Comment to the Editor

A Protein Molecule in a Mixed Solvent: The Preferential Binding Parameter via the Kirkwood-Buff Theory

In a recent article (Schurr, J. M., D. P. Rangel, and S. R. Aragon. 2005. A contribution to the theory of preferential interaction coefficients. *Biophys. J.* 89:2258–2276), a detailed derivation of an expression for the preferential binding coefficient via the Kirkwood-Buff theory of solutions was presented. The authors of this Comment (Shulgin, I. L., and E. Ruckenstein. 2005. A protein molecule in an aqueous mixed solvent: fluctuation theory outlook. *J. Chem. Phys.* 123:054909) also recently established on the basis of the Kirkwood-Buff theory of solutions an equation for the preferential binding of a cosolvent to a protein. There are other publications that relate the preferential binding parameter to the Kirkwood-Buff theory of solutions for protein + binary mixed solvents. The expressions derived in the two articles mentioned above are different because the definitions of the preferential binding parameter are different. However, there are articles in which the definitions of the preferential binding parameter are the same, but the derived equations that relate the preferential binding parameter to the Kirkwood-Buff integrals are different. The goal of this Comment is to examine the various expressions that relate the preferential binding parameter to the Kirkwood-Buff theory.

INTRODUCTION

An important characteristic of a solution of a protein (component 2) in a mixture water (1) + cosolvent (3) is the preferential binding parameter $\Gamma_{23}^{(m)}$ (1–6)

$$\Gamma_{23}^{(m)} \equiv \lim_{m_2 \to 0} (\partial m_3 / \partial m_2)_{T,P,\mu_3}, \tag{1}$$

where m_i is the molality of component i, P is the pressure, T is the absolute temperature, and μ_i is the chemical potential of component i. The preferential binding parameter can be also defined at a molarity scale by

$$\Gamma_{23}^{(c)} \equiv \lim_{c_2 \to 0} (\partial c_3 / \partial c_2)_{T,P,\mu_3}, \tag{2}$$

where c_i is the molar concentration of component i. It should be emphasized that $\Gamma_{23}^{(m)}$ and $\Gamma_{23}^{(c)}$ are defined at infinite protein dilution.

The preferential binding parameter $\Gamma_{23}^{(m)}$ was determined experimentally (5–7) and provides information regarding the interactions between a protein and the components of the mixed solvent. As a rule (1–5), $\Gamma_{23}^{(m)} < 0$, the protein is preferentially hydrated, for cosolvents such as glycerol, sucrose, etc., which can stabilize at high concentrations the protein structure and preserve its enzymatic activity (3–5), and $\Gamma_{23}^{(m)} > 0$, the protein is preferentially solvated by cosolvents (such as urea), which can cause protein denaturation.

In literature (8) a number of different definitions of the preferential binding parameter (coefficient) have been employed. They can be connected by thermodynamic relations for ternary mixtures (8). In this Comment the preferential binding parameter will be mostly defined by Eqs. 1 and 2.

Because the preferential binding parameter is a meaningful physical quantity, attempts have been made to relate it to a general theory of solutions, such as the Kirkwood-Buff theory of solutions (9). Several authors reported results in this direction (10–17). The authors of this Comment derived the following equation for $\Gamma_{23}^{(c)}$ (16):

$$\Gamma_{23}^{(c)} = c_3(G_{23} - G_{13}), \tag{3}$$

where G_{13} and G_{23} are the Kirkwood-Buff integrals defined as (9)

$$G_{\alpha\beta} = \int_0^\infty (g_{\alpha\beta} - 1) 4\pi r^2 dr, \tag{4}$$

where $g_{\alpha\beta}$ is the radial distribution function between species α and β, and r is the distance between the centers of molecules α and β.

Equation 3 differs from the expression of $\Gamma_{23}^{(c)}$ employed in Shimizu (10,11):

$$\Gamma_{23}^{(c)} = c_3(G_{23} - G_{12}). \tag{5}$$

In a recent article in this journal (17), the Kirkwood-Buff theory of solutions was used to express the preferential binding coefficient $\Gamma_3(2)$, defined as

$$\Gamma_3(2) \equiv -\lim_{c_2 \to 0} (\partial \mu_2 / \partial \mu_3)_{T,P,c_2}, \tag{6}$$

in terms of the Kirkwood-Buff integrals. It was found (17) that

$$\Gamma_3(2) = c_3(G_{23} - G_{12}). \tag{7}$$

As noted in Schurr et al. (17) the preferential binding coefficient $\Gamma_3(2)$ defined by Eq. 6 differs from the preferential binding parameter $\Gamma_{23}^{(c)}$ defined by Eq. 2.

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However, Eqs. 3 and 5 are different equations even though they are based on the same definition of the preferential binding parameter and have the same theoretical basis: the Kirkwood-Buff theory of solutions. To make a selection between Eqs. 3 and 5 a simple limiting case, the ideal ternary mixture, will be examined using the traditional thermodynamics, and the results will be compared to those provided by Eqs. 3 and 5.

IDEAL TERNARY MIXTURE

Let us consider an ideal ternary mixture. According to the definition of an ideal mixture (18), the activities of the components (a_i) are equal to their mol fractions (x_i) and their partial molar volumes are equal to those of the pure components ($V_i = V_i^0$).

Because

$$d\mu_3 = \left(\frac{\partial \mu_3}{\partial T}\right)_{P,c_2,c_3} dT + \left(\frac{\partial \mu_3}{\partial P}\right)_{T,c_2,c_3} dP + \left(\frac{\partial \mu_3}{\partial c_2}\right)_{T,P,c_3} dc_2 + \left(\frac{\partial \mu_3}{\partial c_3}\right)_{T,P,c_2} dc_3, \tag{8}$$

one can write for an ideal mixture

$$\Gamma_{23}^{(c)} \equiv \lim_{c_2\to 0} (\partial c_3/\partial c_2)_{T,P,\mu_3} = -\lim_{c_2\to 0} \frac{\left(\frac{\partial \mu_3}{\partial c_2}\right)_{T,P,c_3}}{\left(\frac{\partial \mu_3}{\partial c_3}\right)_{T,P,c_2}} = -\lim_{c_2\to 0} \frac{\left(\frac{\partial \ln x_3}{\partial c_2}\right)_{T,P,c_3}}{\left(\frac{\partial \ln x_3}{\partial c_3}\right)_{T,P,c_2}}. \tag{9}$$

For isothermal-isobaric conditions

$$dc_2 = \frac{dx_2}{V} - \frac{x_2}{V^2}\left(\left(\frac{\partial V}{\partial x_2}\right)_{x_3} dx_2 + \left(\frac{\partial V}{\partial x_3}\right)_{x_2} dx_3\right), \tag{10}$$

and

$$dc_3 = \frac{dx_3}{V} - \frac{x_3}{V^2}\left(\left(\frac{\partial V}{\partial x_2}\right)_{x_3} dx_2 + \left(\frac{\partial V}{\partial x_3}\right)_{x_2} dx_3\right), \tag{11}$$

where V is the molar volume of the ternary mixture.

When c_3 is a constant, Eqs. 10 and 11 lead to

$$\left(\frac{\partial c_2}{\partial x_3}\right)_{c_3} = \frac{V - x_2\left(\frac{\partial V}{\partial x_2}\right)_{x_3} - x_3\left(\frac{\partial V}{\partial x_3}\right)_{x_2}}{x_3 V\left(\frac{\partial V}{\partial x_2}\right)_{x_3}}, \tag{12}$$

and when c_2 is a constant, Eqs. 10 and 11 lead to

$$\left(\frac{\partial c_3}{\partial x_3}\right)_{c_2} = \frac{V - x_2\left(\frac{\partial V}{\partial x_2}\right)_{x_3} - x_3\left(\frac{\partial V}{\partial x_3}\right)_{x_2}}{V\left(V - x_2\left(\frac{\partial V}{\partial x_2}\right)_{x_3}\right)}. \tag{13}$$

By inserting Eqs. 12 and 13 into Eq. 9 at infinite dilution of component 2, one obtains the following expression for $\Gamma_{23}^{(c)}$ of an ideal ternary mixture:

$$\Gamma_{23}^{(c)}(ideal) = c_3(V_1^0 - V_2^0). \tag{14}$$

On the other hand, expressions for $\Gamma_{23}^{(c)}$ for an ideal ternary solution can be also derived by combining Eq. 3 or Eq. 5 with the following Kirkwood-Buff integrals for ideal ternary mixtures (16):

$$G_{12}^{(id)} = kTk_T^{(id)} - V_3^0 c_3(V_1^0 - V_3^0) - V_2^0 \tag{15}$$

$$G_{23}^{(id)} = kTk_T^{(id)} + V_1^0 c_1(V_1^0 - V_3^0) - V_2^0 \tag{16}$$

$$G_{13}^{(id)} = kTk_T^{(id)} - (c_1 + c_3)V_1^0 V_3^0, \tag{17}$$

where k is the Boltzmann constant and k_T is the isothermal compressibility.

Equation 3 leads to

$$\Gamma_{23}^{(c)}(ideal) = c_3(V_1^0 - V_2^0), \tag{18}$$

whereas Eq. 5 to

$$\Gamma_{23}^{(c)}(ideal) = c_3(V_1^0 - V_3^0). \tag{19}$$

DISCUSSION

One can see that the result obtained on the basis of Eq. 3 (Eq. 18) coincides with Eq. 14 derived from general thermodynamic considerations, whereas that based on Eq. 5 does not. The numerical difference between the two expressions is very large because the molar volume of a protein is, usually, much larger than the molar volume of the cosolvent.

Whereas the above discussion involves $\Gamma_{23}^{(c)}$, the quantity $\Gamma_{23}^{(m)}$, which is usually determined experimentally (2–7), is related to $\Gamma_{23}^{(c)}$ through the equation (1,16)

$$\Gamma_{23}^{(c)} = (1 - c_3 V_3)\Gamma_{23}^{(m)} - c_3 V_2^{\infty}, \tag{20}$$

where V_2^{∞} is the partial molar volume of the protein at infinite dilution. V_2^{∞} and V_3 can be expressed at infinite dilution of component 2 in terms of the Kirkwood-Buff integrals as follows (19):

$$V_2^{\infty}=\frac{1+c_1(G_{11}-G_{12})+c_3(G_{33}-G_{23})+c_1c_3(-G_{12}G_{33}+G_{12}G_{13}-G_{13}^2+G_{13}G_{23}+G_{11}G_{33}-G_{11}G_{23})}{c_1+c_3+c_1c_3(G_{11}+G_{33}-2G_{13})}, \tag{21}$$

and (9)

$$V_3=\frac{1+(G_{11}-G_{13})c_1}{c_1+c_3+c_1c_3(G_{11}+G_{33}-2G_{13})}. \tag{22}$$

By combining Eqs. 3, 20, 21, and 22, one obtains after some algebra the following simple expression:

$$\Gamma_{23}^{(\mathrm{m})}=\frac{c_3}{c_1}+c_3(G_{23}-G_{12}+G_{11}-G_{13}). \tag{23}$$

Whereas G_{12} and G_{23} depend on the protein characteristics, G_{11} and G_{13} depend only on the characteristics of the protein-free mixed solvent.

For usual cosolvents (organic solvents, salts, etc.), one can use the following approximation of Eq. 23 in the dilute cosolvent range:

$$\Gamma_{23}^{(\mathrm{m})}\approx c_3(G_{23}-G_{12}). \tag{24}$$

Indeed, $|G_{11}|$ and $|G_{13}|$ are much smaller than the Kirkwood-Buff integrals for the pairs involving the protein ($|G_{12}|$ and $|G_{23}|$). Table 1 provides their values for the system water (1) + lysozyme (2) + urea (3) (pH 7.0, 20°C).

However, when $|G_{11}|$ and $|G_{13}|$ are large, and this occurs when the cosolvent is, for example, a polymer ($G_{13}\approx -1000$ (cm^3/mol) for the system water/polyethylene glycol 2000 at a weight fraction of polyethylene glycol of 0.02 (21)), the complete Eq. 23 should be used. This conclusion is valid for all large cosolvent molecules (polymers, biomolecules, etc.).

Let us consider the biochemical equilibrium between infinitely dilute native (N) and denaturated (D) states of a protein in a mixed solvent. The changes of the preferential binding parameters $\Gamma_{23}^{(\mathrm{c})}$, $\Gamma_{23}^{(\mathrm{m})}$, and $\Gamma_3(2)$ in this process are given by

$$\Delta\Gamma_{23}^{(\mathrm{c})}=c_3(G_{23}(D)-G_{23}(N))=c_3\Delta G_{23} \tag{25}$$

$$\begin{aligned}\Delta\Gamma_{23}^{(\mathrm{m})}&=c_3(G_{23}(D)-G_{23}(N)-G_{12}(D)+G_{12}(N))\\&=c_3(\Delta G_{23}-\Delta G_{12}),\end{aligned} \tag{26}$$

TABLE 1 Numerical values of the Kirkwood-Buff integrals for the water (1) + lysozyme (2) + urea (3) (pH 7.0, 20°C) system

c_3 (mol/l)	$\lvert G_{12}\rvert$ (cm^3/mol) (16)	$\lvert G_{23}\rvert$ (cm^3/mol) (16)	$\lvert G_{11}\rvert$ (cm^3/mol) (20)	$\lvert G_{13}\rvert$ (cm^3/mol) (20)
1	10,350	3700	~16	~42
3	10,630	6180	~12	~45

and

$$\begin{aligned}\Delta\Gamma_3(2)&=c_3(G_{23}(D)-G_{23}(N)-G_{12}(D)+G_{12}(N))\\&=c_3(\Delta G_{23}-\Delta G_{12})=\Delta\Gamma_{23}^{(\mathrm{m})}.\end{aligned} \tag{27}$$

Equations 25 and 26 follow from Eqs. 3 and 23 by taking into account that G_{11} and G_{13} are characteristics of the protein-free mixed solvent at infinite protein dilution.

The equilibrium constant K of biochemical equilibrium between infinitely dilute native (N) and denaturated (D) states of a protein in a mixed solvent can be expressed in terms of $\Delta\Gamma_{23}^{(\mathrm{m})}$ (22)

$$\left(\frac{\partial\ln K}{\partial\ln a_3}\right)_{\mathrm{T,P,m_2}}=\frac{\left(\frac{\partial\mu_3}{\partial m_2}\right)^{\mathrm{N}}_{\mathrm{T,P,m_3}}-\left(\frac{\partial\mu_3}{\partial m_2}\right)^{\mathrm{D}}_{\mathrm{T,P,m_3}}}{\left(\frac{\partial\mu_3}{\partial m_3}\right)_{\mathrm{T,P,m_2}}}=\Delta\Gamma_{23}^{(\mathrm{m})}, \tag{28}$$

where $\Delta\Gamma_{23}^{(\mathrm{m})}$ can be provided by experiment (23).

Using for G_{12} and G_{23} expressions from Shulgin and Ruckenstein (16), Eq. 28 can be also rewritten in the form

$$\left(\frac{\partial\ln K}{\partial\ln a_3}\right)_{\mathrm{T,P,m_2}}=\frac{c_3\Delta J_{21}}{c_1+c_1J_{11}+c_3}, \tag{29}$$

where $J_{11}=\lim_{x_2\to 0}(\partial\ln\gamma_1/\partial x_1)_{x_2}$, $J_{21}=\lim_{x_2\to 0}(\partial\ln\gamma_2/\partial x_1)_{x_2}$, and γ_{i} is the activity coefficient of component i at a mol fraction scale. Let us note that J_{11} is characteristic of the protein-free mixed solvent at infinite protein dilution.

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