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Studies of solute self-association by sedimentation equilibrium: allowance for effects of thermodynamic non-ideality beyond the consequences of nearest-neighbor interactions

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Abstract

A sedimentation equilibrium study of α-chymotrypsin self-association in acetate-chloride buffer, pH 4.1 I 0.05, has been used to illustrate determination of a dimerization constant under conditions where thermodynamic non-ideality is manifested beyond the consequences of nearest-neighbor interactions. Because the expressions for the experimentally determinable interaction parameters comprise a mixture of equilibrium constant and excluded volume terms, the assignment of reasonable magnitudes to the relevant virial coefficients describing non-associative cluster formation is essential for the evaluation of a reliable estimate of the dimerization constant. Determination of these excluded volume parameters by numerical integration over the potential-of-mean-force is shown to be preferable to their calculation by approximate analytical solutions of the integral for this relatively small enzyme monomer with high net charge (+10) under conditions of low ionic strength (0.05 M).

Keywords: Sedimentation equilibrium; Protein self-association; Thermodynamic non-ideality; α-Chymotrypsin

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1. Introduction

The introduction of direct analysis of the radial dependence of solute concentration [1] facilitated greatly the characterization of solute self-association by sedimentation equilibrium [2,3]. Realistic allowance for the effects of thermodynamic nonideality was made initially by incorporating composition-dependent activity coefficients [4,5] assigned on the statistical-mechanical basis of excluded volume [6]. The consequent requirement for iterative analysis was removed subsequently [7] by switching from the conventional expression for solvent chemical potential as a virial expansion in solute concentration to its counterpart [8] in which the virial expansion is in terms of the thermodynamic activity of solute [9]. That development has led to a sedimentation equilibrium expression in which the total solute concentration is described as a specified polynomial of the psi-function (a renormalization of radial distance, the independent experimental variable) in which the coefficients comprise a mixture of equilibrium constant and excluded volume terms.

In the present communication we give further consideration to the assignment of magnitudes to the excluded volume parameters in order that the coefficients of the equation used for non-linear least-squares curve-fitting of the experimental sedimentation equilibrium distribution can be expressed solely in terms of the equilibrium constant(s) and the thermodynamic activity of monomer at some reference radial distance. A sedimentation equilibrium study of α-chymotrypsin dimerization (pH 4.1, I 0.05) is used to illustrate the application of the current theoretical deliberations to an experimental system.

2. Theoretical considerations

2.1. Allowance for effects of thermodynamic nonideality in solute self-association

Although the solute distribution in a sedimentation equilibrium experiment is recorded in terms of concentration, the thermodynamic origins of the data dictate their description in terms of thermodynamic activity [1,2]. For incompressible solutions the pertinent quantity is z_i, the molar thermodynamic activity of solute i defined under conditions of constant temperature and chemical potential of solvent [7,10–13]. Advantage may therefore be taken of theory developed in the context of osmotic pressure, for which the same operational constraints apply to the thermodynamic activity governing its magnitude.

For a solute undergoing reversible self-association the osmotic pressure, Π, is described by the following virial expansion in thermodynamic activity of monomer, z_1 [8,14],

$$\Pi/(RT) = z_1 + (K_2 - B_{11})z_1^2 + (K_3 - K_2B_{12} + 2B_{11}^2 - B_{111}/2)z_1^3 + \ldots \quad (1)$$

where R is the universal gas constant and T the absolute temperature. In this expression K_2 and K_3 are the equilibrium constants (molar scale) describing the respective formation of dimer and trimer from monomer. B_{ij} denotes the potential-of-mean-force contribution [6] for the interaction of the designated species ($i = 1$, monomer, $i = 2$, dimer, etc.) and B_{111} the corresponding contribution from the excluded-volume interaction of three monomers. Combination of Eq. (1) with the relationship [14]

$$\bar{c}/M_1 = z_1\partial[\Pi/(RT)]/\partial z_1 \quad (2)$$

leads to the expression

$$\bar{c}/M_1 = z_1 + 2(K_2 - B_{11})z_1^2 + 3(K_3 - K_2B_{12} + 2B_{11}^2 - B_{111}/2)z_1^3 + \ldots \quad (3)$$

for the description of the base-molar solute concentration (total weight-concentration divided by monomer molecular mass M_1) as a function of the molar thermodynamic activity of monomer.

This expression is readily adapted for the analysis of a sedimentation equilibrium distribution

obtained at angular velocity ω by means of the substitution [7]

$$z_1(r) = z_1(r_F)\psi_1(r) \tag{4a}$$

$$\psi_1(r) = \exp\left[M_1(1 - \bar{v}\rho_s)\omega^2(r^2 - r_F^2)/(2RT)\right] \tag{4b}$$

which describes the thermodynamic activity of monomer at radial distance r in terms of that at some chosen reference radial position r_F: M_1 is the molecular mass of monomer with partial specific volume $\bar{v}$ (assumed to apply to all oligomeric states), and ρ_s is the solvent density. By writing Eq. (3) in the form

$$\begin{aligned}\bar{c}(r)/M_1 = {} & z_1(r_F)\psi_1(r) \\ & + 2(K_2 - B_{11})z_1(r_F)^2\psi_1(r)^2 \\ & + 3(K_3 - K_2B_{12} + 2B_{11}^2 - B_{111}/2)z_1 \\ & \quad (r_F)^3\psi_1(r)^3 \\ & + \ldots \end{aligned} \tag{5}$$

the base-molar concentration of solute now becomes a polynomial expansion with $\psi_1(r)$ as the independent variable [7]. Although $z_1(r_F)$ is of unknown magnitude *ab initio*, it is a constant that may be evaluated as one of the parameters obtained by non-linear least-squares analysis of the sedimentation equilibrium distribution in terms of Eq. (5).

As indicated in Section 1, the coefficients for the quadratic and cubic terms in Eq. (5) reflect contributions from potential-of-mean-force interactions as well as from equilibrium constants for solute self-association. Unambiguous evaluation of K_2 and K_3 is thus conditional upon the specification of magnitudes for B_{11}, the excluded-volume contribution to the quadratic coefficient, as well as B_{12} and B_{111}, the corresponding contributions of monomer-dimer and monomer-monomer-monomer interactions to the term in $\psi_1(r)^3$. We therefore examine the problem of assigning magnitudes to these parameters.

2.2. Evaluation of excluded volume parameters for spherical species

The magnitudes of B_{11} and B_{12} have previously [7,13] been evaluated from relationships of the form [10,15]

$$B_{11} \approx 16\pi NR_1^3/3 + \frac{Z_1^2(1 + 2\kappa R_1)}{4I(1 + \kappa R_1)^2} \tag{6a}$$

$$\begin{aligned} B_{12} \approx {} & 4\pi N(R_1 + R_2)^3/3 \\ & + \frac{Z_1Z_2(1 + \kappa R_1 + \kappa R_2)}{2I(1 + \kappa R_1)(1 + \kappa R_2)} \end{aligned} \tag{6b}$$

where N is Avogadro's number and the charge-charge terms are expressed in standard Debye-Hückel nomenclature. Z_1 and Z_2 are the respective net charges on monomer and dimer ($Z_2 = 2Z_1$ for conditions of charge conservation) with radii R_1 and R_2; and at 20°C the inverse screening length, κ (cm^{-1}), may be calculated as $3.27 \times 10^7\sqrt{I}$, where I is the molar ionic strength. In these expressions, which are based on spherical geometry for monomer and dimer, $R_2 = 2^{1/3}R_1$.

As a check on the adequacy of those estimates, the values of B_{11} and B_{12} have also been determined by numerical integration over the potential energy, $u_{ij}(r_{ij})$, of two spherical molecules i and j specified as a function of the center-to-center separation r_{ij} [4]. The corresponding numerical integration procedure for evaluating B_{111} has also been described [4,7]. We reiterate that numerical integration procedure for the evaluation of B_{12} for spherical monomer and dimer before extending the approach to the evaluation of B_{12} when the dimer is modeled as two spherical monomers in direct contact.

2.3. Evaluation of the second virial coefficient for the monomer–dimer excluded volume interaction by numerical integration

For dissimilar molecules (i and j) whose en-

ergy of interaction depends only on their separation vector $\mathbf{r}_{ij}$ and not explicitly on their relative orientations, the second virial coefficient, B_{ij}, is [16,17]

$$B_{ij} = -N\int f_{ij}(\mathbf{r}_{ij})\mathrm{d}\mathbf{r}_{ij} \tag{7}$$

where $f_{ij}(\mathbf{r}_{ij})$ is the Mayer f-function given by [6]

$$f_{ij}(\mathbf{r}_{ij}) = \exp\left[-u_{ij}(\mathbf{r}_{ij})/(kT)\right] - 1 \tag{8}$$

in which $u_{ij}(\mathbf{r}_{ij})$ specifies the potential energy of the two molecules as a function of the center-to-center separation vector, $\mathbf{r}_{ij}$; and where the integral in Eq. (7) is taken over all relative positions $\mathbf{r}_{ij}$: k is the Boltzmann constant. The potential-of-mean-force $u_{ij}(\mathbf{r}_{ij})$ between compact macromolecules can be divided into hard-sphere (excluded volume) and screened electrostatic contributions that are relevant in different ranges of the separation vector $\mathbf{r}_{ij}$. For two spheres with radii R_1 and R_2, the energy function $u_{12}(\mathbf{r}_{12})$ is spherically symmetric and depends only on the magnitude $|\mathbf{r}_{12}| = r$ of the separation vector. In terms of the Debye-Hückel parameter κ, the dielectric constant of the medium ε, the electronic charge e and the net charge Z_i on each macromolecules is given by

$$u_{12}(r) = \begin{cases} \infty, & r < (R_1 + R_2) \\ \dfrac{Z_1 Z_2 e^2}{\varepsilon(1+\kappa R_1)(1+\kappa R_2)} \dfrac{\exp[-\kappa\{r-(R_1+R_2)\}]}{r}, & r \geq (R_1 + R_2) \end{cases} \tag{9}$$

from which Eq. (6b) can be retrieved after substitution into Eq. (8) [10,15].

We now consider the interaction between a spherical charged monomer (species 1) and a dimer (species 2) consisting of two identical monomers (radius R) which are touching. The charge distribution on each monomer, including those comprising the dimer (signified 'A' and 'B'), is considered to be spherically symmetric. The separation vector $\mathbf{r}_{12}$ relevant to the use of Eq. (8) is now the vector from the center of the monomer to the center of the dimer, but the potential-of-mean-force $u_{12}(\mathbf{r}_{12})$ is expressed as a sum of contributions arising from quasi-independent interactions between the monomer (1) and each of the spheres (A or B) comprising the dimer. An appropriate spherical-polar coordinate (r, ϕ, θ) system is defined in Fig. 1. In particular, ϕ is the azimuthal angle between the separation vector $\mathbf{r}_{12}$ and the axis of the dumbbell dimer. For any azimuthal angle ϕ the monomer is excluded to a distance

$$R_\phi = R\left(\cos\phi + \sqrt{3 + \cos^2\phi}\right) \tag{10}$$

beyond which only the electrostatic part of the energy of interaction is relevant. By using the form of Eq. (9) to sum the contributions due to

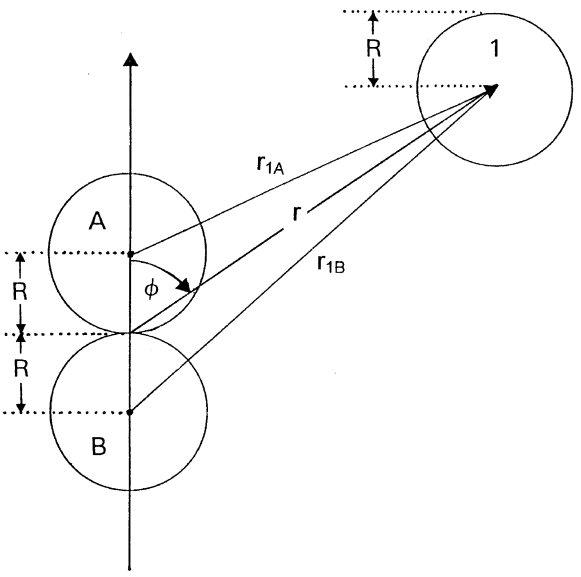

Fig. 1. Specification of nomenclature and coordinate system used in the numerical integration procedure for calculating the excluded volume interaction between a spherical monomer and a dumbbell-shaped dimer.

'1A' and '1B' effects the electrostatic energy of interaction can be written as

$$u_{12}(\mathbf{r}_{12}) = \frac{Z_1^2 e^2}{\varepsilon(1+\kappa R)^2}\left[\frac{\exp[-\kappa(r_{1A}-2R)]}{r_{1A}} + \frac{\exp[-\kappa(r_{1B}-2R)]}{r_{1B}}\right], \quad r_{12} \geq R_\phi \tag{11}$$

where r_{1A} and r_{1B} are the distances from the center of the monomer (1) to the center of each of the spheres (A and B) comprising the dimer, and are expressed in terms of r, R and ϕ as

$$\left.\begin{matrix} r_{1A} \\ r_{1B} \end{matrix}\right\} = \sqrt{r^2 + R^2 \pm 2rR\cos\phi} \tag{12}$$

Finding B_{12} now requires evaluation of the integral

$$B_{12} = -N\int_0^{2\pi} d\theta \int_0^{\pi} \sin\phi \, d\phi \int_0^{\infty} f_{12}(r,\phi,\theta) r^2 \, dr \tag{13}$$

and we note that symmetry allows the integration over θ to be performed independently, yielding a factor of 2π. Integration over r and ϕ can be performed explicitly for the range of the hard-sphere interaction [within which $f_{12}(\mathbf{r}_{12}) = -1$], yielding a contribution of $18\pi NR^3$ to B_{12}. The reflection symmetry of the dumbbell allows for further minor simplification within the range of electrostatic interactions to give

$$B_{12} = N\left[18\pi R^3 - 8\pi\int_0^{\pi/2} \sin\phi \, d\phi \times \int_{R_\phi}^{\infty} f_{el}(r,\phi) r^2 \, dr\right] \tag{14}$$

and the electrostatic Mayer f-function, $f_{el}(r,\phi)$, is obtained by substitution of Eq. (11) into Eq. (7). Eq. (14) does not afford any useful analytical result even in the case $u_{12}(\mathbf{r}_{12}) \ll kT$; but it can be integrated numerically to obtain good estimates of B_{12} from knowledge of the radius and charge of the macromolecular monomer. We note that $f_{el}(r,\phi)$ is negative for positive $u_{12}(\mathbf{r}_{12})$, which is the case here; and therefore that electrostatic interactions make a positive contribution to B_{12}.

3. Experimental

A solution of α-chymotrypsin (Worthington) was prepared by direct dissolution of the crystalline enzyme preparation into acetate-chloride buffer, pH 4.1, I 0.05 (0.01 M sodium acetate-0.04 M sodium chloride, pH adjusted with acetic acid), and dialyzed against the same buffer to establish dialysis equilibrium. Dialyzed enzyme solution (200 μl, approx. 2 mg/ml) was then subjected to centrifugation in a Beckman XL-I ultracentrifuge operating at 20°C and a rotor speed of 30 000 rev./min to generate a sedimentation equilibrium distribution conforming with the meniscus-depletion design of experiment [18]. The resulting sedimentation equilibrium distributions were recorded by the Rayleigh interference optical system and corrected for baseline variation on the basis of a minor linear radial dependence of the absolute fringe displacement. The base-molar enzyme concentration at each radial distance, $\bar{c}(r)/M_1$, was then calculated from the corrected absolute fringe displacement $J_c(r)$ by means of the relationship $\bar{c}(r) = J_c(r)/3.33$ [19] and a molecular mass of 25 000 for monomeric α-chymotrypsin.

Results were then analyzed by non-linear least-squares curve-fitting to Eq. (5) with $r_F = 7.100$ cm, $\bar{v} = 0.736$ ml/g [20] and a measured buffer density of 0.9998 g/ml. For calculation of the excluded volume parameters in Eq. (5) the radius of monomeric α-chymotrypsin was assigned a magnitude of 2.44 nm [5,7], while the valences Z_1 and Z_2 were taken as +10 and +20, respectively [21].

4. Results and discussion

The self-association of α-chymotrypsin in the vicinity of pH 4 is a well-characterized interaction [5,7,21–25] that is restricted to a monomer–dimer equilibrium. Furthermore, the inverse depen-

dence of the dimerization constant upon ionic strength [23] allows the selection of conditions (ionic strength) which should require allowance for effects of thermodynamic non-ideality beyond the consequences of nearest-neighbor interactions. Because the dimerization of α-chymotrypsin in acetate-chloride buffers with ionic strengths of 0.20 and 0.08 M was sufficiently strong to allow its characterization in terms of Eq. (5) truncated at the quadratic term [7], the ionic strength has been lowered to 0.05 M in the present investigation. Even after the simplification that emanates from the confinement of enzyme self-association to a monomer-dimer equilibrium ($K_3 = 0$), any meaningful attempt to determine K_2 is clearly conditional upon the assignment of magnitudes to the three virial coefficients B_{11}, B_{12} and B_{111}. It is therefore appropriate to postpone consideration of the sedimentation equilibrium data until after a discussion of the magnitudes to be assigned to these interaction parameters for 'non-associative' clusters [26].

4.1. Magnitudes of virial coefficients for α-chymotrypsin (pH 4.1, I 0.05)

Although relationships such as Eqs. (6a) and (6b) have been proposed [9,15] as approximate expressions for describing non-associative interactions between two spherical solutes, the adequacy of these truncated series expansions for the charge-charge contribution can be a matter for concern. Indeed, with decreasing ionic strength the approximation that $u_{ij}(\boldsymbol{r}_{ij})/(kT) \ll 1$ becomes increasingly poor at small molecular separations. We have therefore taken the precaution of also determining the magnitudes of the second virial coefficients (B_{11} and B_{12}) by numerical integration of the Mayer *f*-function [Eq. (7)].

Values of the three required virial coefficients obtained by the numerical integration procedure on the basis of spherical geometry for all species [4] are shown in column 2 of Table 1, which also presents the corresponding values of B_{11} and B_{12} calculated from Eqs. (6a) and (6b), respectively. These approximate values overestimate the magnitudes of the virial coefficients listed in column 2, the discrepancy being 21% for B_{11} and 35% for B_{12}. Inasmuch as the charge-charge contribution to either B_{11} or B_{12} in the approximate expressions is restricted to the first term of a power series in Z_1^2 (or $Z_1 Z_2$), extension of the series to include higher-order terms (which alternate in sign) is clearly required for better estimates of virial coefficients to be obtained by this approach for the present system with $R_1 = 2.44$ nm, $Z_1 = +10$ and $I = 0.05$ M. In other words, Eqs. (6a) and (6b) only provide reasonable estimates of the second virial coefficients in situations where the combination of a relatively low charge density and a moderately high ionic strength improve the validity of the inherent assumption that $u_{ij}(\boldsymbol{r}_{ij})/(kT) \ll 1$. We shall therefore employ the values of the various virial coefficients that have been obtained by numerical integration (those listed in column 2 of Table 1).

Table 1
Estimates of virial coefficients for α-chymotrypsin, pH 4.1, I 0.05[a]

Parameter	Exact value[b]	Approx. value[c]
B_{11} (l mol^{-1})	364	441
B_{12} (l mol^{-1})	1140	1535
B_{111} (l^2 mol^{-2})	36 100	

[a] Based on spherical geometry, a radius of 2.44 nm and a net charge of +10 for monomeric α-chymotrypsin.
[b] Value calculated by numerical integration of the Mayer *f*-function [4].
[c] Value calculated from either Eq. (6a) or Eq. (6b).

As well as obtaining B_{12} by numerical integration over the potential energy for the system with the two species modeled as spheres, a value has also been obtained for the excluded volume interaction between a spherical monomer and a dumbbell-shaped dimer. In that regard the procedure for such calculations (Section 2.3) follows the classical procedure of McMillan and Mayer [6]; and thus differs from the approach used by Berg [27] for the same purpose. Although there is a slight disparity between the consequent value of 1057 l/mol for B_{12} and that (1140 l/mol) listed in column 2 of Table 1, its extent (8%) is shown later to be insufficient to have any marked impact on the magnitude of K_2 obtained with allowance for effects of thermodynamic non-ideality.

This relative insensitivity of the magnitude of B_{12} to the specific shape assigned to dimeric α-chymotrypsin is encouraging from the viewpoint of allowing for effects of thermodynamic non-ideality in studies of solute self-association because information is frequently unavailable on the likely shape of putative oligomeric states of a protein. Resort to calculations based on spherical geometry for all species is thus likely to suffice for the incorporation of adequate allowance for effects of thermodynamic non-ideality in studies of solute self-association.

4.2. Evaluation of the dimerization constant for α-chymotrypsin (pH 4.1, I 0.05)

We now present an illustrative (rather than a definitive) application of the present approach to making allowance for the effects of thermodynamic non-ideality in the characterization of α-chymotrypsin dimerization by sedimentation equilibrium. For this purpose an experiment conducted at 30 000 rev./min has yielded an equilibrium distribution with corrected absolute fringe displacement (J_c) ranging from essentially zero to 28 fringes (Fig. 2). Because this translates into a protein concentration range of 0–8.4 mg/ml, incorporation of the cubic term in Eq. (5) should be required to provide adequate allowance for effects of thermodynamic non-ideality in the determination of the dimerization constant at the relatively low ionic strength ($I = 0.05$ M) of this experiment.

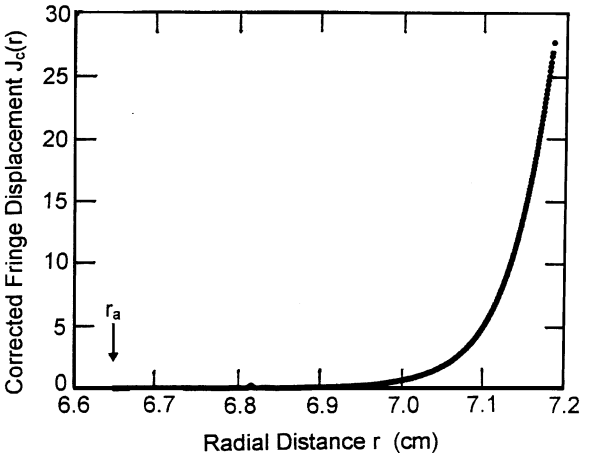

Fig. 2. Rayleigh interferometric record of a high-speed [18] sedimentation equilibrium distribution obtained by ultracentrifugation (20°C, 30 000 rev./min) of α-chymotrypsin (pH 4.1, I 0.05). r_a denotes the position of the air–liquid meniscus.

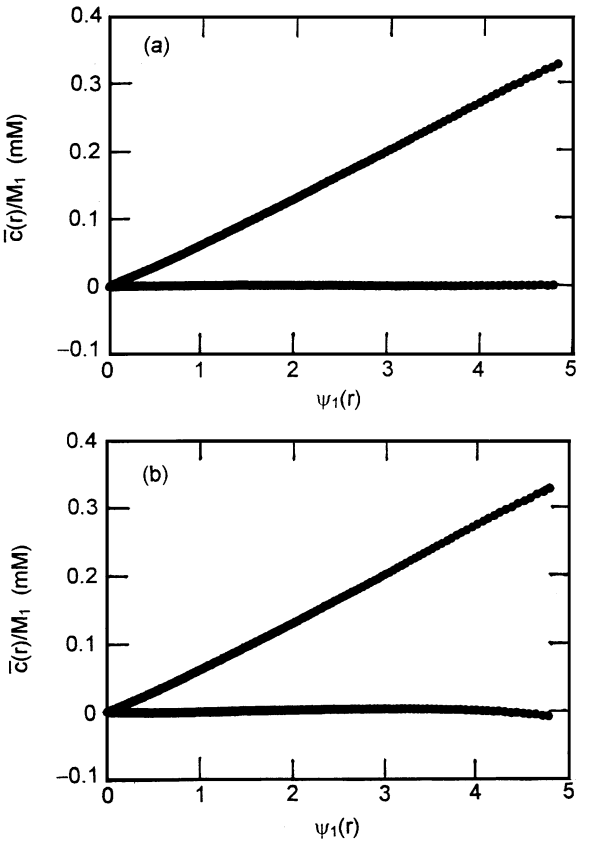

Fig. 3. Evaluation of the dimerization coefficient for α-chymotrypsin in acetate-chloride buffer (pH 4.1, I 0.05) by non-linear least-squares curve-fitting (upper plot in each case) to (a) Eq. (13) and (b) the same expression truncated at the quadratic term. The lower plot in each case shows the distribution of residuals.

Conversion of the radial distribution to one in terms of the psi-function [Eqs. (4a) and (4b)] with $r_F = 7.100$ cm is shown in Fig. 3a (upper curve), where the number of experimental points (440) essentially obliterates any opportunity for visual comparison of their conformity with the best-fit

description obtained by non-linear least-squares curve-fitting in terms of the relationship

$$\bar{c}(r)/M_1 \approx [z_1(r_F)\psi_1(r)] + 2(K_2 - 364)[z_1(r_F)\psi_1(r)]^2 + 3(24\,694 - 1140K_2)[z_1(r_F)\psi_1(r)]^3 \quad (15)$$

which is the particular expression obtained by incorporating the estimates of the virial coefficients (column 2 of Table 1) into Eq. (5). On the grounds that the residuals (lower plot in Fig. 3a) show no discernible deviation from a random distribution about zero, we accept as best-fit estimates the respective values (±2 S.D.) of 1571 (±8) M^{-1} and 53.7 (±0.1) μM for K_2 and $z_1(r_F)$. It should be noted that the S.D. cited in K_2 refers to the statistical precision rather than the accuracy of the estimate of K_2 because no account has been taken of the consequences of any systematic error, or of correlations in the best-fit estimates of the two parameters, K_2 and $z_1(r_F)$.

The present estimate of approximately 1600 M^{-1} for the dimerization constant is larger than that of 1350 M^{-1} inferred from Fig. 1 of an earlier study [23] of α-chymotrypsin self-association under similar conditions (pH 4.1, I 0.05) but slightly higher temperature (25 cf. 20°C). However, because no allowance was made for effects of thermodynamic non-ideality in that analysis of weight-average molecular mass data [23], the parameter designated as the dimerization constant would have been the parameter $(K_2 - B_{11})$ on the basis that the range of enzyme concentration covered (0–3 mg/ml) would be consistent with neglect of the term in $\psi_1(r_F)^3$ [7]. The consequent increase in the estimate of K_2 to approximately 1700 M^{-1} brings the results into reasonable agreement, particularly in the light that the two estimates of K_2 refer to different batches of enzyme studied under similar but not identical conditions.

An attempt to analyze the results in terms of Eq. (13) truncated at the quadratic term is far less satisfactory (Fig. 3b). The best-fit parameters exhibit greater statistical uncertainty [$K_2 = 680$ (±16) M^{-1}; $z_1(r_F) = 59.4$ (±0.3) μM], and the residuals show a systematic departure from zero in the region of highest $\psi_1(r)$ and $\bar{c}(r)/M_1$, where the effect of unjustified neglect of the cubic term in Eq. (13) would be most pronounced. Inability of the truncated version of Eq. (13) to introduce sufficient curvature into the dependence of $\bar{c}(r)/M_1$ upon $\psi_1(r)$ has been compensated by an overestimate (10%) of the best-fit $z_1(r_F)$ value and an underestimate (57%) of the dimerization constant: the values of K_2 and $z_1(r_F)$ are of necessity highly correlated in any attempt to evaluate both parameters by non-linear least-squares curve-fitting. This correlation is also responsible for the gross overestimate of 2574 (±34) M^{-1} that is obtained for the dimerization constant on the basis of the two approximate magnitudes for B_{11} and B_{12} (column 3 of Table 1) — a consequence of the underestimate, 49.4 (±0.2) μM, for $z_1(r_F)$. Use of the numerical integration procedure for determining the second virial coefficients (as well as the third virial coefficient) is thus strongly recommended. Although a software package written in MATLAB for such calculations is available on request, a major obstacle to its application is the requirement of a value for the net charge (valence) of the solute monomer — a parameter which cannot reliably be calculated but which is rarely measured [21].

The above statement (Section 4.1) that the 7% discrepancy between values of B_{12} based on spherical and dumbbell shapes for dimer should have relatively little effect on the determined magnitude of K_2 is supported by repeating the curve-fitting of the sedimentation equilibrium distribution with the dumbbell-based value of 1057 l/mol substituted for the B_{12} of 1140 l/mol in Eq. (13). The consequent estimate of 1452 (±8) M^{-1} for K_2 does differ slightly from that of 1571 M^{-1} obtained from analysis on the basis of spherical geometry for the dimer; but in terms of reaction energetics, the ΔG° values of −4.24 and −4.28 kcal/mol differ trivially.

5. Concluding remarks

This investigation has emphasized the impor-

tance and relative simplicity of making allowance for effects of thermodynamic non-ideality on the characterization of protein self-association by sedimentation equilibrium. With one exception [13] those working in the field have not availed themselves of the rigorous expressions that have been derived for such purposes [7,26]. Admittedly, it is necessary to ascribe magnitudes to the virial coefficients that take into account the consequences of non-associative clustering. However, although the validity of the precise magnitudes assigned to these parameters is invariably open to question, some leeway in the magnitude of B_{12} can seemingly be tolerated without undue influence on the estimate of K_2. It is therefore hoped that this investigation may encourage more researchers involved in the study of protein interactions to abandon the empirical Adams-Fujita approach [28] in favor of taking into account the effects of thermodynamic non-ideality by means of expressions with rigorous statistical-mechanical bases.

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