Internal Condensation of a Single DNA Molecule

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Synopsis

We report a theoretical description of the collapse of a single chain molecule, such as DNA, from a voluminous random coil to a condensed state. With the polymer lattice theory developed by Flory as a starting point, the configurational free energy of a single polymer molecule in solution is expressed as a virial expansion in the polymer segment volume fraction. We have extended the series by one term beyond Flory's analysis and have evaluated the third virial coefficient. Whenever the potential of interaction between two chain segments is attractive, the addition of this new term causes a new minimum to appear in the free energy. The new minimum represents a polymer configuration in which the chain occupies a very small solution volume; we identify this minimum with the collapsed state. There is a critical point for the transition to the collapsed state; above a certain value of the rms end-to-end distance, the change in the polymer solution volume is a sudden one, whereas below this value the transition is gradual. An example of the results of the free energy calculation using parameters from T2 DNA is presented. DNA should show a sudden collapse, but synthetic polymers such as polystyrene should show a gradual collapse.

INTRODUCTION

The condensation of DNA from an extended random coil of low polymer segment density to a smaller, more tightly compacted particle has been described by several workers. Lerman[1] observed that DNA at very low dilutions, ~5 μg/ml, when sedimented through a solution with NaCl and polyoxyethylene (PEO), moved with an anomalously high sedimentation velocity. Further experiments, including CD,[2,3] x-ray scattering,[4] flow birefringence, and fluorescence microscopy,[5] indicate that under these conditions, DNA undergoes a definite configurational change, consistent with an altered tertiary structure of the DNA polymer. Other studies in which DNA condensation was thought to occur have been made using a variety of solution conditions. Electron microscopy studies by Lang[6,7] have shown that DNA exposed to high concentrations of ethanol collapses into small particles, and that the higher the ethanol concentration the greater the degree of collapse. Additional condensing solvent systems include polyamines,[8] acid,[9,10] polypeptides,[11] and other synthetic polymers.[1]

In this paper we report on a theoretical picture that accounts for condensations of this type in any chain molecule. Our description is an extension of the Flory-Huggins polymer solution theory in which a lattice model is used to obtain an expression for the free energy of mixing a polymer and a solvent. Although the lattice model is highly simplified, Flory's

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calculation is found to be adequate in that agreement with experimental data for the osmotic pressure, light scattering, and vapor pressure of polymer solutions at high concentration is good (Ref. 12, Chap. 12; Ref. 13, Chap. 3). Therefore, Flory's model is an attractive one to use to describe the condensation behavior of DNA, since in the collapsed state the local DNA concentration is very high.

We have added an expression for the third virial coefficient to Flory's free energy equation and find that the lattice model does indeed show the phase transition as an abrupt change in the solution volume occupied by a polymer molecule. Furthermore, we show that a critical point exists for this phase transition from the extended polymer configuration to the collapsed form. A polymer whose potential of interaction with the solvent and whose flexibility have values beyond those at the critical point does not undergo a sharp change in solution volume but instead expands and contracts in a continuous fashion.

Other theoretical approaches have been reported which address polymer configurations for solutions of overall high concentration and which consider the expansion of polymers in good solvents.[14–18] The possibility of a collapsed phase has been recognized for years.[5,19–22] A statistical mechanical description of a large polymer in solution with a second, smaller polymer has been recently reported by Naghizadeh and Massih,[23] who found a singularity in the free energy at a critical concentration of the smaller polymer.

While this manuscript was in progress, we received a copy of a manuscript that Frisch and Fesciyan[24] have submitted for publication. Their treatment, also based on the Flory lattice model, appears to differ from ours in a number of ways but is fundamentally similar in its approach.

PROCEDURE

Using a lattice model, Flory some time ago derived an expression for the free energy of mixing for a single polymer molecule with solvent (Ref. 12, Chaps. 12 and 14). The free energy includes the energy from interaction between solvent molecules and polymer segments and is formulated in terms of a dimensionless parameter, χ. The quantity χ is the change in free energy on formation of unlike first-neighbor contacts with the loss of solvent–solvent and segment–segment contacts, divided by kT. The free energy depends on the segment density and is therefore a function of r, the distance of a segment from the center of mass of the polymer. Flory's free energy equation is

$$\frac{\Delta G}{kT} = \int_0^\infty [n_1(r) \ln v_1(r) + \chi n_1(r) v_2(r)]\, 4\pi r^2\, dr + \frac{3(\alpha^2 - 1)}{2} - \ln \alpha^3 \quad (1)$$

where $n_1(r)$ is the number of solvent molecules, and $v_1(r)$ and $v_2(r)$ are

volume fractions of solvent and polymer, respectively, in a spherical shell at distance r. The parameter χ, mentioned above, is more rigorously defined in the Appendix, Eq. (A12), and α is the well-known linear expansion factor, "the factor by which the linear dimensions of the chain molecule are increased owing to intramolecular interactions" (Ref. 12, p. 528). The integrand of Eq. (1) is the contribution to the free energy due to the mixing of segments with solvent and was derived assuming the density is uniform in each spherical shell of radius r. This point is discussed in more detail below. The last two terms of Eq. (1) are included because of the elastic nature of a polymer; they express the entropic contribution to the free energy due to swelling or contraction of the polymer. The quantity $3(\alpha^2 - 1)/2$ is obtained from the probability that the polymer will occur in a configuration consistent with the deformation specified by α. As α describes distances between polymer segments, $\ln \alpha^3$ is seen to be related to the term of ideal solution theory that contains the logarithm of the solute concentration.

Flory minimized the free energy, given by Eq. (1), as a function of α, thereby obtaining the effects of excluded-volume and other intramolecular interactions on the expansion factor. After integrating Eq. (1) and then setting the derivative with respect to α equal to zero, he obtained the well-known equation,

$$\alpha^5 - \alpha^3 = Cz \tag{2}$$

$$z = (\tfrac{1}{2} - \chi)(V_p^2/2^{1/2}V_1)(3/\pi \langle h_0^2 \rangle)^{3/2} \tag{3}$$

where C is a constant; $\langle h_0^2 \rangle^{1/2}$ is an initial value of the rms end-to-end length before intramolecular interaction is considered, i.e., the value in a theta solvent. Also, V_1 is the solvent molecular volume and V_p the polymer molecular volume; $V_p = M_r\bar{v}/N_A$ where M_r is the molecular weight, $\bar{v}$ is the partial specific volume, and N_A is Avogadro's number. The numerical constant C has the value $3^{3/2}/2 = 2.598$ with Flory's model; for further discussion of the value of C from other models, see Stockmayer.[22] Equation (2) is valid only for values of α in the neighborhood of one or greater, i.e., in the range where the segments occupy only a small fraction of the solution volume, as for dissolution in a good solvent.

To describe the condensation of a polymer such as DNA, we follow Flory's method, but extend it to describe the collapse of a single chain into a small compact form. Although the total overall polymer concentration is very low, the collapsed polymer will occupy a significant fraction of the volume within its domain and will have a high segment density. This requires adding a term to Flory's free energy expression to make it applicable to a solution in which the density of segments is high.

We make the same approximations used to obtain Eq. (1). A lattice model is assumed in which both the solvent molecules and the polymer segments occupy equivalent lattice sites. To calculate the free energy, we start with a small volume element which has a uniform segment density. In counting the number of ways the ith polymer segment can be placed on

lattice sites within that volume, it is assumed that the probability that a site is unavailable for occupancy is just equal to the volume fraction of all the previously placed segments. Hence the expectancy of a cell being occupied is approximated by an average, and no specific account is taken of the spatial continuity of the earlier placed segments in the chain.

To determine the free energy of the entire molecule, the polymer domain is divided into spherical shells, each of which has a uniform segment density that is a Gaussian function of the distance r from the center of the molecule. The segment volume fraction is

$$v_2(r) = V_p(3/\alpha\pi^{1/2}\langle h_0^2\rangle^{1/2})^3 \exp[-3^2r^2/\langle h_0{}^2\rangle] \quad (4)$$

To perform the integration in Eq. (1), it is then necessary to replace v_1 by $1 - v_2$ and to expand the logarithm in the power series. To have the free energy behave properly in the collapsed state, it is necessary to go at least as far as the third power of v_2, which is one term beyond that needed by Flory in his original derivation. The following expression is the result, and is the same as Flory's except for an additional term in α^{-6}:

$$\frac{\Delta G}{kT} = \frac{V_p}{V_1}\left((\chi - 1) + \frac{(\frac{1}{2} - \chi)\omega}{2^{3/2}\alpha^3} + \frac{(1 + 12\chi^2/q - 16\chi^3/q^2)\omega^2}{2 \cdot 3^{5/2}\alpha^6}\right) + \frac{3(\alpha^2 - 1)}{2} - \ln \alpha^3 \quad (5)$$

where $\omega = (3/\pi^{1/2}\langle h_0^2\rangle^{1/2})^3 V_p$, and q is the lattice coordination number. The first two terms in brackets are cited by Flory (Ref. 12, p. 522) and are related to the expansion of $\ln v_1$. This can be seen by recognizing that V_p is the polymer molecular volume and that $(\pi^{1/2}\alpha\langle h_0^2\rangle^{1/2}/3)^3$ is proportional to the total solution domain which the polymer encompasses; V_p over this quantity, i.e., ω/α^3, is therefore the polymer volume fraction. Flory's method thus gives the free energy in a virial expansion. Since we have retained third-order terms in our analysis, we have also evaluated the third virial coefficient in order to include the energy of interaction in the α^{-6} term. The derivation is in the Appendix, with the results shown in Eq. (5).

It should be noted that the series obtained from expansion of $\ln v_1$ and integration of the terms over space converges only for $\alpha^3 > \omega$, so Eq. (5) applies only within this range. In physical terms this means that the segment volume fraction must be limited to values less than unity; a value of unity is the pure polymer phase, beyond which further collapse is not possible. In the Results section it is shown that the values of α for the collapsed state are in the valid range.

The "energy of contact" between a DNA segment and the solvent is actually a free energy, that is, a potential of average force of the environment in which the DNA is situated. Solvent, therefore, in the term "solvent–segment interaction," encompasses all constituents of the solution other than DNA. An increase in the concentration of a component such as ethanol, spermidine, or even another polymer such as PEO would result in an

increase in the average free energy of solvent–DNA interaction, corresponding to a higher value of χ.

An expression for α at the minimum in free energy can also be obtained from Eq. (5) by differentiation as before. The result is as follows:

$$\alpha^8 - \alpha^6 - Cz\alpha^3 = y \tag{6}$$

$$y = 3^{1/2}(1 + 12\chi^2/q - 16\chi^3/q^2)(3V_p/\pi\langle h_0^2\rangle V_1^{1/3})^3 \tag{7}$$

Equation (6), which has also been presented by de Gennes,[20] has two parameters, z and y. Values of z and y depend on three physical characteristics of the polymer—chain flexibility, the interaction energy with the solvent, and molecular weight. A measure of chain flexibility can be obtained in the following way: defining an effective segment length a by

$$a^2 \equiv \langle h_0^2\rangle V_1/V_p \tag{8a}$$

gives the result

$$V_p^3/\langle h_0^2\rangle^3 V_1 = V_1^2/a^6 \equiv w^6 \tag{8b}$$

The quantity $w = V_1^{1/3}/a$ is recognized to be a measure of flexibility, since $V_1^{1/3}$ is the actual intersegment spacing and a is its effective spacing. From Eq. (7), then, y is seen to contain two contributions: (1) the terms involving χ and q, which were derived for the third virial coefficient, are due to the interaction between polymer and solvent; and (2) the polymer flexibility as given by w^6. The value of y changes very little with χ and q over the ranges of χ and q which are of interest; y is therefore sensitive mostly to changes in flexibility.

The parameter z is a function of all three polymer characteristics. This can be shown by substitution of Eq. (8b) into Eq. (3):

$$z = 2^{-1/2}(3/\pi)^{3/2}(\tfrac{1}{2} - \chi)N^{1/2}w^3 \tag{9}$$

where $N \equiv V_p/V_1$. This expression for z contains the interaction term from the second virial coefficient, $\tfrac{1}{2} - \chi$, and it contains the measure of flexibility, w. The molecular-weight dependence appears in the ratio of molecular volumes N. The variable to which z is most sensitive is $\tfrac{1}{2} - \chi$; as χ changes from less than $\tfrac{1}{2}$ to greater than $\tfrac{1}{2}$, z changes from positive to negative.

RESULTS

The free energy of T2 phage DNA, for fixed q and χ, is plotted in Fig. 1 as a function of the linear expansion factor, α. The curves are calculated from Eq. (5) with $M_r = 1.24 \times 10^8$ and $\langle h_0^2\rangle^{1/2} = 2.52 \times 10^{-4}$ cm. The value for $\langle h_0^2\rangle^{1/2}$ is determined from the viscosity data of T2 DNA in a 0.2M NaCl solution.[25] These are not the theta conditions for DNA, but we do not expect the small difference to be serious. For small values of χ, a single minimum in the free energy is observed at a value of $\alpha_{\min}$ near 1.0. Dissolution of the polymer in a good solvent, where $\chi < \tfrac{1}{2}$, or a solvent with

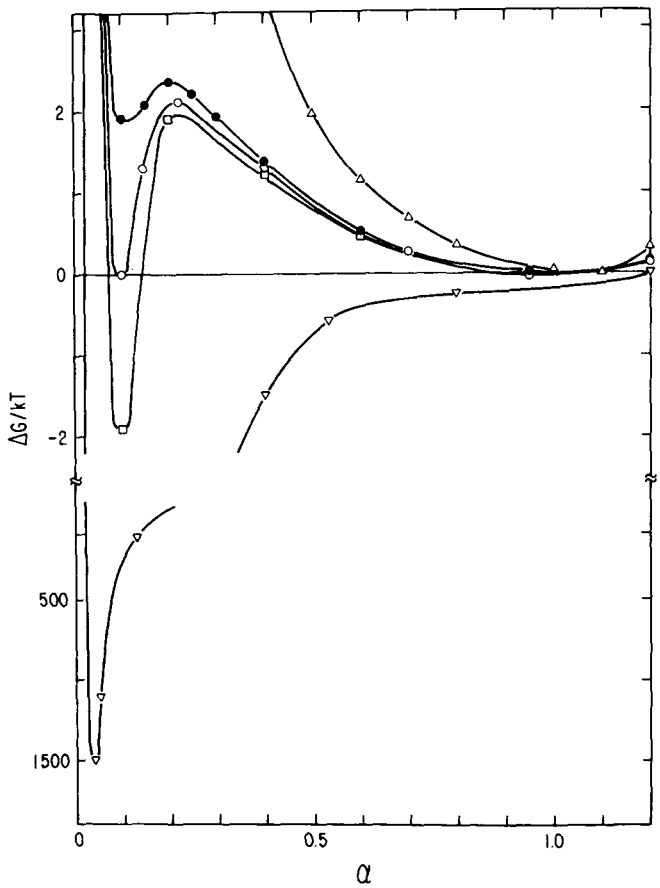

Fig. 1. Free energy divided by kT for T2 DNA plotted as a function of the linear expansion factor for several values of χ. The values of the parameters are $M_r = 1.24 \times 10^8$ g/mol, $\langle h_0^2 \rangle^{1/2} = 2.52 \times 10^{-4}$ cm, $V_p/V_1 = 10^5$, and $q = 10$. The values of χ are (△) 0.4000; (●) 0.5075, (○) 0.5085; (□) 0.5100; and (▽) 0.6500.

only a slightly positive energy of interaction, $\chi \approx \frac{1}{2}$, results in expansion, i.e., $\alpha_{\min} > 1.0$, due to excluded-volume effects, or in a small contraction, i.e., $\alpha_{\min}$ slightly less than 1.0, respectively. For small values of χ the free energy calculated from Eq. (5) is the same as that stated by Flory. However, as the polymer–solvent interaction becomes more unfavorable, χ increases, and a second minimum appears in the free energy at $\alpha \ll 1.0$. This is the collapsed state. At a certain "condensation" value of χ, slightly greater than $\frac{1}{2}$, the two minima become equal in free energy. For χ greater than the condensation value, the minimum at $\alpha \ll 1.0$ is deeper, and the collapsed state becomes the stable conformation. As χ continues to increase, the value of α at the "collapsed minimum" decreases. For example, for $\chi = 0.51$, we have $\alpha_{\min} = 0.097$, and for $\chi = 0.70$, we have $\alpha_{\min} = 0.037$.

Addition of the α^{-6} term results in the free energy becoming rapidly

positive for very small values of α. Without this term, the free energy function becomes more and more negative for small α when $\chi > 1/2$; that is, there is no real and positive root for α which satisfies Flory's Eq. (2) for large χ.

Changing the values chosen for q and for the ratio of the molecular volumes, V_p/V_1, did not significantly alter the results of the free energy calculation. For $q = 8$ and 12 the minimum in free energy of the collapsed state was at $\alpha_{min} = 0.057$ and 0.056, respectively. Similarly, setting $V_p/V_1 = 10^5$ and 10^7 gave $\alpha_{min} = 0.097$ and 0.095, respectively. The condensation value of χ was also found to change with q and V_p/V_1 but, again, not significantly. Including the interaction parameter χ in the α^{-6} term, as compared with having only the excluded-volume portion of that term, has some effect on α_{min}; for $\chi = 0.510$, without the interaction, the collapsed value of α is 0.086, whereas with the interaction it is 0.097.

The density of segments is markedly increased on condensation of the DNA molecule. To take an example of the predicted collapsed state of DNA, we find that for $\chi = 0.55$, the value of α at the minimum in free energy is 0.056. This corresponds to a reduction in the domain occupied by a single DNA molecule, as measured by $\alpha^3\langle h_0^2\rangle^{3/2}$, by a factor of 6000. On collapse the DNA domain changes from one with about 0.003% of the volume occupied by polymer segments to one with about 20% so occupied. (This value of α is within the limits for convergence as mentioned previously.)

Though experimental results have not been reported for actual values of χ for DNA solutions, other polymer binary systems show a small linear dependence of χ on polymer concentration (Ref. 12, p. 512). That is, instead of χ being a constant for a given solvent and temperature, it is found experimentally that in poor solvents χ increases linearly as the polymer concentration increases. (For good solvents the data show that χ decreases as polymer concentration increases, i.e., χ is always $< 1/2$; and no collapse is expected.) We have considered how a linear increase in χ would affect our results. Equation (5) shows that the free energy minimum of the collapsed state becomes deeper at large values of χ. Thus if empirically χ increases as the density of segments goes up, then the collapsed state becomes even more stable relative to the extended form.

To illustrate the collapse of DNA for molecules of various molecular weights, we have plotted the values of α_{min} as a function of χ (Fig. 2). The real and positive roots of Eq. (6) were calculated using the Newton-Raphson method to find the values of α at the minima in the free energy. The root at which the free energy has the deeper minimum is α_{min}. The phenomenon is seen to be similar over the whole range of sizes within which DNA can be reasonably considered to be a random coil. (We have not taken thermal fluctuations from the lower to the higher minimum into account, which would tend to make the collapse somewhat diffuse, but the values of $\Delta G/kT$ in Fig. 1 suggest that this effect would make only a minor correction.)

The theoretical values of α_{min} shown in Fig. 2 can be used to calculate

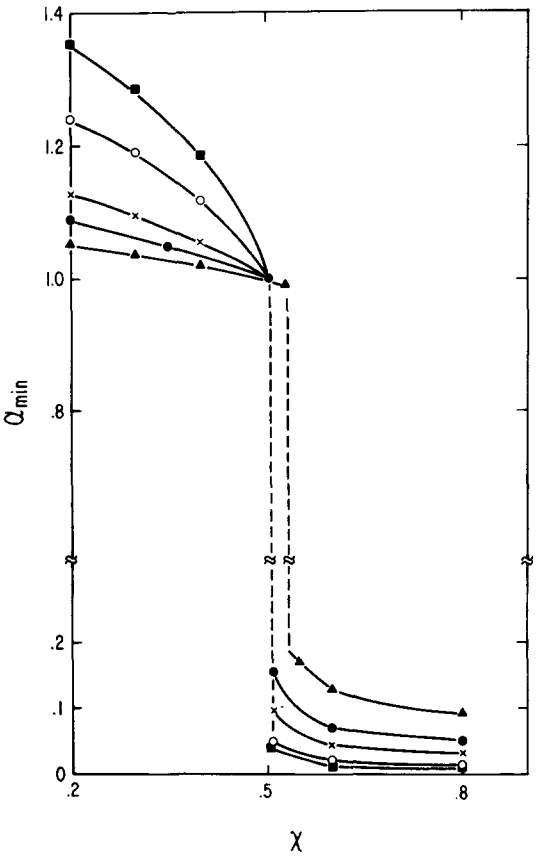

Fig. 2. Values of linear expansion factor, α_{min}, are shown as a function of the DNA–solvent interaction parameter χ for different molecular weights of DNA. The positions of the minima were calculated from Eq. (6), which was solved numerically by the Newton-Raphson method. In the case of three real and positive roots satisfying Eq. (6), the value of α_{min} plotted corresponds to the deeper minimum in the free energy of Eq. (4). The curves are for $q = 10$ and for (■) $M_r = 2.0 \times 10^{10}$, $\langle h_0^2 \rangle^{1/2} = 4.26 \times 10^{-3}$ cm, $V_p/V_1 = 1.6 \times 10^7$; (○) $M_r = 2.5 \times 10^9$, $\langle h_0^2 \rangle^{1/2} = 1.34 \times 10^{-3}$ cm, $V_p/V_1 = 2 \times 10^6$; (×) T2 with $M_r = 1.24 \times 10^8$, $\langle h_0^2 \rangle^{1/2} = 2.52 \times 10^{-4}$ cm, $V_p/V_1 = 1 \times 10^5$; (●) T7 with $M_r = 2.5 \times 10^7$, $\langle h_0^2 \rangle^{1/2} = 1.02 \times 10^{-4}$ cm, $V_p/V_1 = 2 \times 10^4$; and (▲) $M_r = 2 \times 10^6$, $\langle h_0^2 \rangle^{1/2} = 2.3 \times 10^{-5}$ cm, $V_p/V_1 = 1.6 \times 10^3$.

a diameter of the collapsed DNA and this diameter compared with that determined by experiment. Dore et al.[9,10] obtained a diameter from scattering data assuming spheres. They report a value of 0.15 μm for the diameter of T2 DNA just after immersion in acid solution. We compare this with the calculated diameters of 0.08 μm for $\alpha_{min} = 0.04$ and 0.2 μm for $\alpha_{min} = 0.1$ (see Fig. 1) to find reasonable agreement.

The collapse resembles a phase transition, such as that between gas and liquid, with α_{min} analogous to volume and χ (or z) to temperature. Looking at Eq. (6), we see that there is a third parameter, y, the flexibility parameter. This raises the question of whether varying y would cause the collapse

transition to disappear at a critical point, analogous to the critical point of a gas.

Figures 3 and 4 illustrate that there is indeed critical behavior in this system. Values of α at the deeper minimum in free energy are related to the solvent–segment interaction parameter χ, as shown in Fig. 3, and are related to the parameter y, as shown in Fig. 4. The tie lines on the graphs indicate the point at which the deeper minimum in free energy changes from the root at α close to 1.0 to the one at α close to 0. The graphs show a critical point. The values of Cz and y at the critical point are approximately -0.2464 and 0.0227. (We state the critical values in terms of Cz and Y because in these terms they are independent of molecular weight, etc. For T2 DNA, the corresponding values are $\chi = 0.5041$ and $\langle h_0^2 \rangle^{1/2} = 7.02 \times 10^{-5}$ cm.) As can be seen in Fig. 3, the collapse is continuous, not discontinuous, as χ is changed for values of y greater than the critical value.

de Gennes[20] also recognized the existence of a critical point; however,

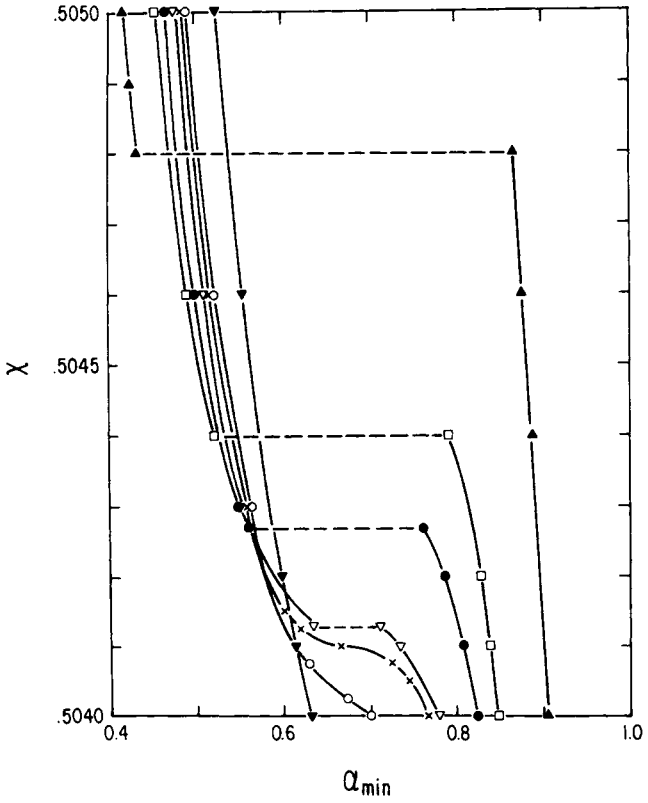

Fig. 3. Phase transition to the polymer collapsed state for T2 DNA. Holding $\langle h_0^2 \rangle^{1/2}$ constant for each curve, the value of α_{min} was determined as in Fig. 2 for various χ. M_r, q, and V_p/V_1 are the same as in Fig. 1. The values of $\langle h_0^2 \rangle^{1/2}$ and y are (▲) 8.24×10^{-5} cm, 0.0087; (□) 7.47×10^{-5} cm, 0.0156; (●) 7.27×10^{-5} cm, 0.0184; (▽) 7.06×10^{-5} cm, 0.0219; (×) 7.02×10^{-5} cm, 0.0227; (○) 6.90×10^{-5} cm, 0.0251; and (▼) 6.35×10^{-5} cm, 0.0414.

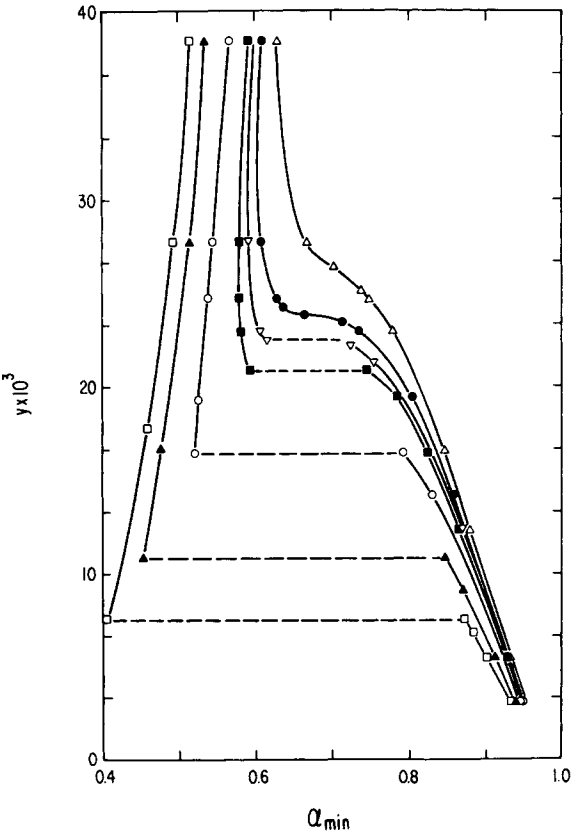

Fig. 4. Phase transition to the polymer collapsed state for T2 DNA. Holding χ constant for each curve, α_{min} was determined as in Fig. 2 for various values of the flexibility parameter y. M_r, q, and V_p/V_1 are the same as in Fig. 1. The values of χ are (△) 0.50400; (●) 0.50410; (▽) 0.50415; (■) 0.50420; (○) 0.50440; (▲) 0.50470; and (□) 0.50500.

the critical value of y which he reported is 0.038, slightly larger than our value. We do not know the cause of this.

We should note that in our analysis y is varied by changing the flexibility parameter w, which also leads to a change in the value of Cz. That is, since y and z are related through Eqs. (7)–(9), we cannot vary them independently to obtain the critical values.

DISCUSSION

Intramolecular association between polymer segments is greater on dissolution in a poor solvent than on dissolution in a good solvent. We distinguish between two possible consequences for poor solvent systems. When the polymer concentration is such that the density of molecules is high and the frequency of bimolecular encounters is substantial, the polymer will tend to associate with other polymer molecules and form a separate, more concentrated phase.

When the polymer is at very, very low concentrations, the same solvent conditions which result in this macroscopic phase separation lead to the monomolecular collapse of a chain. At these high dilutions the probability of one polymer domain overlapping a second domain is small. Therefore, intramolecular associations, in which one segment interacts with a second segment removed in chain sequence, but near in space, are more probable than intermolecular contacts. In order to reduce the frequency of solvent–polymer contacts in poor solvent conditions, the polymer segments join together, resulting in a large decrease in the surface area available to the solvent. If the flexibility of the chain is low, as in DNA, the result is a phase transition in which there is a discontinuous change in the hydrodynamic volume; the open, extended polymer configuration changes abruptly into a small, compact form with high density of segments. Of course these compact forms may be metastable with respect to further aggregation[10]; whether or not this is so is not clear.

To describe the phase transition, we have formulated an expression for the free energy of a chain molecule with a high segment density based on the polymer solution theory developed by Flory. The model used assumes a Gaussian distribution of segments in both the expanded and collapsed form. It is unlikely that the collapsed DNA configuration is Gaussian; for example, the collapsed state may have some crystalline order.[5] However, description of a condensed phase using some other model would be of dubious value until further information about the actual configuration in the collapsed state is available. Flory's model tends to ignore details concerned with the fact that segments of the chain are connected rather than being distributed in spherical shells of uniform density. Nevertheless, the formulation fits the experimental data on concentrated polymer solutions well and appears to be suitable to model the condensation behavior of DNA.

In our analysis of the collapsed state of DNA, no consideration is given to the molecular nature of solvent. The condensation caused by PEO, or any other polymer, is treated in the same manner as that caused by the addition of a poor solvent composed of small molecules. That is, in both cases an attraction between polymer segments is induced by the preference of the solvent molecules to be in contact with other solvent molecules, resulting in a transition to a collapsed DNA particle at the condensation value of χ. This assumption can be somewhat justified by the behavior of other ternary systems composed of solvent and two polymers; the addition of the second polymer brings about a phase transition similar to that caused by the addition of a poor solvent, or caused by lowering the temperature (Ref. 12, Chap. 13; Ref. 26, Chap. 2)

Figure 3 shows that a more flexible polymer, having a larger y value, undergoes collapse at a lower value of χ. We found that the transition to the collapsed state has a critical point; for highly flexible chains, whose value of y is greater than the critical value, there is only a gradual change in the size of the polymer domain. DNA, which is a rather inflexible chain, shows a pronounced two-phase region. The actual value of y for T2 DNA is 1.06

$\times 10^{-5}$ (assuming $V_1 = 1.2 \times 10^{-21}$ cm^3, equivalent to a base pair); this is much smaller than the critical value of 0.0227. In contrast we may take a typical synthetic polymer such as polystyrene. Using $M_r = 1.74 \times 10^6$, $V_1 = 1.67 \times 10^{-22}$ cm^3 (a styrene unit), $\chi = 0.5$, and $h_0 = 900$ Å,[27] we find that $y = 0.53$, much larger than the critical value. Hence polystyrene should show only a gradual collapse. Single-chain polynucleotides are an intermediate case. These molecules at neutral pH and high salt have values of y not much less than the critical value. Considering that such a simple theory is not expected to be quantitatively accurate near the critical point, it is not possible to predict whether the transition with single-chain polynucleotides is continuous or discontinuous.

Experiments have shown that a collapsed state of DNA does occur, but no data have been reported which may be used to ascertain the accuracy of the free energy calculation presented here. On the other hand, no discontinuous collapse transition has been reported for a synthetic polymer, as far as we know. Mazur and McIntyre[28] and Nierlich et al.[29] have measured α by scattering from polystyrene samples of high and low molecular weights as a function of temperature through the theta point; although they find a rapid change near $T = \theta$, the data actually appear to follow smooth curves without apparent breaks, which is in qualitative accord with the theory reported here.

APPENDIX: DETERMINATION OF THE THIRD VIRIAL COEFFICIENT

By Flory's method, evaluation of the configurational free energy of a single polymer molecule requires expansion of $\ln(1 - v_2)$. As the polymer collapses, the segment density increases substantially, and intersegmental contacts become frequent. It is therefore necessary to add the term in the square of the volume fraction, i.e., α^{-6}, to the original free energy function used by Flory. We accomplish this by using the virial expansion theory to calculate the third virial coefficient. (The next few higher powers of the volume fraction seem to be unimportant.[20])

First, we show that Flory's expression can indeed be derived from a virial expansion. The virial expansion of the osmotic pressure gives an expression for the partial free energy of the solvent, $\overline{G}_1$. Using the Gibbs-Duhem relation, we obtain the partial free energy of the solute $\overline{G}_2$, which, for this purpose, is the free energy of the polymer segments. These expressions are

$$\overline{G}_1 - G_1^0 = -V_1\Pi = -(kTV_1/V_2)(v_2 + B_2v_2^2 + B_3v_2^3 + \cdots) \quad \text{(A1)}$$

$$\overline{G}_2 = -kT[-\ln v_2 + (1 - 2B_2)v_2 + (B_2 - 3B_3/2)v_2^2 + B_3v_2{}^3 + \cdots] + C_1 \quad \text{(A2)}$$

B_2 and B_3 are the second and third virial coefficients, respectively; V_1 and V_2 are molecular volumes of the solvent and segment, respectively; and v_2 is the segment volume fraction. In Eq. (A2), C_1 is a constant of integration. The free energy of mixing n_1 solvent molecules with n_2 segments is

$$\Delta G_m = n_1(\overline{G}_1 - G_1^0) + n_2(\overline{G}_2 - G_2^0) \tag{A3}$$

Substitution of Eqs. (A1) and (A2) into Eq. (A3) and collecting terms in equal powers of the segment volume fraction gives the final equation for mixing:

$$\Delta G_m/kT = n_2(\ln v_2 - 1 + B_2 v_2 + (1/2)B_3 v_2^2 + C_1 - G_2^0) \tag{A4}$$

Henceforth, n_2, the number of polymer segments, is to be considered as constant, while v_2, and hence n_1, is variable.

We wish to compare Eq. (A4) with the mixing portion of the free energy found by Flory. [In deriving the expression for the configurational free energy of a single polymer molecule, Eq. (1), Flory distinguished between the free energy due to mixing the solvent and the polymer and that due to the elastic behavior of the polymer. The contributions from mixing are the terms inside the brackets of Eq. (1), and those from the rubber elasticity theory are $3(\alpha^2 - 1)/2 - \ln \alpha^3$. See Ref. 12, Chap. 12 for details.] For the case of a single polymer molecule, the expression Flory derived for the free energy of mixing did not include $n_2 \ln v_2$. That is, for the case of $\chi = 0$, the integrand in Eq. (1) is

$$\begin{aligned} \Delta G_F/kT &= n_1 \ln v_1 \\ &= \Delta G_m/kT - n_2 \ln v_2 \end{aligned} \tag{A5}$$

To make the transition from the ideal solution case to the polymer lattice theory, the polymer is considered as a cloud of noninteracting segments. In the ideal solution case, $n_2 \ln v_2$ represents the change in the number of configurations available to the solute when the volume changes. However, for a polymer molecule, the solute consists of connected segments, and the change in the number of configurations accompanying a change in volume is not the same as that for nonconnected molecules (segments). Thus $n_2 \ln v_2$ does not explicitly appear in the polymer free energy but is replaced by the elasticity terms. Therefore, to equate terms in the free energy from Eq. (A4) with the terms in brackets in Eq. (1), we subtract the $n_2 \ln v_2$ from Eq. (A4). We compare

$$\Delta G_m/kT - n_2 \ln v_2 = n_2(-1 + B_2 v_2 + (1/2)B_3 v_2^2 + C_1 - G_2^0) \tag{A6}$$

and

$$\begin{aligned} \Delta G_F/kT &= n_1(\chi - 1)v_2 - n_1(v_2^2/2 + v_2^3/3 + \cdots) \\ &= n_2[\chi - 1 + (1/2 - \chi)v_2 + v_2^2/6 + \cdots] \end{aligned} \tag{A7}$$

In the last line we have substituted $n_1 = n_2(1 - v_2)/v_2$, which is true if $V_1 = V_2$.

We now proceed to calculate the virial coefficients from a simple lattice model. Using a standard formula, we have

$$B_2 = \frac{-1}{2V_2} \int_0^\infty \left[\exp\left(\frac{-w_{ij}}{kT}\right) - 1\right] d\mathbf{R}_{ij} \tag{A8}$$

where w_{ij} is the potential of average force between segments i and j.[30] With the lattice model we replace the integral by a sum and write

$$B_2 = -\frac{1}{2}\sum_j g_{ij} \tag{A9}$$

where g_{ij} represents the expression in brackets in Eq. (A8) over V_2 and where the summation is over all positions of a segment j on sites in the neighborhood of the site occupied by segment i. The potential w_{ij} is infinity when i and j are coincident, and since the volume of a site is V_2, g_{ii} becomes -1. We assume that w_{ij} has a small negative value when j is adjacent to i (an attractive potential), so that in this case $g_{ij} = \zeta$, where ζ measures the strength of the attraction. We further assume that w_{ij} and g_{ij} are zero elsewhere.

In this way we get

$$B_2 = \tfrac{1}{2}(1 - q\zeta) \tag{A10}$$

where q is the lattice coordination number.

Now we compare the terms in Eqs. (A6) and (A7) with the same power of v_2. We find that the two are the same if

$$B_2 = \tfrac{1}{2} - \chi \tag{A11}$$

Substitution of Eq. (A10) for B_2 leads us to identify χ as

$$\chi = q\zeta/2 \tag{A12}$$

This is reasonable, since χ was originally introduced with the heat of mixing, which must result from the attractive forces represented by ζ.

Using the same potentials as for B_2, the expression for the third virial coefficient is

$$\begin{aligned} B_3 &= \frac{-1}{3}\sum_j \sum_k g_{ij}g_{ik}g_{jk} \\ &= \tfrac{1}{3}\,(1 + 3q\zeta^2 - 2q\zeta^3) \end{aligned} \tag{A13}$$

Substituting Eq. (A12) into Eq. (A13), we have the third virial coefficient as it appears in Eq. (5):

$$B_3 = \tfrac{1}{3}(1 + 12\chi^2/q - 16\chi^3/q^2) \tag{A14}$$

Comparing Eq. (A14) to the coefficient of the v_2^2 term in Eq. (A7), we see that the terms are the same, but that we have now, in addition, a contribution resulting from intersegment attraction.

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