DETERMINATION OF THE EQUILIBRIUM CONSTANTS OF ASSOCIATING PROTEIN SYSTEMS

VII. THERMODYNAMIC ANALYSIS OF LINEAR AND HELICAL ASSOCIATION

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ABSTRACT The expression of free energy change for linear and helical association based on Casper's definition as applied to Oosawa and Kasai's model is described. Redefinition of the thermodynamic quantity ζ indicates that it is dependent on Δf_B, the free energy change in the formation of B-bonds, rather than mean distortion energy as previously believed.

INTRODUCTION

The work done by Fraenkel-Conrat and Williams (1955) on the disassociation and reassembly of tobacco mosaic virus (TMV) protein and in vitro reassembly of this virus from coat protein and RNA introduced the concept of self-assembly of macromolecules which now dominates research on the morphogenesis of viruses. The well-documented reassembly of TMV virus has led to the two major theories on the nature of the self-assembly process (Casper, 1963; Lauffer, 1966 *a*, 1966 *b*), in which the model systems differ only on the question of the specificity of the intersubunit bonds.

Casper and Klug (1962) proposed that the most stable minimum energy designs for surface crystals are tubular cylinder or helical, and icosahedral symmetry. A structure with cylindrical symmetry must have an integral number of units in each turn and the helical segments are determined only by state of lowest energy bonding between the subunits (Casper, 1963).

Statistical thermodynamics of polymerization of protein into helical structure have been presented by Oosawa and Kasai (1962) and Oosawa and Higashi (1967). Based on the total concentration for helical association as expressed as $C_{ih} = \sum_i i\zeta(k')^{i-1}C_1{}^i$, 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. The significance of ζ in plotting the curve of $M_1/M_{w\,app}$ vs. concentration for linear and helical association has been described in the preceding paper (Chun, 1970).

This communication describes a redefinition of the thermodynamic quantity ζ which is based upon free energy change under conditions of helical association, and which differs markedly from Oosawa and Kasai's consideration of the quantity. Linear association is represented in terms of $(i - 1)$ B-bonds and helical association by $(i - 1)$ A-bonds and $(i - 3)$ B-bonds, as seen in Fig. 1. This theoretical derivation suggests that it is the relative strength of these B-bonds which determines the value of ζ.

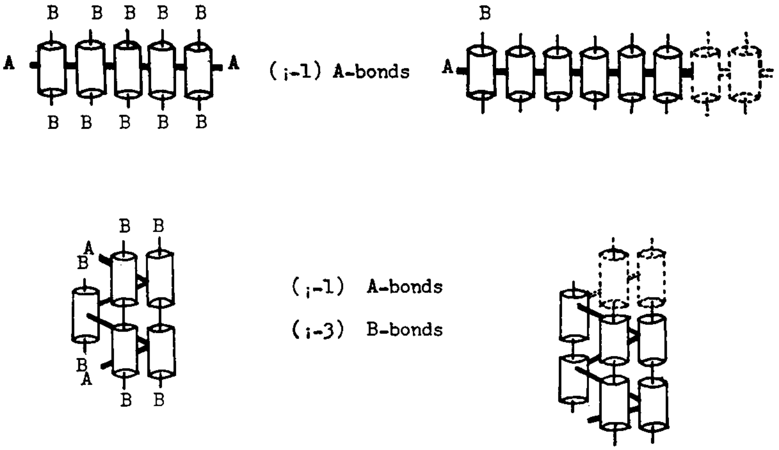

FIGURE 1 Schematic diagram of the number of possible configurational bonds in linear and helical aggregates.

Expression of Free Energy Change[1] *for Linear and Helical Association*

Necessary assumptions in the derivation of an expression for free energy change in linear and helical association are (*a*) that no degenerate state $[G_n{}^i]$ (number of geometrically distinct configurations of n-mer with i-bonds formed) exists, (*b*) that the mean distortion energies (Casper, 1963) per subunit of all helical segments are the same, i.e., $[\Delta D_n{}^{A-(i-1)}]$ for linear and $[\Delta D_n{}^{A-(i-1),\ B-(i-3)}]$ for helical when $n > 3$.

Casper's Model (1963). The free energy change in forming linear aggregates of i-mer with $(i - 1)$ A-bonds can be expressed as

$$\Delta F_{il} = (i - 1)\Delta f_A + [i\Delta D_n{}^{A-(i-1)}] = (i - 1)\Delta f_A + i\Delta D_l\,. \qquad (1)$$

The distortion energy in forming an i-mer of linear aggregates with $(i - 1)$ A-bonds is represented by $i\Delta D_l$. (All bonds are assumed to have equal energy.) This distor-

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

tion energy term includes the strain energy in forming the i-mer and the repulsion energy between nonbonded neighbors, and the value differs from that of $i\Delta D_h$, or helical distortion energy ($i\Delta D_l \not+ i\Delta D_h$). Casper (1963) gives the free energy change in forming helical aggregates of i-mer with $(i - 1)$ A-bonds and $(i - 3)$ B-bonds as

$$\begin{aligned}\Delta F_{ih} &= (i-1)\Delta f_A + (i-3)\Delta f_B + [i\Delta {D_h}^{A=(i-1),\ B=(i-3)}] \\ &= (i-1)\Delta f_A + (i-3)\Delta f_B + i\Delta D_h\,. \qquad (2)\end{aligned}$$

Modification of Casper's Definition as Applied to Oosawa and Kasai's Linear and Helical Models

In the case of simple end-to-end aggregates in which each monomer is bound to two neighboring monomers, the concentration (mole/liter) of such linear association is expressed as

$$[P_{il}] = k^{i-1}[P_1]^i. \qquad (3)^2$$

Since $\Delta F_{il} = -RT \ln [P_{il}]/[P_1]^i$, equation 3 becomes

$$[P_{il}] = [P_1]^i e^{-\Delta F_i/RT}. \qquad (3\,a)$$

Substitution of equation 1 into equation 3 *a* yields

$$[P_{il}] = [P_1]^i e^{-[(i-1)\Delta f_A + i\Delta D_l]/RT}. \qquad (4)$$

From equations 3 and 4

$$k = [e^{-(i-1)(\Delta f_A + i\Delta D_l)/RT}]^{[1/(i-1)]} = e^{-[\Delta f_A + [i/(i-1)]\Delta D_l]/RT}. \qquad (5)$$

The intrinsic equilibrium constant, k, in equation 3 is constant whereas k in equation 5 is not, being still dependent on the value of $i/(i - 1)$. Thus equations 3 and 5 are not comparable quantities, although they should be equivalent. Consider a further example, solving for k:

$$\begin{aligned}[P_2] &= k[P_1]^2 = [P_1]^2 e^{-(\Delta f_A + 2\Delta D_l)/RT} \\ k &= e^{-(\Delta f_A + 2\Delta D_l)/RT} \qquad (05)\end{aligned}$$

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[2] Equation 3 can be expressed in terms of concentration (g/liter) as follows:

$$\begin{aligned}C_i &= [P_i]M_i\text{, where } [P_i] = C_i/iM_1 \\ C_i &= i[P_i]M_1 = ik^{i-1}M_1[P_1]^i.\end{aligned}$$

Squaring equation (05),

$$k^2 = e^{-(2\Delta f_A + 4\Delta D_l)/RT} \tag{06}$$

$$[P_3] = k^2[P_1]^3 = [P_1]^3 e^{-(2\Delta f_A + 3\Delta D_l)/RT}$$

$$k^2 = e^{-(2\Delta f_A + 3\Delta D_l)/RT}. \qquad (0\,7$$

Again equations 06 and 07, although both solve for k^2, are not equivalent.

Returning to Casper's original definition of ΔF_{il} or ΔF_{ih}, let ΔD_l and ΔD_h be the average values of change in distortion energy per additional unit and redefine Casper's distortion energy using $[(i - 1)\Delta D_n{}^{A-(i-1),\ B-(i-3)}]$, which satisfies both expressions. Equations 1 and 2 are redefined as

$$\Delta F_{il} = (i - 1)(\Delta f_A + \Delta D_l) \tag{6}$$

$$\begin{aligned} \Delta F_{ih} &= (i - 1)(\Delta f_A + \Delta D_h) + (i - 3)\Delta f_B \\ &= (i - 3)(\Delta f_A + \Delta f_B + \Delta D_h) + 2(\Delta f_A + \Delta D_h),\ i \geqslant 4. \end{aligned} \tag{6 a}$$

Expressing ΔF_i for linear or helical polymers as follows:

ΔF_{il}	ΔF_{ih}, $i \geqslant 4$
P_{2l}, $\Delta F_{2l} = (\Delta f_A + \Delta D_l)$	P_{4h}, $\Delta F_{4h} = 3\Delta f_A + \Delta f_B + 3\Delta D_h$
P_{3l}, $\Delta F_{3l} = 2(\Delta f_A + \Delta D_l)$	P_{5h}, $\Delta F_{5h} = 4\Delta f_A + 2\Delta f_B + 4\Delta D_h$
$\vdots$	$\vdots$
P_{il}, $\Delta F_{il} = (i - 1)(\Delta f_A + \Delta D_l)$	P_{ih}, $\Delta F_{ih} = (i - 1)(\Delta f_A + \Delta D_h)$
	$+ (i - 3)\Delta f_B$

Substituting equations 6 and 6 *a* into equation 3 *a*

$$[P_{il}] = [P_1]^i e^{-[(i-1)(\Delta f_A + \Delta D_l)]/RT} \tag{7}$$

$$\begin{aligned} [P_{ih}] &= [P_1]^i e^{-[(i-1)(\Delta f_A + \Delta D_h) + (i-3)\Delta f_B]/RT} \\ &= [P_1]^i \{e^{-(i-3)(\Delta f_A + \Delta f_B + \Delta D_h)/RT}\}\{e^{-2(\Delta f_A + \Delta D_h)/RT}\}. \end{aligned} \tag{7 a}$$

Since $[P_{il}] = k^{i-1}[P_1]^i$, the intrinsic equilibrium constant k for linear association becomes

$$[P_1]^i e^{-\{(i-1)(\Delta f_A + \Delta D_l)\}/RT} = k^{i-1}[P_1]^i$$

$$k = e^{-(\Delta f_A + \Delta D_l)/RT}. \tag{8}$$

And $[P_{i\mathrm{h}}] = \zeta(k')^{i-1}[P_1]^i$ can be substituted into equation 7 *a*.

$$\zeta(k')^{i-1}[P_1]^i = [P_1]^i\{e^{-(i-3)(\Delta f_\mathrm{A}+\Delta f_\mathrm{B}+\Delta D_\mathrm{h})/RT}\}\{e^{-2(\Delta f_\mathrm{A}+\Delta D_\mathrm{h})/RT}\}$$

$$\zeta(k')^{i-1} = \{e^{-(i-3)(\Delta f_\mathrm{A}+\Delta f_\mathrm{B}+\Delta D_\mathrm{h})/RT}\}\{e^{-2(\Delta f_\mathrm{A}+\Delta D_\mathrm{l})/RT}\}\{e^{-2(\Delta D_\mathrm{h}-\Delta D_\mathrm{l})/RT}\}. \quad (9)$$

From equation 9, one obtains the intrinsic equilibrium constant k' for helical association.

$$k = e^{-(\Delta f_\mathrm{A}+\Delta D_\mathrm{l})/RT}$$

$$S = e^{-2(\Delta D_\mathrm{h}-\Delta D_\mathrm{l})/RT}$$

$$k' = e^{-(\Delta f_\mathrm{A}+\Delta f_\mathrm{B}+\Delta D_\mathrm{h})/RT}. \quad (9\,a)$$

Thus,

$$\frac{k}{k'} = \frac{e^{-(\Delta f_\mathrm{A}+\Delta D_\mathrm{l})/RT}}{e^{-(\Delta f_\mathrm{A}+\Delta f_\mathrm{B}+\Delta D_\mathrm{h})/RT}} = e^{\Delta f_\mathrm{B}/RT}\cdot\frac{1}{\sqrt{S}}$$

$$\zeta = S\left(\frac{k}{k'}\right)^2 = \frac{[e^{-(\Delta D_\mathrm{h}-\Delta D_\mathrm{l})/RT}][e^{-2(\Delta f_\mathrm{A}+\Delta D_\mathrm{l})/RT}]}{[e^{-2(\Delta f_\mathrm{A}+\Delta f_\mathrm{B}+\Delta D_\mathrm{h})/RT}]} = e^{2\Delta f_\mathrm{B}/RT}. \quad (10)$$

Comparing the redefined values of ζ, S, and k/k' with Oosawa and Kasai's original presentation, the deviation in value is marked.

Oosawa and Kasai (1962)	Chun (1970)
$S = e^{-\Delta f'/RT}$	$S = e^{-2(\Delta D_\mathrm{h}-\Delta D_\mathrm{l})/RT}$
$\frac{k}{k'} = e^{-\Delta f/RT}$	$\frac{k}{k'} = e^{\Delta f_\mathrm{B}/RT}\cdot\frac{1}{\sqrt{S}}$
$\zeta = S\left(\frac{k}{k'}\right)^2 = e^{-(\Delta f'+2\Delta f)/RT}$	$\zeta = e^{2\Delta f_\mathrm{B}/RT}$

The redefinition of ζ leads to the conclusion that the quantity is determined by Δf_B, or the strength of the B-bonds of helical association rather than a ratio of k/k' and S as proposed by Oosawa and Kasai (1962). For a given value of Δf_B, the value of $(k/k')^2$ is inversely proportional to S.

Thermodynamic Conditions for Helical Association

Using the redefined value of ζ, it is possible to determine the conditions for helical association. The effect of ζ on the curve of $M_1/M_{w\mathrm{app}}$ vs. concentration has been extensively described in the preceding paper (Chun, 1970).

In using the basic quantities defined by equations 9 *a* and 10, let

$$(\Delta f_A + \Delta D_l)/RT = \epsilon_A$$

$$(\Delta D_h - \Delta D_l)/RT = \delta$$

$$\Delta f_B/RT = \epsilon_B .$$

Then equation 9 *a* becomes

$$k = e^{-\epsilon_A}$$

$$k' = e^{-(\epsilon_A + \epsilon_B + \delta)}$$

$$S = e^{-2\delta}, \qquad (10\,a)$$

and

$$\zeta = e^{2\epsilon_B}.$$

Assuming the free energy change per formation of B-bond is greater than the mean distortion energy required to distort a linear polymer into a helical polymer, then the concentration of the helical polymer will be greater than that of the linear polymer, that is, $[P_{ih}]/[P_{il}] > 1$

$$\frac{[P_{ih}]}{[P_{il}]} = \frac{\zeta (k')^{i-1}}{k^{i-1}} = e^{-[(i-1)\delta + (i-3)\epsilon_B]} > 1, \qquad (11)$$

when $i > 3$,

$$|\epsilon_B| > \left(\frac{i-1}{i-3}\right)\delta. \qquad (11\,a)$$

For large values of i, equation 11 *a* becomes

$$|\epsilon_B| > \delta. \qquad (12)$$

This equation satisfies the conditions for helical association, i.e., $\zeta = e^{2\epsilon_B}$. If $|\epsilon_B|$ is large, the value of ζ is quite small, resulting in a sharp transition in the curve of $M_1/M_{w\mathrm{app}}$ vs. C, an observation which is confirmed by our preceding paper.

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