Biophysical Chemistry, 34 (1989) 143–153
Elsevier

BIOCHE 01387

Osmometric analysis of quasi-indefinite mixed associations of the first kind

E.T. Adams, Jr

Chemistry Department, Texas A&M University, College Station, TX 77843, U.S.A.

Received 17 April 1989
Accepted 28 June 1989

Membrane osmometry; Vapor pressure osmometry; Binding degree, number average; Molecular weight, number average; Osmotic coefficient; Binding polynomial; Equilibrium constant; Nonideal term

Mixed associations of the type $A+B \leftrightarrows AB$, $A+AB \leftrightarrows A_2B$, ..., $A+A_{i-1}B \leftrightarrows A_iB$, ... are readily analyzed by osmometric methods. The equilibrium molar concentration of A, m_A, is obtained very simply from $m_A = m^{eq} - m_B^0$; here $m^{eq} = c/M_n^{eq}$ is the equilibrium molar concentration of all associating species and m_B^0 denotes the stoichiometric or original molar concentration of B. The quantity m_B can then be obtained from methods developed by Steiner. The value of the binding polynomial λ is given by $\lambda = m_B^0/m_B$; λ is a function of m_A only. In principle, one can evaluate the equilibrium constants ($k_{A,B}$, etc.) by fitting λ to the appropriate polynomial in m_A of degree n ($n = 2, 3, \ldots$). The binding polynomial λ is analogous to polynomials encountered in the analysis of self-associations. By making some simple assumptions one can develop four analogs of two sequential, equal equilibrium constant (SEK) or two attenuated equilibrium constant (AK) models. With the aid of r (the number average degree of binding), g (the osmotic coefficient), λ, as well as m_A and m_B, one can evaluate the equilibrium constant or constants. The methods developed here can be extended to the nonideal case.

1. Introduction

Noncovalent (weak) interactions play an important role in biology and biochemistry [1]. Membrane components and also lipoproteins are held together by noncovalent interactions [2–6]. Mixed associations between unlike molecules are due to noncovalent interactions and are of great importance. Examples of these mixed associations are antigen-antibody reactions [7,8], binding of smaller molecules to larger ones [9,10], and various heterogeneous protein-protein interactions [11–16]. Mixed associations also occur between smaller molecules; for example, in $CHCl_3$ solutions cholesterol and methyl cholate [17] and 1-cyclohexyluracil and 9-ethyladenine undergo mixed associations [18]. In aqueous solutions the mixed association between purine and uridine has been studied by vapor pressure osmometry [19].

Mixed associations can be studied by a variety of methods: sedimentation equilibrium [15,16,20], elastic light scattering [20,21], analytical gel chromatography [22,23], and membrane or vapor pressure osmometry [14,17–19,24] have been used. Osmometric methods offer an advantage in studying mixed associations, since one does not need to know refractive index increments (which are needed in light scattering and some sedimentation equilibrium experiments), nor does one need to know density increments or apparent partial specific volumes (these are needed for sedimentation equilibrium experiments) [20]. The analysis of mixed associations by osmometric (colligative) methods is simpler [24,25], and some very elegant

Correspondence address: E.T. Adams, Jr, Chemistry Department, Texas A&M University, College Station, TX 77843, U.S.A.

0301-4622/89/$03.50

methods have been developed for analyzing ideal or nonideal mixed associations [24,26–28].

Here, a re-examination of mixed associations of the type

$$\begin{aligned} &\mathrm{A + B \leftrightharpoons AB} \\ &\mathrm{A + AB \leftrightharpoons A_2B} \\ &\dots \qquad \dots \\ &\mathrm{A + A_{\mathit{i}-1}B \leftrightharpoons A_{\mathit{i}}B} \\ &\dots \qquad \dots \end{aligned} \tag{1}$$

will be considered. These associations are analogous to binding equilibria. Firstly, the general case will be re-examined in light of new developments. Then a special case will be considered. Thirdly, the treatment of nonideality will be considered. Then a discussion of four types of indefinite mixed associations will be presented, and some methods for analyzing them will be shown. These methods are similar to those used in the analysis of indefinite self-associations [24,29,30]. The treatment presented here begins with some background information and a discussion of previous studies.

2. Background

At constant temperature and pressure the condition for chemical equilibrium is [24]

$$n\mu_{\mathrm{A}} + \mu_{\mathrm{B}} = \mu_{\mathrm{A}_n\mathrm{B}}, \quad n = 1, 2, \dots \tag{2}$$

for a mixed association of the type

$$n\mathrm{A} + \mathrm{B} \leftrightharpoons \mathrm{A}_n\mathrm{B} \tag{3}$$

It will be assumed in the development that follows that the natural logarithms of the activity coefficients of the reactants can be expressed as

$$\ln y_i = M_i \sum B_{ik}^* c_k^0, \quad i, k = \mathrm{A} \text{ or } \mathrm{B} \tag{4}$$

c_k^0 = initial concentration (in g/l) of component k

$$B_{ik}^* = \frac{1}{M_i} \frac{(\partial \ln y_i)}{(\partial c_k^0)_{T,P,c_i^0 \neq k}}$$

It is also assumed that

$$\ln y_{\mathrm{A}_n\mathrm{B}} = n \ln y_{\mathrm{A}} + \ln y_{\mathrm{B}} \tag{5}$$

It should be noted that $B_{ik}^* = B_{ki}^*$. The osmotic pressure equation becomes

$$\frac{\pi}{RT} = \frac{c}{M_{\mathrm{na}}} = \frac{c}{M_{\mathrm{n}}^{\mathrm{eq}}} + \tfrac{1}{2} \sum_i \sum_k B_{ik} c_i^0 c_k^0 \tag{6}$$

where (assuming the partial specific volumes, $\bar{v}_k$, are equal)

$$B_{ik} = B_{ik}^* + (\bar{v}/1000 M_k) \tag{7}$$

The analogous equation for vapor pressure osmometry (VPO) is [24]

$$E = K_{\mathrm{VP}}(c/M_{\mathrm{na}}) \tag{8}$$

where E is the imbalance (μV) on the Wheatstone bridge circuit of the VPO and K_{VP} the operational instrumental constant for the VPO (see refs. 17, 24 and 25 for more details on VPO). The quantity c/M_{na} is the apparent number of moles of solute at chemical equilibrium, c the total concentration of associating solute (in g/l) and M_{na} the apparent number average molecular weight. For the ideal case $B_{ik} = 0$ and $M_{\mathrm{na}} = M_{\mathrm{n}}^{\mathrm{eq}}$, the number average molecular weight. If no chemical equilibrium is present, then $c/M_{\mathrm{n}}^{\mathrm{eq}} = c/M_{\mathrm{n}}^0$, the stoichiometric number of moles of the solute. Here, M_{n}^0 is the number average molecular weight that would be measured if no chemical equilibrium is present; one notes that

$$m^0 = \frac{c}{M_{\mathrm{n}}^0} = \frac{c_{\mathrm{A}}^0}{M_{\mathrm{A}}} + \frac{c_{\mathrm{B}}^0}{M_{\mathrm{B}}} = m_{\mathrm{A}}^0 + m_{\mathrm{B}}^0 \tag{9}$$

where m^0 is the total molar concentration of the solutes, m_i^0 (i = A or B) the total molar concentration of each reactant and c_i^0 the total concentration (g/l) of each reactant. It is convenient to perform each series of experiments under conditions of constant β; here

$$\beta = \frac{m_{\mathrm{B}}^0}{m_{\mathrm{A}}^0} = (c_{\mathrm{B}}^0/c_{\mathrm{A}}^0)(M_{\mathrm{A}}^0/M_{\mathrm{B}}^0) \tag{10}$$

Stock solutions of pure A and pure B are prepared; dialysis is carried out in the appropriate medium on each stock solution, if necessary. The concentration of each stock solution is determined, and separate osmotic pressure (or VPO) experiments are made on each stock solution in order to determine M_A, M_B, B_{AA} and B_{BB} (the osmotic pressure second virial coefficients for A and B). The stock solutions of A and B are mixed in the desired ratio β to make a working stock solution; dilutions are made from this working stock so that a series of osmotic pressure (or VPO) experiments can be performed at constant β.

For the nonideal case

$$\begin{aligned}\frac{c}{M_{na}} &= \frac{c}{M_n^{eq}} + \frac{B_{AA}\left(c_A^0\right)^2}{2} + \frac{B_{BB}\left(c_B^0\right)^2}{2} + B_{AB}c_A^0c_B^0 \\ &= \frac{c}{M_n^{eq}} + \frac{B_{AA}M_A^2\left(m_A^0\right)^2}{2} + \frac{B_{BB}M_B^2\left(m_B^0\right)^2}{2} \\ &\quad + B_{AB}M_AM_Bm_A^0m_B^0 \\ &= m_{app} \end{aligned} \tag{11}$$

Here $c/M_n^{eq} = \Sigma c_i/M_i = \Sigma m_i$, $m_i = c_i/M_i$ is the number of moles of reacting species i at chemical equilibrium, and $m_i^0 = c_i^0/M_i$ denotes initial molar concentration of reactant i. This formulation is consistent with previous treatments. There may appear to be some confusion here since Steiner uses (1/2) $B_{AB}c_A^0c_B^0$; however, (see p. 4272, 2nd column, of ref. 28), Steiner's $B_{ii} = 2\beta_{ii}/M_i^2$ and his $B_{AB} = 4\beta_{AB}/M_AM_B$, so our formulation of c/M_{na} is consistent with his. Eq. 11 can be rearranged to give

$$\begin{aligned}\frac{c}{M_{na}^*} &= \frac{c}{M_{na}} - \frac{B_{AA}\left(c_A^0\right)^2}{2} - \frac{B_{BB}\left(c_B^0\right)^2}{2} \\ &= \frac{c}{M_n^{eq}} + B_{AB}c_A^0c_B^0 = m^* \end{aligned} \tag{12}$$

For the quasi-ideal cases B_{AA} and B_{BB} are nonzero, but $B_{AB} = 0$.

2.1. Previous status

Here we follow Steiner's [21,26,28] treatment for various equilibria of the type

$$\begin{aligned} &A + B \leftrightarrows AB && m_{AB} = k_{A,B}m_Am_B \\ &A + AB \leftrightarrows A_2B && m_{A_2B} = k_{A,AB}k_{A,B}m_A^2m_B \\ &A + A_{n-1}B \leftrightarrows A_nB && m_{A_iB} = \prod_{n=0}^{n=i-1} k_{A_nB,A}m_A^im_B \end{aligned} \tag{13}$$

The following relations apply:

$$\begin{aligned} m_A^0 &= c_A^0/M_A \\ &= m_A + k_{A,B}m_Am_B + 2k_{A,B}k_{A,AB}m_A^2m_B + \ldots \\ &= m_A + \sum_i im_{A_iB} \end{aligned} \tag{14}$$

$$\begin{aligned} m_B^0 &= c_B^0/M_B \\ &= m_B + k_{A,B}m_Am_B + k_{A,B}k_{A,AB}m_A^2m_B + \ldots \\ &= \sum_i m_{A_iB} \end{aligned} \tag{15}$$

The total number of moles of reacting solute at chemical equilibrium is

$$\begin{aligned} m^{eq} = \frac{c}{M_n^{eq}} = \sum_i \frac{c_i}{M_i} &= m_A + m_B + k_{A,B}m_Am_B \\ &\quad + k_{A,B}k_{A,AB}m_A^2m_B + \ldots = m_A + m_B^0 \end{aligned} \tag{16}$$

Here

$$M_n^{eq} = \frac{\Sigma m_iM_i}{\Sigma m_i} = \frac{c}{\Sigma c_i/M_i} \tag{17}$$

The stoichiometric total number of moles of reacting solute is

$$\begin{aligned} m^0 = \frac{c}{M_n^0} &= \frac{c_A^0}{M_A} + \frac{c_B^0}{M_B} = m_A^0 + m_B^0 \\ &= m_A + m_B + 2k_{A,B}m_Am_B \\ &\quad + 3k_{A,B}k_{A,AB}m_A^2m_B + \ldots \\ &= m_A + \sum_{i=0}^{q} (i+1)m_{A_iB} \end{aligned} \tag{18}$$

The number average number of moles of A combined with one mole of B is r, which is defined by

$$\begin{aligned} r = \frac{\sum im_{A_iB}}{\sum m_{A_iB}} &= \frac{\left(\partial \ln m_B^0\right)}{\left(\partial m_A\right)_{m_B}} = \frac{d \ln \lambda}{d \ln m_A} \\ &= \left\{k_{A,B}m_A + 2k_{A,B}k_{A,AB}m_A^2 \right. \\ &\quad \left. + 3k_{A,B}k_{A,AB}k_{A,A_2B}m_A^3 + \ldots\right\} \\ &\quad \times \left\{1 + k_{A,B}m_A + k_{A,B}k_{A,AB}m_A^2 \right. \\ &\quad \left. + k_{A,B}k_{A,AB}k_{A,A_2B}m_A^3 + \ldots\right\}^{-1} \end{aligned} \tag{19}$$

Since (see eq. 16)

$$c/M_n^{eq} = m^{eq} = m_A + m_B^0 = m_A^0 + m_B^0 - \sum i m_{A_iB} \tag{20}$$

r is related to m^{eq} by

$$\begin{aligned} r &= 1 + \left(M_B/c_B^0\right)\left[\left(c_A^0/M_A\right) - \left(c/M_n^{eq}\right)\right] \\ &= 1 + \frac{\left(m_A^0 - m^{eq}\right)}{m_B^0} \\ &= 1 + (1/\beta) - \left(m^{eq}/m_B^0\right) \end{aligned} \tag{21}$$

This relation between r and c/M_n^{eq} (or m^{eq}) was pointed out by Steiner [21]. The binding polynomial λ is defined by

$$\lambda(m_A) = 1 + k_{A,B}m_A + k_{A,B}k_{A,AB}m_A^2 + \ldots \tag{22}$$

Steiner [21] showed that one could obtain ln λ from

$$\ln \lambda = \int_0^{m_A} (r/m_A)\, \mathrm{d}m_A \tag{23}$$

Once λ was known, then one could use it to obtain the equilibrium constants $k_{A,B}$, $k_{A,AB}$, etc. Subsequent developments have shown that there is an alternative way to obtain λ.

In 1968, Steiner [26] developed a relationship between x_A and x_B, the equilibrium number fractions of A and B; his relation is

$$\begin{aligned} \Phi &= \ln x_A + \beta \ln x_B \\ &= (1+\beta)\int_0^{m^{eq}} (g-1)\, \mathrm{d}m^{eq}/m^{eq} \\ &\quad + \ln\left[\frac{1}{1+\beta}\right] + \beta \ln\left[\frac{\beta}{1+\beta}\right] \end{aligned} \tag{24}$$

Here g is the osmotic coefficient (Steiner used α_n^{-1} in place of g); it is defined by

$$g = \frac{\pi(\text{real})}{\pi(\text{ideal})} = \frac{M_n^0}{M_n^{eq}} = \frac{m^{eq}}{m^0} \tag{25}$$

Two years later Steiner developed another relation [28] between z_A and z_B, the stoichiometric number fractions of A and B; this relation is

$$\begin{aligned} \psi &= \ln z_A + \beta \ln z_B = (1+\beta)(g-1) \\ &\quad + (1+\beta)\int_0^{m^0} (g-1)\frac{\mathrm{d}m^0}{m^0} + \ln\left[\frac{1}{1+\beta}\right] \\ &\quad + \beta \ln\left[\frac{\beta}{1+\beta}\right] \end{aligned} \tag{26}$$

One can use eq. 16 to obtain m_A; then this can be converted to x_A or z_A, so that one can use eq. 24 or 26 to obtain x_B or m_B; and these can be converted to m_B. Table 1 summarizes these steps. The equations for Φ and ψ (see eqs. 24 and 26) can be extended to the nonideal case [28].

3. General case

For the general case, $m^{eq} = c/M_n^{eq}$ is described by eq. 16, $m^0 = c/M_n^0$ is described by eq. 18 and g, r and λ are given by eqs. 25, 21 and 22, respectively. For the ideal or quasi-ideal case (after correction for the nonideal terms) m_A and m_B can be obtained as described in table 1. In addition one notes that λ is simply defined by

$$\lambda = m_B^0/m_B = 1 + k_{A,B}m_A + k_{A,B}k_{A,AB}m_A^2 + k_{A,B}k_{A,AB}k_{A,A_2B}m_A^3 + \ldots \tag{27}$$

This is an alternative to the use of eq. 23. Furthermore, g and r are interrelated; eq. 21 can be rewritten as

$$\begin{aligned} r &= 1 + (1/\beta) - \left(m^{eq}/m^0\right)\left(m^0/m_B^0\right) \\ &= 1 + (1/\beta) - g\left(m^0/m_B^0\right) \end{aligned} \tag{28}$$

The relations for m_A, m_B and λ also apply to the nonideal case after it has been corrected for nonideal behavior.

In principle, one can fit λ to a polynomial in m_A and obtain the association constants $k_{A,B}$,

$k_{A,AB}$, etc. from the regression coefficients of the polynomial. One would have to use a statistical test, such as the F test [31], in order to determine if there is a significant improvement in fit as the degree of the polynomial is increased.

Table 1

Procedure for evaluation of m_A and m_B

Evaluation of m_A

Use eq. 16 to evaluate m_A, since $m_A = m^{eq} - m_B^0 =$ equilibrium molar concentration of A

Evaluation of m_B from Φ

$$\Phi = \ln x_A + \beta \ln x_B = (1+\beta)\int_0^{m^{eq}} (g-1)\, dm^{eq}/m^{eq} + \ln\left[\frac{1}{1+\beta}\right] + \beta \ln\left[\frac{\beta}{1+\beta}\right] \tag{24}$$

(1) Convert m_A to x_A

$$x_i = m_i/m^{eq} = \text{number fraction of reactant } i \quad (i = \text{A or B}) \tag{24a}$$

(2) Obtain x_B from Φ; convert it to m_B

$m_B = x_B m^{eq} =$ equilibrium molar concentration of B

$$g = m^{eq}/m^0 = M_n^0/M_n^{eq} = \text{osmotic coefficient} \tag{25}$$

$$m_i^0 = c_i^0/M_i = \text{original molar concentration of reactant } i \quad (i = \text{A or B}) \tag{24b}$$

$$m^0 = m_A^0 + m_B^0 = \text{total molar concentration of the reactants} \tag{18}$$

$$\beta = m_B^0/m_A^0 \tag{10}$$

Alternative procedure for the evaluation of m_B

(1) Obtain m_A from eq. 16; convert it to z_A

(2) Evaluate ψ. Then obtain z_B and convert it to m_B.

$$\psi = \ln z_A + \beta \ln z_B = (1+\beta)(g-1) + (1+\beta)\int_0^{m^0}(g-1)\frac{dm^0}{m^0} + \ln\left[\frac{1}{1+\beta}\right] + \beta \ln\left[\frac{\beta}{1+\beta}\right] \tag{26}$$

$$z_i = m_i/m^0 = \text{stoichiometric number fraction of reactant } i \quad (i = \text{A or B}) \tag{26a}$$

$$z_i = g x_i \tag{26b}$$

$$m_i = z_i m^0 = \text{equilibrium molar concentration of reactant } i \quad (i = \text{A or B}) \tag{26c}$$

---

4. Limited number of sites with all equilibrium constants equal

Let $x = km_A$, and let there be n sites that are identical and completely independent of each other. For this case, it has been shown that [9,10]

$$r = \frac{x(d/dx)[(1+x)^n]}{(1+x)^n} = \frac{nx}{1+x} = \frac{nkm_A}{1+km_A} \tag{29}$$

Here

$$x = km_A = \frac{r}{n-r} \tag{29a}$$

and

$$n = \frac{r(x+1)}{x} \tag{29b}$$

Consequently, M_n^{eq} (see eqs. 20 and 21) is given by

$$M_n^{eq} = \frac{c}{m_B^0[1 + (1/\beta) - r]} = \frac{m_A^0 M_A + m_B^0 M_B}{m_A + m_B^0}$$
$$= \left\{ M_A\left[m_A + nm_B x(1+x)^{n-1}\right] + M_B m_B (1+x)^n \right\}\left\{m_A + m_B(1+x)^n\right\}^{-1} \tag{30}$$

since

$$m_A^0 = m_A + nm_B x(1+x)^{n-1} \tag{30a}$$

and

$$m_B^0 = m_B(1+x)^n \tag{30b}$$

The binding polynomial λ and the osmotic coefficient g are given by

$$\lambda = m_B^0/m_B = (1+x)^n \tag{31}$$

and

$$g = \frac{M_n^0}{M_n^{eq}} = \frac{m^{eq}}{m^0} = \frac{m_A + m_B(1+x)^n}{m_A + nm_B x(1+x)^{n-1} + m_B(1+x)^n} = \frac{(1+x) + (m_A/m^0)nx}{x(n+1)+1} \tag{32}$$

Now note that m_A and m_B are available (see table 1), so that

$$\ln \lambda = n \ln(1+x) = n \ln[n/(n-r)]$$

Because r and λ are known, one can solve for n ($n = 1, 2, \ldots$) by successive approximations. Once n is known then x can be obtained from g (eq. 32), λ (eq. 31), r (eq. 29) or M_n^{eq} (eq. 30). A plot of x vs. m_A will have an intercept of zero and a slope of k. Alternatively, once m_A is known, one can make a plot based on the reciprocal of eq. 29; by plotting $1/r$ vs. $1/m_A$, the slope is $1/nk$ and the intercept $1/n$ [32].

5. Nonideal case

The treatment presented here can be applied to the two previously presented cases, all well as the special cases that follow. For the nonideal case $m^* = c/M_{na^*}$ (see eq. 12). The equations for Φ and ψ can be extended to the nonideal case [28]; thus

$$\begin{aligned}\Phi^* &= \ln x_A^* + \beta \ln x_B^* \\ &= (1+\beta)\int_0^{m^*}(g^*-1)\frac{dm^*}{m^*} \\ &\quad + \ln\left[\frac{1}{1+\beta}\right] + \beta \ln\left[\frac{\beta}{1+\beta}\right] \qquad (33)\end{aligned}$$

and

$$\begin{aligned}\psi^* &= \ln z_A^* + \beta \ln z_B^* = (1+\beta)(g^*-1) \\ &\quad + (1+\beta)\int_0^{m^0}(g^*-1)\frac{dm^0}{m^0} + \ln\left[\frac{1}{1+\beta}\right] \\ &\quad + \beta \ln\left[\frac{\beta}{1+\beta}\right] \qquad (34)\end{aligned}$$

Here,

$$g^* = M_n^0/M_{na}^* = m^*/m^0 \qquad (35)$$

$$x_A^* = x_A \exp\{B_{AB}M_AM_Bm_B^0\} \qquad (36)$$

$$x_B^* = x_B \exp\{B_{AB}M_AM_Bm_A^0\} \qquad (37)$$

and

$$z_i^* = g^*x_i^*, \quad i = \text{A or B} \qquad (38)$$

Steiner [28] showed that if one performed several series of experiments, at constant β for each series, then at constant m^*

$$\ln x_B^* = (\partial\Phi^*/\partial\beta)_{m^*} \qquad (39)$$

Once x_B^* is known, then one obtains x_A^* from eq. 33. For the nonideal case, eq. 16 is replaced by eq. 12 and

$$m^* - m_B^0 = m_A + B_{AB}M_AM_Bm_A^0m_B^0 \qquad (40)$$

which leads to

$$\begin{aligned}&(m^* - m_B^0)/m_A^0 \\ &\quad = (m_A/m_A^0) + B_{AB}M_AM_Bm_B^0 \\ &\quad = x_A^*g^*(m^0/m_A^0) + B_{AB}M_AM_Bm_B^0 \qquad (41)\end{aligned}$$

From eq. 36 one obtains

$$\ln x_A^* = \ln x_A + B_{AB}M_AM_Bm_B^0 \qquad (42)$$

Combining eqs. 41 and 42 results in

$$\begin{aligned}\ln x_A &= x_A^*g^*(m^0/m_A^0) + \ln x_A^* \\ &\quad - [(m^* - m_B^0)/m_A^0] \qquad (43)\end{aligned}$$

which contains only one unknown, $\ln x_A$. Once $\ln x_A$ is known, one also knows $B_{AB}M_AM_Bm_B^0$ (see eq. 42) as well as $B_{AB}M_AM_Bm_A^0m_B^0$. Then, from eq. 12 $m^{eq} = m^* - B_{AB}M_AM_Bm_A^0m_B^0$. At this point one can do the rest of the analysis as if the system were ideal. The main difficulty with this procedure is that one needs to carry out several series of experiments, with each series having a different value of β, so that one can make the plots required by eq. 38. The procedure is general, and it can be applied to all the examples described in this paper.

6. Analogy between mixed associations and self-associations

In the general model, it was pointed out that λ was a polynomial, and that the equilibrium constants could be obtained from the coefficients of the polynomial. Tobolsky and Thach [33] reanalyzed some self-association data of White and Kilpatrick [34], who had used cryoscopy in their studies of the self-association of 2-*n*-butylbenzimidazole and benzotriazole in benzene. White and Kilpatrick [34] found that their data required eight equilibrium constants. Tobolsky and Thach [33] found that the data could be fitted to about the same degree of precision with a two-equilibrium constant, indefinite self-association (a type III SEK model). Similar considerations should apply to the mixed associations described here. If one makes some simple assumptions then one can develop analogs of some of the SEK (sequential, equal equilibrium constant) or the AK (attenuated equilibrium constant) models [29,30]. Here the ideal case is illustrated. The quasi-ideal case, after the data are corrected for the B_{AA} and B_{BB} terms, can be treated as if it were ideal. For the nonideal case, one will also have to correct for the B_{AB} term, as described earlier; then one can proceed with the analysis as if the system were ideal.

7. Analogs of the SEK association

7.1. Noncooperative model

If it is assumed in eqs. 13–16 that $k_{A,B} = k_{A,AB} = \ldots = k$, then at constant β

$$\begin{aligned} m_A^0 = c_A^0/M_A &= m_A + k m_A m_B + 2k^2 m_A^2 m_B \\ &\quad + 3k^3 m_A^3 m_B + \ldots \\ &= m_A\left\{1 + \left[k m_B/(1 - k m_A)^2\right]\right\} \\ &= m_A\left\{1 + \left[k m_B/(1 - x)^2\right]\right\}, \quad \text{if } k m_A < 1 \end{aligned} \tag{44}$$

Here

$$x = k m_A \tag{45}$$

The quantity $m_B^0 = c_B^0/M_B$ is given by

$$\begin{aligned} m_B^0 &= m_B + k m_A m_B + k^2 m_A^2 m_B + k^3 m_A^3 m_B + \ldots \\ &= m_B(1 + x + x^2 + x^3 + \ldots) \\ &= m_B/(1 - x) = \lambda m_B, \quad \text{if } x < 1 \end{aligned} \tag{46}$$

Here

$$\lambda \equiv m_B^0/m_B = 1/(1 - x) \tag{47}$$

$$m^{eq} = c/M_n^{eq} = m_A + m_B^0 \tag{16}$$

$$r = x/(1 - x) \tag{48}$$

and

$$g = 1 + x\left[\left(m_A/m^0\right) - 1\right] \tag{49}$$

One obtains m_A from eq. 16; then one can obtain x from eq. 48 or 49 for r or g, and k is obtained from the slope of a plot of x vs. m_A (see eq. 45). Once m_A and x are known m_B can be obtained from eq. 46; alternatively, one can obtain m_B from eq. 24 or 25.

7.2. Cooperative model

For the cooperative model it is assumed that (see eqs. 13–16) $k_{A,B} \neq k_{A,AB}$, etc., but that $k_{A,AB} = k_{A,A_2B} = \ldots = k$. Then, at constant β and if $x = k m_A < 1$, the following relations apply:

$$\begin{aligned} m_A^0 &= m_A\left\{1 + \left[k_{A,B} m_B/(1 - k m_A)^2\right]\right\} \\ &= m_A\left\{1 + \left[y/(1 - x)^2\right]\right\} \end{aligned} \tag{50}$$

$$\begin{aligned} m_B^0 &= m_B\left\{1 + \left[k_{A,B} m_B/(1 - k m_A)\right]\right\} \\ &= m_B\left\{1 + \left[y/(1 - x)\right]\right\} = \lambda m_B \end{aligned} \tag{51}$$

Here

$$y = k_{A,B} m_B \tag{52}$$

$$x = k m_A (0 < x < 1) \tag{53}$$

$$\lambda \equiv m_B^0/m_B = 1 + y/(1 - x) \tag{54}$$

$$m^{eq} = m_A + m_B^0 \tag{16}$$

The quantity m_A is available from eq. 16; it can be converted to x_A or z_A (see table 1) to obtain x_B or z_B, which can then be converted to m_B. Now note that

$$R_1 = \frac{\left(m_B^0/m_B\right) - 1}{\left(m_A^0/m_A\right) - 1} = \frac{\lambda - 1}{\left(m_A^0/m_A\right) - 1} = 1 - x \tag{55}$$

A plot of R_1 vs. m_A will have a slope of $-k$ and an intercept of 1. Then note that

$$R_2 = R_1(\lambda - 1) = y = k_{A,B} m_B \tag{56}$$

A plot of R_2 vs. m_B will have a slope of $k_{A,B}$. The quantity r is given by

$$\begin{aligned} r &= \frac{k_{A,B} m_A + 2k_{A,B} k m_A^2 + 3k_{A,B} k^2 m_A^2 + \ldots}{1 + k_{A,B} m_A + k_{A,B} k m_A + k_{A,B} k^2 m_A^3 + \ldots} \\ &= \frac{k_{A,B} m_A}{(1-km_A)^2} \left\{ \frac{1}{1 + [k_{A,B} m_A/(1-km_A)]} \right\} \\ &= \frac{k_{A,B} m_A}{(1-km_A)(1-km_A+k_{A,B} m_A)} \\ &= \frac{y}{(1-x)(1-x+y)} \end{aligned} \tag{57}$$

and g is given by

$$\begin{aligned} g &= \frac{m_A + m_B\{1 + y/(1-x)\}}{m_A\left[1 + y/(1-x)^2\right] + m_B[1 + y/(1-x)]} \\ &= \frac{m_B(1-x) + m_A(m_A + m_B)}{m_A + m_B(1-x)} \end{aligned} \tag{58}$$

This can be rearranged to give

$$x = 1 - \frac{m_A[m_A + m_B - g]}{g m_B - m_A} \tag{59}$$

which gives a very simple way to evaluate x.

8. Analogs of the AK indefinite self-association

8.1. Noncooperative variant

Here it will be assumed that the molar equilibrium constants (see eq. 13) may be written as $k_{A,B} = k/2$; $k_{A,AB} = k/3$; $k_{A,A_2B} = k/4$, etc. From these relations, it follows that

$$\begin{aligned} m_A^0 &= m_A + (k/2) m_A m_B + (2k^2/2 \cdot 3) m_A^2 m_B \\ &\quad + (3k^3/2 \cdot 3 \cdot 4) \cdot m_A^3 m_B + \ldots \\ &= m_A + m_B\{(e^x(x-1)+1)/x\} = c_A^0/M_A \end{aligned} \tag{60}$$

$$x = k m_A \quad (0 < x < \infty) \tag{61}$$

Similarly, one notes that

$$m_B^0 = c_B^0/M_B = (m_B/x)(e^x - 1) = \lambda m_B \tag{62}$$

and

$$\begin{aligned} m^{eq} &= c/M_n^{eq} \\ &= m_A + m_B[1 + (x/2!) + (x^2/3!) + \ldots] \\ &= m_A + m_B^0 \end{aligned} \tag{63}$$

The quantities r and g are given by

$$\begin{aligned} r &= \frac{(x/2!) + (2x^2/3!) + (3x^3/4!) + \ldots}{1 + (x/2!) + (x^2/3!) + (x^3/4!) + \ldots} \\ &= \frac{e^x(x-1)+1}{e^x - 1} \end{aligned} \tag{64}$$

and

$$g = \frac{m^{eq}}{m^0} = \frac{(e^x - 1)(1 - [m_A/m^0])}{x e^x (1 - m_A)} \tag{65}$$

The quantity m_A is available from eq. 16 and m_B can be obtained from the relations in table 1. One can obtain x by successive approximations from g, r or λ. Here (see eq. 62)

$$\lambda = m_B^0/m_B = (e^x - 1)/x \tag{66}$$

The values of x can be plotted against m_A to obtain k from the slope.

8.2. Cooperative variant

Here it is assumed (see eq. 13) that $k_{A,B} \neq k/2$, but that $k_{A,AB} = k/3$, $k_{A,A_2B} = k/4$, etc. This leads to

$$\begin{aligned} m_A^0 &= m_A + k_{A,B} m_A m_B + [2k_{A,B} k/3] m_A^2 m_B \\ &\quad + \left[3k_{A,B} k^2/3 \cdot 4\right] \cdot m_A^3 m_B + \ldots \\ &= m_A + k_{A,B} m_A m_B\left[1 + (2k/3) m_A \right. \\ &\quad \left. + (3k^2/3 \cdot 4) m_A^2 + (4k^3/3 \cdot 4 \cdot 5) m_A^3 + \ldots\right] \\ &= m_A + 2 m_B y\left[(e^x(x-1)+1)/x^2\right] \end{aligned} \tag{67}$$

$$\begin{aligned} m_B^0 &= m_B + 2 m_B y\left[(1/2) + (x/3!) + (x^2/4!) \right. \\ &\quad \left. + (x^3/5!) + \ldots\right] \\ &= m_B\left[1 + 2y\{(e^x - x - 1)/x^2\}\right] = \lambda m_B \end{aligned} \tag{68}$$

Here

$$x = km_A \quad (0 < x < \infty) \tag{69}$$

$$y = k_{A,B} m_A \tag{70}$$

$$\lambda = 1 + 2y\{(e^x - x - 1)/x^2\} \tag{71}$$

$$m^{eq} = m_A + m_B^0 \tag{16}$$

The quantities r and g are given by

$$\begin{aligned} r &= [k_{A,B} m_A\{1 + (2k/3)m_A + (3k^2/3 \cdot 4)m_A^2 \\ &\quad + \ldots\}][1 + k_{A,B} m_A\{1 + (k/3)m_A \\ &\quad + (k^2/3 \cdot 4)m_A^2 + \ldots\}]^{-1} \\ &= \frac{2k_{A,B} m_A[e^x(x-1)+1]}{x^2 + 2k_{A,B} m_A[e^x - x - 1]} \\ &= \frac{2y[e^x(x-1)+1]}{x^2 + 2y[e^x - x - 1]} \end{aligned} \tag{72}$$

and

$$\begin{aligned} g = \frac{m^{eq}}{m^0} &= \{(e^x - x + 1) \\ &\quad + [(m_A + m_B)/m^0](xe^x - e^x + 1)\} \\ &\quad \times \{x(e^x - 1)\}^{-1} \end{aligned} \tag{73}$$

One obtains m_A from eq. 16; then m_B can be obtained from Φ or ψ (see table 1). Now note that eqs. 67 and 68 can be used to obtain

$$\frac{m_A^0 - m_A}{m_B^0 - m_B} = \frac{e^x(x-1)+1}{e^x - x - 1} \tag{74}$$

Here x can be obtained by successive approximations; alternatively, eq. 73 for g could be used. Once x is known, y can be obtained from eq. 67 or 71. A plot of x vs. m_A has k as a slope and 0 as an intercept. Similarly, a plot of y vs. m_A has $k_{A,B}$ as a slope and 0 as an intercept.

9. Discussion

It is evident from the preceding treatment that the special binding type mixed associations can be expressed as infinite series that can be summed. The assumptions used in this treatment are the same as those used in analyzing indefinite self-associations [29,30]. Although the general procedure initiated by Steiner [21,26–28] has been modified somewhat, it may be that one obtains, within experimental error, the same or better precision by using indefinite models. These have the advantage that only one or two equilibrium constants plus the nonideal term are needed to describe indefinite mixed associations.

The integrals required by Steiner's equations for ψ and Φ (see table 1) must be performed numerically. It is quite likely that at low concentrations there may be quite a bit of scatter in the plot of $(g-1)/m$ vs. m. However, it should be noted that the integrals in eqs. 24 and 25 are identical to those used in the evaluation of activity coefficients from osmotic coefficients [35]. For nonelectrolytes the natural logarithm of the activity coefficient of the solute ($\ln \gamma_2$) is given by

$$\ln \gamma_2 = (g-1) + \int_0^m \frac{(g-1)}{m}\, dm$$

Some books use ϕ instead of g for the osmotic coefficient. Noggle [35] mentions the problem of scatter in the data, and he suggests that this problem may be overcome by fitting $(g-1)/m$ to a polynomial in m. A similar procedure exists for evaluating mean ionic activity coefficients in electrolyte solutions (for more details see pp. 366–371 of ref. 35). The procedure described above might be applied to the Steiner equations for Φ and ψ (see table 1); it might also prove useful in the evaluation of $\ln \lambda$ from eq. 23.

One fault with the SEK (sequential, equal equilibrium constant) indefinite self-associations was that some felt it overcompensated for the proportion of larger aggregates. This overcompensation for self-associations was overcome by the development of the AK (attenuated equilibrium constant) model [30,36]. Here only ΔH^0 is constant, and both ΔG^0 and ΔS^0 vary with the size of the aggregate. Although the range of $x = km_A$ in the AK model is such that $0 < x < \infty$, the attenuation factor k/n makes it unlikely that large aggregates will predominate. For instance with the species $A_{10}B$, the equilibrium constants are attenuated so that $m_{A_{10}B} = (k^{10}/10!)m_A^{10} m_B$, and $10! = 3\,628\,000$. The factorial becomes larger and larger as the aggregate size increases; the effect of this would be to limit the size of the aggregate. This limit would depend on k and n; beyond a certain size, the concentration of the larger aggregates becomes vanishingly small.

Steiner [21] pointed out the relation between the average binding number, r and the number average molecular weight, M_n^{eq}. Here I have pointed out the relationship between r, g, and λ for the general case, the special case and the four special cases that might be described by infinite series. Interestingly, for the ideal case one always obtains m_A from $m^{eq} - m_B^0$ for all the examples given here (had the associations described by eq. 1 been written as $A + B \leftrightarrows AB$, $B + AB \leftrightarrows AB_2$, $\ldots$, $B + A_{n-1}B \leftrightarrows AB_n$, then one would obtain m_B from $m^{eq} - m_A^0$). Because of the Steiner relations for Φ and ψ (see table 1), once m_A (or m_B) is known, then one can calculate m_B (or m_A) and λ.

For the quasi-ideal case, one need only correct for the nonideal terms $B_{AA}(c_A^0)^2/2$ and $B_{BB}(c_B^0)^2/2$ only. Once this has been done, then m^* becomes m^{eq} and the analysis can be done as if the associations were ideal. For the more complicated nonideal case where a term $B_{AB}c_A^0c_B^0$ needs to be included, the analysis at present is more complicated. Nonetheless, a method for obtaining this term has been presented.

One consequence of the Steiner equations for Φ and ψ is the ease with which λ is available once m_A and m_B are known. It is also possible to evaluate $\ln \lambda$ from eq. 23, and the values of λ obtained by the two methods can be compared. Since the osmotic coefficient g is available from the experimental data, the utility of the equations for Φ and ψ (see table 1) is obvious. An interesting feature of this work is the utility of the osmotic coefficient g in the analysis. For the four special indefinite cases, once m_A and m_B are known, then g has only one unknown, $x = km_A$. For the two noncooperative variants one does not need to know m_B in order to relate g to x. Note that $y = k_{AB}m_A$ drops out in the expression for g in the two cooperative variants. Even for the case of limited sites with all equilibrium constants equal, g does not depend explicitly on m_B. In this work, I have tied together a number of developments which may help in the analysis of these mixed associations.

References

1 J.D. Watson, N.H. Hopkins, J.W. Roberts, J.A. Steitz and A.M. Weiner, Molecular biology of the gene, 4th edn. (Benjamin/Cummings, Menlo Park, CA, 1987).

2 M.J. Gurr and A.T. James, Lipid biochemistry: an introduction, 3rd edn. (Chapman and Hall, London, 1980).

3 T.M. Devlin, Textbook of biochemistry with chemical correlations (Wiley, New York, 1982).

4 J.C. Osborne, Jr and H.B. Brewer, Jr, Adv. Protein Chem. 31 (1977) 253.

5 A.M. Scanu, C. Edelstein and B.W. Shen, in: Lipid-protein interactions, eds. P. Jost and O.H. Griffith (Wiley, New York, 1982) vol. 1, ch. 6.

6 A.M. Scanu, in: Dietary fats and health, eds. E.G. Perkins and W.J. Visek (American Oil Chemist's Society, Champaign, IL, 1983) p. 530.

7 R.F. Steiner, Arch. Biochem. Biophys. 55 (1955) 235.

8 S.N. Timasheff, in: Electromagnetic scattering, ed. M. Kerker (MacMillan, New York, 1963) p. 337.

9 C. Tanford, Physical chemistry of macromolecules (Wiley, New York, 1961) ch. 8.

10 C.R. Cantor and P.R. Schimmel, Biophysical chemistry (Freeman, San Francisco, 1981) part III, ch. 15.

11 R.F. Steiner, Biopolymers 9 (1970) 1465.

12 R. Huber, in: Protein-protein interactions, eds. R. Jaenicke and E. Helmreich (Springer, New York, 1972) p. 111.

13 M. Lazdumski and J.P. Vincent, in: Protein-protein interactions, eds. R. Jaenicke and E. Helmreich (Springer, New York, 1972) p. 129.

14 B.W. McCarty and E.T. Adams, Jr, Biophys. Chem. 28 (1987) 149.

15 M.S. Lewis and R.J. Youle, J. Biol. Chem. 261 (1986) 11571.

16 P. Hensley, M.C. O'Keefe, C.J. Spangler, J.C. Osborne, Jr and C.-W. Vogel, J. Biol. Chem. 261 (1986) 11038.

17 B.W. Foster, R.L. Huggins, J. Robeson and E.T. Adams, Jr, Biophys. Chem. 16 (1982) 317.

18 G.M. Nagel and S. Hanlon, Biochemistry 11 (1972) 823.

19 R.F. Steiner, Biochim. Biophys. Acta 247 (1971) 22.

20 A.H. Pekar, P.J. Wan and E.T. Adams, Jr, Adv. Chem. Ser. No. 125 (1973) 260.

21 R.F. Steiner, Arch. Biochem. Biophys. 47 (1953) 56.

22 L.W. Nichol, A.G. Ogston and D.J. Winzor, Arch. Biochem. Biophys. 121 (1953) 517.

23 G.K. Ackers, Adv. Protein Chem. 24 (1970) 343.

24 E.T. Adams, Jr, P.J. Wan and E.F. Crawford, Methods Enzymol. 48 (1978) 69.

25 E.T. Adams, Jr, J. Lipid Res. 27 (1986) 1233.

26 R.F. Steiner, Biochemistry 7 (1968) 2201.

27 R.F. Steiner, Biochemistry 9 (1970) 1375.

28 R.F. Steiner, Biochemistry 9 (1970) 4268.

29 L.-H. Tang, D.R. Powell, B.M. Escott and E.T. Adams, Jr, Biophys. Chem. 7 (1977) 121.
30 J.M. Beckerdite, C.C. Wan and E.T. Adams, Jr, Biophys. Chem. 12 (1980) 199.
31 L. Ott, An introduction to statistical method and data analysis, 2nd edn. (Duxbury, Boston, 1984).
32 N.C. Price and R.A. Dwek, Principles and problems in physical chemistry for biochemists (Clarendon, Oxford, 1974).
33 A.V. Tobolsky and R.E. Thach, J. Colloid Sci. 17 (1962) 410.
34 N.E. White and M. Kilpatrick, J. Phys. Chem. 59 (1955) 1044.
35 J.H. Noggle, Physical chemistry (Little Brown, Boston, 1985).
36 F. Garland and S.D. Christian, J. Phys. Chem. 79 (1975) 1447.