Tactoidal state and phase transitions in systems of linear polymers of variable length

(entropy driven/polymer alignment/liquid crystals/length control/hemoglobin S)

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ABSTRACT The tactoidal state in systems containing long, rod-like molecules consists of partially aligned solute molecules in equilibrium with and at a concentration not much higher than that in the conjugate isotropic phase. Under the liquid lattice model of Flory [*Proc. R. Soc. London Ser. A*, (1956) 234, 73-89], as well as under other models, tactoid formation by molecules of fixed axial ratio depends on nonideality induced by excluded volumes; the process is wholly entropy driven and requires no direct interactions between rods.

Many rod-like biological polymers exhibit reversible polymerization, so that axial ratio and length are not fixed. Polymerization and rod length will then not only induce nonideality, alignment, and phase separation, but will be affected by these. In this work these interrelations are treated under the model of Flory, modified to include a free energy of polymerization and to permit reversible changes in rod length. The primary conclusion is that, in contrast to the situation for fixed lengths, excluded volume-dependent nonideality alone does not suffice to induce a tactoidal phase separation. In the absence of attractions or repulsions between rods the anisotropic phase is highly concentrated. This phase only becomes tactoidal when a minimal level of repulsive interaction between rods is reached. Under this model, tactoid formation in systems such as deoxygenated hemoglobin S and tobacco mosaic virus depends on repulsive interactions or metastability or both. As a secondary result it is shown that rod length in the anisotropic phase is much greater than in the conjugate isotropic phase.

In their classical studies of tobacco mosaic virus, Bernal and Fankuchen (1, 2) demonstrated the existence of birefringent spindle-shaped bodies, designated tactoids (3, 4), consisting of long, rod-like virus particles aligned in almost parallel array. The particles could separate spontaneously into two phases, one less dense and isotropic, and the other more dense and anisotropic, showing spontaneous birefringence and the presence of tactoids.

Since then, tubulin, flagellin under some conditions, actin, and deoxygenated sickle hemoglobin (hemoglobin S), as well as tobacco mosaic virus, have been shown to exhibit reversible helical polymerizations which produce very long, rod-like particles. In hemoglobin S, the polymerization and resultant phase transition provide the pathogenesis for sickle cell disease (5).

The theoretical basis for tactoid formation and phase separation in systems of rod-like molecules or particles of fixed length has been elucidated by Onsager (6), Ishihara (7), Flory (8), DiMarzio (9) and, as applied to hemoglobin S, by Minton (10). Large excluded volumes resulting from particle asymmetry induce high degrees of nonideality as solute concentration increases. At sufficient concentration, a local entropy maximum and free energy minimum are achieved when the

particles are in a state of partial alignment. At a critical concentration, C_c , above this level the new phase becomes more stable than the isotropic condition, in which the rods are more likely to abut and exclude each other. The phase transition at C_c is then bridged by a separation into isotropic and anisotropic conjugate phases at concentrations C^* and C^{**} , respectively, where $C^* < C_c < C^{**}$. Characteristic of the tactoidal phase, and in contrast to a phase change associated with simple solubility, the more concentrated phase has a solute concentration that is only slightly higher than that of its conjugate. This process is altogether entropy driven. Direct interactions between rods have a major effect only if they are sufficiently attractive to induce a solubility transition in which a highly concentrated and more highly aligned anisotropic phase replaces the tactoidal state as the more concentrated conjugate phase.

Onsager (6) and Ishihara (7) considered the effects of shape through analysis of first-order excluded-volume effects, reflected in second virial coefficients. Flory (8) and DiMarzio (9) employed liquid lattice models to permit analysis of systems at much higher solute concentration. Minton (10), in applying the analysis to hemoglobin S, included equilibria between rods and their monomeric constituents, but in his analysis, as in the others, the permitted rods were of fixed length. In hemoglobin S and in other biological polymerizing systems, this is not generally the case. There need not be, and usually is not, a mechanism that regulates length to a specific value and, in consequence, average polymer length will depend on conditions. Among the factors controlling length will then be the nonideality associated with high concentrations of solute and, in particular, with shape asymmetry. Length itself, however, will affect nonideality so that there must exist interrelations between rod length and the polymerization equilibrium on the one hand and nonideality, alignment, and phase separation on the other. It is the purpose of this work to extend the liquid lattice formulation of asymmetry-dependent entropically driven phase separations to the situation existing in most biological systems, that in which polymer length and thus axial ratio can vary. The results bear on (i) the modifications required in the stem-shaped phase diagrams of Flory (8) and Minton (10), (ii) the effect of phase transition and phase separation on polymer length, and (iii) the stability of the tactoidal state.

In the ensuing discussion, the overall class of anisotropic states will be subdivided as follows: Tactoidal, applying originally to a description of the macroscopic shape of arrays of polymers, will be taken, by extension, to indicate a phase in which the polymers are partially aligned and at a concentration that is usually not a great deal higher than that of the solute in the conjugate isotropic phase, and in any case is not as high as that in a closely packed crystalline or quasi-crystalline array. In the tactoidal phase, alternatively called the mesophase, the alignment, although not completely parallel, is great. However, in order to distinguish the even greater or complete alignment seen

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in quasi-crystalline and crystalline phases, the term "highly aligned" is reserved for these last two states. Quasi-crystalline (11, 12) implies a phase of very high solute concentration in which the polymers are highly aligned. Crystalline implies a phase with complete alignment of rods of infinite length. Monomer refers to a constituent unit of the linear polymer; this unit may or may not correspond to a single biological macromolecule.

METHODS AND RESULTS

In this analysis, we have modified the model of Flory (8) to allow for variation in polymer length by adding a term in the expression for free energy of mixing that reflects the interaction of monomers in the polymer, and by permitting polymer axial ratio, reflecting length, to vary. In a companion work (in preparation), the model of DiMarzio (9) and Minton (10) is similarly modified with the same essential results.

Two restrictive assumptions are made: (i) The free energy of monomer-monomer interaction in the polymer is independent of polymer length and (ii) the solute is homogeneous, so that all solute particles in a given phase have the same length and axial ratio. This second assumption neglects the presence of free monomers coexisting with polymers as well as prohibiting heterogeneity of polymer lengths. The analysis, therefore, permits only variation in number of polymers, under the constraint that the total mass of solute is fixed. Justification that these assumptions either conform to reality or do not alter the fundamental conclusions is given in the *Discussion*.

The fundamental expression in Flory's analysis for systems of fixed rod length is that for the free energy of mixing, ΔG_M ,

$$\frac{\Delta G_M}{kT} = n_1 \ln v_1 + n_2 \ln v_2 - (n_1 + y n_2) \ln \left(v_1 + \frac{y}{x} v_2 \right) - n_2 [\ln(x y^2) - y + 1] + \chi_s x n_2 v_1 \quad [1]$$

where x is the polymer axial ratio (a constant), y is an orientation parameter, n_1 and n_2 are the numbers of solvent and solute molecules, respectively, $v_1 = n_1/(n_1 + x n_2)$ and $v_2 = x n_2/(n_1 + x n_2)$ are the volume fractions of solvent and solute, respectively, and χ_s is a parameter, given in units of kT , that reflects the free energy of interaction between solvent and solute per monomeric segment; $\chi_s < 0$ implies attractive interaction between solute and solvent or, equivalently, and as used here, repulsive interaction between solute particles. The parameter y varies from x for isotropically oriented rods to one for full parallel orientation. Fig. 1, summarizing the results of Flory (8), shows phase diagrams for this system.

If the rods consist of monomers that aggregate reversibly in linear array, the free energy of polymerization, consisting of monomer-monomer interactions in the rods, is $(x - 1)n_2\chi_a$ where χ_a is the interaction free energy of association in units of kT . The addition of this term and division of Eq. 1 by $(n_1 + x n_2)$ gives f , the free energy of mixing per unit of lattice or solution,

$$f = v_1 \ln v_1 + \frac{v_2}{x} \ln v_2 - \left(v_1 + \frac{y}{x} v_2 \right) \ln \left(v_1 + \frac{y}{x} v_2 \right) - \frac{v_2}{x} \ln(x y^2) + \frac{v_2}{x} (y - 1) + v_1 v_2 \chi_s + \frac{v_2}{x} (x - 1) \chi_a \quad [2]$$

In this system, x as well as y is variable.

In the variable rod length system the isotropic phase, defined as in the domain $x = y \geq 1$, occurs at the value of x within this

FIG. 1. Phase diagrams for rods of fixed length according to model of Flory (8). The axial ratios, x , are: ····, $x = 15$; ---, $x = 30$; - - -, $x = 50$; - · - ·, $x = 100$. For each diagram, the region between the ordinate and the left-hand curve corresponds to isotropically oriented rods, and the region between the right-hand curve and $v_2 = 1$ corresponds to anisotropic orientation. The region between the curves consists of the two conjugate phases, the compositions of which, v_2^* and $v_2'^*$, are shown by the curves. For χ_s values in the stem region of each diagram, the more concentrated conjugate phase is tactoidal, whereas below the shoulder $v_2'^*$ is high and the phase has been called quasi-crystalline (11, 12). The tactoidal phase can exist when $\chi_s \leq 0$ and at low values of rod-rod attraction ($\chi_s > 0$).

field for which f in Eq. 2 is a minimum. The anisotropic phase, defined as in the region $x > y \geq 1$, occurs if there is local minimum in f or when a minimum occurs along the line $y = 1$ subject to $\partial f / \partial x > 0$. The phase that is absolutely stable at each v_2 is then ascertained from the absolute minimum in f . A phase separation occurs across the value of v_2 at which the identity of the most stable phase changes. A distinct miscibility (solubility) phase separation may also occur as a result of the van Laar term (10), $v_1 v_2 \chi_s$, in the free energy.

In a manner similar to that employed by Flory (8), Eq. 2 and the chemical potentials derived from it were used to obtain v_2^* and $v_2'^*$, the volume fractions of solute in the isotropic and anisotropic conjugate phases, respectively, for this reversibly polymerizing system. § Fig. 2 shows resultant phase diagrams and, with Fig. 1, serves to compare the variable and fixed length systems as follows: (i) The salient feature of both systems is a stem-like phase diagram. In the stem region, the conjugate phases do not differ greatly in solute concentration and the upper, anisotropic, conjugate phase is tactoidal. (ii) In the fixed x system, the stem begins in the region of rod-rod attractions, extends through the athermal state ($\chi_s = 0$), and includes the entirety of the region of rod-rod repulsions. For variable x , however, the stem begins at small negative values of χ_s and is confined to the region of rod-rod repulsions. The upper conjugate phase in the presence of very weak repulsions as well as for $\chi_s \geq 0$ is highly concentrated and highly aligned rather than tactoidal. That the tactoidal state at equilibrium requires repulsive interactions between rods in the variable length system constitutes the principal difference between it and the fixed length system.

Also, (iii) in the fixed x system, below the shoulder of the

§ The solvent chemical potential, μ_1 , as obtained from Eq. 2, approaches a finite limit rather than $-\infty$ at $v_2 = 1$ (where $y = 1$) if rod length is permitted to increase without limit. This unrealistic aspect of the model may be eliminated by imposing the constraint that μ_1 falls to $-\infty$ at $v_2 = 1$ or, equivalently, by imposing a large but arbitrary limit on rod length.

FIG. 2. Phase diagrams for variable length rods for different values of χ_a . ····, $\chi_a = -5$; - · - ·, $\chi_a = -7$; —, $\chi_a = -10$; ---, $\chi_a = -15$. In all the diagrams the tactoidal phase exists only when $\chi_s < 0$. As χ_a becomes less negative, the maximum value of χ_s at which the tactoidal phase can exist becomes more negative and the size of the anisotropic region in the phase diagram decreases. For clarity the identical boundaries of the two-phase regions at $\nu_2 = 1$ are shown with a single line.

phase diagram, where the tactoidal transition is "overwhelmed" by the miscibility transition (10), the anisotropic conjugate phase is quasi-crystalline, with $\nu_2 < 1$. In the variable x system, in this region, ν_2 for the anisotropic conjugate phase approaches 1 and the polymers approach infinite length; this phase might therefore be more appropriately called crystalline. (iv) Although not necessarily of physical significance, there is, for high values of x , in the fixed but not in the variable x system a narrow range of χ_s at the base of the stem in which the tactoidal and miscibility transitions exist separately. (v) Decreasing rod length and weakening polymerization interaction, in the respective systems, result in a broader stem which moves to higher ν_2 values. On the other hand, these same actions on rod length and χ_a permit the tactoidal phase to exist at even more positive χ_s in the fixed length system while requiring even more negative χ_s to induce tactoids in the variable length system.

Fig. 3 repeats the phase diagram for $\chi_a = -10$ and shows that rod length is much longer in the anisotropic phase, whether tactoidal or highly concentrated, than in its conjugate isotropic phase. This leads to the second major conclusion for systems of variable rod length: Interaction between nonideality and phase separation on the one hand with polymerization and rod length on the other results in a marked enhancement of polymerization of solute as it enters the anisotropic phase from the isotropic phase.

The phase diagrams of Fig. 2 are cross sections of a three-dimensional phase diagram that may also be cut perpendicular to the χ_s axis, giving phase diagrams such as those shown in Fig. 4. Each of the phase diagrams under conditions of rod-rod repulsion shows that strong polymerization interaction can induce the tactoidal phase; weaker polymerization results in an anisotropic conjugate phase consisting of pure solute, and very weak polymerization produces only the isotropic phase up to $\nu_2 = 1$. The phase diagram for $\chi_s > 0$ shows that the tactoidal phase cannot exist under conditions of rod-rod attraction.

Fig. 4 may also be viewed as a family of phase diagrams: As χ_s increases toward 0, more negative values of χ_a are required to induce the tactoidal phase until, at $\chi_s = 0$, the tactoidal region completely vanishes, a conclusion which can also be drawn from Fig. 2.

FIG. 3. Superimposed on the phase diagram for $\chi_a = -10$ (solid line, taken from Fig. 2), and using the scale at the top of the figure, are graphs of the values of $\log x$ and $\log y$ for the conjugate phases as functions of χ_s , where x is rod axial ratio and y is the order parameter; the conjugate phases are designated by subscripts, I for the isotropic and A for the anisotropic phase. ····, $\log x_I = \log y_I$; - · - ·, $\log x_A$; ---, $\log y_A$. Rod length is greater in the tactoidal phase by a factor of 17 at $\chi_s = -0.7$, the ratio increasing as χ_s becomes less negative. The arrow indicates that x_A becomes infinite in the pure solute phase.

Fig. 5 repeats the phase diagram for $\chi_s = -0.02$ and shows the effect of phase separation on polymerization. Taken together, Figs. 5 and 3 show that the enhancement of polymerization is greatest when rod-rod repulsions are weak for constant χ_a and when the polymerization interaction is weak at a constant level of rod-rod repulsion. At high levels of repulsion and polymerization interaction, changes in χ_s and χ_a do not affect the ratio of rod lengths in the conjugate phases greatly. Finally, Fig. 5 shows that there exists a value of χ_a at which x_A has a minimum: For strong polymerization interaction, x_A expectedly increases, but the tendency toward tactoid rather than

FIG. 4. Phase diagrams for variable length rods for different values of χ_s . ····, $\chi_s = -0.5$; —, $\chi_s = -0.02$; - · - ·, $\chi_s = -0.001$; ····, $\chi_s = +0.2$. Portions of the left-hand boundaries of the phase regions for various values of χ_s are omitted for reasons of clarity because they lie very close to the corresponding boundary for $\chi_s = -0.02$, which is shown. Also, for clarity the identical boundaries of two phase regions at $\nu_2 = 1$ are shown only with a single line. The arrow indicates that the upper conjugate phase for $\chi_s > 0$ is always the pure solute; tactoids cannot exist. In each diagram for $\chi_s < 0$ the tactoidal phase can exist only below a critical value of χ_a (for $\chi_s = -0.02$ this is $\chi_a = -8.4$); at less negative χ_a the upper conjugate phase is pure solute. At values of χ_a more positive than -1.3 only the isotropic phase exists for all ν_2 .

FIG. 5. Superimposed on phase diagram for $\chi_s = -0.02$ (solid line, taken from Fig. 4) are plots of $\log x$ and $\log y$, shown on the upper scale as in Fig. 3 as functions of χ_a . $\cdots$, $\log x_I = \log y_I$; $---$, $\log x_A$; $---$, $\log y_A$. x_A is about 500 times greater than x_I in the conjugate phase in the range $-30 < \chi_a < -15$. x_A attains a minimum near $\chi_a = -11$.

crystal formation is also increased; as the interaction weakens, crystal rather than tactoid formation is favored, thus producing rods of great and eventually infinite length.

The effect of nonideality and phase separation on rod length is considered in more detail in Fig. 6. Considering each phase independently and prior to addressing questions of relative stability and phase separation, x is a function of χ_a and v_2 , but not of χ_s because the van Laar term operates equally on free monomers and polymers of any length. The figure shows curves

FIG. 6. Rod lengths expressed as axial ratio x in isotropic and tactoidal phases for different values of χ_a (shown on curves). Each continuous solid curve consists of a left-hand region, which represents the isotropic phase, a right-hand region, which represents the anisotropic phase, and a vertical line joining these regions at the v_2 value for which the two phases are equally stable. The dashed extensions of the solid curves indicate that, to the left of the vertical line, the anisotropic phase is unstable relative to the isotropic and, to the right of the line, the converse is true. The leftward extension of each anisotropic phase curve terminates at the minimum v_2 value for which there exists a local minimum in f . The curves are independent of χ_s . The conjugate phases, however, do depend on χ_s . Conjugate phases (v_2^* and $v_2^{*'}$) lie on either side of the vertical lines and are indicated by symbols for different values of χ_s . $\bullet$, $\chi_s = -0.5$; $\ominus$, $\chi_s = -0.1$; $\circ$, $\chi_s = -0.01$. The vertical arrow indicates that the conjugate phases lie at $v_2 = 1$, where x is infinite.

for x modified to show which portions represent states of absolute stability. Two effects contribute to the enhancement of length associated with anisotropy: (i) At the concentration of equal stability of the two phases, each curve jumps vertically associated with the phase transition. (ii) In addition, a phase separation, which is dependent on χ_s , bridges this concentration to produce conjugate phases such that the ratio of length in the anisotropic to that in the isotropic phase is made larger.

Fig. 6 also shows rod lengths in systems consisting of a single phase, either isotropic or anisotropic, corresponding to $v_2 < v_2^*$ and $v_2^{*'} < v_2$, respectively.

DISCUSSION

Validity of Model. Miller and coworkers (13, 14) have shown that rigid rod-like molecules of poly(γ -benzyl-L-glutamate) and polycarbobenzoxylysine give the phase properties predicted by the theory of Flory (8), supporting the validity of the model for rods of fixed length. The extension of the model employed in the current work involves only the assumption of a free energy of interaction between monomeric units of the rod-like molecule. The current model, therefore, has the same justification for systems of varying rod length as that of Flory has for fixed length systems, provided that (i) the polymerization interaction free energy is independent of rod length, (ii) all rods are of the same length (which is also assumed in Flory's model), and (iii) only rods need be considered, the free monomer-rod interaction being neglected (this is a special case of restriction ii). The first condition is a minor restriction, most systems of interest exhibiting a constant free energy of monomer addition once the nucleation stage (if such exists) is passed. The second condition probably does not alter the fundamental results in respect to the use of an average polymer length, although the current work does not demonstrate this analytically. The third condition is not relevant if the concentration of monomers is low so that the mass of polymerized material is constant, the polymers being permitted to change only in average length to achieve a free energy minimum. The justification for the current model is weakest in systems with high free monomer concentration. In such systems, (i) the effect of the free monomers on nonideality and (ii) the monomer-polymer equilibria must be considered. The former problem is in part avoided because the Flory model treats solvent "molecules" as equal in volume to one unit of polymer length; thus, the presence of a fixed number of free monomers can be assigned as portion of the solvent, the effective solute consisting only of polymers. The latter problem is most marked when free monomers are present in very high concentration and when there is significant change in the masses of monomeric and polymerized material as x and y change in the process of minimizing free energy.

Existence of Tactoids. The current analysis predicts that the tactoidal state will be bypassed in athermal solutions and that any separating phase will be highly ordered and very concentrated. Tactoids will exist only in the presence of repulsive interactions or in a metastable state.

Tactoids that depend on repulsive forces might be expected to be labile to small changes in these repulsions if χ_s lies near the level of the shoulder of the phase diagram, particularly, as occurs with the more negative values of χ_a in Fig. 2, when the shoulder is almost flat. At this critical level of χ_s , small changes in solvent conditions could induce grossly polymorphic forms of the condensate.

Tactoids that depend on metastability might occur transiently if rod lengths remained temporarily fixed due to a kinetic barrier. Such barriers exist, for example, in polymerizing systems in which a slow nucleation step is followed by rapid

fiber growth (15–18); prior to reequilibration to shorter lengths, the fibers exhibit a transient overshoot in length. If stirring facilitates length reequilibration, the presence of a kinetic barrier is one possible basis for the observation of Pumphrey and Steinhardt (19, 20) that stirred deoxyhemoglobin S assumes a crystalline form in the condensed state instead of the more familiar gel seen in the absence of stirring. Our observation (21) that deoxyhemoglobin S in 1.7 M phosphate can form a gel or a turbid but less viscous suspension, as a function of agitation and rate of temperature change, could also depend on such a mechanism. Alternatively, the existence of these metastable gels might also result from a slow process of fiber alignment. In this circumstance shearing by stirring would facilitate alignment and accelerate arrival at the equilibrium state.

Length Control in Polymerizing Systems. In reversibly polymerizing linear and helical systems lengths may obey a random distribution. They may also be governed by a length-determining factor or template, by a cumulated strain (22) or vernier mechanism (23), or by overshoot (15–18). The current work indicates that nonideality through its expression in phase transition and phase separation can be a powerful factor in governing the lengths of rod-like polymers.

Possible Applications to Sickle Cell Disease and Other Biological Systems. In sickle cell disease pathological events arise from the inability of the erythrocyte to transit small capillaries which, in turn, depends on increased rigidity of the cell. Under the concept of Harris (5), the sickled erythrocyte is a membrane covered tactoid, so that the tactoidal state is considered to be responsible for the disease. Under models for rods of fixed length, tactoid formation requires only entropic factors resulting from excluded volumes so that pathogenesis does not depend on the presence of any direct interactions between fibers of deoxyhemoglobin S. The current model is limited in its application to the hemoglobin S system, in which free monomer concentrations are high. However, the model is still preferable to that for fixed rod lengths. If the primary qualitative conclusion of the current model, that stable tactoids do not occur in the athermal state, is applicable to hemoglobin S, then the classical analysis of pathogenesis must be reconsidered. (a) If it is still assumed that the disease depends on tactoid formation, either (i) repulsive interactions are required or (ii) pathogenic events occur only in metastable states. In the former case, small changes in rod-rod interactions resulting from small alterations in the intraerythrocytic milieu could eliminate the pathogenesis. In the latter, appropriate diversion of the condensed state to the absolutely stable condition, even as suggested by recent experimental results (19–21), could do the same. (b) On the other hand, it may also be considered that the tactoid is not the pathogenic state. In this situation, also, small changes in χ_s and the existence of metastable states could enhance or decrease pathological events depending on which of the three polymerized states, isotropic, tactoidal, or highly aligned and concentrated, were the most pathogenic.

The interrelation between polymerization and alignment, in which each, through phase separation, enhances the other may be of interest in other biological systems. If functional or structural requirements are better satisfied by high degrees of alignment in polymerizing proteins, the present mechanism could provide this. For example, polymerization and alignment of tubulin during cell division might depend in part on non-ideality in the manner described here.

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