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Rigorous analysis of static light scattering measurements on buffered protein solutions

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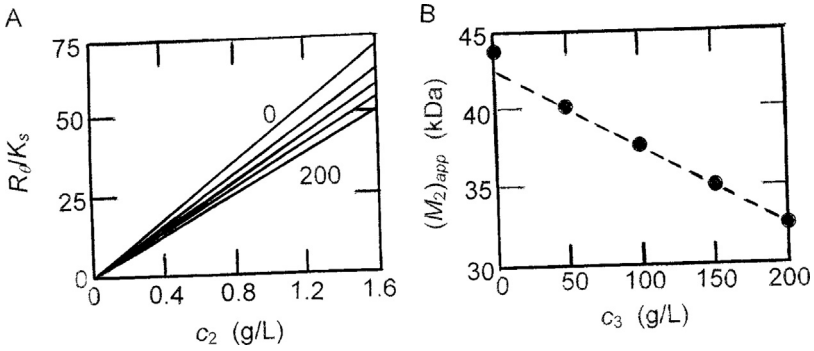
HIGHLIGHTS

- Expressions for rigorous thermodynamic analysis of static light scattering measurements on buffered protein solutions
- Buffer components and supporting electrolytes must be regarded as additional cosolutes rather than part of the solvent
- Effects of small cosolute inclusion on molecular mass estimation are evident in published light scattering data
- Allowance for nonideality by currently used single-solute theory is of questionable value

GRAPHICAL ABSTRACT



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ABSTRACT

Attention is drawn to the thermodynamic invalidity of the current practice of analyzing static light scattering measurements on globular proteins in terms of theory for a single solute because of its disregard of the need to consider small species such as buffer components as additional cosolutes rather than as part of the solvent. This practice continues despite its demonstrated inadequacy in studies of sucrose-supplemented protein solutions, where the aberrant behavior was recognized to be a consequence of physical protein interaction with the small cosolute. Failure to take into account the consequences of small cosolute effects renders extremely difficult any attempt to obtain a rigorous thermodynamic characterization of protein interactions by this empirical technique.

1. Introduction

Thermodynamic nonideality of protein solutions can certainly be quantified in terms of the second virial coefficient obtained from light scattering experiments. However, the parameter derived therefrom (A_2) is not identical to the second virial coefficient for protein self-interaction (B_{22}) that emanates from osmotic pressure, sedimentation equilibrium and size-exclusion chromatography measurements on buffered protein solutions [1]. We were first alerted to this problem by reports of negative B_{22} values from light scattering studies of protein solutions supplemented with high salt concentrations [2–4] – values incompatible with the statistical-mechanical concept of the osmotic second virial coefficient for protein self-interaction as an excluded volume [5,6]. The anomaly has been traced to disregard of the role of buffer and supporting electrolyte components, as if these cosolutes have no effect on the overall intensity of light scattering, even though such effects were described more than fifty years ago [7,8]. Reconsideration of how cosolutes affect the way macromolecules scatter light [9–11] has explained the negative light scattering second virial coefficients [2–4], reflecting situations in which the B_{22} contribution is outweighed by an

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opposing protein–cosolute counterpart at high cosolute concentration.

The popularity of static light scattering for the characterization of protein interactions has been boosted considerably by the development of an automated procedure [12,13] for measuring the concentration dependence of the excess light scattering (R_θ) at set angle θ relative to the incident laser beam. However, results obtained by using this technique, termed composition gradient multi-angle light scattering (CG-MALS), on nonassociating proteins continue to be interpreted in terms of single-solute theory [14] and hence on the assumption that buffer components can be regarded as part of the solvent. The present communication examines the consequences of this approximation by subjecting reported CG-MALS results [13,15] to closer thermodynamic scrutiny; and a protocol for the correct interpretation of Debye plots is presented.

2. Theoretical considerations

As re-emphasized recently [1], the inclusion of a single nonassociating protein in solvent at constant temperature gives rise to one of two situations: that in which the protein chemical potential is being monitored under the additional constraint of constant solvent chemical potential, and that in which constant pressure is the second constraint – a distinction that is overlooked in standard textbooks and most experimental studies. Studies performed under the former constraint, which applies in osmometry and size-exclusion chromatography, are the simplest to consider because small partitioning solutes (buffer components and electrolytes) can justifiably be regarded as part of the solvent (species 1).

2.1. Solute chemical potential under the constraint of constant solvent chemical potential

For these simpler situations the pertinent measure of the thermodynamic activity of the protein (species 2 with molecular mass M_2) is defined in terms of its weight per unit volume concentration c_2 by the expression [16]

$$(\mu_2)_{T,\mu_1} = (\mu_2^o)_{T,\mu_1} + RT\ln z_2 = (\mu_2^o)_{T,\mu_1} + RT\ln(\gamma_2 c_2/M_2) \tag{1}$$

in which the thermodynamic activity (z_2) of the protein is a molar quantity and therefore written as the product of its molar concentration (c_2/M_2) and a corresponding molar activity coefficient (γ_2). By a purely thermodynamic argument it has been shown [6] that

$$\ln\gamma_2 = 2B_{22}c_2/M_2 + \ldots \tag{2}$$

where B_{22}, the osmotic second virial coefficient for protein self-interaction, is a rigorously defined parameter that can be described on the statistical-mechanical basis of physical interaction between pairs of protein molecules [5,6]. For globular proteins in a buffer medium with moderate ionic strength ($I \geq 0.1$ M) a reasonably reliable estimate of the osmotic second virial coefficient for protein self-interaction can be obtained from the expression [17,18]

$$B_{22} = \frac{16\pi N_A R_2^3}{3} + \frac{Z_2^2(1+2\kappa R_2)}{4I(1+\kappa R_2)^2} - \frac{Z_2^4(1000\kappa^3)}{128\pi N_A I^2(1+\kappa R_2)^4} + \ldots \tag{3}$$

where the first term is the hard-sphere contribution for a protein with pair-exclusion diameter $2R_2$; and where the subsequent terms account for the exclusion of one solute molecule from further space around another arising from charge–charge repulsion between them, each of which has a symmetrically distributed net charge Z_2. The appearance of the 1000 factor in the last term reflects calculation of the Debye–Hückel inverse screening length κ (in cm^{-1}) as $3.27\times10^7\sqrt{I}$ from the ionic strength I, which also appears as a numerical factor with implicit molar units (M) in the denominator. Avogadro's number (N_A) is included to convert the virial coefficient from a molecular to a molar basis. An alternative procedure for evaluating B_{22} entails an adaptation of scaled-particle theory [19,20] for which the counterpart of Eq. (3) is

$$B_{22} = \frac{16\pi N_A R_{eff}^3}{3} \tag{4}$$

in which the effective radius of the molecule (R_{eff}) is increased to take into account the excluded volume contributions arising from the charge-charge repulsion terms in Eq. (3) [21]. This effective size is determined as the effective specific volume v_{eff}, from which the second virial coefficient is calculated as $B_{22} = 4M_2v_{eff}$.

Although not properly applicable, the above theory is also used to interpret static light scattering measurements in the mistaken belief that the same definition of solute chemical potential (Eq. (1)) applies without further consideration of which thermodynamic variables are chosen to be independent.

2.2. Solute chemical potential under the constraint of constant pressure

In common with most physicochemical situations, the second constraint applying to static light scattering measurements is constant pressure, whereupon the expression for the thermodynamic activity of the protein becomes more complicated. Under the constraints of constant temperature and pressure the thermodynamic activity of a single nonassociating macromolecular solute (a_2) needs to be written as [16]

$$(\mu_2)_{T,P} = (\mu_2^o)_{T,P} + RT\ln a_2 = (\mu_2^o)_{T,P} + RT\ln(y_2w_2/M_2) \tag{5}$$

where a_2 is the molal activity that is most logically expressed in terms of the molal concentration (w_2/M_2) with w_2 defined in terms of g per kg of solvent and the corresponding molal activity coefficient (y_2). Furthermore, the relevant expression for chemical potential in terms of a virial expansion is now [16]

$$-\frac{(\mu_1)_{T,P} - (\mu_1^0)_{T,P}}{RT} = (w_2/M_2) + C_{22}(w_2/M_2)^2 + \ldots \tag{6}$$

and the counterpart of Eq. (2) becomes

$$\ln y_2 = 2C_{22}(w_2/M_2) + \ldots \tag{7}$$

The molal second virial coefficient (C_{22}) is not amenable to simple statistical-mechanical rationalization except for incompressible solutions of solute in a single component solvent. Under those restrictive circumstances the molal and molar second virial coefficients for solute self-interaction are related by the expression

$$C_{22}/\rho_1 = B_{22} - M_2\bar{v}_2 \tag{8}$$

where $\bar{v}_2$ is the partial specific volume of the protein, independent of concentration; and where ρ_1, the solvent density, is required to convert the units of C_{22} (mol per kg solvent) into those of the osmotic second virial coefficient for self-interaction and the molar volume (mol per liter of solution). After replacement of w_2 in Eq. (7) by its more commonly used counterpart c_2 via the relationship $w_2 = c_2/[\rho_1(1-\bar{v}_2c_2)]$, applicable to incompressible solutions, the expression for the molal activity coefficient becomes, correct to linear order in protein concentration,

$$\ln y_2 = (2B_{22} - M_2\bar{v}_2)(c_2/M_2) + \ldots \tag{9}$$

which differs only slightly from its counterpart for the molar activity coefficient (Eq. (2)).

Unfortunately, physicochemical studies of aqueous protein solutions require supplementation of the solvent (water) with low molecular mass buffer and supporting electrolyte components. Whereas these small species could be regarded as part of the solvent in osmometry and size-exclusion chromatography, the experimental constraint of constant pressure (rather than constant solvent chemical potential) necessitates their consideration as additional cosolutes [7,8]. To simplify nomenclature we shall regard them as a single “buffer” component (species 3) present at molar concentration c_3/M_3. The counterpart of Eq. (6) must now be written as