Determination of the Equilibrium Constants of Associating Protein Systems. IV. The Application of the Weight-Average Partition Coefficient to Analysis of BM_1 Nonideality Term (As Applied to Bovine Liver L-Glutamate Dehydrogenase)*

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ABSTRACT: In numerical evaluation of the equilibrium constants of associating protein systems of the type $qP_1 \rightleftharpoons tP_l + rP_m + sP_n$, $n > m > l > 1$, or $qP_1 \rightleftharpoons qP_i + tP_l + rP_m + sP_n$, $n > m > l > i > 1$, the more readily obtainable weight-average partition coefficients can be substituted for the quantities

$$-\left(\sum_i C_i M_i^2\right)/M_1^2$$

as proposed by Adams (Adams, E. T., Jr. (1967), *Biochemistry 6*, 1864) and

$$M_1^2 \sum_i (C_i/M_i^2)$$

(Adams, E. T., Jr., and Lewis, M. S. (1968), *Biochemistry 7*, 1044). The resulting equations from a combination of the weight-average partition coefficient and molecular weight data allow a more simplified evaluation of BM_1 (nonideality term) and the weight fraction of the monomer. Bovine liver L-glutamate dehydrogenase was used in experimental applications. Results confirmed the linear indefinite association described in detail in part III (Chun, P. W., Kim, S. J., Stanley, C. A., and Ackers, G. K. (1969), *Biochemistry 8*, 1625 (this issue; preceding paper)).

In part III of this research communication (Chun *et al.* (1969)), it was shown that when the weight fraction of monomer from the weight-average partition coefficients of more than four species are involved in chemical equilibrium, quantitative evaluation becomes both tedious and involved.

It is also true that Adams' (1967) evaluation of the quantities

$$-\frac{\sum C_i M_i^2}{M_1^2} \text{ and } M_1^2 \sum_i C_i/M_i^2$$

from molecular weight data is cumbersome. Adams (1967) pointed out that the determination of the quantity could be complicated by experimental error

$$-\frac{\sum C_i M_i^2}{M_1^2}$$

in the vicinity of zero concentration. In addition, the quantity

$$M_1^2 \sum_i C_i/M_i^2$$

is a rather unwieldy term due to the required double integration in its determination. Any error introduced in the first integration is multiplied by the second.

In this paper a quantitative evaluation of BM_1 (nonideality term) and the weight fraction of monomer based on a combination of molecular sieve partition coefficient and molecular weight data will be proposed as a method of checking the calculation of these values at low concentration. The application of two independent methods furnishes the needed quantity BM_1 and the equilibrium constant of associating protein systems when more than four species are involved in the chemical equilibrium. Thus, a correlation of these two approaches permits the verification of previous calculations of the equilibrium constant and nonideality term. Application of both methods provides a dual check on the mode of association and weight fraction of monomer in the associating protein model selected. Molecular sieve chromatography has a special advantage in the studies at low concentration (Ackers and Thompson, 1965; Ackers 1967a,b, 1968) in that it avoids the introduction of the two more cumbersome terms derived by Adams (1967) and Adams and Lewis (1968) which are the basis of the equilibrium sedimentation method.

The theoretical derivation which follows may be applied to various model systems, providing that partition and molecular weight data are collected under identical conditions.

Elementary Column Matrix Transformation for Evaluation of BM_1 and the Weight Fraction of Monomer. BASIC EQUATIONS AND ASSUMPTIONS. The assumptions

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that are used in the analysis of associating protein systems of all types based on molecular weight data are (1) that the partial specific volumes of all species are the same, (2) that the refractive index increments of all species are equal, (3) it is assumed that the logarithm of the activity coefficient on a concentration scale for each species i is described by $\ln \gamma_i = iBM_1C + 0(C^2)$, $i = 1, 2, 3, \ldots$ As the result of this assumption, the total concentration for the associating protein system is given by (Adams and Fujita, 1963)

$$C_n = K_n C_1^n \tag{1}$$

$$C = \sum_i K_i C_1^i = C_1 + K_2 C_1^2 + K_3 C_1^3 + \cdots \tag{2}$$

By definition, the weight-average molecular weight (Goldberg, 1953; Adams and Williams, 1964) for the associating system is

$$\frac{M_1}{M_{\text{wapp}}} = \frac{M_1}{M_{w(C)}} + BM_1C \tag{3}$$

By using eq 1–3, Adams (1965a) derived

$$\Phi_{(w)} = \frac{1}{\left(\frac{M_1}{CM_{\text{wapp}}} - BM_1\right)} = \sum_i iK_iC_1^i = C_1 + 2K_2C_1^2 + \cdots \tag{4}$$

Adams (1965a) has shown that one can obtain the apparent number-average molecular weight, M_{napp}, from a series of sedimentation equilibrium experiments at different initial concentrations, using the equation

$$\frac{CM_1}{M_{\text{napp}}} = \int_0^C \frac{M_1}{M_{\text{wapp}}}dC = \int_0^C \left(\frac{M_1}{M_{w(C)}} + BM_1C\right)dC = \sum_i \frac{K_iC_1^i}{i} + \frac{BM_1C^2}{2} = C_1 + \frac{K_2C_1^2}{2} + \frac{K_3C_1^3}{3} + \cdots + \frac{BM_1C^2}{2} \tag{5}$$

The apparent concentration of monomer for a non-ideal solution as expressed by Adams and Williams (1964) is $C_1 = \alpha e^{-BM_1C}$, where

$$\alpha = Ce^{\int_1^C \left(\frac{M_1}{M\text{wapp}} - 1\right)\frac{dC}{C}} \tag{6}$$

α is obtained from the integration of $(M_1/M_{\text{wapp}} - 1)$ *vs.* C, where $C \to 0$, $C \to C$. Basic equations given to this point will suffice for the determination of the non-ideality term BM_1, equilibrium constant, and the weight fraction of monomer when as many as three species are involved in chemical equilibrium.

In dealing with four or five species in chemical equilibrium, Adams (1967) and Adams and Lewis (1968) derived two additional quantities, $-\sum C_iM_i^2/M_1^2$ and $M_1^2 \sum_i C_i/M_i^2$.

$$\varphi = -\frac{\sum C_iM_i^2}{M_1^2} = \frac{\frac{d}{dC}\left(\frac{M_1}{CM_{\text{wapp}}}\right)}{\left[\frac{M_1}{CM_{\text{wapp}}} - BM_1\right]^3} = -[C_1 + 4K_2C_1^2 + 9K_3C_1^3 + \cdots] = -\sum_i i^2K_iC_1^i \tag{7}$$

and

$$\left(M_1^2\sum_i C_i/M_i^2\right)_{\text{app}} = \int_0^C \frac{M_1}{M_{\text{napp}}M_{\text{wapp}}}dC = BM_1\int_0^C \frac{CM_1}{M_{\text{napp}}}dC + \frac{BM_1}{2}\int_0^C \frac{CM_1}{M_{\text{wapp}}}dC - \tfrac{1}{6}(BM_1)^2C^3 + \sum_i \frac{K_iC_1^i}{i} \tag{8}$$

The necessity for numerical evaluation of the derivative of eq 7 and double integration of eq 8 increases the chance of errors in calculation even though the equations themselves are valid. These intricate calculations can be eliminated by introducing the weight-average partition coefficient in combination with molecular weight data. As described in part III (Chun *et al.*, 1969) of this series, the weight-average partition coefficient, $\bar{\sigma}_w$, at low concentration is expressed as (Ackers, 1967a,b, 1968)

$$\bar{\sigma}_w = \sum_i \sigma_i f_i + 0(C)^1 \tag{9}$$

The first-order term, $\beta_iC_if_i = 0$, is linear at low concentration of the molecular sieve partition isotherm. The weight-average partition coefficient is evaluated from the following expression

$$\sigma_i = erfc\left[\frac{A_i - A_0}{b_0}\right] \text{ (Ackers, 1967a,b)} \tag{9a}$$

Substitution of $f_i = C_i/C$ into eq 9 gives

$$\bar{\sigma}_w = \sum_i \sigma_i f_i = \sum_i \frac{\sigma_iK_iC_1^i}{C} \tag{9b}$$

Combining eq 9b with the definition of $M_{w(C)}$ (Adams, 1965a,b) gives

$$\frac{M_1}{M_{w(C)}}\bar{\sigma}_w dC = \sum_i \sigma_iK_iC_1^{i-1}dC_1 \tag{10}$$

where

$$\frac{dC_1}{C_1} = \frac{M_1}{M_{w(C)}}\frac{dC}{C} \tag{11}$$

Integrating eq 10 gives

$$\int_0^C \frac{M_1}{M_{w(C)}}\bar{\sigma}_w dC = \int_0^{C_1} \sum_i \sigma_iK_iC_1^{i-1}dC_1 = \sum_i \frac{\sigma_iK_iC_1^i}{i} \tag{11a}$$

[1] Cases in which the first order term, $\gamma C \neq 0$, in equation $\bar{\sigma}_{\text{wapp}} = \bar{\sigma}_{w(c)} + \gamma C$ are discussed in detail in Appendix II.

$$\int_0^C \frac{M_1}{M_{wapp}}\bar{\sigma}_w dC = \int_0^C \left(\frac{M_1}{M_{w(C)}} + BM_1C\right)\bar{\sigma}_w dC = \sum_i \frac{\sigma_i K_i C_1{}^i}{i} + BM_1 \int_0^C \bar{\sigma}_w C dC \qquad (12)$$

letting

$$\lambda = \int_0^C \frac{M_1}{M_{wapp}}\bar{\sigma}_w dC - BM_1 \int_0^C \bar{\sigma}_w C dC = \sum_i \frac{\sigma_i K_i C_1{}^i}{i} \qquad (13)$$

Note that by substitution of simplified eq 9a for previous eq 7 and 8, the involved calculations have been eliminated.

It is also possible to establish an empirical relationship, $\sigma_i = \sigma_1 - A \ln i$, which is based on the constant A, the slope of a plot of σ_i *vs.* $\ln i$. Substitution of $\sigma_i = \sigma_1 - A \ln i$ into eq 9 gives

$$\bar{\sigma}_w = \sigma_1 - A\sum_i f_i \ln i \qquad (14)$$

Equation 10 becomes

$$\zeta_{(C)} = \frac{\sigma_1 - \bar{\sigma}_w}{A} = \sum_i f_i \ln i = \frac{\sum K_i C_1{}^i \ln i}{C} \qquad (14a)$$

Combining eq 14a with eq 11 gives

$$\frac{M_1}{M_{w(C)}}\zeta dC = \sum_i K_i C_1^{i-1} (\ln i) dC_1 \qquad (14b)$$

Integrating eq 14b

$$\int_0^C \frac{M_1}{M_{w(C)}}\zeta dC = \int_0^{C_1} \sum_i K_i C_1^{i-1} \ln i dC_1 = \sum_i \frac{K_i C_1{}^i}{i} \ln i \qquad (14c)$$

Equation 14c can be expressed in terms of M_1/M_{wapp} as follows.

$$\int_0^C \frac{M_1}{M_{wapp}}\zeta dC = \int_0^C \left(\frac{M_1}{M_{w(C)}} + BM_1C\right)\zeta dC = \sum_i \frac{K_i C_1{}^i}{i} \ln i + BM_1 \int_0^C C\zeta dC \qquad (14d)$$

letting

$$\zeta = \int_0^C \frac{M_1}{M_{wapp}}\zeta dC - BM_1 \int_0^C C\zeta dC$$

Hence

$$\zeta = \sum_i \frac{K_i C_1{}^i}{i} \ln i \qquad (15)$$

Note that eq 14 is an empirical equation based on $\sigma_i = -A \ln M_i + B$. This empirical relationship between the partition coefficient and molecular weight of i-mer may not hold true for all cases. Until more exacting measurements of the constant A may be made, use of the empirical method involves a certain risk. Therefore, the theoretical method based on eq 9b is preferable for application to other associating protein model systems.

Six basic equations (2, 4, 5, 6, 9b, and 13) which incorporate the weight-average partition coefficient can be used for discrete association when four or more species are in chemical equilibrium. In addition, the empirically derived eq 14a and 15 can be applied, with the precaution that they are less dependable than the other six. In order to illustrate the use of the basic equations outlined here, their application to several discrete cases will be considered. Their use in cases involving indefinite association is described as applied to bovine liver L-glutamate dehydrogenase. It was found that only results for indefinite association fit this particular protein system.

The evaluations which follow are arrayed in matrix form, due to their adaptability to computer analysis and ease of application to various model systems.

$$qP_1 \rightleftharpoons rP_n \qquad n > 1 \qquad (A)$$

Method 1A. Two species are involved in chemical equilibrium. The nonideality term, BM_1, and the weight fraction of monomer are obtained by the following procedure. Combining eq 2 and 9b

$$C = C_1 + K_n C_1{}^n$$
$$C\bar{\sigma}_w = C_1\sigma_1 + \sigma_n K_n C_1{}^n \qquad (16)$$

By determinants (Harris, 1964), the solution to equation set (eq 16) is given by eq 16a, $C_1 = D_1/D$, $C_n = D_2/D$, where

$$D = \begin{vmatrix} 1 & 1 \\ \sigma_1 & \sigma_n \end{vmatrix} \quad D_1 = \begin{vmatrix} C & 1 \\ C\bar{\sigma}_w & \sigma_n \end{vmatrix} \quad D_2 = \begin{vmatrix} 1 & C \\ \sigma_1 & C\bar{\sigma}_w \end{vmatrix} \qquad (16a)$$

Hence, eq 16a becomes

$$C_1 = \begin{vmatrix} C & 1 \\ C\bar{\sigma}_w & \sigma_n \end{vmatrix} \Big/ \begin{vmatrix} 1 & 1 \\ \sigma_1 & \sigma_n \end{vmatrix} = C(\sigma_n - \bar{\sigma}_w)/(\sigma_n - \sigma_1) \qquad (16b)$$

$$C_n = K_n C_1{}^n = D_2/D = C(\bar{\sigma}_w - \sigma_1)/(\sigma_n - \sigma_1)$$

Since the apparent concentration of monomer for a nonideal solution as expressed by Adams and Williams (1964) in eq 6 is $C_1 = \alpha e^{-BM_1C}$. Using this expression and

$$\alpha = Ce^{\int_0^C \left(\frac{M_1}{M_{wapp}} - 1\right)\frac{dC}{C}}$$

the nonideality term BM_1 can be evaluated from C_1 or from $C = C_1 + K_n C_1{}^n$ using $BM_1 = 1/C \ln (\alpha/C_1)$. The equilibrium constant of monomer–n-mer association may be evaluated from

$$K_n = \frac{C - C_1}{C_1{}^n} \text{ or } C_n = K_n C_1{}^n \qquad (17)$$

Method 2A. For monomer–n-mer association, Adams (1965a,b) has shown that $C = C_1 + K_n C_1{}^n$ and $\Phi_{(w)} = C_1 + nK_n C_1{}^n$. By Kramer's rule and using (6)

$$C_1 = \alpha e^{-BM_1C} = \frac{1}{D}\begin{vmatrix} C & 1 \\ \Phi_{(w)} & n \end{vmatrix} =$$

$$\frac{1}{D}\begin{vmatrix} C & 1 \\ \dfrac{1}{\left(\dfrac{M}{CM_{wapp}} - BM_1\right)} & n \end{vmatrix} \quad (18)$$

$$C_n = K_nC_1^n = \frac{1}{D}\begin{vmatrix} 1 & C \\ 1 & \Phi_{(w)} \end{vmatrix} =$$

$$\frac{1}{D}\begin{vmatrix} 1 & C \\ 1 & \dfrac{1}{\left(\dfrac{M_1}{CM_{wapp}} - BM_1\right)} \end{vmatrix} \quad (18a)$$

where

$$D = \begin{vmatrix} 1 & 1 \\ 1 & n \end{vmatrix} = (n - 1) \quad (18b)$$

Proper substitution of eq 18b into eq 18 yields

$$(n - 1)\alpha e^{-BM_1C} = \left| nC - \frac{1}{\dfrac{M_1}{CM_{wapp}} - BM_1} \right| \quad (18c)$$

Rearrangement of eq 18c gives

$$[nC - (n - 1)\alpha e^{-BM_1C}]\left[\frac{M_1}{M_{wapp}} - BM_1C\right] = 0 \quad (18d)$$

Equation 18d can be used to evaluate the nonideality term BM_1 by successive approximation. Subroutines by interaction and elimination for matrix analyses such as these are readily available for use with computers. Notice that eq 18d is identical with that of Adams (1965a), as is shown in the appendix.

Method A3. Combining the empirical relationship of eq 14a with eq 2 gives the basic equations

$$\begin{aligned} C &= C_1 + K_nC_1^n \\ C &= (\ln n)K_nC_1^n \end{aligned} \quad (19)$$

Knowing $C_1 = \alpha e^{-BM_1C}$, eq 19 becomes

$$C_1 = \alpha e^{-BM_1C} = \frac{C}{D}\begin{vmatrix} 1 & 1 \\ \zeta & \ln n \end{vmatrix} = C\left(1 - \frac{\zeta}{\ln n}\right) \quad (20)$$

$$C_n = K_nC_1^n = C\zeta/\ln n \quad (20a)$$

The nonideality terms (BM_1) of methods 2A and 3A can be determined by applying eq 18 and 20, respectively. The equilibrium constant of this model system is evaluated from eq 17.

$$qP_1 \rightleftharpoons sP_m + rP_n \qquad n > m > 1 \quad (B)$$

Method 1B. Modified procedure of Adams (1965a,b). When three species are involved in chemical equilibrium, the following equations (eq 2, 4, and 5) are solved as simultaneous equations

$$\begin{aligned} C &= C_1 + K_mC_1^m + K_nC_1^n \\ \Phi_{(w)} &= C_1 + mK_mC_1^m + nK_nC_1^n \\ \frac{CM_1}{M_{napp}} &= C_1 + \frac{1}{m}K_mC_1^m + \frac{1}{n}K_nC_1^n + \frac{BM_1^2}{2} \end{aligned} \quad (21)$$

Applying column matrix transformation, one obtains

$$\begin{bmatrix} 1 & 1 & 1 \\ 1 & m & n \\ 1 & \frac{1}{m} & \frac{1}{n} \end{bmatrix}\begin{bmatrix} C_1 \\ C_m \\ C_n \end{bmatrix} = \begin{bmatrix} C \\ \Phi_{(w)} \\ \left(\dfrac{CM_1}{M_{napp}} - \dfrac{BM_1^2}{2}\right) \end{bmatrix} \quad (21a)$$

Note that a matrix array is distinguished from some other array by the brackets as shown here. By Kramer's rule, eq 21a becomes $C_1 = D_1/D$, $C_m = D_2/D$, and $C_n = D_3/D$, where

$$D_1 = \begin{vmatrix} C & 1 & 1 \\ \Phi_{(w)} & m & n \\ \left(\dfrac{CM_1}{M_{napp}} - \dfrac{BM_1C^2}{2}\right) & \frac{1}{m} & \frac{1}{n} \end{vmatrix}$$

$$D_2 = \begin{vmatrix} 1 & C & 1 \\ 1 & \Phi_{(w)} & n \\ 1 & \left(\dfrac{CM_1}{M_{napp}} - \dfrac{BM_1C^2}{2}\right) & \frac{1}{n} \end{vmatrix}$$

$$D_3 = \begin{vmatrix} 1 & 1 & C \\ 1 & m & \Phi_{(w)} \\ 1 & \frac{1}{m} & \left(\dfrac{CM_1}{M_{napp}} - \dfrac{BM_1C^2}{2}\right) \end{vmatrix}$$

$$D = \begin{vmatrix} 1 & 1 & 1 \\ 1 & m & n \\ 1 & \frac{1}{m} & \frac{1}{n} \end{vmatrix} \quad (21b)$$

Hence

$$C_1 = \alpha e^{-BM_1C} = \frac{1}{D}\begin{vmatrix} C & 1 & 1 \\ \Phi_{(w)} & m & n \\ \left(\dfrac{CM_1}{M_{napp}} - \dfrac{BM_1C^2}{2}\right) & \frac{1}{m} & \frac{1}{n} \end{vmatrix} \quad (21c)$$

$$C_m = K_mC_1^m = \frac{1}{D}\begin{vmatrix} 1 & C & 1 \\ 1 & \Phi_{(w)} & n \\ 1 & \left(\dfrac{CM_1}{M_{napp}} - \dfrac{BM_1C^2}{2}\right) & \frac{1}{n} \end{vmatrix} \quad (21d)$$

$$C_n = K_nC_1^n = C - C_1 - C_m \quad (21e)$$

The nonideality term, BM_1, is obtainable from eq 21c and C_1, C_m, K_m, C_n, and K_n are computed by simple substitution. The quantities $\Phi_{(w)}$ and $\Phi_{(N)}$ can be obtained from the curve of M_1/M_{wapp} *vs.* C.

$$\Phi_{(w)} = \left[\frac{1}{\dfrac{M_1}{CM_{wapp}} - BM_1}\right], \Phi_{(N)} = \left[\frac{CM_1}{M_{napp}} - \frac{BM_1C^2}{2}\right] \quad (21f)$$

Method 2B. When the weight-average partition coefficient is employed, the following sets of equations (eq 2, 4, and 14) can be transformed into matrix form

$$C = C_1 + K_mC_1^m + K_nC_1^n$$
$$\Phi_{(w)} = C_1 + mK_mC_1^m + nK_nC_1^n \quad (22)$$
$$C\bar{\sigma}_w = \sigma_1C_1 + \sigma_mK_mC_1^m + \sigma_nK_nC_1^n$$

The concentration of monomer and the nonideality term is expressed as

$$C_1 = \alpha e^{-BM_1C} = \frac{1}{D}\begin{vmatrix} C & 1 & 1 \\ \Phi_{(w)} & m & n \\ C\bar{\sigma}_w & \sigma_m & \sigma_n \end{vmatrix} \quad (22a)$$

$$C_m = K_mC_1^m = \frac{1}{D}\begin{vmatrix} 1 & C & 1 \\ 1 & \Phi_{(w)} & n \\ \sigma_1 & C\bar{\sigma}_w & \sigma_n \end{vmatrix} \quad (22b)$$

$$C_n = K_nC_1^n = \frac{1}{D}\begin{vmatrix} 1 & 1 & C \\ 1 & m & \Phi_{(w)} \\ \sigma_1 & \sigma_m & C\bar{\sigma}_w \end{vmatrix} = C - C_1 - C_m \quad (22c)$$

where determinant

$$D = \begin{vmatrix} 1 & 1 & 1 \\ 1 & m & n \\ \sigma_1 & \sigma_m & \sigma_n \end{vmatrix}$$

Method 2B is analogous to the Adams' procedure as defined in method 1B with the exception that the partition coefficient becomes a variable. It is also possible to derive two additional sets of three simultaneous equations which provide alternate procedures for evaluating BM_1.

Method 3B. The third simultaneous equation used with the two basic equations (eq 2 and 4) is based on the term λ as defined in eq 13.

$$C = C_1 + K_mC_1^m + K_nC_1^n$$
$$\Phi_{(w)} = C_1 + mK_mC_1^m + nK_nC_1^n \quad (23)$$
$$\lambda = C_1\sigma_1 + \frac{\sigma_mK_mC_1^m}{m} + \frac{\sigma_nK_nC_1^n}{n}$$

Setting up the determinants in matrix form

$$C_1 = \alpha e^{-BM_1C} = \frac{1}{D}\begin{vmatrix} C & 1 & 1 \\ \Phi_{(w)} & m & n \\ \lambda & \frac{\sigma_m}{m} & \frac{\sigma_n}{n} \end{vmatrix} \quad (23a)$$

$$C_m = K_mC_1^m = \frac{1}{D}\begin{vmatrix} 1 & C & 1 \\ 1 & \Phi_{(w)} & n \\ \sigma_1 & \lambda & \frac{\sigma_n}{n} \end{vmatrix} \quad (23b)$$

$$C_n = K_nC_1^n = \frac{1}{D}\begin{vmatrix} 1 & 1 & C \\ 1 & m & \Phi_{(w)} \\ \sigma_1 & \frac{\sigma_m}{m} & \lambda \end{vmatrix} \quad (23c)$$

or $C_n = C - C_1 - C_m$.

The equilibrium constants in this case are evaluated from eq 17 having first determined BM_1 and the weight fractions.

Method 4B. This method is empirically derived based on eq 14a. It is absolutely essential to note that the value of A in eq 14 is based on the slope of plot of σ_i *vs.* ln M_i. Because this slope is not always linear, a greater possibility of error is introduced when this method is employed.

In cases where an accurate determination of the A value can be made, then this method is theoretically valid.

$$C = C_1 + K_mC_1^m + K_nC_1^n$$
$$(w) = C_1 + mK_mC_1^m + nK_nC_1^n \quad (24)$$
$$C\zeta = 0 + (\ln m)K_mC_1^m + (\ln n)K_nC_1^n$$

Establishing matrices

$$C_1 = \alpha e^{-BM_1C} = \frac{1}{D}\begin{vmatrix} C & 1 & 1 \\ \Phi_{(w)} & m & n \\ C\zeta & \ln m & \ln n \end{vmatrix} \quad (24a)$$

$$C_m = k_mC_1^m + \frac{1}{D}\begin{vmatrix} 1 & C & 1 \\ 1 & \Phi_{(w)} & n \\ 0 & C\zeta & \ln n \end{vmatrix} \quad (24b)$$

$$C_n = K_nC_1^n = C - C_1 - C_n$$

to be solved for BM_1 and the weight fractions.

$$qP_1 \rightleftharpoons tP_l + rP_m + sP_n \qquad n > m > l > 1 \quad (C)$$

When four species are involved in chemical equilibrium, a combination of the molecular weight and weight-average partition data allows elimination of the quantities

$$-\frac{\sum C_iM_i^2}{M_1^2} \text{ and } M_1^2\sum_i(C_i/M_i^2)$$

As was described in detail in case B, four procedures can be developed from the basic eq 2, 4, and 5 solved simultaneously with equations from the partition isotherm.

Method 1C. Based on eq 14

$$C = C_1 + KC_1 + K_mC_1^m + K_nC_1^n$$
$$\Phi_{(w)} = C_1 + KC_1 + mK_mC_1^m + nK_nC_1^n$$
$$\Phi_{(N)} = C_1 + \frac{K_lC_1^l}{l} + \frac{K_mC_1^m}{m} + \frac{K_nC_1^n}{n} \quad (25)$$
$$C\bar{\sigma}_w = \sigma_1C_1 + \sigma_lK_lC_1^l + \sigma_mK_mC_1^m + \sigma_nK_nC_1^n$$

Setting up matrices

$$C_1 = \alpha e^{-BM_1C} = \frac{1}{C}\begin{vmatrix} C & 1 & 1 & 1 \\ \Phi_{(w)} & l & m & n \\ \Phi_{(N)} & \frac{1}{l} & \frac{1}{m} & \frac{1}{n} \\ C\bar{\sigma}_w & \sigma_l & \sigma_m & \sigma_n \end{vmatrix} \quad (25a)$$

It is apparent that eq 25a can be used to evaluate both BM_1 and C_1.

$$C_l = K_lC_1 = \frac{1}{D}\begin{vmatrix} 1 & C & 1 & 1 \\ 1 & \Phi_{(w)} & m & n \\ 1 & \Phi_{(N)} & \frac{1}{m} & \frac{1}{n} \\ \sigma_1 & C\bar{\sigma}_w & \sigma_m & \sigma_n \end{vmatrix} \quad (25b)$$

$$C_m = K_m C_1^m = \frac{1}{D}\begin{vmatrix} 1 & 1 & C & 1 \\ 1 & l & \Phi_{(w)} & n \\ 1 & \frac{1}{l} & \Phi_{(N)} & \frac{1}{n} \\ \sigma_1 & \sigma_l & C\bar{\sigma}_w & \sigma_n \end{vmatrix} \quad (25c)$$

$$C_n = K_n C_1^n = \frac{1}{D}\begin{vmatrix} 1 & 1 & 1 & C \\ 1 & l & m & \Phi_{(w)} \\ 1 & \frac{1}{l} & \frac{1}{m} & \Phi_{(N)} \\ \sigma_1 & \sigma_l & \sigma_m & C\bar{\sigma}_w \end{vmatrix} \quad (25d)$$

$$K_n = [C - C_1 - C_l - C_m]/C_n \quad (26)$$

Method 2C. In the four simultaneous equations in method 1C, the equation based on $C\bar{\sigma}_w$ (eq 9b) is replaced by one based on λ (eq 13), that is

$$\lambda = C_1\sigma_1 + \frac{\sigma_l K_l C_1}{l} + \frac{\sigma_m K_m C_1^m}{m} + \frac{\sigma_n K_n C_1^n}{n}$$

The concentration of monomer and BM_1 in the matrix form becomes

$$C_1 = \alpha e^{-BM_1 C} = \frac{1}{D}\begin{vmatrix} C & 1 & 1 & 1 \\ \Phi_{(w)} & l & m & n \\ \Phi_{(N)} & \frac{1}{l} & \frac{1}{m} & \frac{1}{n} \\ \lambda & \frac{\sigma_l}{l} & \frac{\sigma_m}{m} & \frac{\sigma_n}{n} \end{vmatrix} \quad (27)$$

Method 3C. Empirical eq 14a becomes the fourth variable equation to be simultaneously solved with basic eq 2, 4, and 5.

$$C\zeta = 0 + (\ln l)K_l C_1^l + (\ln m)K_m C_1^m + (\ln n)K_n C_1^n$$

In this case, C_1 term is expressed as

$$C_1 = \alpha e^{-BM_1 C} = \frac{1}{D}\begin{vmatrix} C & 1 & 1 & 1 \\ \Phi_{(w)} & l & m & n \\ \Phi_{(N)} & \frac{1}{l} & \frac{1}{m} & \frac{1}{n} \\ C\zeta & \ln l & \ln m & \ln n \end{vmatrix} \quad (28)$$

Matrices for C_l, C_m, and C_n are also established, where D is

$$D = \begin{vmatrix} 1 & 1 & 1 & 1 \\ 1 & l & m & n \\ 1 & \frac{1}{l} & \frac{1}{m} & \frac{1}{n} \\ 0 & \ln l & \ln m & \ln n \end{vmatrix}$$

Method 4C. This method employs the two empirical equations (14a and 15), one of which also incorporates M_1/M_{wapp}.

$$C\zeta = 0 + (\ln l)K_l C_1^l + (\ln m)K_m C_1^m + (\ln n)K_n C_1^n = 0 + \frac{(\ln l)}{l}K_l C_1^l + \frac{\ln m}{m}K_m C_1^m + \frac{\ln n}{n}K_n C_1^n$$

Setting up matrices

$$C_1 = \alpha e^{-BM_1 C} = \frac{1}{D}\begin{vmatrix} C & 1 & 1 & 1 \\ \Phi_{(w)} & l & m & n \\ C\zeta & \ln l & \ln m & \ln n \\ \xi & \frac{\ln 1}{l} & \frac{\ln m}{m} & \frac{\ln n}{n} \end{vmatrix} \quad (29)$$

$$\alpha e^{-BM_1 C}\begin{vmatrix} 1 & 1 & 1 & 1 \\ 1 & l & m & n \\ 0 & \ln l & \ln m & \ln n \\ 0 & \frac{\ln l}{l} & \frac{\ln m}{m} & \frac{\ln n}{n} \end{vmatrix} =$$

$$\begin{vmatrix} C & 1 & 1 & 1 \\ \Phi_{(w)} & l & m & n \\ C\zeta & \ln l & \ln m & \ln n \\ \xi & \frac{\ln l}{l} & \frac{\ln m}{m} & \frac{\ln n}{n} \end{vmatrix} \quad (29a)$$

$$C_l = \frac{1}{D}\begin{vmatrix} 1 & C & 1 & 1 \\ 1 & \Phi_{(w)} & m & n \\ 0 & C\zeta & \ln m & \ln n \\ 0 & \xi & \frac{\ln m}{m} & \frac{\ln n}{n} \end{vmatrix} \quad (29b)$$

$$C_m = \frac{1}{D}\begin{vmatrix} 1 & 1 & C & 1 \\ 1 & l & \Phi_{(w)} & n \\ 0 & \ln l & C\zeta & \ln n \\ 0 & \frac{\ln l}{l} & \xi & \frac{\ln n}{n} \end{vmatrix} \quad (29c)$$

$$C_n = \frac{1}{D}\begin{vmatrix} 1 & 1 & 1 & C \\ 1 & l & m & \Phi_{(w)} \\ 0 & \ln l & \ln m & C\zeta \\ 0 & \frac{\ln l}{l} & \frac{\ln m}{m} & \xi \end{vmatrix} \quad (29d)$$

Having shown how to obtain the quantities α and BM_1, the equilibrium constants are evaluated from eq 29e.

$$K_i = C_i/C_1^i = \frac{C_i/C}{C_1^i/C} = \frac{f_i}{f_1 C_1^{i-1}} = \frac{f_i}{f_1}(\alpha e^{-BM_1 C})^{(-i-1)} \quad (29e)$$

Although this method can be theoretically derived, it is our opinion that it demands more refined empirical measurement for A values before it can be widely applied to model systems.

$$qP_1 \rightleftharpoons qP_j + tP_l + rP_m + sP_n \qquad n > m > l > j > 1 \quad (D)$$

The methods in case D, when five species are in chemical equilibrium, employ molecular weight equations 2, 4, and 5 of Adams (1965a,b) and two additional variable equations (9b, 13, 14a, and 15).

Method 1D. This method employs eq 9b and 13 with

eq 2, 4, and 5. The equations and the determinants in matrix form are as follows.

$$C = C_1 + K_jC_1^{\,j} + K_lC_1^{\,l} + K_mC_1^{\,m} + K_nC_1^{\,n}$$

$$\Phi_{(w)} = C_1 + jK_jC_1^{\,j} + K_lC_1^{\,l} + mK_mC_1^{\,m} + nK_nC_1^{\,n}$$

$$\Phi_{(N)} = C_1 + \frac{K_jC_1^{\,j}}{j} + \frac{K_lC_1^{\,l}}{l} + \frac{K_mC_1^{\,m}}{m} + \frac{K_nC_1^{\,n}}{n} \quad (30)$$

$$C\bar{\sigma}_w = \sigma_1C_1 + \sigma_jK_jC_1^{\,j} + \sigma_lK_lC_1^{\,l} + \sigma_mK_mC_1^{\,m} + \sigma_nK_nC_1^{\,n}$$

$$\lambda = \sigma_1C_1 + \frac{\sigma_jK_jC_1^{\,j}}{j} + \frac{\sigma_lK_lC_1^{\,l}}{l} + \frac{\sigma_mK_mC_1^{\,m}}{m} + \frac{\sigma_nK_nC_1^{\,n}}{n}$$

$$C_1 = \alpha e^{-BM_1C} = \frac{1}{D}\begin{vmatrix} C & 1 & 1 & 1 & 1 \\ \Phi_{(w)} & j & l & m & n \\ \Phi_{(N)} & \frac{1}{j} & \frac{1}{l} & \frac{1}{m} & \frac{1}{n} \\ C\bar{\sigma}_w & \sigma_j & \sigma_l & \sigma_m & \sigma_n \\ \lambda & \frac{\sigma_j}{j} & \frac{\sigma_l}{l} & \frac{\sigma_m}{m} & \frac{\sigma_n}{n} \end{vmatrix} \quad (30a)$$

Method 2D. Equation 15 is substituted for equation λ from eq 30. Note that ξ is empirically derived. Resulting determinant of C_1 becomes

$$C_1 = \alpha e^{-BM_1C} = \frac{1}{D}\begin{vmatrix} C & 1 & 1 & 1 & 1 \\ \Phi_{(w)} & j & l & m & n \\ \Phi_{(N)} & \frac{1}{j} & \frac{1}{l} & \frac{1}{m} & \frac{1}{n} \\ C\bar{\sigma}_w & \sigma_j & \sigma_l & \sigma_m & \sigma_n \\ \xi & \frac{\ln j}{j} & \frac{\ln l}{l} & \frac{\ln m}{m} & \frac{\ln n}{n} \end{vmatrix} \quad (30b)$$

Method 3D. This method is based on two empirical eq 14 and 15. BM_1 and C_1 are evaluated as

$$C_1 = \alpha e^{-BM_1C} = \frac{1}{D}\begin{vmatrix} C & 1 & 1 & 1 & 1 \\ \Phi_{(w)} & j & l & m & n \\ \Phi_{(N)} & \frac{1}{j} & \frac{1}{l} & \frac{1}{m} & \frac{1}{n} \\ C\zeta & \ln j & \ln l & \ln m & \ln n \\ \xi & \frac{\ln j}{j} & \frac{\ln l}{l} & \frac{\ln m}{m} & \frac{\ln n}{n} \end{vmatrix} \quad (30c)$$

In addition, in each method of case D determinants for C_j, C_l, C_m, and C_n must be evaluated in matrix form. When BM_1 and these values are known, the equilibrium constants are evaluated from eq 29e. As with previous cases, particular care must be exercised in using such empirical equations.

The theory and analytical methods presented in cases A–D can be used as a check on the values of BM_1 obtained from molecular weight data alone (see Appendix I).

INDEFINITE ASSOCIATION. The methods to be used in the theoretical consideration of indefinite association are identical with the procedure outlined in part III (Chun *et al.*, 1969) of this series and by Adams (1965a,b).

Method 1. In part III (Chun *et al.*, 1969), a method for obtaining the weight fraction of monomer from the weight-average partition coefficient has been demonstrated. After the weight fraction of monomer f_1 is evaluated from the partition coefficient, intrinsic equilibrium constants can be obtained using

$$\bar{\sigma}_w = \sum_i i\sigma_i f_1(1 - \sqrt{f_1})^{i-1}$$

$$k = \frac{1}{C_1}(1 - \sqrt{f_1}) = \frac{1}{Cf_1}(1 - \sqrt{f_1}) \quad (31)$$

The resulting intrinsic equilibrium constant, denoting quantities consistent with the Adams notation, may be compared with that obtained from Adams and Lewis (1968) using the expression

$$kC_1 = 1 - \frac{M_1}{M_{napp}} + \frac{\hat{B}M_1C}{2} \quad (32)$$

Equation 31 can be combined with $M_1/M_{napp} = ((1 - kC)/(1 + kC_1)) + \hat{B}M_1C$ in order to evaluate the nonideality term $\hat{B}M_1$.

$$\hat{B}M_1 = \frac{1}{C}\left[\frac{M_1}{M_{wapp}} + \frac{1}{1 - \frac{2}{\sqrt{f_1}}}\right] \quad (33)$$

Method 2. This method employs an empirical equation as given in part III (Chun *et al.*, 1969).

$$\bar{\sigma}_w = \sigma_1 - A\sum_i i \ln i[f_1(1 - \sqrt{f_1})^{i-1}] \quad (34)$$

Letting $Z(f_1) = (\sigma_1 - \bar{\sigma}_w)/A$, eq 34 becomes

$$Z(f_1) = \sum_i i \ln i[f_1(1 - \sqrt{f_1})^{i-1}] \quad (35)$$

Hence

$$f_1 = Z^{-1}(\sigma_1 - \bar{\sigma}_w)/A \quad (35a)$$

Using Table I of part III (Chun *et al.*, 1969), f_1 may be obtained. Then the intrinsic equilibrium constant is computed from f_1 using the following expression.

$$K = \frac{1}{CZ^{-1}\left(\frac{\sigma_1 - \bar{\sigma}_w}{A}\right)}\left[1 - \sqrt{Z^{-1}\left(\frac{\sigma_1 - \bar{\sigma}_w}{A}\right)}\right] \quad (36)$$

Thus,

$$\hat{B}M_1 = \frac{1}{C}\left|\frac{M_1}{M_{wapp}} + \frac{1}{1 - \frac{2}{\sqrt{Z^{-1}\left(\frac{\sigma_1 - \bar{\sigma}_w}{A}\right)}}}\right| \quad (37)$$

TABLE I: Values of BM_1, the Nonideality Term, for Discrete Association Models Based on Molecular Weight *vs.* Concentration from Eisenberg and Tomkins (1968). pH 7.0, 0.2 M Phosphate Buffer–10^{-3} M EDTA.[a]

C (g/l.)	M_1/M_{wapp}	M_1/M_{napp}	α	$\frac{d}{dc}\left(\frac{M_1}{CM_{wapp}}\right)$	BM_1 Value of Discrete Assocns					
					1–6	1–7	1–8	1–9	1–3–6	1–4–8
0	1.0000									
0.1	0.7351	0.8050	0.0754							
0.3	0.5513	0.7042	0.1450							
0.5	0.5110	0.6470	0.2023	−0.50						
1.0	0.3763	0.5325	0.2746	−5.39	0.1700	0.2000	0.2220	0.2390	−1.880	
1.5	0.3084	0.4677	0.3144	−2.03	0.0739	0.0927	0.1068	0.1178	−1.510	−1.4329
2.0	0.2739	0.4241	0.3432	−1.00	0.0410	0.0547	0.0650	0.0729	−1.2735	−1.2128
3.0	0.2348	0.3673	0.3813	−0.360	0.0165	0.0253	0.0319	0.0370	−0.9874	−0.9449
4.0	0.2153	0.3314	0.4092	−0.189	0.0084	0.0149	0.0197	0.0236	−0.8142	−0.7813
5.0	0.2026	0.3072	0.4335	−0.129	0.0050	0.0088	0.0136	0.0166	−0.6960	−0.6690
6.0	0.1957	0.2893	0.4488	−0.068	0.0030	0.0072	0.0104	0.0129	−0.6127	−0.5901
7.0	0.1892	0.2755	0.4718	−0.048	0.0018	0.0054	0.0081	0.0102	−0.5447	−0.5251
8.0	0.1835	0.2645	0.4880	−0.040	0.0010	0.0041	0.0065	0.0083	−0.4930	−0.4575

[a] The BM_1 values of 1-2-3-4 and 1-2-3-4-5 discrete association were also computed, along with the modes of association represented above. The equilibrium constants of discrete cases were found to be inconsistent. Only in the cases of indefinite association was a unit of BM_1 values evident.

The quantity needed to solve eq 33 and 37 is

$$\frac{M_1}{M_{napp}} = \frac{1}{C}\int_0^C \frac{M_1}{M_{wapp}}dC$$

and the weight fraction from the weight-average partition coefficient.

Bovine Liver L-*Glutamate Dehydrogenase.* In Table I, it is shown how the BM_1 values test for the various possible types of association that are thought to be present. The equations for the various types of discrete association are described separately in Appendix I. In a recent publication, Sund and Burchard (1968) have proposed that bovine liver L-glutamate dehydrogenase at 20° in pH 7.6, 0.15 M potassium–sodium phosphate buffer undergoes a stepwise association–dissociation equilibrium of eight subunits. Coleman and Frieden (1966) proposed a 1–8 association without intermediate steps. A glance at the BM_1 values for 1–8 association (Table I) will show inconsistency which would eliminate it as the mode of association in operation here. Such a conclusion is further supported by a comparison of the sigmoidal curve calculated by Sund and Burchard with that generated by our own results (Figure 2 of Chun *et al.*, 1969). The curve for indefinite association fits much more precisely to the experimental curve than that for 1–8 discrete association.

Our results gave us confidence that the possibility of discrete association may be eliminted. The nonideality term BM_1 and intrinsic equilibrium constant for bovine liver L-glutamate dehydrogenase evaluated by Adams' procedure for indefinite association are shown in Table II. The results are consistent with those obtained using partition coefficient data shown in Table III. Our computations demonstrated that both the nonideality terms from molecular weight and partition data show a striking degree of correlation. However, the partition coefficient as a function of concentration generated from the empirical equation does not fit the experimentally observed curve, evidence of the fact that eq 37 must be used with reservation. The correlation of eq 33, however, with molecular weight data is very good. All values for BM_1 and equilibrium constants as determined from both molecular weight and partition data strongly support the conclusion that the model enzyme undergoes linear aggregation, as described in detail in Chun *et al.* (1969). The theoretical evaluation of interaction parameters here derived furnishes two methods for the determination and rechecking of nonideality terms, equilibrium constants, and mode of association of various model systems.

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Appendix[2] I

Evaluation of nonideality term BM_1 based on the sedimentation equilibrium and light-scattering experiments varies with the mode of association as summarized below (further elucidation of the terms of

[2] A stepwise regression analysis by computer to determine the mode of association and the equilibrium constants of reversibly interacting protein systems in part II (Chun *et al.*, 1968) using molecular weight as a function of concentration is described.

TABLE II: Values of BM_1, the Nonideality Term, for Bovine Liver L-Glutamate Dehydrogenase Based on Molecular Weight Data for Indefinite Association. $\overline{M}_w$ *vs.* C data were Taken from Eisenberg and Tomkins (1968).[a]

		Indefinite Association					
C (g/l.)	C (g/ml)	BM_1	k	C_1	$(M_1/M_{wapp})_{calcd}$	$(M_1/M_{napp})_{calcd}$	$(\alpha) \times 10^{-3}$
0	0						
0.1	10^{-4}			0.76×10^{-4}	0.7756	0.8735	0.76×10^{-4}
0.3	3×10^{-4}			1.61×10^{-4}	0.5797	0.7334	1.6×10^{-4}
0.5	5×10^{-4}			2.11×10^{-4}	0.4839	0.6510	2.1×10^{-4}
1.0	1.0×10^{-3}			2.82×10^{-4}	0.3677	0.5342	2.8×10^{-4}
1.5	1.5×10^{-3}			3.23×10^{-4}	0.3111	0.4684	3.2×10^{-4}
2.0	2×10^{-3}			3.50×10^{-4}	0.2768	0.4244	3.5×10^{-4}
3.0	3×10^{-3}	5.23	1652.4	3.86×10^{-4}	0.2370	0.3678	3.9×10^{-4}
4.0	4×10^{-3}	6.48	1680.2	4.09×10^{-4}	0.2150	0.3321	4.2×10^{-4}
5.0	5×10^{-3}	6.46	1674.0	4.26×10^{-4}	0.2017	0.3073	4.4×10^{-4}
6.0	6×10^{-3}	6.69	1680.2	4.4×10^{-4}	0.1933	0.2889	4.5×10^{-4}
7.0	7×10^{-3}	6.30	1660.6	4.5×10^{-4}	0.1881	0.2749	4.7×10^{-4}
8.0	8×10^{-3}	5.78	1627.4	4.6×10^{-4}	0.1855	0.2638	4.8×10^{-4}

[a] $BM_1 = 6.16$, $k = 1660$ ml/g.

these equations is found in Adams and Williams (1964) in Adams (1965a,b, 1967) and in Adams and Lewis (1968)).

$$nP_1 \rightleftharpoons P_n \qquad n = 2, 3, 4 \ldots \qquad \text{(A-1)}$$

$$[nC - (n-1)\alpha e^{-BM_1C}]\left[\frac{M_1}{M_{wapp}} - BM_1C\right] - C = 0$$

$$nP_1 \rightleftharpoons qP_m + rP \qquad l > m > 1,\ m = 2, 3, 4 \ldots \qquad \text{(A-2)}$$

$$lm\left[\frac{CM_1}{M_{napp}} - \frac{BM_1C^2}{2}\right] + \left[\frac{1}{\frac{M_1}{CM_{wapp}} - BM_1}\right] - (l+m)C = (l-1)(m-1)\alpha e^{-BM_1C}$$

$$nP_1 \rightleftharpoons qP_m + rP + sP_j \qquad j > l > m,\ m = 2, 3, 4 \ldots \qquad \text{(A-3)}$$

$$lmj\left[\frac{M_1C}{M_{napp}} - \frac{BM_1C^2}{2}\right] - (lm + mj + jl)C + (m + l + j)\Phi_{(w)} + \varphi = (l-1)(j-1) \times (m-1)\alpha e^{-BM_1C}$$

where

$$\Phi_{(w)} = \frac{1}{\left[\frac{M_1}{CM_{wapp}} - BM_1\right]} \quad \text{and}$$

$$\varphi = -\frac{\Sigma C_i M_i^2}{M_1^2} = \frac{\frac{d}{dC}\left[\frac{M_1}{CM_{wapp}}\right]}{\left[\frac{M_1}{CM_{wapp}} - BM_1\right]^3}$$

$$nP_1 \rightleftharpoons qP_m + rP_l + sP_j + tP_i \qquad i > j > l > m > 1,\ m = 2, 3, 4 \ldots \qquad \text{(A-4)}$$

TABLE III: Values of BM_1, the Nonideality Term, for Bovine Liver L-Glutamate Dehydrogenase, Based on the Combination of Molecular Weight and Partition Data.[a]

C (g/ml)	M_1/M_{wapp}	$\bar{\sigma}_w$	f_1	BM_1
1.0	0.3763	0.5330	0.280	6.50
2.0	0.2739	0.470	0.180	6.01
3.0	0.2348	0.428	0.135	5.95
4.0	0.2153	0.402	0.112	6.10

[a] $BM_1 = 6.14$; $k = 1650$ (ml/g). The weight-average partition coefficient as a function of concentration data was run at pH 7.0, 0.2 M sodium phosphate buffer–10^{-3} M EDTA at 25°. Column gel was composed of Sepharose-4B.

$$\hat{B}M_1 = \frac{1}{C}\left[\frac{M_1}{M_{wapp}} + \frac{1}{1 - \frac{2}{\sqrt{f_1}}}\right]$$

$$ijml\left\{\int_0^C \frac{M_1^2 dC}{M_{wapp}M_{napp}} + \frac{(BM_1)^2C^3}{6} - BM_1 \times \int_0^C \left[\frac{M_1}{M_{napp}} - \frac{M_1}{2M_{wapp}}\right]CdC\right\} - (ijl + jlm + lmi + mij) \times \left[\frac{CM_1}{M_{napp}} + (ij + il + jl + im + jm + lm)C - (i + j + l + m)\Phi_{(w)} - \varphi - (i-1)(j-1) \times (l-1)(m-1)\alpha e^{-BM_1C}\right] = 0$$

For example, in 1-2-3-4-5 association, $m = 2$, $l = 3$, $j = 4$, $i = 5$, the term for evaluating BM_1 becomes

$$120\tau - 154\frac{CM_1}{M_{\text{napp}}} - 71C + 14\Phi_{(w)} + \varphi - 24\alpha e^{-BM_1C} = 0$$

where

$$\tau = \left\{\int_0^C \frac{M_1{}^2 dC}{M_{\text{wapp}}M_{\text{napp}}} + \frac{(BM_1)^2C^3}{6} - BM_1\int_0^C\left[\frac{M_1}{M_{\text{napp}}} - \frac{M_1}{2M_{\text{wapp}}}\right]CdC\right\}$$

Indefinite Association. The product of the intrinsic equilibrium constant and concentration is less than 1, $kC_1 < 1$.

$$\left(\frac{2}{\rho} - 1\right)\left(\frac{M_1}{M_{\text{wapp}}} - \hat{B}M_1C\right) - 1 = 0 \quad \text{(A-5)}$$

where

$$\rho = \left(\frac{M_1}{M_{\text{napp}}} - \frac{\hat{B}M_1C}{2}\right)$$

Appendix II

The evaluation of the nonideality term BM_1 based on the combination of the weight-average partition coefficient and molecular weight experiments when γC does not equal zero is more involved than when this term is minute enough to be ignored at low concentration. The quantity to be measured experimentally thus becomes not $\tilde{\sigma}_w$ but $\tilde{\sigma}_{\text{wapp}} = \tilde{\sigma}_{w(c)} + \gamma C$.

$$C\tilde{\sigma}_{\text{wapp}} - \gamma C^2 = \sum_i \sigma_i K_i C_1{}^i \quad (38)$$

Combining eq 38 with the definition of eq 10

$$\int_0^C \frac{M_1}{M_{w(c)}}\tilde{\sigma}_{w(c)}dC = \int_0^C \frac{M_1}{M_{\text{wapp}}}\tilde{\sigma}_{\text{wapp}}dC - \gamma\int_0^C \frac{M_1}{M_{\text{wapp}}}CdC - BM_1\int_0^C \tilde{\sigma}_{\text{wapp}}CdC + \frac{\gamma BM_1C^3}{3} = A_1 - \gamma A_2 - BM_1A_3 + \frac{\gamma BM_1C^3}{3} \quad (39)$$

where

$$A_1 = \int_0^C \frac{M_1}{M_{\text{wapp}}}\tilde{\sigma}_{\text{wapp}}dC$$

$$A_2 = \int_0^C \frac{M_1}{M_{\text{wapp}}}CdC$$

$$A_3 = \int_0^C \tilde{\sigma}_{\text{wapp}}CdC$$

From eq 11a and 39

$$\sum_i \frac{\sigma_i K_i C_1{}^i}{i} = A_1 - \gamma\left(A_2 - \frac{C^2}{3}BM_1\right) - BM_1A_3$$

$$\gamma C^2 = \left[(A_1 - BM_1A_3)\Big/\left(\frac{A_2}{C^2} - \frac{1}{3}BM_1\right)\right] - \left[\frac{1}{\left(\frac{A_2}{C^2} - \frac{1}{3}BM_1\right)}\sum_i \frac{\sigma_i K_i C_1{}^i}{i}\right] \quad (40)$$

Substituting eq 40 into eq 38

$$C\tilde{\sigma}_{\text{wapp}} - \left[(A_1 - BM_1A_3)\Big/\left(\frac{A_2}{C^2} - \frac{1}{3}BM_1\right)\right] = \sum_i\left[1 - \frac{1}{i\left[\frac{A_2}{C^2} - \frac{1}{3}BM_1\right]}\right]\sigma_i K_i C_1{}^i \quad (41)$$

Letting

$$\tau = \sum_i \eta_i K_i C_1{}^i$$

$$\tau = \tilde{\sigma}_{\text{wapp}}\left(\frac{A_2}{C} - \frac{1}{3}BM_1C\right) - A_1 + BM_1A_3 \quad (42)$$

$$\eta_i = \left(\frac{A_2}{C^2} - \frac{1}{3}BM_1 - \frac{1}{i}\right)\sigma_i$$

τ is introduced to eliminate the γ term from eq 38.

$$qP_1 \rightleftharpoons rP_n \qquad n > 1 \quad \text{(I)}$$

$$C_1 = \alpha e^{-BM_1C} = \begin{vmatrix} C & 1 \\ \tau & \eta_n \end{vmatrix}\Big/\begin{vmatrix} 1 & 1 \\ \eta_1 & \eta_n \end{vmatrix} = (C\eta_n - \tau)/(\eta_n - \eta_1)$$

$$qP_1 \rightleftharpoons sP_m + \gamma P_n \qquad n > m > 1 \quad \text{(II)}$$

$$C_1 = \alpha e^{-BM_1C} = \frac{1}{D}\begin{vmatrix} C & 1 & 1 \\ \Phi_{(w)} & m & n \\ \tau & \eta_m & \eta_n \end{vmatrix} \quad D = \begin{vmatrix} 1 & 1 & 1 \\ 1 & m & n \\ \eta_1 & \eta_m & \eta_n \end{vmatrix}$$

$$C_m = K_mC_1{}^m = \frac{1}{D}\begin{vmatrix} 1 & C & 1 \\ 1 & \Phi_{(w)} & n \\ \eta_1 & \tau & \eta_n \end{vmatrix} C_n = K_nC_1{}^n = C - C_1 - C_m$$

$$qP_1 \rightleftharpoons tP_l + \gamma P_m + sP_n \quad \text{(III)}$$

$$C_1 = \alpha e^{-BM_1C} = \frac{1}{D}\begin{vmatrix} C & 1 & 1 & 1 \\ \Phi_{(w)} & l & m & n \\ \Phi_{(N)} & \frac{1}{l} & \frac{1}{m} & \frac{1}{n} \\ \tau & \eta_l & \eta_m & \eta_n \end{vmatrix}$$

$$D = \begin{vmatrix} 1 & 1 & 1 & 1 \\ 1 & l & m & n \\ 1 & \frac{1}{l} & \frac{1}{m} & \frac{1}{n} \\ \eta_1 & \eta_l & \eta_m & \eta_n \end{vmatrix}$$

$$C_j = K_l C_1{}^j = \frac{1}{D}\begin{vmatrix} 1 & C & 1 & 1 \\ 1 & \Phi_{(w)} & m & n \\ 1 & \Phi_{(N)} & \frac{1}{m} & \frac{1}{n} \\ \eta_1 & \tau & \eta_m & \eta_n \end{vmatrix}$$

$$C_m = K_m C_1{}^m = \frac{1}{D}\begin{vmatrix} 1 & 1 & C & 1 \\ 1 & l & \Phi_{(w)} & n \\ 1 & \frac{1}{l} & \Phi_{(N)} & \frac{1}{n} \\ \eta_1 & \eta_l & \tau & \eta_n \end{vmatrix}$$

$$C_n = K_n C_1{}^n = C - C_1 - C_l - C_m$$

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Phenylalanine Transfer Ribonucleic Acid from *Escherichia coli* B. Isolation and Characterization of Oligonucleotides from Ribonuclease T_1 and Ribonuclease A Hydrolysates*

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ABSTRACT: Phenylalanine transfer ribonucleic acid of *Escherichia coli* B, exhaustively hydrolyzed by either ribonuclease T_1 or ribonuclease A, yields a total of 34 oligonucleotides. These have been isolated and characterized, and account for all the bases present in the molecule, including 4-thiouracil, which is apparently in an unstable state. The oligonucleotide from each enzyme hydrolysate that contains the single 7-methylguanosine residue is routinely isolated in only 60–70% yield.

E. coli B phenylalanine transfer ribonucleic acid has an unique anticodon region, A-ψ-U-G-G-A-A-A-ψ, that includes three possible anticodons in an overlapping configuration. The unexpected anticodon triplet GGA is in the most favored position stereochemically. The full potential of the remaining overlaps may be realized if the protein-synthesizing complex undergoes a conformational shift so as to utilize AAA for the UUU codon and GAA for the UUC codon. On the other hand, as in yeast phenylalanine transfer ribonucleic acid, only the GAA sequence is needed to fulfill the anticodon requirements.

The large number of known tRNA sequences has permitted a number of structural comparisons (Holley *et al.*, 1965; Goodman *et al.*, 1968; Dube *et al.*, 1968; Bayev *et al.*, 1967; RajBhandary and Chang, 1968; Zachau *et al.*, 1966; Madison and Kung, 1967; Takemura *et al.*, 1968; Madison, 1968). By judicious choice of deleted regions it is possible to align all the known tRNA sequences so that there are a large number of sequence positions where the same base occurs (Jukes, 1966; Madison, 1968). Attempts to correlate these structural features unequivocally with the variety of biological roles attributed to tRNA have been hampered by the absence of independent measures of function. The location of the amino acid acceptor site has been readily proven because of the relative ease of isolation of covalently linked aminoacyl ester. However, the enzyme recognition site and the topological interactions of the tRNA with ribosomes and mRNA are

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