix radius and the mean maximum radius of $(EO)_n$ (5.5 nm), and if we assume also that $(EO)_n$ moves freely elsewhere, a partition coefficient may be calculated representing the ratio of the average concentration within the RMS radius of a DNA molecule and the bulk. The RMS radius of $(EO)_n$, MW 6000, is calculated from the intrinsic viscosity and its molecular weight dependence (3), and converted to mean maximum radius (14). The $\log_e$ of the calculated partition coefficient is then -3.6×10^{-4}, obviously quite small, reflecting the large open spaces within the DNA domain in which $(EO)_n$ is unimpeded. A similar value, $\log_e k = -2.3 \times 10^{-4}$, is given by Ogston's relation (15) for the penetration of spheres into a random assembly of fibers, where the fibers correspond to persistence length segments of DNA spaced according to their mean density within the RMS radius of T4 DNA, and the spheres have the RMS radius of $(EO)_n$. With this value, neglecting the second virial coefficient of $(EO)_n$, and the use of an effective DNA concentration equivalent to that within the RMS radius of T4 DNA, I find that instability is expected at about 45 mg/ml $(EO)_n$, not far from the value indicated in Fig. 5. In view of the guesswork, the agreement indicates only an order-of-magnitude plausibility for the exclusion interaction. The molecular weight dependence shown in the $(VP)_n$ experiments is also in qualitative agreement. While the calculation has obviously disregarded the cation requirement, it may be supposed that in the absence of adequate salt, intramolecular electrostatic repulsions are too great to permit a collapse.

It may be noted that the upper concentration limit for concentration-independent sedimentation rates corresponds to an intermolecular separation at which collisions are marginally probable during the time that the DNA remains concentrated in a narrow zone in the polymer solution.

These results suggest that phage DNA readily undergoes a transition in its secondary or tertiary structure in aqueous solution, allowing each molecule to collapse into a compact particle approaching the dimensions of a phage head. Frictional coefficients greater than that of a head-size sphere may derive from asymmetry of the particles or from the straggling of loops and tails. The conditions inducing this transition are similar to the intracellular environment in one aspect—the intracellular environment, like the concentrated polymer solutions, is densely crowded with macromolecules. Although the critical concentration of cations required for the transition has not been examined in detail, it is much lower for divalent ions than for sodium and is within the limits of intracellular concentrations. It would appear that the capacity to adopt a compact structure does not require additional intramolecular constraints, as can be presumed to accompany the complexing of DNA with basic proteins and related substances.

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