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Size Distribution of Protein Polymers

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Theoretical analyses were made on the size distribution of tubular or helical polymers of globular protein molecules in the reversible polymerization. In the true final equilibrium, polymerization approximates to a kind of crystallization and the average degree of polymerization becomes very large; nevertheless, the size distribution is of a simple exponential type, the same as found in macromolecular chemistry (Flory, 1953). Even when spontaneous nucleation is inhibited and the number of polymers is given, the final distribution must tend towards the exponential type. Free energy due to deviation of the size distribution from the true equilibrium was calculated. It is very much smaller than the main free energy coming from the monomer-polymer equilibrium.

Kinetics showed that polymerization having characteristics of crystallization usually consists of three stages—nucleation, growth and redistribution of polymer size. In the first and second stages where the rate of depolymerization is negligible, the concentration of monomers approaches closely to the equilibrium value. In the third stage, where both polymerization and depolymerization take place nearly at the same rate, the size distribution is slowly transformed into the exponential type. The relaxation time for such redistribution was estimated as a function of rate constants and the average degree of polymerization under various conditions.

All of the theoretical results are quantitatively in good agreement with experimental data on polymerization of globular proteins such as actin and flagellin. Brief analyses were added on the size distribution of two-dimensional membraneous polymers and distorted polymers.

1. Introduction

In biological systems there are many examples of polymers of globular protein molecules. In the simplest case polymers are composed of only one kind of protein molecules, structural units. Some of them have been proved to be reversibly reconstituted *in vitro*. The polymerization is a self-assembly process (Casper & Klug, 1962; Casper, 1963). Helical or tubular polymers of tobacco mosaic virus protein (Casper & Klug, 1962; Casper, 1963), bacterial flagellin (Asakura, Eguchi & Iino, 1964, 1966),

muscle protein actin (Straub, 1942; Hayashi & Rosenbluth, 1960; Kasai, Nakano & Oosawa, 1965), mitotic spindle protein (Stephens, 1968), etc., are those examples. The fundamental structure of reconstituted polymers is the same as found *in vivo*. However, there is an additional point to be noted in the comparison between the structures *in vivo* and *in vitro*; that is the size and shape or the size distribution of polymers. Bacterial flagella in a cell grow up to the same definite length; actin filaments in a muscle cell also have a uniform length. How is the size of polymers distributed in the *in vitro* polymerization? Is any special mechanism needed to regulate the size *in vivo*? The determination of this level of the structure, which in a sense corresponds to size and habit of inorganic crystals, is often very important for biological systems to exhibit their functions.

In this paper statistical thermodynamic analysis is presented on the size distribution of protein polymers in the reversible polymerization under various conditions, and on some related problems. The analysis is concerned mainly with helical or tubular polymers which show essentially one-dimensional growth and in addition, with distorted polymers and membraneous or two-dimensional polymers. A part of the present theory is simple translation of the polymerization theory of linear high polymers developed in the field of macromolecular chemistry (for example, Flory, 1953). Nevertheless, since experimental data have begun to be accumulated on polymerization of globular protein molecules, it is valuable to point out that in some respects it has characters similar to the synthesis of linear high polymers.

2. True Equilibrium

For the convenience of later calculations some results in previous papers on helical polymerization of protein molecules are described here (Oosawa, Asakura & Ooi, 1960; Oosawa & Kasai, 1962; Oosawa & Higashi, 1967). Let us consider a solution of globular protein molecules in the polymerization equilibrium. The number concentration of polymers composed of i monomers (i-mers) is denoted by c_i (the number of polymers/number of solvent molecules). The number concentration of dispersed monomers is denoted by c_1 and the total number concentration of monomers including those in polymers by c_0. If almost all monomers in a polymer are equivalent to each other, the total interaction free energy of monomers in an i-mer is approximately expressed as:

$$E_i = -i\varepsilon + \delta \tag{1}$$

where $-\varepsilon$ is the interaction free energy of a monomer added to the end of an $i-1$ mer to form an i-mer and assumed to be independent of i for sufficiently large i's. In the case of helical or tubular polymers, the correction

term δ comes from monomers at the ends of polymers which have smaller numbers of neighbouring monomers.

According to the mass action law, the equilibrium number concentration of i-mers is given by

$$c_i = c_1^i \exp(-E_i/kT) = AK^{-1}(Kc_1)^i \tag{2}$$

where

$$K = \exp(\varepsilon/kT) \quad \text{and} \quad A = \exp((\varepsilon-\delta)/kT)$$

For polymers composed of a small number of monomers (smaller than the polymer nucleus), the above expression is not applicable. Instead of (2) it is better to apply

$$c_i = K_i c_1^i, \quad \text{for } i > i_0 \tag{2'}$$

where i_0 means the number of monomers in the nucleus. However, the contribution of such small polymers to the total number of polymers is very small when the polymerization takes place as a kind of crystallization (Oosawa & Kasai, 1962).

The total number concentration of polymers m and the total number concentration of monomers participating in polymers c_p are given by the summation $\sum c_i$ and $\sum ic_i$, respectively; where the summation is performed from i_0 to ∞, or approximately the summation may be extended from unity to ∞ with c_i expressed in form (2) for all i's if the value of constant A is very much smaller than unity. Thus, we have the approximate formulae:

$$m = Ac_1/(1-Kc_1) \tag{3}$$

$$c_p = c_0 - c_1 = Ac_1/(1-Kc_1)^2. \tag{4}$$

In (4), the monomer concentration c_1 can be solved as a function of A, K and c_0.

As described in previous papers (Oosawa & Kasai, 1962; Oosawa & Higashi, 1967), in the case of helical or tubular polymers where each monomer interacts with many neighboring monomers, the value of A is very much smaller than unity. Then, the polymerization can be regarded, approximately, as a kind of crystallization. When the total concentration c_0 is increased, polymers are formed only above the critical concentration determined by the condition $c_0K = 1$. Above this concentration, polymers coexist with monomers of a constant concentration $c_1 = K^{-1}$. [A constant concentration of small polymers may also coexist (Oosawa & Kasai, 1962).] Strictly speaking, however, c_1K is a little smaller than unity and approaches unity with increasing total concentration c_0 (see Fig. 1).

The equation (2) gives the number concentration of i-mers as a function of i, the size. It has a form λ^i or $\exp(-\alpha i)$, $(\alpha = -\ln \lambda)$; where $\lambda(= Kc_1)$

is a little smaller than unity or α is a small positive number. Therefore, the number concentration exponentially decreases with i, as shown in Fig. 2. This distribution is the same as discussed by Flory in the high polymer theory where $c_1 K$ corresponds to the probability parameter (Flory, 1953).

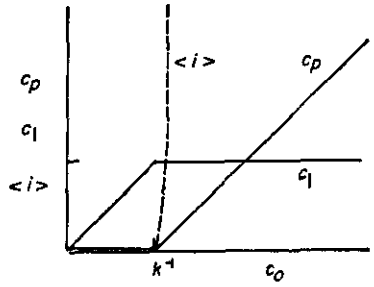

FIG. 1. Helical or tubular polymerization as a kind of condensation or crystallization. The abscissa is the total concentration of protein molecules (c_0) and the ordinate gives the total amount of polymers (c_p), the concentration of dispersed monomers (c_1) and the average length of polymers ($\langle i \rangle$).

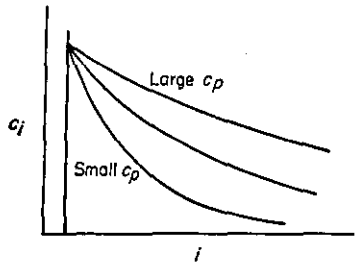

FIG. 2. The size distribution of helical and tubular polymers in the true equilibrium at different total amounts of polymers (c_p) and a constant solvent condition. Numbers of small polymers are little changed but numbers of large polymers are greatly increased by the increase of c_p. The average degree of polymerization $\langle i \rangle$ is proportional to $c_p^{1/2}$.

The average degree of polymerization $\langle i \rangle$ is given by

$$\langle i \rangle = c_p/m = 1/(1-Kc_1) = (c_p/c_1 A)^{1/2}. \tag{5}$$

In Fig. 2, the distribution is compared at different total amounts of polymers c_p. The average square deviation of the degree of polymerization $\langle \delta i^2 \rangle$ defined as $\langle i^2 \rangle - \langle i \rangle^2$ is given by

$$\langle \delta i^2 \rangle \fallingdotseq \langle i \rangle^2 \tag{6}$$

if $\langle i \rangle \gg 1$. The broad exponential distribution decreasing with the size is not inconsistent with the character of polymerization as crystallization, because,

on account of a very small value of A, the average size $\langle i \rangle$ given by (5) suddenly becomes very large above the critical concentration (Oosawa & Kasai, 1962; Oosawa & Higashi, 1967).

The experimental analysis to be compared with these results has been carried out in the polymerization of muscle protein actin (Maruyama & Kawamura, 1967). The size distribution of actin polymers (F-actin) in the equilibrium was found by electronmicroscopy to be expressed just in the form $\exp(-\alpha i)$. The relation $\langle \delta i^2 \rangle \fallingdotseq \langle i \rangle^2$ was also confirmed. In spite of such a broad distribution, the equilibrium between monomers (G-actin) and polymers (F-actin) is well described as a kind of condensation or crystallization (Oosawa, Asakura, Hotta, Imai & Ooi, 1959; Kasai, Asakura & Oosawa, 1962).

3. Equilibrium at a Fixed Number of Polymers

The true equilibrium state was treated in the above section. In practice, there are some difficulties to establish such a state. In most cases where A is very much smaller than unity, the nucleation process is rate limiting. If the spontaneous nucleation is practically inhibited, the number of polymers formed is determined by the number of nuclei or seeds added. The *in vitro* polymerization of bacterial flagellin into flagella gave an example of such a case (Asakura *et al.*, 1964). Here, the equilibrium is analysed under the limitation that the total number of polymers is fixed.

In this case also, the mass action law can give the size distribution. However, in order to see the effect of the size distribution on the free energy, we start from the total free energy of a solution of monomers and polymers F expressed as (e.g. Landau & Lifschitz, 1951);

$$F = n_0 kT(\sum c_i \ln c_i/e + \sum c_i(-i\varepsilon+\delta)/kT + c_1 \ln c_1/e) \tag{7}$$

(n_0 is the total number of solvent molecules). Minimization of this free energy with respect to the distribution c_1 and c_i's under the condition that

$$\sum c_i = m \tag{8}$$

and

$$\sum i c_i = c_0 - c_1 \tag{9}$$

gives the equilibrium. (The same remarks as in the previous case must be made on the range of summation in these equations.) The solution of minimization is given by

$$c_i = aAK^{-1}(c_1 K)^i \tag{10}$$

where a and c_1 are determined from (8) and (9). The previous case corresponds to the case $a = 1$. It is easily found that similarly to the previous case, c_1 is nearly equal to c_0 below the critical concentration given by $c_0 K = 1$ and is kept nearly constant at K^{-1} above this critical concentration.

The size distribution (10) has the same form as (2); the number concentration of i-mers simply decreases with i, as shown in Fig. 3; where the distribution is compared at different amounts of added nuclei or m. The average

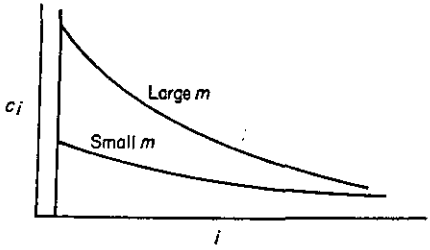

FIG. 3. The size distribution of helical and tubular polymers in the equilibrium at different fixed values of the total number of polymers (m) and a constant amount of polymers (c_p). The number of smallest polymers c_{i_0} is proportional to m^2 and the average degree of polymerization $\langle i \rangle$ is proportional to $1/m$. Therefore, numbers of small polymers are decreased but numbers of very large polymers are increased by the decrease of m.

value of i, $\langle i \rangle$, is given by a similar relation to (5). The only difference is that in the previous case, m, $\langle i \rangle$ and c_1 are determined by A, K and c_0, while in the present case, m is given independently, and $\langle i \rangle$ and c_1 are determined by A, K, c_0 and m. Let us distinguish the value of m, $\langle i \rangle$, c_1 and c_p in the true equilibrium by writing as m^0, $\langle i \rangle^0$, c_1^0 and c_p^0. Even when the value of m is fixed at a value different from m^0, the polymerization takes place as a kind of crystallization, and the critical concentration c_1 is nearly equal to c_1^0. It is easily proved that at the same value of c_0 above the critical concentration, there is a small difference between c_1 and c_1^0 given approximately by

$$(c_1 - c_1^0)/c_1^0 = 1/\langle i \rangle - 1/\langle i \rangle^0 = m^0/c_p^0 - m/c_p. \tag{11}$$

The monomer concentration co-existing with polymers increases slightly with decreasing number of polymers. However, the relative difference is negligible since both $\langle i \rangle^0$ and $\langle i \rangle$ are very much larger than unity in ordinary cases.

Recently, the polymerization equilibrium of actin was investigated under sonic vibration (Nakaoka & Kasai, 1969). The vibration breaks long actin polymers into short ones. Nevertheless, the presence of the critical actin concentration was confirmed; its value is nearly equal to that without sonic vibration. In the case of bacterial flagellin also, the polymerization equilibrium was found within a limited range of temperature, where both polymerization and depolymerization take place, and the critical concentration was

independent of the number of added nuclei (Gerber, Asakura & Oosawa, unpublished). This independence was considered to be one of the evidences that the polymerization can be regarded as crystallization.

The size distribution of actin polymers under sonic vibration was not very different from the exponential one (Kawamura & Maruyama, 1969). On the other hand, flagellin polymers in the equilibrium have a size distribution somewhat different from (10). This problem is discussed later again.

4. Free Energy of the Size Distribution

The free energy in the equilibrium at a fixed number of polymers F must be larger than that in the true equilibrium F^0. This difference of the free energy must be small because, as shown in the above section, the equilibrium between monomers and polymers is little changed by the change in the number of polymers. Actually, the excess free energy due to the fixation of the number of polymers can be calculated by putting the result (10) into (7), where $a \approx 1$ in the true equilibrium. Then we have:

$$F - F^0 = 2n_0 kT(m \ln (m/m^0) - (m - m^0)). \tag{12}$$

When m is not equal to m^0, F is always larger than F^0. That is, F has a minimum at $m = m^0$. Thus, the excess free energy was expressed as a function of m only. The change in the number concentration of polymers produces the change of the free energy of the order of $n_0 mkT$. Since usually the number concentration of polymers m is very much smaller than the number concentration of monomers, the excess free energy is very much smaller than F or F^0 itself.

From (12), the average square deviation of the number of polymers in the true equilibrium is found to be given by:

$$\begin{aligned} \langle \delta m^2 \rangle / \langle m \rangle^2 &= (\langle m^2 \rangle - \langle m \rangle^2)/\langle m \rangle^2 \\ &= 1/n_0 m. \end{aligned} \tag{13}$$

Therefore, the relative deviation of the number of polymers is very much larger than the relative deviation of the number of dispersed monomers, which is of the order of $1/n_0 c_1$, under ordinary conditions.

At a fixed number of polymers, the size distribution at the final state must be of type (10). In actual processes, however, it often takes too long time to establish such a distribution. As shown later, the equilibrium between monomers and polymers is attained much faster and the Poisson distribution of the polymer size is formed tentatively but rather stably. The free energy in this distribution must be a little higher than that in the final distribution (10). This excess free energy due to the size distribution difference at the same values of m and c_p or $\langle i \rangle$ is estimated to be approximately of the order of $n_0 mkT \ln \langle i \rangle$.

Thus, "the free energy of the size distribution of polymers" is very small. This is one of the main causes of the slowing down phenomenon in the distribution regulation.

5. Kinetics of the Size Distribution

The helical or tubular polymerization consists of two processes, nucleation and growth. In such a case, from the other standpoint, the polymerization equilibrium is established in two steps; the number concentration of dispersed monomers approaches equilibrium, and then the number and size distribution of polymers attain equilibrium. This situation is analysed below. This kind of problem has been treated extensively in the field of macromolecular chemistry (e.g. Miyake & Stochmayer, 1965). Here, the kinetics of size distribution is treated by a simplest method of calculation.

The number concentrations of dispersed monomers and polymers are all functions of time t. The change of concentration of i-mers with time due to the growth of polymers is assumed to be given by the equation (Oosawa & Kasai, 1962; Oosawa & Higashi, 1967):

$$dc_i/dt = k_+ c_1 c_{i-1} - k_- c_i - k_+ c_1 c_i + k_- c_{i+1}, \quad i > i_0 \tag{14}$$

where k_+ and k_- are kinetic constants for binding of a monomer to the end of a polymer and for removing a monomer from the end of a polymer, respectively; they are assumed to be independent of i. It is also assumed that neither fragmentation nor association of polymers takes place. On the other hand, for the change of the number concentration of nuclei composed of i_0 monomers, we put

$$dc_{i_0}/dt = -k_+ c_1 c_{i_0} + k_- c_{i_0+1} + k^* c_1^{i_0}, \tag{15}$$

where the last term of the right-hand side gives the rate of production of nuclei, which was assumed to be proportional to the i_0th power of the monomer concentration.

As an extreme case, suppose that spontaneous nucleation is practically inhibited, i.e. k^* is negligible, and a definite number of nuclei is added to a solution of monomers to induce polymerization. Polymerization takes place only through addition of monomers to these pre-existing nuclei which may be made of other substances. Then, the total number of polymers is kept constant. Denoting the number concentration of polymers composed of i monomers added to nuclei by c_i^* we have equations similar to (14) for all i's ($i \geqslant 1$) and an equation similar to (15) (without the last term) giving the change of the number concentration of nuclei having no added monomers c_0^*. Summing up these equations for dc_i^*/dt ($i \geqslant 0$), we can find

$$\begin{aligned} d\sum i c_i^*/dt &= -dc_1/dt = dc_p/dt \\ &= (k_+ c_1 - k_-) m + k_- c_0^* \end{aligned} \tag{16}$$

and

$$\mathrm{d}\sum i^2 c_i^*/\mathrm{d}t = 2(k_+ c_1 - k_-)c_p + (k_+ c_1 + k_-)m - k_- c_0^* \qquad (17)$$

where kinetic constants for association and dissociation between monomers and nuclei were assumed to be equal to those between monomers and long polymers.

The first term of the right-hand side of (16) is very much larger than the second term, except at the beginning of polymerization where c_0^* is nearly equal to m and at the final state of polymerization. Therefore, the final equilibrium of the monomer concentration is given approximately by the condition that $k_+ c_1 - k_- = 0$ or $c_1 = k_-/k_+ \ (= K^{-1})$, although rigorously, c_1 must be a little smaller than k_-/k_+, as expected from (16). This agrees with the result in the previous equilibrium theory.

In order to see qualitatively the size distribution during polymerization, calculate the change of the mean square deviation of the polymerization degree with time. From (16) and (17) it is readily derived that

$$\mathrm{d}/\mathrm{d}t(\langle i^2\rangle - \langle i\rangle^2) = \mathrm{d}\langle i\rangle/\mathrm{d}t + 2k_-(1-(c_0^*/m)(1+\langle i\rangle)). \qquad (18)$$

This is rewritten as:

$$\langle i^2\rangle - \langle i\rangle^2 = \langle i\rangle + 2k_- \int_0^t (1-(c_0^*/m)(1+\langle i\rangle))\,\mathrm{d}t. \qquad (19)$$

Usually, except at the final stage of polymerization, the rate of polymerization $k_+ c_1$ is very much faster than that of the reverse reaction k_-. Then the second term of (19) can be neglected and the relation:

$$\langle \delta i^2\rangle = \langle i^2\rangle - \langle i\rangle^2 = \langle i\rangle \qquad (20)$$

which is satisfied by the Poisson distribution, holds during polymerization, as shown in Fig. 4. The distribution has a sharp maximum and the relative

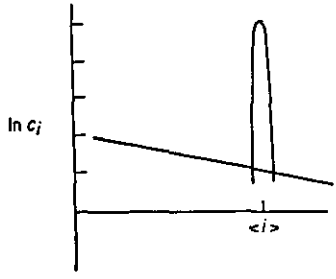

FIG. 4. A Poisson-type size distribution and a simple exponential type size distribution are compared at the same values of the average size $\langle i\rangle$ and the total number of polymers m. The ordinate gives the logarithm of the number of i-mers. At $i = \langle i\rangle$, c_i is of the order of $m/\langle i\rangle^{1/2}$ for the Poisson distribution and it is of the order of $m/\langle i\rangle$ for the exponential one.

deviation becomes smaller and smaller with polymerization, in proportion to $1/\langle i\rangle^{1/2}$. Thus, the condition $k_+ c_1 \gg k_-$ lead to the Poisson distribution. The integration of the equation (16), if the second term $k_- c_0^*$ can be neglected, shows that the time of approach of the concentration of dispersed monomers to the final value, τ_1, is of the order of $1/k_+ m$ or:

$$\tau_1 \simeq \frac{1}{k_-}\left(\frac{c_1}{c_p}\right)\langle i\rangle. \tag{21}$$

Now, in the present case where the polymerization takes place as the addition of monomers to pre-existing seeds only, the final equilibrium distribution must satisfy the relation

$$\langle \delta i^2\rangle = \langle i\rangle^2 + \langle i\rangle$$

independently of the number of seeds m. Therefore, the second term of the right-hand side of (19) must become:

$$2k_- \int_0^\infty (1-(c_0^*/m)(1+\langle i\rangle))\,\mathrm{d}t = \langle i\rangle^2. \tag{22}$$

It is easily confirmed that the integrand becomes zero at the equilibrium given by the distribution (10). Soon after the polymerization begins, the ratio c_0^*/m becomes very much smaller than unity (smaller than $1/\langle i\rangle$) and during polymerization the integrand is nearly unity except at the final stage. As the size distribution finally changes from the Poisson type to the simple exponential type, the integrand tends to zero. Therefore, the time of approach of the distribution to the final equilibrium one, τ_2, is of the same order as the time of approach of the integrand from unity to zero. From the above equation (22), this time τ_2 is given approximately by

$$\tau_2 \simeq \langle i\rangle^2/2k_-. \tag{23}$$

As mentioned above, before the redistribution of the polymer size, the concentration of dispersed monomers is already nearly equal to the final equilibrium one. Then, the rate of polymerization reaction $k_+ c_1$ is nearly equal to the rate of the reverse reaction k_-. Consequently, the change of the length of each polymer takes place just like a diffusion process. Equation (23) is interpreted as giving the time of diffusion necessary for the distance $\langle i\rangle$ with the diffusion constant k_- or $k_+ c_1$. A result equivalent to (23) has been derived already in the theory of reversible polymerization of "living" high polymers (Miyake & Stockmayer, 1965).

If the number of polymers m is just equal to the number m_0 in the true equilibrium, the average degree of polymerization $\langle i\rangle$ is given by (5). Consequently, the time τ_2 is rewritten

$$\tau_2 = \langle i\rangle/(k_- - k_+ c_1). \tag{23'}$$

This is interpreted as giving the time necessary for a reaction to proceed by the distance $\langle i \rangle$ with the rate constant $k_- - k_+ c_1$.

Comparing the two relaxation times τ_1 and τ_2, it is found that

$$\tau_1/\tau_2 \simeq (c_1/c_p)(1/\langle i \rangle). \tag{24}$$

Since $\langle i \rangle$ is very much larger than unity, τ_2 is very much larger than τ_1. In Fig. 4, two types of distribution are compared.

For example, in the case of actin at a certain condition of temperature, pH and salt concentration, the time of depolymerization ($\langle i \rangle / k_-$) was found to be of the order of five minutes for polymers of the average length of 1 μ, which corresponds to $\langle i \rangle = 400$ (Kasai, 1969). Then, the time of redistribution of the polymer size to the equilibrium is expected to be of the order of five minutes × 400 ≃ 30 hours. This is reasonable in comparison with experimental data (Kawamura & Maruyama, 1969). In the case of bacterial flagellin polymerizing on seeds added, the rate of depolymerization k_- is very small, except at the intermediate temperature near 40°C or at higher temperature where the polymerization can not happen. The average degree of polymerization is very large, more than thousands. Thus, the time for redistribution is of the order of several weeks or months. The Poisson distribution once formed during polymerization cannot be corrected practically (Asakura *et al.*, 1964). In such a case, polymers of the uniform length are stably formed, whose length is determined by the total numbers of nuclei and monomers.

6. Spontaneous Nucleation and Growth

Let us return to the case where nuclei are formed spontaneously by interaction among i_0 monomers and the rate of the nucleus formation is proportional to the i_0th power of the monomer concentration as shown in (15). Equations (14) and (15), if simplified under the condition that the depolymerization rate k_- is negligible, can be readily integrated (Oosawa & Kasai, 1962; Wakabayashi, Hotani & Asakura, 1969). Here only the size distribution of polymers is discussed at the final stage of polymerization which proceeded without depolymerization reaction.

According to equations (14) and (15), the rate of increase of the number of polymers is given by

$$\mathrm{d} \sum c_i / \mathrm{d}t = \mathrm{d}m/\mathrm{d}t = k^* c_1^{i_0}. \tag{25}$$

Since the rate of growth of polymers is expressed, from (14), by

$$-\mathrm{d}c_1/\mathrm{d}t = \mathrm{d} \sum i c_i / \mathrm{d}t = k_+ c_1 m, \tag{26}$$

a nucleus produced at time t has the average degree of polymerization given by

$$i_\infty(t) = \int_t^\infty k_+ c_1 \, dt \tag{27}$$

after full polymerization. Therefore, the number of i-mers at the final stage of polymerization is given by

$$c_i = (dm/dt)/(di_\infty(t)/dt) = (k^*/k_+)c_1^{i_0-1} \tag{28}$$

where c_1 in the right-hand side is the monomer concentration at time t which is connected with the size i by equation (27). Equation (28) means that the final concentration of i-mers c_i decreases with i because c_1 decreases with time t. That is, the size distribution of polymers has a long tail in the side of short lengths.

By integrating (25) and (26) and using the result (28), $\sum c_i$, $\sum ic_i$ and $\sum i^2 c_i$ can be calculated as functions of kinetic constants and the monomer concentration c_0. Finally it is found that the average length $\langle i \rangle$ is proportional to $c_0^{1-i_0/2}$ and the relative mean square deviation of the length after full polymerization is approximately given by:

$$\langle \delta i^2 \rangle / \langle i \rangle^2 = 4G(i_0)/i_0 - 1 \tag{29}$$

where

$$G = \int_0^\infty (\cosh x)^{-m}(1-(\cosh x)^{-m}) \, dx, \quad m = 2/i_0.$$

The right-hand side of (29) is about 0·026 for $i_0 = 4$ and about 0·017 for $i_0 = 8$. It is remarkable that the relative deviation is independent of kinetic constants and monomer concentration, and determined by the number i_0.

The time course of spontaneous polymerization of actin and flagellin is well understood by the present scheme (Kasai, 1969). However, the analysis of the final size distribution showed that even in the case of flagellin the number concentration c_i decreases with increasing i (Wakabayashi, Hotani & Asakura, 1969). This probably suggests that the depolymerization rate k_- is not negligible when spontaneous polymerization takes place. If so, the distribution must become of type (2).

7. Fragmentation and Association of Polymers

In the previous section it was assumed that approach of the size distribution to the equilibrium takes place only through polymerization and depolymerization of monomers at the ends of polymers. The size distribution, however, may be changed also through fragmentation and association of polymers. Here, a rough estimation of the time necessary for such process is made. For the change of the number of polymers it may be assumed that

$$d(\sum c_i)/dt = k'_-(\sum ic_i) - k'_+(\sum c_i)^2/2 \tag{30}$$

where k'_+ and k'_- are rate constants for association and fragmentation of polymers. If the concentration of monomers co-existing with polymers were in equilibrium in advance, it is found by integration of (30) that the relaxation time for approach of the total number of polymers to equilibrium is of the order of $1/(2k'_+ k'_-(\sum ic_i))^{1/2}$, which is rewritten as $1/2k'_-\langle i\rangle$. The same order of time is also estimated for the process of fragmentation if a long polymer is broken at random with rate constant k'_- into fragments of the average length $\langle i\rangle$. Random fragmentation produces the size distribution of type (2). Therefore, the time $1/2k'_-\langle i\rangle$ is considered to give the relaxation time τ_3 for approach of the size distribution and the total number of polymers to equilibrium through fragmentation and association of polymers.

This relaxation time τ_3 must be compared with the time τ_1 and τ_2. The ratios are given by

$$\tau_1/\tau_3 \simeq (k'_-/k_-)(c_1/c_p)\langle i\rangle^2 = (k'_+/k_+) \tag{31a}$$

$$\tau_2/\tau_3 \simeq (k'_-/k_-)\langle i\rangle^3 = (k'_+/k_+)(c_p/c_1)\langle i\rangle \tag{31b}$$

where the relations $(k'_-/k_-) = A(k'_+/k_+)$ and $\langle i\rangle = (c_p/c_1 A)^{1/2}$ were used.

In the case of actin, the rate of association of short polymers k'_+ was estimated by following the viscosity increase after stopping sonic vibration which produced short polymers (Nakaoka & Kasai, 1969). The ratio (k'_+/k_+) is considered to be of the order of 1/10 to 1/100. In the case of flagellin, end-to-end association of short polymers to complete long polymers was never observed (Asakura, Eguchi & Iino, 1968). Equation (31b) shows that comparison between the ratio (k'_+/k_+) and the average length $\langle i\rangle$ determines which process is more important, fragmentation and association of polymers or depolymerization and polymerization of monomers at the ends of polymers, for establishing the final equilibrium size distribution. In actin, fragmentation and association of polymers probably makes important contribution, while in flagellin it can hardly take place.

8. Two-dimensional Polymers

In the case of two-dimensional or membraneous polymers, a different situation appears concerning the size distribution of polymers. For comparison with tubular polymers, brief analyses are added here, although the results are similar to those pointed out in three-dimensional condensation (for example, Frenkel, 1946; Oosawa, 1956). For sufficiently large two-dimensional polymers, the total interaction free energy of monomers in a polymer composed of i monomers is given approximately by:

$$E_i = -i\varepsilon + \sqrt{i}\cdot\delta + \delta'. \tag{32}$$

Then, the number concentration of i-mers in the true equilibrium is given by:

$$c_i = bB^{\sqrt{i}}(Kc_1)^i \tag{33}$$

where $b = \exp(-\delta'/kT)$, $B = \exp(-\delta/kT)$ and $K = \exp(\varepsilon/kT)$. In general $bB^{\sqrt{i}}$ is very much smaller than unity. Similarly to the previous case, the polymer concentration is negligible before the total concentration approaches K^{-1} very closely. In the present case, however, c_1 can become just equal to K^{-1}. Even when $Kc_1 = 1$, c_i decreases with i and the total concentration of monomers in polymers c_p remains finite, as shown by integration:

$$c_p = \sum ic_i = b\sum iB^{\sqrt{i}} = b\int_{x_0}^{\infty} x\exp(-\delta\sqrt{x}/kT)\,dx$$
$$= c_1 b(kT/\delta)^4(12+12z+6z^2+2z^3)\exp(-z) \qquad (34a)$$

where $z = (\delta/kT)i_0$. This equation with the condition

$$c_0 = c_1 + c_p \qquad (34b)$$

determines the concentrations of dispersed monomers c_1 and polymers c_p just below the critical point. If $i_0 = 10$, $\delta/kT = 3$ and $b = 1$, equation (34a) gives $c_p/c_1 = 10^{-3}$. If the total concentration c_0 exceeds the value given by (34b) with (34a), the condition (34b) cannot be satisfied with any values of c_1 in the range $Kc_1 \leqslant 1$. The value of Kc_1 must exceed unity. However, if it exceeds unity, the expression of c_i of (33) deverges with increasing i. This means that macroscopic polymers begin to appear at c_0 determined by (34b) with (34a). With further increase of c_0, macroscopic polymers increase and grow, while the concentration of dispersed monomers is kept constant at K^{-1}. Polymers of the intermediate size do not exist stably, except a small amount given by (34a) (Fig. 5). Thus, the two-dimensional polymerization

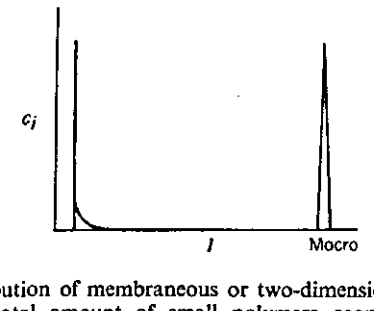

FIG. 5. The size distribution of membraneous or two-dimensional polymers above the critical condition. The total amount of small polymers coexisting with macroscopic polymers is independent of the amount of macroscopic polymers. In ordinary conditions, it is very much smaller than the amount of dispersed monomers, according to (34a).

is a true crystallization phenomenon, as a natural consequence. In such a case, it is difficult to make reasonable estimation on the free energy of the size and shape distribution of polymers.

9. Distorted Polymers

When globular macromolecules are polymerized into a regular crystal structure, each pair of neighbouring monomers cannot always be arranged in a way most favourable to the interaction between them. It is accidental that neighbouring three monomers in a large regular polymer occupy the same relative positions as three monomers in a trimer separated from the polymer. If the relative positions are different, the inter-monomer structure in a polymer depends on its size. Some stress due to distortion is buried in the polymer structure. Such distortion, which is generally expected in polymers of globular protein molecules having no symmetry in themselves (Takahashi, 1966), has influences on the equilibrium size distribution of polymers. A simple example is described below.

Let us consider a polymer of a curved band-like or ribbon-like structure, as shown in Fig. 6. It is formed by piling strands composed of l monomers.

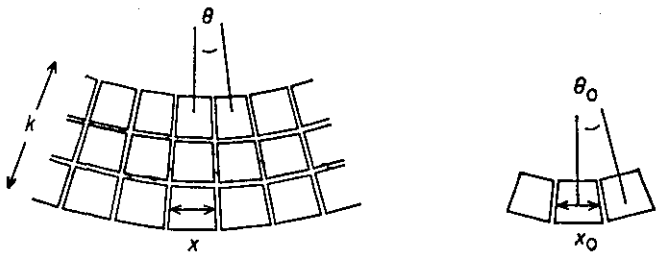

FIG. 6. A part of a curved band-like polymer.

The most stable position of two neighbouring monomers in a single strand makes a definite angle θ_0. If many strands are piled on with angle θ all fixed at θ_0, the distance x between neighbouring monomers in these strands can not be maintained at the most stable distance x_0 in an independent strand. The structure of the polymer is determined by the balance between the interaction free energy between neighbouring strands and the distortion free energy of strands. Suppose an i-mer composed of k strands ($i = lk$) and assume that the angle θ is common to all pairs of neighbouring monomers in all strands and consequently, the distance x_j in the jth strand is a linear function of j; that is, $x_j = x^0 + j\delta x$ ($j = -k/2, \ldots, +k/2$). The deviation δx is proportional to the angle θ; that is, $\delta x = \theta y$, where y is the thickness of the strand.

The excess free energy of the i-mer due to deviation of θ and x_j from θ_0 and x_0 contains a term proportional to l,

$$l \sum_j (x_j - x_0)^2 = l \sum_j ((x^0 - x_0) + j\,\delta x)^2.$$

For large values of k, this term increases in proportion to $ik^2\theta^2$. At the free energy minimum of the i-mer, the angle θ must tend to zero with increasing k. It is easily proved that for sufficiently large values of k, the angle θ is inversely proportional to k^2. Generally speaking, the deviation from equivalence is inversely proportional to the square of the thickness (Takahashi, 1966).

In the stable structure the excess free energy of the i-mer depends on both i and k. The critical concentration for the growth of polymers of thickness k is a function of k. In some cases, the critical concentration has a minimum at a certain value of k; then, polymers of this thickness are formed, and those of other thickness cannot be formed even at high total concentrations of monomers.

As another example, we can consider membraneous polymers in which monomers are arranged in a square lattice having some curvatures in two directions. The curvatures are determined by the balance between the interaction free energy and the distortion free energy, depending on the size of polymers. Infinite growth in any direction is made possible only by elimination of one of the curvatures. Under some conditions polymers of intermediate size can be most stable and the size distribution can have a maximum.

10. Concluding Remarks

Globular protein molecules form regular polymers in which all (or almost all) monomers are in equivalent positions, making bonds with many neighbouring monomers. Formation of these polymers takes place as a kind of crystallization. Statistical thermodynamics of crystallization has been mainly concentrated in the equilibrium between the crystal and the dispersed monomers. The condition determining the size, shape, and number of crystals is not easy to analyse, theoretically. The free energy of the system is insensitive to their size, shape and number. There are many states of different size distributions, among which the difference in the free energy is negligible. In the case of true crystallization, three-dimensional or two-dimensional, where macroscopic crystals must be formed, one cannot expect to find a simple general theory which gives significant results on the determination of their size, shape and number.

In the case of helical and tubular polymers which are very often found in biological systems, however, the theoretical treatment of this kind of problem is possible to a certain extent. In this paper, the size distribution was determined in equilibrium under various conditions and the free energy of the distribution pattern was calculated, which is very small as compared with the total crystallization free energy, as expected. The relaxation time of the size distribution change was also estimated.

The present theory was based on several assumptions to simplify calculation. The free energy of pairwise interaction between n monomers was assumed to be constant and the total free energy of a polymer was put to be proportional to the number of monomers except a constant correction term. The kinetic constants were assumed to be independent of the size of polymers and nuclei were treated as if they have a definite number of monomers and a definite structure. Actually, however, the interaction energy was found to depend on the size of polymers, for example, in the case of TMV protein (Casper, 1963). One of the causes of such dependence was discussed in the previous section. Long range interaction between charges on monomers may have a similar effect (Oosawa, 1957). Configurational entropy also must be taken into consideration if polymers were flexible. These factors, however, would not significantly alter qualitative conclusions derived in the simple theory.

In the kinetics of polymerization of flagellin into flagella, Asakura (1968) found the transconformation process of flagellin molecules attached to the end of flagella. Such a process was not assumed in the present theory on the kinetics of polymer size distribution. However, it is likely that inclusion of transconformation of monomers has no great effect on the behaviour of the size distribution during polymerization. Some analyses of this problem were made in a previous paper (Oosawa & Higashi, 1967).

Biological systems may have special mechanisms to select the distribution pattern of protein polymers in accordance with their purposes. Several mechanisms have been proposed to make polymers of the definite length (Casper, 1966; Huxley & Brown, 1967; Maruyama & Kawamura, 1968); hidden distortion in polymers, interaction with other protein systems or production of definite numbers of monomers and seeds. In order to see whether any special mechanism is needed or not, the simple and basic analysis presented here must be useful.

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