DETERMINATION OF THE EQUILIBRIUM CONSTANTS OF ASSOCIATING PROTEIN SYSTEMS

VI. Evaluation of M_1/M_{wapp} vs. C Curves for Linear and Helical Association

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ABSTRACT Procedures for the determination of equilibrium constants of linear and helical associations based on molecular weight data have been derived. Theoretical construction of curves for M_1/M_{wapp} vs. C reveals two clearly identifiable patterns for linear and helical association. The change in molecular conformation between linear and helical association is evidenced by a recurring pattern of irregular curves which obviously differ from those of an indefinite stacking model.

INTRODUCTION

The systematic investigation of the formation of intermolecular helical structures by protein macromolecules has been relatively neglected by researchers, with the exception of the theoretical analyses of Oosawa and Kasai (1962), Zimm and Bragg (1959), Oosawa and Higashi (1967), Casper and Klug (1962), and Casper (1963). Their results suggest that the transition from dispersed monomers to helical aggregates both in vivo and in vitro is a condensation phenomenon. Such association may be either linear or helical. If the association is strictly linear, the association would be similar to that proposed in Lauffer's model of tobacco mosaic virus protein (multiples of stacked trimer discs [1962, 1966]). If the association is helical, the model would be similar to that described by Casper (1963), that is, the grouping of four or more monomers to form a helix.

This communication describes considerations for analysis of linear and helical associations based on molecular weight as a function of concentration, applying Oosawa and Kasai's model (1962). The manipulations which follow can be applied to numerous model systems in order to determine whether the mode of association operating in that system is one of linear stacking or helical aggregation.

Theoretical Derivation of Interaction Parameters of Linear and Helical Association.

Helical Association. The assumptions that are used in deriving helical and linear association based on molecular weight data are (*a*) that the partial specific volumes of all species are equal, (*b*) that the refractive index increments of all species are the same, and (*c*) that the logarithm of the activity coefficient on a concentration scale for each species i is described by $\ln\gamma_i = iBM_1C + 0(C^2)$, $i = 1, 2, 3\cdots$. Based on this last assumption, the total concentration for linear association is given by Adams (1967 *a*). The quantity kC is < 1, where k is the intrinsic equilibrium constant denoting quantities consistent with the Adams notation (Chun, Kim, Stanley, and Ackers, 1969).

$$C = \sum_i C_i = \sum_i ik^{i-1}C_1^{\,i} = \frac{C_1}{(1 - kC_1)^2}, \qquad i \geqslant 1 \tag{1}$$

The total concentration for helical association is expressed as

$$C_{ih} = i\zeta k'^{(i-1)}C_1^{\,i}, \; i > 3, \tag{2}$$

where $\zeta = S(k/k')^2$. Oosawa and Kasai (1962) express the equilibrium constant for helical formation as k'. Thus,

$$S = e^{-\Delta f'/RT}, \qquad \frac{k}{k'} = e^{-\Delta f/RT}. \tag{2a}$$

$\Delta f'$ is the free energy[1] increment necessary for the special conformation between linear and helical association. Δf is the free energy increment necessary for forming linear or helical aggregates from the n to $(n + 1)$ degree. In order to satisfy the conditions of equation 2 *a*, it must be assumed that $S \ll 1$, $k/k' \ll 1$. Then the total concentration for linear and helical association can be expressed as

$$\begin{aligned} C &= \sum_i C_i + \sum_{i=3} C_{ih} = \sum_i ik^{i-1}C_1^{\,i} + \sum_{i=3} (k')^{i-1}C_1^{\,i}i\zeta \\ &= \sum_i i(k^{i-1} + \zeta k'^{i-1})C_1^{\,i} - [\zeta C_1 + 2\zeta k'C_1^2] \\ &= C_1\left[\frac{1}{(1 - kC_1)^2} + \frac{\zeta}{(1 - k'C_1)^2} - \zeta(1 + 2k'C_1)\right]. \end{aligned} \tag{3}$$

As the value of ζ is extremely small (Oosawa and Kasai, 1962), the final term of equation 3 becomes negligible and may be disregarded. It should be noted that

[1] The term free energy as used here denotes standard free energy.

$1/(1 - kC_1)^2$ is greater than 1 and equation 3 becomes

$$C = C_1\left[\frac{1}{(1 - kC_1)^2} + \frac{\zeta}{(1 - k'C_1)^2}\right]. \qquad (3\,a)$$

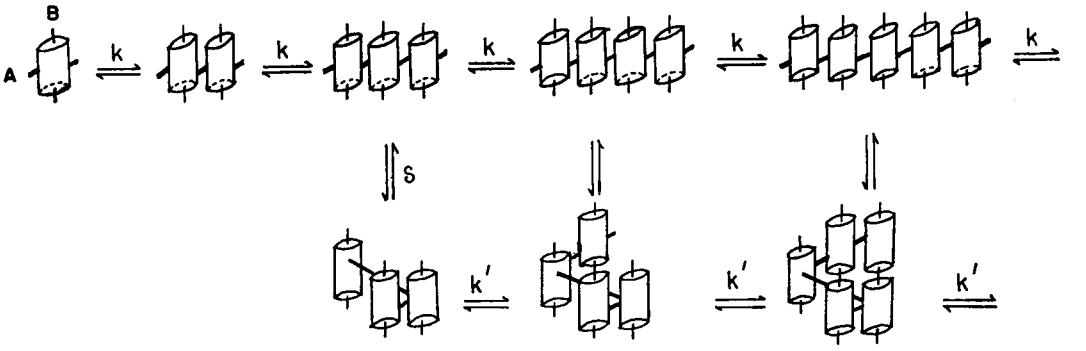

FIGURE 1 Schematic diagram of equilibrium between linear and helical association.

By definition, the weight average molecular weight for the associating system is

$$M_{w(c)} = \frac{\sum_i C_{it} M_i}{C} = \frac{[\sum_i iC_i + \sum_i iC_{ih}]_{M_i}}{C}$$

$$= \frac{M_1 C_1}{C}[\sum_i i^2 k^{i-1} C_1^{i-1} + \zeta \sum_i i^2 k'^{i-1} C_1^{i-1}]$$

$$= \frac{M_1 C_1}{C}\left[\frac{1 + kC_1}{(1 - kC_1)^3} + \zeta \frac{1 + k'C_1}{(1 - k'C_1)^3}\right]$$

$$\frac{M_1}{M_{w(c)}} = \frac{C}{C_1}\Big/\left[\frac{1 + kC_1}{(1 - kC_1)^3} + \zeta \frac{1 + k'C_1}{(1 - k'C_1)^3}\right]. \qquad (4)$$

By substitution of equation 4 into the apparent weight average molecular weight definition (Goldberg, 1953; Adams and Williams, 1964), then

$$\frac{M_1}{CM_{wapp}} = \frac{1}{C_1\left[\frac{1 + kC_1}{(1 - kC_1)^3} + \zeta \frac{1 + k'C_1}{(1 - k'C_1)^3}\right]} + \hat{B}M_1 \qquad (5)$$

Adams (1967 *a*, *b*), and Elias and Lys (1966) have shown that one can obtain the apparent number average molecular weight, $M_{n\text{app}}$, from a series of sedimentation equilibrium experiments at different initial concentrations.

$$M_{n(o)} = \frac{CM_1}{\sum_i (C_{it}/i)} = \frac{CM_1}{\sum_i k^{i-1}C_1{}^i + \sum_i k'^{i-1}C_1}$$

$$= \frac{C}{C_1}\left[\frac{M_1}{\sum_i k^{i-1}C_1^{i-1} + \zeta \sum_i k'^{i-1}C_1^{i-1}}\right] = \frac{C}{C_1}\left[\frac{M_1}{\frac{1}{1-kC_1} + \frac{\zeta}{1-k'C_1}}\right]$$

$$\frac{M_1}{M_{n(o)}} = \frac{C_1}{C}\left[\frac{1}{1-kC_1} + \frac{\zeta}{1-k'C_1}\right] \tag{6}$$

For $M_1/M_{n\text{app}}$ equation 6 becomes

$$\frac{CM_1}{M_{n\text{app}}} = C_1\left(\frac{1}{1-kC_1} + \frac{\zeta}{1-k'C_1}\right) + \frac{\hat{B}M_1C^2}{2} \tag{7}$$

$$\frac{M_1}{M_{n\text{app}}} = \frac{1}{C}\int_0^C \frac{M_1}{M_{w\text{app}}}\,dC. \tag{7a}$$

The apparent concentration of monomer for a nonideal solution as expressed by Adams and Williams (1964) is $C_1 = \alpha e^{-\hat{B}M_1C}$, where

$$\alpha = Ce^{\int_0^C([M_1/M_{w\text{app}}]-1)dC/C}. \tag{8}$$

α is obtained from the integration of $([M_1/M_{w\text{app}}] - 1)$ vs. C, where $C \to 0$, $C \to C$. Basic equations given to this point will suffice for the determination of the nonideality term $\hat{B}M_1$, the equilibrium constant, and the weight fraction of monomer. In dealing with four or five species in chemical equilibrium, Adams (1967 *b*) and Adams and Lewis (1968) derived two additional quantities, $-\sum_i C_iM_i^2/M_1^2$ and $M_1^2\sum_i C_i/M_i^2$. The quantity, $-\psi = \sum_i C_{it}M_i^2/M_1^2$ can be expressed as

$$-\psi = \sum_i C_{it}(i)^2 = \sum_i i^3k^{i-1}C_1{}^i + \sum_i \zeta i^3k'^{i-1}C_1{}^i$$

$$= \sum_i i^3(k^{i-1} + \zeta k'^{i-1})C_1{}^i. \tag{9}$$

In order to evaluate the quantity $\sum_i i^3k^{i-1}C_1{}^i = C_1\sum_i i^3(kC_1)^{i-1}$, note that

$$\sum_{i=1}^{\infty}(kC_1)^{i-1} = \frac{1}{1-kC_1}$$

$$\sum_{i=1}^{\infty} i(kC_1)^{i-1} = \frac{1}{(1-kC_1)^2}$$

$$\sum_{i=1}^{\infty} i^2(kC_1)^{i-1} = \frac{1+kC_1}{(1-kC_1)^3}. \tag{9a}$$

Equation 9 *a* is multiplied by kC_1, and differentiated.

$$\frac{1}{d(kC_1)} \sum_{i=1} i^2(kC_1)^i = \sum_{i=1} i^3(kC_1)^{i-1} = \frac{d}{d(kC_1)}\left[\frac{kC_1\,(1 + kC_1)}{(1 - kC_1)^3}\right]$$

$$= \frac{1 + 4kC_1 + (kC_1)^2}{(1 - kC_1)^4}.$$

Therefore, the expression becomes

$$C_1 \sum_i i^3\,(kC_1)^{i-1} = C_1\left[\frac{1 + 4kC_1 + (kC_1)^2}{(1 - kC_1)^4}\right]. \qquad (10)$$

Similarly, the quantity $\sum_i \zeta i^3 k'^{i-1} C_1{}^i$ yields

$$\sum_i \zeta i^3 k'^{i-1} C_1{}^i = \zeta C_1 \sum_i i^3 k'^{i-1} C_1{}^{i-1} = \zeta C_1 \sum_i i^3 (kC_1)^{i-1}$$

$$= \zeta C_1\left[\frac{1 + 4k'C_1 + (k'C_1)^2}{(1 - k'C_1)^4}\right]. \qquad (10\,a)$$

And that for linear and helical association, the quantity ψ becomes

$$-\psi = -\frac{\dfrac{d}{dc}\,[M_1/CM_{\text{wapp}}]}{[M_1/CM_{\text{wapp}} - \hat{B}M_1]}$$

$$= C_1\left[\frac{1 + 4kC_1 + (kC_1)^2}{(1 - kC_1)^4} + \zeta\,\frac{1 + 4k'C_1 + (k'C_1)^2}{(1 - kC_1)^4}\right]. \qquad (11)$$

Adams' (1967 *b*) evaluation of the quantities $-\sum_i C_i M_i{}^2/M_1{}^2$, $M_1{}^2 \sum_i C_i/M_i{}^2$ and $M_1{}^q \sum_i C_i/M_i{}^q (q = 3, 4\cdots)$ from molecular weight data can become cumbersome, and Chun and Kim (1969) point out that the determination may be complicated by experimental error in the vicinity of zero concentration. Therefore it is advisable to use four basic equations, (3 *a*, 5, 7, 8) in analysis of helical association. When the values of S, ζ, or $\hat{B}M_1$ vary from the basic assumptions outlined earlier, the four equations may be modified for that particular system, as follows. With the considerations which follow, it is possible to evaluate all interaction parameters necessary to differentiate between linear and helical association.

Special considerations when $S = 0$ or $\zeta = 0$. When $S = 0$ or $\zeta = 0$, equations 3, 5, 7, and 8 can be reduced to the expression for linear indefinite association of protein subunits described by Adams and Lewis (1968).

Special considerations when $\hat{B}M_1 = 0$, *ideal association.* In this case, the apparent

concentration of monomer for an ideal solution is directly obtainable from

$$C_1 = Ce^{\int_0^C([M_1/M_{wapp}]-1)dC/C}$$

Note that equations 3 *a*, 4, and 6 are given by

$$\left(\frac{C}{C_1}\right)\frac{M_1}{M_{n(o)}} = \frac{1}{(1 - kC_1)} + \frac{\zeta}{(1 - k'C_1)} \tag{12}$$

$$\frac{1}{\left[\frac{C_1}{C}\frac{M_1}{M_{w(o)}}\right]} = \frac{1 + kC_1}{(1 - kC_1)^3} + \zeta\frac{1 + k'C_1}{(1 - k'C_1)^3} \tag{13}$$

$$\zeta = \left[\frac{C}{C_1} - \frac{1}{(1 - kC_1)^2}\right](1 - k'C_1)^2. \tag{14}$$

Substituting equation 14 into equations 12 and 13 yields

$$\left[\frac{C}{C_1}\frac{M_1}{M_{n(o)}}\right] = \frac{1}{(1 - kC_1)} + \left[\frac{C}{C_1} - \frac{1}{(1 - kC_1)^2}\right](1 - k'C_1) \tag{15}$$

$$\frac{1}{\frac{C_1M_1}{CM_{w(o)}}} = \frac{1 + kC_1}{(1 - kC_1)^3} + \left[\frac{C}{C_1} - \frac{1}{(1 - kC_1)^2}\right]\frac{1 + k'C_1}{1 - k'C_1} \tag{16}$$

since $(1 + k'C_1)/(1 - k'C_1) = [2/(1 - k'C)] - 1$ and

$$(1 - k'C) = \left[\frac{CM_1}{C_1M_{n(o)}} - \frac{1}{(1 - kC_1)}\right] \Big/ \left[\frac{C}{C_1} - \frac{1}{(1 - kC_1)^2}\right] \tag{15 a}$$

Equation 16 takes the following form:

$$\frac{1}{\left[\frac{C_1M_1}{CM_{w(o)}}\right]} = \frac{1 + kC_1}{(1 - kC_1)^3} + \left[\frac{C}{C_1} - \frac{1}{(1 - kC_1)^2}\right]\left[2\frac{\frac{C}{C_1} - \frac{1}{(1 - kC_1)^2}}{\frac{CM_1}{C_1M_{n(o)}} - \frac{1}{(1 - kC_1)}} - 1\right] \tag{16 a}$$

Equation 16 *a* can be expressed as:

$$\left[\frac{M_1f}{M_{w(o)}}\right]^{-1} = \frac{2 - \xi}{\xi^2} + \left[\frac{1}{f} - \frac{1}{\xi^2}\right]\left[\frac{2\left(\frac{1}{f} - \frac{1}{\xi^2}\right)}{\left(\frac{M_1}{fM_{n(o)}} - \frac{1}{\xi}\right)} - 1\right], \tag{16 b}$$

where $(1 - kC_1) = \xi$ and $C_1/C = f$. Equation 16 *a* can be used to evaluate ξ from molecular weight data. Once ξ is known, the intrinsic equilibrium constant k is obtainable from $k = (1 - \xi)/C_1$. Equation 15 *a* yields

$$k' = \frac{1}{C_1}\left[1 - \frac{\left(\frac{M_1}{fM_{n(c)}} - \frac{1}{\xi}\right)}{\left(\frac{1}{f} - \frac{1}{\xi^2}\right)}\right]. \tag{17}$$

From equation 14, ζ and S can be readily computed, as values for C_1, k, and k' are known.

$$\zeta = S\left(\frac{k}{k'}\right)^2 = \left[\frac{C}{C_1} - \frac{1}{(1 - kC_1)^2}\right][1 - k'C_1^2] \tag{17 a}$$

$$S = \left(\frac{k'}{k}\right)^2 \zeta = \left(\frac{k'}{k}\right)^2 \left(\frac{1}{f} - \frac{1}{\xi^2}\right)\left[\frac{\frac{M_1}{fM_{n(c)}} - \frac{1}{\xi}}{\frac{1}{f} - \frac{1}{\xi^2}}\right]^2 \tag{18}$$

General solutions to helical association. From equation 5 and letting

$$\frac{1}{f} = \left[\frac{1}{(1 - kC_1)^2} + \frac{\zeta}{(1 - k'C_1)^2}\right]$$

then

$$\Phi_w = \frac{1}{\left[\frac{M_1}{M_{wapp}} - \hat{B}M_1C\right]f} = \left[\frac{1 + kC_1}{(1 - kC_1)^3} + \zeta\frac{1 + k'C_1}{(1 - k'C_1)^3}\right] \tag{18 a}$$

$$\Phi_N = \frac{1}{f}\left[\frac{M_1}{M_{napp}} - \frac{\hat{B}M_1C}{2}\right] = \left[\frac{1}{1 - kC_1} + \frac{\zeta}{1 - k'C_1}\right]. \tag{18 b}$$

From equation 18 *b*

$$\frac{\zeta}{1 - k'C} = \left[\Phi_N - \frac{1}{1 - kC_1}\right]. \tag{18 c}$$

Substituting equation 18 *c* into $1/f$ gives

$$\frac{1}{f} = \left\{\frac{1}{(1 - kC_1)^2} + \left[\Phi_N - \frac{1}{(1 - kC_1)}\right]\left[\frac{1}{1 - k'C_1}\right]\right\}$$

$$\left[\frac{1}{f} - \frac{1}{(1 - kC_1)^2}\right] = \left[\Phi_N - \frac{1}{(1 - kC_1)}\right]\left[\frac{1}{1 - k'C_1}\right] \tag{18 d}$$

Rearrangement of equation 18 *d* yields

$$k'C_1 = \left[1 - \frac{\Phi_N - \dfrac{1}{(1 - kC_1)}}{\dfrac{1}{f} - \dfrac{1}{(1 - kC_1)}}\right] \tag{18 e}$$

Letting $\eta = 1/(1 - kC_1)$, equation 18 *e* becomes

$$k'C_1 = \left[1 - \frac{\Phi_N - \eta}{\dfrac{1}{f} - \eta^2}\right]. \tag{18 f}$$

Substituting $k'C_1$ into equation 18 *a*, the equation for Φ_w,

$$\frac{1}{\left[\dfrac{M_1}{M_{wapp}} - \hat{B}M_1C\right]f} = \eta^2(2\eta - 1) + (\Phi_N - \eta)\left[\frac{2 - \dfrac{(\Phi_N - \eta)}{(1/f - \eta^2)}}{\dfrac{(\Phi_N - \eta)}{(1/f - \eta^2)}}\right]. \tag{19}$$

Solution of equation 19 yields k. Substituting k into equation 18 *f* gives a value for k', and solution of equation 19 *a* yields ζ. It is essential that various test values of $\hat{B}M_1$ be used in equation 19, until the resulting values of k, k', and ζ show constancy. Computer analysis at this point is the most efficient technique for determining such constant values of k, k', and ζ, assuming a given set of values of $\hat{B}M_1$.

$$\zeta = [1 - k'C_1\alpha e^{-\hat{B}M_1C}][\Phi_N - \eta] \tag{19 a}$$

Indefinite Linear Association (Linear Stacking Model)

When indefinite linear association by P_1, P_{3h}, P_{6h} $\cdots$ $P_{n(3h)}$ aggregates without limit, two possible stacking models must be considered. These are:

$$P_1 \rightleftarrows P_{3h} \rightleftarrows P_{6h} \rightleftarrows \cdots \rightleftarrows P_{i(3h)} \rightleftarrows \cdots \tag{i}$$

$$P_{3h} \rightleftarrows P_{6h} \rightleftarrows P_{9h} \rightleftarrows \cdots \rightleftarrows P_{i(3h)} \rightleftarrows \cdots \tag{ii}$$

The assumptions made in this analysis are the same as described by Adams and Lewis (1968) for indefinite association. The stacking monomer in this case is the trimer P_{3h}. Concentration of the monomer P_1 is only detectable at the initial stage of association. Thus the total concentration C in case (i) can be expressed as

$$C = C_1 + \sum_i ik^{i-1}C_{3h}{}^i = C_1 + \frac{C_{3h}}{(1 - kC_{3h})^2}. \tag{20}$$

If the trimer P_{3h} is the smallest monomeric unit at the beginning of association then

the concentration of the system becomes

$$C = \sum_i i\hat{k}^{i-1}C_{3h}{}^i = \frac{C_{3h}}{(1 - \hat{k}C_{3h})^2},$$

as has been described by Adams and Lewis (1968) for indefinite self-association. The weight average molecular weight for the associating system in case (i) is

$$M_{w(c)} = \frac{\sum_i C_{it} M_i}{C} = \frac{1}{C}[M_1 C_1 + \sum_i i\hat{k}^{i-1}C_{3h}{}^i(iM_3)]$$

$$= \frac{C_1 M_1}{C} + \frac{C_{3h} M_3}{C}\sum_i i^2\hat{k}^{i-1}C_{3h}{}^{i-1} = \frac{C_1 M_1}{C} + \frac{C_{3h} M_3}{C}\left[\frac{1 + \hat{k}C_{3h}}{(1 + \hat{k}C_{3h})^3}\right]$$

$$= \frac{C_1 M_1}{C}\left[1 + 3C_{3h}\frac{1 + \hat{k}C_{3h}}{(1 - \hat{k}C_{3h})^3}\right] \tag{21}$$

$$\frac{M_1}{M_{w(c)}} = \left[\frac{C}{C_1}\right]\Big/\left[1 + 3C_{3h}\frac{(1 + \hat{k}C_{3h})}{(1 - \hat{k}C_{3h})^3}\right]$$

$$= \frac{C}{\left[C_1 + 3C_{3h}\dfrac{(1 + \hat{k}C_{3h})}{(1 - \hat{k}C_{3h})^3}\right]} \tag{21 a}$$

The apparent weight average molecular weight becomes

$$\frac{M_1}{CM_{wapp}} = \frac{1}{\left[C_1 + C_{3h}\dfrac{(1 + \hat{k}C_{3h})}{(1 - \hat{k}C_{3h})^3}\right]} + \hat{B}M_1 . \tag{22}$$

The number average molecular weight, as described in the previous section, is obtainable as follows:

$$M_{n(c)} = \frac{CM_1}{\sum_i C_{it}/i} = \frac{CM_1}{\left[C_1 + \sum_i \dfrac{i\hat{k}^{i-1}C_{3h}{}^i}{3i}\right]} = \frac{CM_1}{\left[C_1 + \dfrac{C_{3h}}{3}\sum_i \hat{k}^{i-1}C_{3h}{}^{i-1}\right]}$$

$$= \frac{CM_1}{\left[C_1 + \dfrac{C_{3h}}{3}\left(\dfrac{1}{1 - \hat{k}C_{3h}}\right)\right]} \tag{23}$$

$$\frac{CM_1}{M_{napp}} = \left[C_1 + \frac{C_{3h}}{3}\left(\frac{1}{1 - \hat{k}C_{3h}}\right)\right] + \frac{\hat{B}M_1 C^2}{2} . \tag{24}$$

Evaluation of $\hat{B}M_1$, Nonideality Term. Letting $(1 - \hat{k}C_{3h}) = \lambda$, $[M_1/$

$CM_{\text{wapp}}] - \hat{B}M_1 = \rho_w$, and $[CM_1/M_{n\text{app}}] - [\hat{B}M_1C^2/2] = \rho_N$, equations 20, 22, and 24 become:

$$C = C_1 + \frac{C_{2h}}{\lambda^2} \tag{24 a}$$

$$\frac{1}{\rho_w} = C_1 + 3C_{3h}\frac{(2-\lambda)}{\lambda^2} \tag{24 b}$$

$$\rho_N = C_1 + \frac{C_{3h}}{3\lambda}. \tag{24 c}$$

From equations 24 *c* and 24 *a*:

$$\frac{C_{3h}}{\lambda} = 3(\rho_N - C_1) \tag{24 d}$$

$$(C - C_1) = \frac{C_{3h}}{\lambda}\cdot\frac{1}{\lambda} = \frac{3(\rho_N - C_1)}{\lambda}$$

$$\lambda = (C - C_1)/3(\rho_N - C_1). \tag{24 e}$$

With a proper substitution of equation 24 *e* into equation 24 *b*

$$\left(\frac{1}{\rho_w} - C_1\right) = \left[\frac{3C_{3h}}{\lambda}\right]\left[\frac{2-\lambda}{\lambda^2}\right] = 9(\rho_N - C_1)\frac{\left[2 - \dfrac{C - C_1}{3(\rho_N - C_1)}\right]}{\left[\dfrac{C - C_1}{3(\rho_N - C_1)}\right]^2} \tag{24 f}$$

$$\left(\frac{1}{\rho_w} - \alpha e^{-\hat{B}M_1C}\right) = \frac{18[\rho_N - \alpha e^{-\hat{B}M_1C}] - 3[C - \alpha e^{-\hat{B}M_1C}]}{\left[\dfrac{C - \alpha e^{-\hat{B}M_1C}}{3(\rho_N - \alpha e^{-\hat{B}M_1C})}\right]^2}. \tag{24 g}$$

The nonideality term, $\hat{B}M_1$, can be evaluated from equation 24 *g*. C_1 is evaluated from $C_1 = \alpha e^{-\hat{B}M_1C}$ and C_{3h} from

$$C_{3h} = \frac{9\left[\dfrac{CM_1}{M_{n\text{app}}} - \dfrac{\hat{B}M_1C^2}{2} - C_1\right]^2}{[C - C_1]}. \tag{24 h}$$

Once C_{3h} is known, it is possible to determine the equilibrium constant $\hat{k}$ and λ where $\lambda = (1 - \hat{k}C_{3h})$. In those cases when $C_1 = 0$ or $\hat{B}M_1 = 0$ for the stacking model, the evaluation of the interaction parameters becomes a simple matter which is briefly outlined as follows.

Special considerations when $C_1 = 0$. When $C_1 = 0$, the system undergoes $P_{3h} \rightleftarrows$

$P_{6h} \rightleftarrows P_{n(3h)} \rightleftarrows \cdots$, a linear association of trimers. Equations 20, 22, and 24 become

$$C = \frac{C_{3h}}{(1 - \hat{k}C_{3h})^2}$$

$$\frac{M_3}{M_{wapp}} = \frac{(1 - \hat{k}C_{3h})}{(1 + \hat{k}C_{3h})} + \hat{B}M_3\,C$$

$$\frac{CM_3}{M_{napp}} = \frac{C_{3h}}{(1 - \hat{k}C_{3h})} + \frac{\hat{B}M_3\,C}{2}$$

$$C_{3h} = \alpha' e^{-\hat{B}M_3 C} \quad \text{where} \quad \alpha' = Ce^{\int_0^C ([M_3/M_{wapp}]-1)dC/C}.$$

Special considerations when $\hat{B}M_1 = 0$.

$$C = \left[C_1 + \frac{C_{3h}}{(1 - \hat{k}C_{3h})^2}\right]$$

$$\frac{M_1}{CM_{wapp}} = \frac{1}{\left[C_1 + 3C_{3h}\dfrac{(1 + \hat{k}C_{3h})}{(1 - \hat{k}C_{3h})^3}\right]}$$

$$\frac{CM_1}{M_{napp}} = \left[C_1 + \frac{C_{3h}}{3}\left(\frac{1}{1 - \hat{k}C_{3h}}\right)\right]$$

$$C_{3h} = \frac{9\left[\dfrac{CM_1}{M_{napp}} - C_1\right]^2}{[C - C_1]}, \quad \text{where} \quad \alpha = Ce^{\int_0^C ([M_1/M_{wapp}]-1)dC/C}.$$

Behavior of a Curve of M_1/M_{wapp} *as a Function of Concentration as Defined by* k, k', *and* ζ

When $\hat{B}M_1 = 0$ and $k < k'$, then equation 5 becomes

$$\frac{M_1}{M_{wapp}} = \frac{M_1}{M_{w(c)}} = \frac{C}{C_1\left[\dfrac{1 + kC_1}{(1 - kC_1)^3} + \zeta\dfrac{1 + k'C_1}{(1 - k'C_1)^3}\right]} \tag{25}$$

and

$$C = C_1\left[\frac{1}{(1 - kC_1)^2} + \zeta\frac{1}{(1 - k'C_1)^2}\right]. \tag{3 a}$$

Substitution of equation 3 *a* into equation 25 gives

$$\frac{M_1}{M_{wapp}} = \frac{\left[\dfrac{1}{(1 - kC_1)^2} + \zeta\dfrac{1}{(1 - k'C_1)^2}\right]}{\left[\dfrac{1 + kC_1}{(1 - kC_1)^3} + \zeta\dfrac{1 + k'C_1}{(1 - k'C_1)^3}\right]} \tag{26}$$

Linear Association where $\zeta/(1 - k'C_1)^3 \ll 1$. Since ζ is much smaller than 1, the value of M_1/M_{wapp} of equation 25 will be determined by kC_1 as $C_1 \ll (1 - \zeta^{1/3})/k'$. Thus equation 26 becomes

$$\frac{M_1}{M_{wapp}} = \frac{\left[\frac{1}{(1 - kC_1)^2}\right]}{\left[\frac{1 + kC_1}{(1 - kC_1)^3}\right]} = \frac{(1 - kC_1)}{(1 + kC_1)}. \qquad (26\,a)$$

The resulting equation gives a curve identical to that of indefinite association when $C \rightarrow 0$ (Fig. 2).

Helical Association Where $\zeta/(1 - k'C_1)^3 \gg 1$, $C_1 \gg (1 - \zeta^{1/3})/k'$. As the value of C_1 increases, $k'C_1$ approaches 1. Equation 26 then becomes

$$\frac{M_1}{M_{wapp}} = \frac{\left[\frac{\zeta}{(1 - k'C_1)^2}\right]}{\left[\frac{(1 + k'C_1)}{(1 - k'C_1)^3}\right]} = \frac{(1 - k'C_1)}{(1 + k'C_1)}. \qquad (26\,b)$$

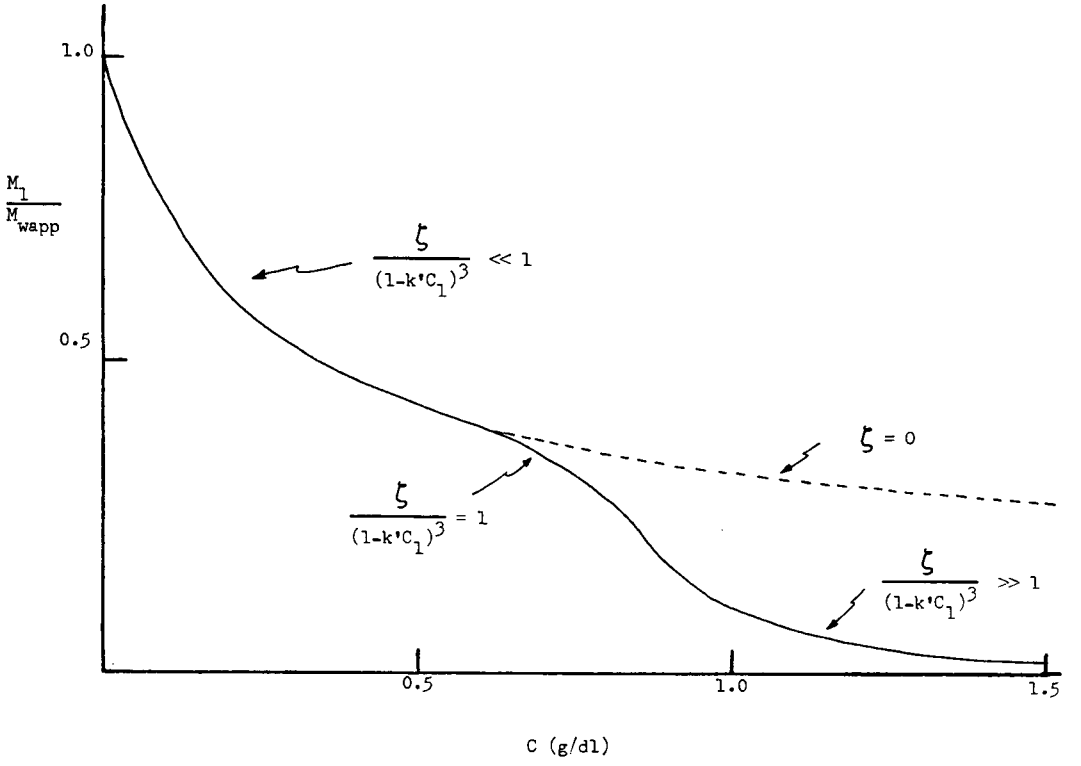

FIGURE 2 Plot of M_1/M_{wapp} vs. concentration curve showing conditions for linear and helical association.

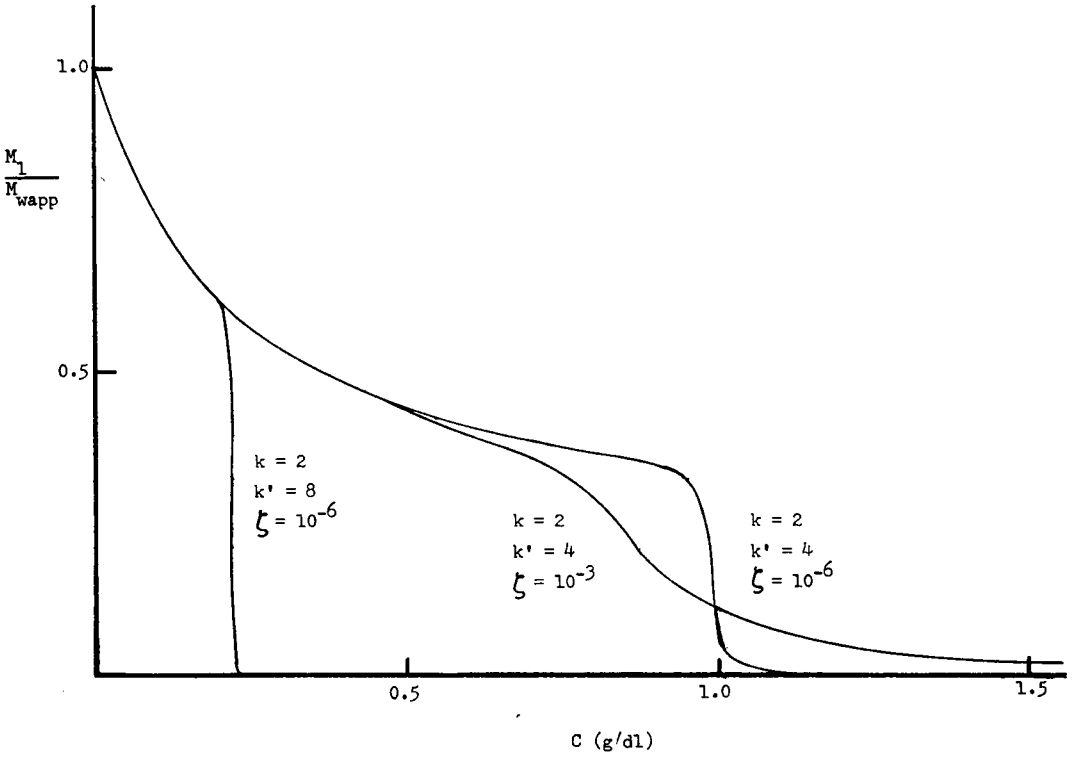

FIGURE 3 Plot of M_1/M_{wapp} vs. concentration curve showing the effect of either ζ of k' on sharpness of transition.

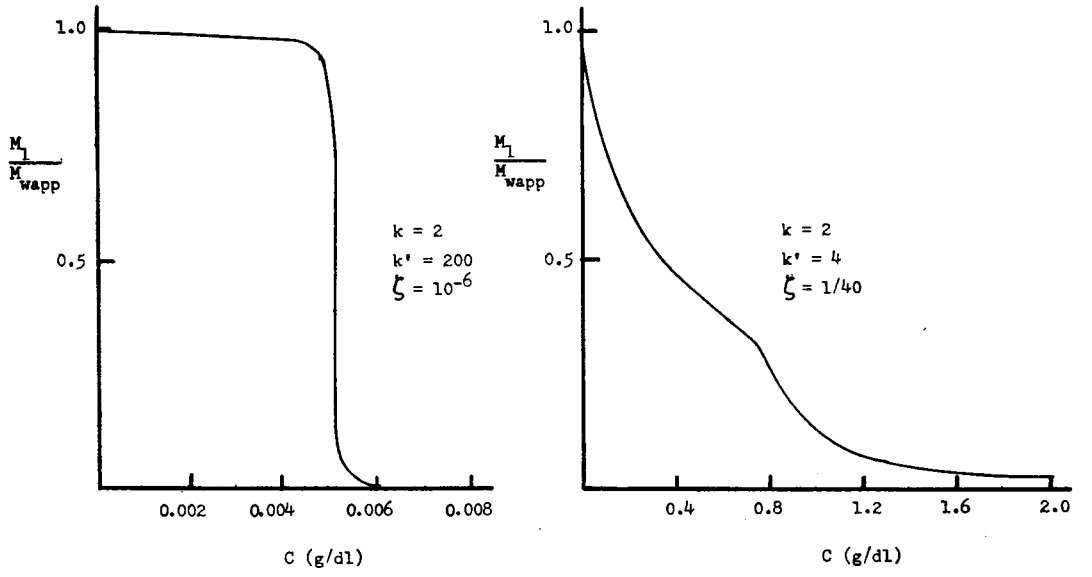

FIGURE 4 Plot of M_1/M_{wapp} vs. concentration showing the combined effect of ζ and k/k'.

The resulting curve of $M_1/M_{w_{app}}$ vs. C is identical to that of helical association [Fig. 2).

Linear-Helical Transition Stage, where $\zeta/(1 - k'C_1)^3 = 1$. In plotting a curve for $M_1/M_{w_{app}}$ vs. C, when $\zeta/(1 - k'C_1)^3 \ll 1$, the curve will be identical to that of indefinite linear association; when $\zeta/(1 - 1'C_1)^3 \gg 1$, the results show helical association; and when $\zeta/(1 - k'C_1)^3 = 1$, the curve shows a transition stage between linear and helical association. The steepness of the transition is determined by ζ, and the point at which the transition begins is determined by k', as may be seen in Fig. 3. The curve in this particular case will show three distinct regions which correspond to the states of association.

Based on the total concentration for helical association as expressed as $C_{ih} = \sum_i i\zeta(k')^{i-1}C_1^i$ (equation 2), it has been suggested that the value of ζ is dependent on the distortion energy described by Casper (1963) and which Oosawa and Kasai (1962) denoted by S. However, thermodynamic analysis reveals that ζ is solely dependent on the relative strength of the B-bonds, linear association being represented in terms of $(i - 1)$ A-bonds and helical association by $(i - 1)$ A-bonds and $(i - 3)$ B-bonds. The combined effect of ζ and k/k' upon transition may be seen in Fig. 4. The energetics of the transition between linear and helical association will be discussed in detail in the succeeding paper.

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