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Molecular Weight Dependence of the Rotor Speed Induced Aggregation of Deoxyribonucleic Acid*

Joel Rosenbloom and Verne Schumaker

ABSTRACT: The molecular weight dependence of the rotor speed induced aggregation phenomenon has been studied using deoxyribonucleic acids (DNAs) isolated from the T_3, T_4, T_5, and T_7 bacteriophages. A linear relation between the reciprocal of the monomer concentration and the square of the rotor speed has been found in each case. This relation may be termed the pseudo-phase-transition line. Both the slope and the intercept of the pseudo-phase-transition line are functions of the molecular weight of the DNA, and the slope appears to depend upon the three-halves power of the molecular weight. A quantitative theory has been developed in an attempt to explain the behavior of these molecules in the centrifuge.

The anomalous rotor speed dependence of the sedimentation coefficient of high molecular weight DNA isolated from the T-even bacteriophage (Hearst and Vinograd, 1961; Burgi and Hershey, 1961; Crothers and Zimm, 1965; Aten and Cohen, 1965) and bacteria (Eigner *et al.*, 1962) is caused by an aggregation process which is dependent upon the angular velocity of the ultracentrifuge (Rosenbloom and Schumaker, 1963). When such bacteriophage DNA preparations are sedimented in the analytical ultracentrifuge in density gradients to prevent convection, the following events are noted. (1) A variable fraction of the DNA, depending upon the initial concentration and the rotor speed, behaves like a precipitate and sediments rapidly out of solution. (2) The concentration of DNA which remains in solution and sediments normally is inversely proportional to the square of the rotor speed and independent of the initial concentration. (3) The sedimentation coefficient of the DNA remaining in solution is not a function of the rotor speed, but is dependent upon the concentration of DNA present during the remainder of the run.

In order to explain these observations, Rosenbloom and Schumaker (1963) have suggested that the collision rate between small aggregates, formed initially by thermal motion, and single DNA molecules is increased as the sedimentation velocity increases. This increased collision frequency leads to the formation of larger aggregates and ultimately to the separation of a solid phase which precipitates from solution. The DNA remaining in solution sediments normally with a sedimentation coefficient which is independent of rotor speed but markedly dependent upon the concentration of DNA existing during the run. Since the nature of the forces involved in the molecular interaction are probably those of steric entanglement, the precipitation phenomenon should depend upon the molecular weight of the DNA. In order to test this hypothesis we have studied the DNAs isolated from bacteriophage T_5, containing DNA of 84,000,000 molecular weight (Hershey *et al.*, 1962); T_7 and T_3 both having DNA of molecular weights of 25,000,000 (Crothers and Zimm, 1965; Davidson and Freifelder, 1962); and preparations of T_4 DNA sheared to a molecular weight of 65,000,000. We have found that the precipitation

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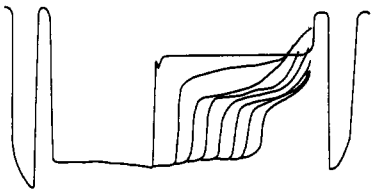

FIGURE 1: Composite densitometer tracings for a run with T_7 DNA. Centrifugation at 59,780 rpm; initial DNA concentration, 48.4 μg/ml.

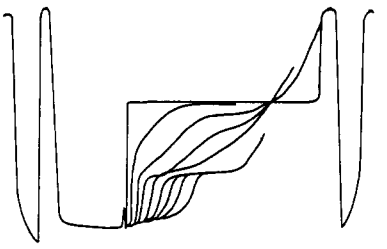

FIGURE 2: Composite densitometer tracings for a run with T_5 DNA. Centrifugation at 35,600; initial DNA concentration, 40 μg/ml.

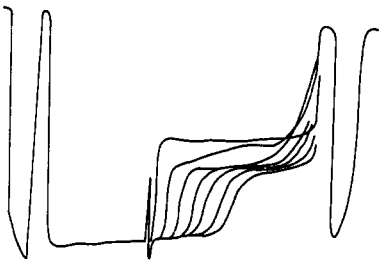

FIGURE 3: Composite densitometer tracings for a run with half-molecules of T_4 DNA. Centrifugation at 35,600 rpm; initial DNA concentration, 30 μg/ml.

effect increases with increasing molecular weight. For a given molecular weight, however, the concentration of DNA which remains in solution is inversely proportional to the square of the rotor speed. The theory previously developed to account for the dependence of the precipitation phenomenon upon the rotor speed has been revised and extended to account for the dependence of the precipitation phenomenon upon the molecular weight of the DNA.

Materials and Methods

Growth of Phage.

T_4, T_3, and T_7 bacteriophages were prepared by the plate lysate method of Cohen and Arbogast (1950). The crude lysate was freed of bacterial debris by low-speed sedimentation and the supernatant solution was treated with 1 μg/ml of deoxyribonuclease for 30 min at room temperature in 0.85 g/100 ml of NaCl, 0.002 M phosphate, pH 7.2, and 0.01 M $MgCl_2$. The phages were then purified by differential sedimentation.

T_5 was grown in liquid broth media as described by Adams (1950). The phages were purified as above.

Preparation of DNA. The DNA was isolated from the purified phage preparations by the phenol extraction method of Mandell and Hershey (1960), using a ground glass stoppered vial. After removal of phenol by ether extraction, the DNA solution was dialyzed against 0.05 M phosphate, pH 7.2, and 0.2 M NaCl. All subsequent dilutions of the concentrated DNA solutions were performed by pouring and weighing. An extinction coefficient of 18.1 cm^2/mg of sodium nucleate was used to convert optical density measurements to concentration for T_4 DNA (Rubenstein *et al.*, 1961). An extinction coefficient of 19.8 cm^2/mg (Crothers and Zimm, 1965) was used for T_3 and T_7 DNA, while a value of 18.1 cm^2/mg was chosen for T_5 DNA.

Preparation of T_4 Half-Molecules. T_4 DNA was sheared in a Virtis mixer using stainless steel blades at such a speed that a relatively homogeneous preparation of half-molecules was obtained (Hershey *et al.*, 1962; Burgi and Hershey, 1963).

Characterization of DNA by Ultracentrifugation. All DNA preparations were studied in the ultracentrifuge at four or five concentrations over the range 2–10 μg/ml in order to determine $s^0_{20,w}$. The runs were made either at 25,600 or 29,500 rpm in a 30-mm Kel-F centerpiece using preformed sucrose gradients of 4–6% to prevent disturbance of the boundary. The following values were found for $s^0_{20,w}$: T_5, 48.5 S; T_3, 31.5 S; T_7, 31 S; T_4 half-molecules, 41.5 S.

Gradient Centrifugation. The DNA, at an initially uniform concentration, was centrifuged in preformed 4–6% sucrose gradients as previously described (Rosenbloom and Schumaker, 1963). A 12-mm Epon-filled centerpiece was used except at concentrations greater than 50 μg/ml where a 3-mm Epon-filled centerpiece was used.

Results

The general plan of these studies was as follows. Concentrated solutions of each DNA preparation were centrifuged at various speeds over the range 14,290–59,780 rpm. The concentration of DNA remaining in solution after any rapid loss was then estimated from the pen height of the densitometer tracing, assuming a linear relationship between pen height and concentration. The inverse of this concentration was then plotted as a function of the square of the rotor speed. In all cases, the DNA remaining in solution sedimented normally. In this way phase diagrams for each species of DNA were defined.

Figures 1–3 are composite densitometer tracings of typical runs using whole DNA molecules of T_7 and T_5, and half-molecules of T_4. In each of these experiments a fraction of the DNA sediments rapidly to the bottom of the cell, while the remainder sediments in a normal manner. We had previously shown that this phenomenon was not affected by wide variation of

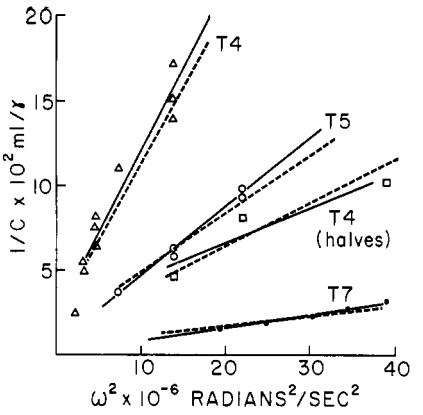

FIGURE 4: Phase diagram for the four species of DNA which were studied. The experimental points are the reciprocal of the DNA concentration which remained in solution after any rapid loss. Triangles, T_4; open circles, T_5; rectangles, T_4 half-molecules; closed circles, T_7. The solid lines are the regression lines for the data for each of the DNAs. The dashed lines are the phase lines which have been constructed using eq 10.

ionic strength or the presence of chelating agents. Table I shows that it is insensitive to temperature or pH variation. The last entry indicates that the substance

TABLE I: Additional Experiments with Whole T_4 DNA.[a]

DNA Initial (μg/ml)	DNA Remaining (μg/ml)	Special Feature
25.9	6.3	Control
27.6	6.4	pH 11.0
25.9	6.3	31°
25.9	6.2	7°
30.4	7.1	0–28% D_2O gradient

[a] All runs in a preformed gradient are at 35,600 rpm.

used to provide the density gradient is unimportant, since an equivalent density gradient of deuterium oxide was as effective as the sucrose gradient.

Table II contains representative data obtained with the various DNA preparations. Two generalizations may be deduced from this group of experiments. (1) The concentration of DNA remaining in solution after any loss is independent of the initial concentration. (2) For a given rotor speed the soluble concentration decreases with increasing molecular weight of the DNA. In order to illustrate these two points more effectively, we have constructed Figure 4. In this figure the ordinate is the reciprocal of the final concentration remaining after any aggregated

TABLE II: Pseudo-Phase-Transition Data for T_5, T_7, and T_3 DNA.[a]

Species	ω (rpm)	DNA Initial (μg/ml)	DNA Remaining (μg/ml)	Aggregation
T_5	20,410	40.0	40.0	–
T_5	25,980	40.0	27.2	+
T_5	25,980	26.4	26.4	–
T_5	35,600	40	16.0	+
T_5	35,600	26.4	17.1	+
T_5	44,770	40	10.2	+
T_5	44,770	26.4	10.7	+
T_7	42,040	82.9	65.5	+
T_7	47,660	82.9	52.1	+
T_7	47,660	48.4	48.4	–
T_7	52,640	48.4	42.9	+
T_7	56,100	48.4	37.3	+
T_7	59,780	48.4	31.7	+
T_3	52,640	50.2	50.2	–
T_3	56,100	50.2	43.0	+
T_3	59,780	50.2	37.1	+
T_4 half-molecules	20,410	30.0	30.0	–
T_4 half-molecules	35,600	30.0	21.9	+
T_4 half-molecules	35,600	15.0	15.0	–
T_4 half-molecules	44,770	15.0	12.2	+
T_4 half-molecules	59,780	15.0	9.1	+

[a] The T_5 had an $s^0_{20,w}$ of 48.5 S and a molecular weight of 84,000,000. The T_7 had an $s^0_{20,w}$ of 31.0 S and a molecular weight of 25,000,000. The T_4 half-molecules had an $s^0_{20,w}$ of 41.3 and an assumed molecular weight of 65,000,000.

material has sedimented; the abscissa is the square of the rotor speed. The solid lines which have been drawn define two regions for each specie of DNA, a region below the line in which the concentration of DNA is sufficiently high that the DNA separates into two phases and a region above the line in which all the DNA remains in solution and sediments normally. We call such lines "pseudo-phase-transition lines." The initial concentrations of DNA have been omitted for clarity of presentation. Data for whole molecules of T_4 have been included for comparison.

The molecular weight dependence of the slopes of the pseudo-phase-transition line is approximately that of $M^{3/2}$. The dashed lines drawn on Figure 4 are calculated according to eq 10 in which this molecular weight dependence is assumed. It can be seen that the fit is reasonably close.

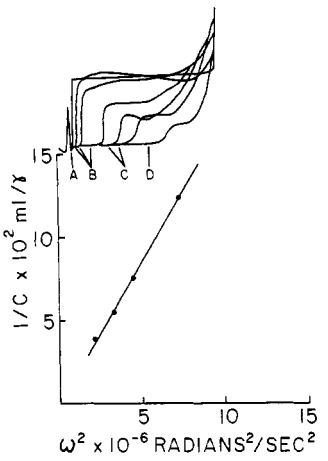

FIGURE 5: Determination of phase line during single centrifuge run. Composite densitometer tracings are shown in the top portion. These graphically demonstrate the rapid loss of material and reappearance of a plateau region as the angular velocity is increased by discrete steps. In the bottom portion the reciprocal of the concentration which is stable at a given speed is plotted as a function of the square of that speed. The initial T_4 DNA concentration is 25.9 μg/ml. A = 14,290 rpm; B = 17,250 rpm; C = 20,410 rpm; D = 25,980 rpm.

Such pseudo-phase-transition lines may be obtained during a single run by beginning with a concentrated DNA solution at a low rotor speed. The run is allowed to proceed until any aggregated material has precipitated and the plateau region has re-formed. At this point the rotor speed is increased and the procedure is repeated (Figure 5).

Discussion

We have previously postulated that the rotor speed dependent phase transition was initiated by Brownian motion (Rosenbloom and Schumaker, 1963). Collision between monomer molecules results in the formation of dimers and trimers. These small aggregates probably are held together only by the steric forces of molecular entanglement and presumably have a brief life span. As the rotor speed is increased, the dimers and trimers move through the solution appreciably faster than the monomers. Consequently, the collision rate between the monomers and the aggregates is increased, and the latter may grow in size. By performing experiments at a variety of initial concentrations and rotor speeds, we showed that the concentration of monomer which would remain unassociated with aggregate is independent of the initial concentration, but it is a function of rotor speed. We suggested a simple mathematical model from which it could be predicted that the reciprocal of the concentration of the unassociated monomer should be a linear function of the square of the rotor speed. Experiments reported in that communication, as well as those reported here, seem to support this conclusion very well. We have called that function, which relates the reciprocal of the concentration of unassociated monomer to the square of the rotor speed, the "pseudo-phase-transition line."

In the following discussion, a more detailed treatment will be presented which leads to the same conclusion. Moreover, the molecular weight dependence of the pseudo-phase-transition line is incorporated into the treatment.

Mathematical Preliminaries. The chemical equation between monomers, dimers, trimers, etc., in equilibrium is

$$m + m \underset{}{\overset{K_1}{\rightleftharpoons}} A_2 + m \overset{K_2}{\rightleftharpoons} A_3 + m \overset{K_3}{\rightleftharpoons} \ldots \overset{K_{i-1}}{\rightleftharpoons} A_i + m \overset{K_i}{\rightleftharpoons} A_{i+1} + m \overset{K_{i+1}}{\rightleftharpoons} \ldots \quad (1)$$

where m and A_i are the concentration of monomer and polymer containing i monomer units, and where the K_i are the respective equilibrium constants.

If the total molar concentration of monomeric units (free and associated) in solution is given the symbol, m_0, then

$$m_0 = m + 2A_2 + \ldots iA_i + \ldots \quad (2)$$

which by virtue of the separate equilibria of eq 1 may be written

$$m_0 = m + 2K_1m^2 + 3K_1K_2m^3 + \ldots + i(K_1K_2 \ldots K_{i-1})\, m^i + \ldots \quad (3)$$

For reasons which will become apparent later, we wish to consider the system for which all of the equilibrium constants except K_1 and K_2 are equal.

$$K = K_3 = K_4 = K_5 = \ldots = K_i = \ldots \quad (4)$$

Then eq 3 may be rearranged to yield

$$m_0 = m + K_1m^2 + 3K_1K_2m^3 + (K_1K_2/K^2)m[(1 + 2Km + 4(Km)^2 + 3(Km)^3 + \ldots) - (1 + 2Km + 3(Km)^2)] \quad (5)$$

The infinite series contained in parentheses within eq 5 is convergent and has a value of $1/(1 - Km)^2$, for $Km < 1$. Therefore, we may write

$$m_0 = m + 2K_1m^2 + 3K_1K_2m^3 + (K_1K_2/K^2) \times m[1/(1 - Km)^2 - (1 + 2Km + 3(Km)^2)] \quad (6)$$

This equation will now be used to describe the formation of the monomer plateau.

Formation of the Monomer Plateau. The essential characteristic of a phase transition between a soluble phase and undissolved material is that at low concentrations all of the substance must remain in the unassociated (monomeric) form. Therefore, if a plot is

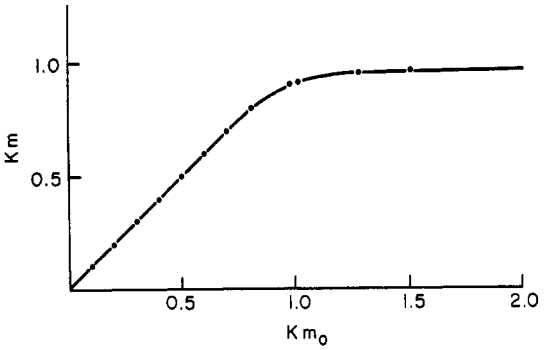

FIGURE 6: Plot of Km *vs.* Km_0. The curve was constructed using eq 6 and shows that the fraction of DNA appearing as monomer undergoes a sharp decline as the total concentration of DNA is raised.

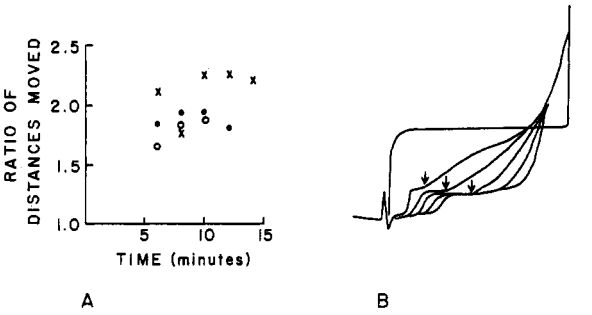

FIGURE 7: On the left is a plot of the ratio of the sedimentation coefficient of the trailing edge of the aggregate boundary to the sedimentation coefficient of the monomer as a function of time. These data were taken from three experiments signified by the ×, O, and ●, using T_4 DNA at a rotor speed of 35,600 rpm. Similar results were obtained using T_5 and T_7 DNA, although the trailing edge was not as clearly defined. The average value of s_A/s_M is 1.96. For tetramer a ratio of $4^{0.455} = 2.05$ should be obtained. On the right are shown the concentration distributions observed during the experiment corresponding to the x points.

constructed of total concentration, m_0, as a function of the amount of monomer present, m, then a 45° line will be obtained at low concentrations. At some critical concentration however, aggregation begins, and the amount of monomer will not increase further. Thus the 45° line abruptly levels off. This behavior is characteristic of a phase transition. Equation 6 may be plotted in this manner. Since the quantity K has units of liters per mole, it is most convenient to plot concentration in terms of reciprocal K units. Furthermore, if we make the assumption that $100K_1 = 10K_2 = K$, then eq 6 yields the curve drawn in Figure 6. The sharpness of the transition is determined by the ratio $K_1K_2:K^2$, and the transition becomes sharper as this ratio decreases.

As shown in Figure 6 the maximum value for the monomer concentration is approached asymptotically. This maximum value would then represent the concentration found at the pseudo-phase-transition line. This concentration is determined by the maximum value of the term $1/(1 - Km)^2$ which appears on the right-hand side of eq 6. This term reaches a maximum value when $Km_p = 1$. Hence, the molar concentration of monomer in the plateau, m_p, is equal to the reciprocal of the equilibrium constant, $m_p = 1/K$.

Relative Values of the Equilibrium Constants. The assumption that $K_1K_2 << K^2$ implies that dimers and trimers are considerably less stable than larger aggregates. We believe that this may hold for the rotor speed dependent phase transition of high molecular weight DNA, as described below.

In general large aggregates, crystals, or droplets tend to be more stable than those which are smaller. Surface energy, which favors the dissolving of the aggregate, represents a smaller fraction of the total energy of interaction for the large aggregate.

We suggest that a similar situation may exist for the dimers and trimers which form between DNA molecules. Since the interaction energy is apparently due to molecular entanglement, the magnitude of this energy should be related to the number and size of the regions of contact between molecules. Each monomeric subunit will have one contact region in the dimeric form, two contact regions in the trimeric form, and three regions in the tetrameric form. Larger aggregates will have some of the surface subunits held by three contact regions, and these will determine the aggregate stability. Therefore, we suggest that $K_1 < K_2 < K_3$ and that $K_3 = K_4 = K_5 =$ etc., for the system being studied.

Some estimate of the size of the smallest stable aggregates is obtained from measurement of the sedimentation coefficient of the trailing edge of the aggregate boundary. As is shown in Figure 7, the ratio between the sedimentation coefficient of the monomer boundary and the trailing edge is of the order of 2. Since the sedimentation coefficient of the aggregates, s_A, is related to that of the monomer, s_M, by the expression $s_A = s_M n^{0.455}$ (Crothers and Zimm, 1965), where n is the number of monomer units in the aggregate, we estimate that the tetramer is the trailing species found in appreciable quantity.

Development of an Empirical Equation for the Pseudo-Phase-Transition Line. In the preceding sections, we have attempted to show how the concentration in the monomer plateau is related to the equilibrium constant. It is now our purpose to develop an equation which will relate the monomer concentration, and hence the equilibrium constant, to the angular velocity of the ultracentrifuge.

Since our experimental data are measured in terms of weight concentration, c, it will be easier to continue the treatment in weight concentration units. The conversion, $c = Mm_p$, may always be made.

It has been found experimentally that the relationship between the reciprocal of the concentration in the monomer plateau and the rotor speed is linear. This

is called the pseudo-phase-transition line, and its equation may be written as

$$\frac{1}{c} = A(1 + B\omega^2) \tag{7}$$

where A and B are constants. It can be seen that the constant A must be equal to the reciprocal of the maximum monomer concentration in the absence of a centrifugal field, $\omega^2 = 0$. In theory, this constant could be determined from the intercept of the pseudo-phase-transition line. However, the data presented in Figure 5 are not of sufficient accuracy to give reliable values for this quantity. (This is particularly true since the origin of the vertical axis is equal to an infinite concentration.) We feel it is plausible to work backwards, assuming reasonable values for this maximum concentration and then testing these values to see if they fit the experimental data. To do this, the assumption is made that in solution the DNA molecule may be represented by an enormous random coil with a radius of gyration which may be calculated from the molecular weight and the stiffness of the DNA molecule. Because of collisions between outer chain segments, the centers of the two random coils will not approach much closer than twice this distance and, therefore, an aggregate of many such random coils may have a structure approximated by a close-packed collection of spheres. The radii of these spheres will be proportional to the radius of gyration of the random coils. The mass per unit length figure for the B form of DNA is about 200 daltons/A, and since the length of the Kuhn statistical segment is 717 A (Hearst and Stockmayer, 1962), the radius of gyration may be computed as a function of the molecular weight from the formula

$$\text{radius of gyration} = [717M/200]^{1/2}/\sqrt{6} \tag{8}$$

where M is the molecular weight of the DNA. If these molecules were crowded together to give the configuration of a close-packed collection of spheres, the volume occupied would by 74% of the total volume of the solution. Therefore, we can calculate the reciprocal of the maximum concentration in units of milliliters per gram.

$$\frac{1}{c_0} = A = (4/3\pi r^3)N/(0.74M) \tag{9}$$

where r is the radius of closest approach (assumed equal to the radius of gyration).

In order to evaluate the second constant which appears in eq 7, we advance the following argument. The term $B\omega^2$ probably represents, among other things, the strength of the centrifugal field. Therefore, we would expect that the terms $B\omega^2$ would include the term $\omega^2 x$, where x is the distance from the axis of rotation. During acceleration it is frequently seen that the aggregation phenomenon first occurs at the bottom

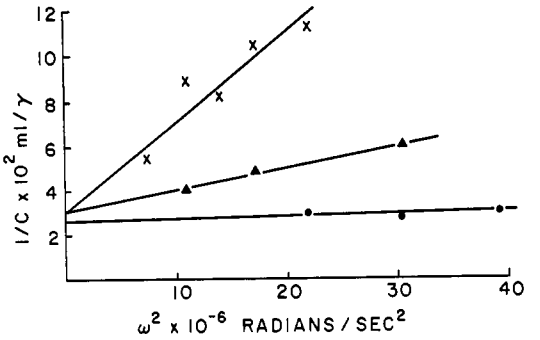

FIGURE 8: Pseudo-phase-transition lines for T_5 DNA at different density values in CsCl solution. The crosses, triangles, and circles refer to, respectively, solutions of 3.36, 33, and 50% CsCl in 4–6% sucrose gradients, yielding solutions of average densities of 1.04, 1.34, and 1.60 g/ml.

of the cell, which provides some additional justification for including this term. Now if $B\omega^2$ contains the term $\omega^2 x$, it is logical to expect that the centrifugal field will operate on a mass. Moreover, it is reasonable to expect that this will be the bouyant mass of the macromolecules, $M(1 - \bar{v}\rho)$. The justification is twofold. In the first place, it will complete the observed dependence of the slope on the molecular weight; since the term A includes the molecular weight to the one-half power, the total dependence of the slope of the pseudo-phase-transition line upon the molecular weight will be to the three-halves power. Second, we can test for the existence of the $(1 - \bar{v}\rho)$ term by performing experiments in concentrated cesium chloride solutions which will increase the density without much affecting the viscosity. It is found in such experiments (Figure 8) that increasing the density causes a marked decrease in the slope of the pseudo-phase-transition line.[1] (This decrease is somewhat larger than would be predicted by the $(1 - \bar{v}\rho)$ term, which possibly may be explained as a decrease in radius of gyration due to damping of charge by the very high salt concentrations employed.)

Now if $B\omega^2$ contains the complex term $M(1 - \bar{v}\rho)\omega^2 x$, it is reasonable to ask what quantities might be expected to appear in the denominator. Familiarity with this grouping of symbols which frequently occurs in centrifuge theory suggests that the denominator contains either Nf, where N is Avogadro's number and f is the translational frictional coefficient, or else the denominator would be expected to include the term RT. It is possible to distinguish between these because the magnitude of the translational frictional coefficient is proportional to the viscosity of the solution. By adding sucrose the solution viscosity can be in-

[1] The *inverse* relationship between density and the degree of aggregation seems to us to rule out the hypothesis that hydrostatic pressure is the cause of this pseudo phase transition.

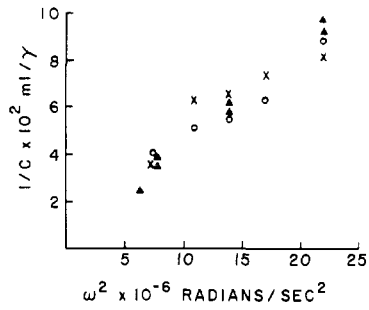

FIGURE 9: Experimental points determined at different viscosities to illustrate independence of the pseudo-phase-transition line upon solution viscosity. T_5 DNA was used in these experiments, and the triangles, crosses, and circles respectively represent data obtained in solutions of average sucrose concentrations of 3, 8.5, and 17.5% having relative viscosities of 1.10, 1.29, and 1.77. The salt concentration was 0.2 M NaCl.

creased without much increasing the density. These experiments have been performed and the results are given in Figure 9, where it is shown that there is little or no dependence of the slope of the pseudo-phase-transition line upon solution viscosity. Therefore, the term appearing in the denominator is most likely RT.

The quantity $B\omega^2$ must have no units, for it is added as a dimensionless number to 1. However, the quantity $M(1 - \bar{v}\rho)\omega^2 x/RT$ has dimensions which are given in reciprocal centimeters. Therefore, it must be multiplied by a characteristic length Δx, to convert it to a pure number. The final equation then assumes the form

$$\frac{1}{c} = \frac{1}{c_0}\left[1 + \frac{M(1 - \bar{v}\rho)\omega^2 x \Delta x}{RT}\right] \tag{10}$$

where c_0 is given by eq 9.

The four dashed lines drawn on Figure 5 have been computed using eq 10 with a single value of 2500 A for Δx. These lines fit the data surprisingly well and should enable us to predict with some confidence the pseudo-phase-transition behavior for DNA preparations of intermediate molecular weights. Hopefully, the validity of eq 10 may extend beyond these limits.

Equation 10 may be rewritten as

$$\frac{1}{c}\frac{\Delta c}{\Delta x} = \frac{M(1 - \bar{v}\rho)\omega^2 x}{RT} \tag{11}$$

where $\Delta c = c_0 - c$. Formally, this equation is analogous to the sedimentation equilibrium equation and also to the Archibald equation. It implies that at the surface of the moving aggregate, the concentration of monomer is c_0, and that this concentration drops to the value of c over the distance Δx, which may thus be called the thickness of the layer of concentration buildup around the aggregate.

Concluding Remarks

To explain the observed pseudo phase transition, we have presented an aggregation–dissociation hypothesis and have attempted to show that it is consistent with the formation of a monomer plateau. According to this hypothesis the aggregation can be described by an equilibrium constant, K, and the molar concentration remaining in the monomer plateau is equal to the reciprocal of the equilibrium constant, $m_1 = 1/K$. Next, we developed empirical equations relating the weight concentration of monomer in the plateau, $c = Mm_1$, to the molecular weight, rotor speed, density, etc. according to eq 10. It is now possible to eliminate c and m_1 to yield an expression for the equilibrium constant

$$K = \frac{M}{c_0}\frac{(1 + M(1 - \bar{v}\rho)\omega^2 x \Delta x)}{RT} \tag{12}$$

where c_0 is given by eq 9.

From eq 12 it would be possible to derive pseudo-thermodynamic parameters for the aggregation of DNA molecules of a particular molecular weight at a given rotor speed, density, etc. The most serious deficiency in the treatment we have presented above is the lack of a detailed theoretical account of molecular flows around the surface of the aggregates. However, we suggest the following events occur. Dimers are formed in low concentration by collision between monomers due to Brownian motion. In a centrifugal field the dimers move faster than the monomers and collide with the latter, causing the formation of larger aggregates. At the surface of the moving aggregates, the effective monomer concentration is increased, and the aggregates grow in size at the expense of the monomer concentration. Eventually, the monomer concentration at the aggregate surface drops to a value of c_0, at which point the rate of association at the surface is balanced by the rate of release of monomer. This event establishes the concentration of monomer. Because the aggregate molecules sediment appreciably faster, a monomer plateau is uncovered.

We wish to close this discussion with a word of caution. Although the arguments presented above appear reasonable, and the equations developed describe the phenomenon very well indeed, we do not believe that the mechanism we have presented above should be considered proved. It is possible that a much different explanation might account for the pseudo phase transition. For example, liquid crystal formation involving relatively long-range electrical forces such as has been observed with concentrated TMV solutions has been suggested to us by many other workers. Such liquid crystals should be very sensitive to low ionic strengths, and we are currently engaged in such studies. At the present, however, we feel that the explanation advanced above renders the most plausible description of this fascinating and novel system.

Summary

A detailed study has been made of the precipitation phenomenon observed with high molecular weight DNA in the analytical ultracentrifuge. The dependence of the phenomenon upon molecular weight, density, viscosity, and rotor speed has been studied and an equation developed which fits the experimental data closely. The phenomenon can be described by a pseudo-phase-transition line relating ω^2 to the reciprocal of the monomer concentration. The slope of this line appears to be a function of $M^{3/2}$ and $(1 - \bar{v}\rho)$ as well as ω^2, but independent of the viscosity of the solution.

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Evidence for Ribonucleic Acid Molecules Restricted to the Cell Nucleus*

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ABSTRACT: The molecules of ribonucleic acid (RNA) present in the mouse L-cell nucleus and cytoplasm were compared by competition for complementary sites on mouse deoxyribonucleic acid (DNA). All sequences of RNA in the cytoplasm were also represented in the nucleus. However, cytoplasmic ribonucleic acid (cRNA) was unable to compete against a fraction of the nuclear ribonucleic acid (nRNA) molecules, indicating that these are not present in the cytoplasm. Saturation of the homologous DNA showed that nRNA is complementary to about five times as much of the genome as is cRNA, further confirming the existence of RNA molecules present only in the nucleus. Turnover of the unique sequences was examined by measurement of the accumulation of label in the unique fraction and its rate of breakdown after the labeled precursor was removed from the medium. A maximum was reached after 100 min of label, indicating a short lifetime for the RNA molecules which are limited to the nucleus.

The label (80% of it) was lost after 150 min in unlabeled medium, and no label remained in the unique nuclear fraction after 20 hr. Competition of cRNA against nRNA was repeated using rabbit kidney cells, to test the possibility that the above results are peculiar to heteroploid cells or to cells in culture. Unique nRNA was again found. It was concluded that although cytoplasmic messenger ribonucleic acid (mRNA) originates in the nucleus, approximately 80% of the base sequences being transcribed into RNA in L cells are in unstable molecules which are retained in the nucleus.

The nucleus of mammalian cells has been shown to be the site of origin of at least the bulk of cRNA[1] (Prescott, 1964; Girard *et al.*, 1965). However, there is evidence that the nucleus is also the site of synthesis of RNA with a specifically nuclear function. Kinetic studies have indicated a high rate of turnover of RNA within the nucleus (Harris, 1963). An RNA of unusual base composition has been found complexed to histones

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[1] Abbreviations used: SDS, sodium dodecyl sulfate; SSC, standard saline citrate (0.15 M NaCl–0.015 M sodium citrate); cRNA and nRNA, cytoplasmic and nuclear ribonucleic acids, respectively.