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Quantitative Assessment of the Relative Contributions of Steric Repulsion and Chemical Interactions to Macromolecular Crowding

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ABSTRACT:

The term "macromolecular crowding" denotes the combined effects of high volume fractions of nominally unrelated macromolecules upon the equilibrium and transport properties of all macrosolutes, dilute as well as concentrated, in the crowded medium. We present a formal partitioning of the total crowding effect into contributions from steric exclusion (excluded volume) and weak, nonspecific attractive interactions between a concentrated "crowding agent" and reactant and product species present at trace concentration. A numerical example of the combined effect of both steric and chemical interactions between crowder and tracer upon the reversible dimerization of tracer is presented, based upon reasonable estimates of the magnitude of both repulsive and attractive interactions between tracer and crowder species. Published 2012 Wiley Periodicals, Inc. Biopolymers 99: 239–244, 2013.*

Keywords: excluded volume; steric repulsion; weak binding; equivalent hard particle model

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INTRODUCTION

The stability, reactivity, and transport properties of dilute proteins and other macromolecules have been found to be significantly affected by the presence of high concentrations of nominally unrelated and presumably unreactive proteins and polymers that occupy a substantial fraction of total solution volume.[1–3] These effects have been referred to as consequences of "macromolecular crowding." Many effects of crowding upon reaction equilibria involving isomerization or association of dilute "test" macromolecules may be accounted for qualitatively and even semi-quantitatively by simple statistical-thermodynamic models invoking entropic changes resulting from the size- and shape-dependent reduction in solution volume available to the test molecule by the concentrated "background" macromolecules due to mutual impenetrability.[4]

Although it has long been recognized that long-ranged chemical interactions between background and test molecules may in principle and in certain cases do contribute significantly to crowding effects,[5] until recently attention has been focused upon the excluded volume consequences of crowding due to their ubiquity and generality, since excluded volume effects do not depend upon the chemical identity of test and background molecules, but only upon their relative sizes and shapes. However, in recent years additional examples of phenomena that cannot be satisfactorily accounted for on the basis of excluded volume alone have been reported.[6–8] These findings emphasize a need to provide a quantitative treatment of chemical interactions between tracer and background molecules to supplement the existing treatment of excluded volume interactions, and hence provide a more comprehensive framework for understanding and interpreting the thermodynamic consequences of macromolecular crowding. In what follows, we shall develop this treatment as it applies to a simple association equilibrium. Generalizations to more complex and isomerization equilibria will become evident as we proceed.

This work is dedicated to the memory of Professor Henryk Eisenberg, under whose guidance the author was first introduced to the field of physical biochemistry. The interest in obtaining a quantitative understanding of the behavior of biological macromolecules that he inspired in me endures to this day.

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GENERAL STATISTICAL-THERMODYNAMIC CONSIDERATIONS

Consider the specific association of two protein monomers in solution to form a homodimer:

$$2A_1 \rightleftarrows A_2 \tag{1}$$

Under a given set of experimental conditions (temperature, pressure, and solvent composition), the conversion of two moles of A_1 to one mole of A_2 in dilute (thermodynamically ideal) solution will result in a change in the Gibbs free energy of the solution amounting to $\Delta G^0_{12,\text{ideal}}$, a quantity referred to as the standard state free energy change or the standard state free energy of the reaction in ideal solution. Under the same set of experimental conditions, the conversion of two moles of A to one mole of A_2 in a "crowded" (thermodynamically nonideal) solution containing a concentration c_B of a background species will result in a corresponding standard state free energy change $\Delta G^0_{12,\text{nonideal}}$. According to the thermodynamic cycle depicted in Figure 1,

$$\Delta G^0_{12,\text{nonideal}} - \Delta G^0_{12,\text{ideal}} = \Delta G^0_{\text{transfer},2} - 2\Delta G^0_{\text{transfer},1} \tag{2}$$

where $\Delta G^0_{\text{transfer},i}$ denotes the free energy change accompanying the transfer of one mole of A_i from a fixed position in the ideal dilute solution to a fixed position in the nonideal solution containing concentration c_B of the background species B, referred to as the standard state transfer free energy. This quantity is related to the thermodynamic activity coefficient of each species by

$$\Delta G^0_{\text{transfer},i} = RT\ln\gamma_i \tag{3}$$

It follows from Eqs. (2) and (3) that

$$\ln K = \ln K_o + 2\ln\gamma_1 - \ln\gamma_2 \tag{4}$$

where K_o and K denote the equilibrium association constant c_2/c_1^2 in the ideal and nonideal solutions, respectively. It is stressed that while K_o is truly constant at constant T, P, and solvent conditions, K is subject to variation with c_B as shown below. We refer to the effect of changing c_B upon the equilibrium association constant as the "crowding effect," and define a quantitative measure of the crowding effect called the "crowding factor"[9]:

$$\Gamma = \frac{K}{K_o} = \frac{\gamma_1^2}{\gamma_2} \tag{5}$$

One may conceptually decompose the process of transferring a molecule of solute species i from an ideal to a nonideal solution into the following steps:

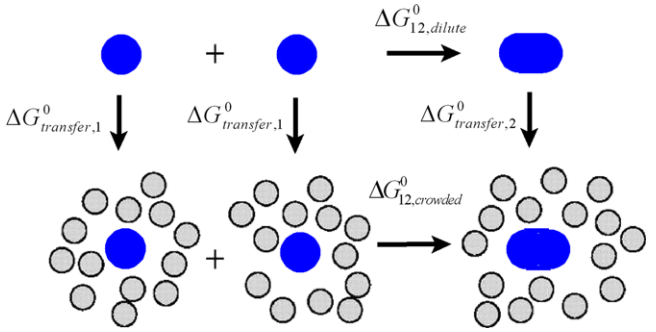

FIGURE 1 Thermodynamic cycle indicating free energy changes accompanying dimerization in dilute and crowded media, and transfer of monomer and dimer from a dilute to a crowded medium.

1. Turn off all chemical interactions in the nonideal solution. This has the effect of turning background molecules into hard particles and is associated with a free energy change of $-\Delta G^0_{BB,\text{preinsertion}}$.
2. Create a cavity in the hard particle fluid into which a molecule of species i can fit without overlapping any part of any background molecule. This is associated with a purely negentropic free energy change of $\Delta G^0_{\text{cavity},i} = -T\Delta S^0_{\text{cavity},i}$ which is always > 0.
3. Take a molecule from a fixed location in the dilute solution and place it in the cavity formed in step 2. This step may be accomplished with no free energy change since in the absence of chemical interactions (step 1) there is no interaction between the molecule of species i in the cavity and any background molecule.
4. Turn on chemical interactions in the nonideal solution, which include interactions between the newly introduced molecule and background molecules and interactions between the background molecules themselves. This process is thus associated with a free energy change of $\Delta G^0_{i,B} + \Delta G^0_{\text{BB,postinsertion}}$.

Thus the total transfer free energy is as follows:

$$\Delta G^0_{\text{transfer},i} = \Delta G^0_{\text{cavity},i} + \Delta G^0_{\text{chem},i} \tag{6a}$$

where

$$\Delta G^0_{\text{chem},i} = \Delta G^0_{i,B} + \Delta G^0_{BB,\text{postinsertion}} - \Delta G^0_{BB,\text{preinsertion}} \tag{6b}$$

Thus, the thermodynamic activity coefficient may be partitioned into contributions from excluded volume and from chemical interactions

$$\ln\gamma_i = \ln\gamma_{\text{exvol},i} + \ln\gamma_{\text{chem},i} \tag{7a}$$

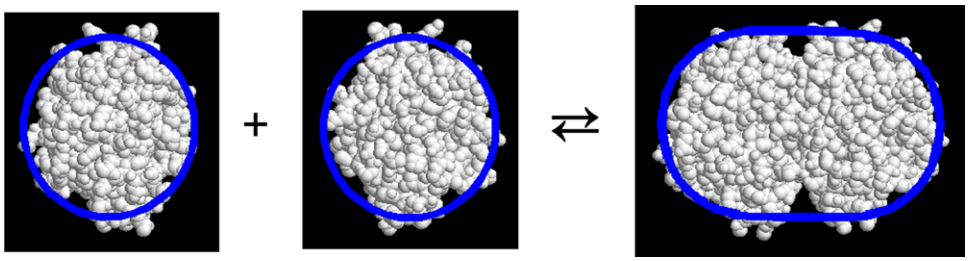

FIGURE 2 Equivalent convex particles described in text superimposed on molecular models of monomeric and dimeric alpha-chymotrypsin (PDB 4CHA).

where

$$\ln\gamma_{\mathrm{exvol},i} = \Delta G^0_{\mathrm{cavity},i}/RT \tag{7b}$$

and

$$\ln\gamma_{\mathrm{chem},i} = \Delta G^0_{\mathrm{chem},i}/RT \tag{7c}$$

It is evident that when chemical interactions between the test species i and the background molecules are predominantly attractive ($\Delta G^0_{\mathrm{chem},i} < 0$), then $\ln\gamma_i < \ln\gamma_{\mathrm{exvol},i}$, i.e., attractive chemical interactions can compensate excluded volume effects to a greater or lesser degree.

Equation (7) may be expanded in terms of enthalpic and entropic contributions to the transfer free energy:

$$\ln\gamma_i = \frac{\Delta G^0_{\mathrm{transfer},i}}{RT} = \frac{\Delta H^0_{\mathrm{chem},i}}{R}\frac{1}{T} - \frac{(\Delta S^0_{\mathrm{exvol},i} + \Delta S^0_{\mathrm{chem},i})}{R} \tag{8}$$

where $\Delta S^0_{\mathrm{exvol},i} = \Delta S^0_{\mathrm{cavity},i}$. Equation (8) informs us that a significant dependence of ln γ_i upon temperature is a hallmark of significant chemical interactions between species i and the background molecules present in solution.

Combination of Eqs. (5) and (7) yields as follows:

$$\ln\Gamma = \ln\Gamma_{\mathrm{exvol}} + \ln\Gamma_{\mathrm{chem}} \tag{9}$$

where

$$\ln\Gamma_{\mathrm{exvol}} = 2\ln\gamma_{\mathrm{exvol},1} - \ln\gamma_{\mathrm{exvol},2}$$

and

$$\ln\Gamma_{\mathrm{chem}} = 2\ln\gamma_{\mathrm{chem},1} - \ln\gamma_{\mathrm{chem},2}$$

A NUMERICAL EXAMPLE BASED UPON SIMPLIFIED STRUCTURAL MODELS AND "REALISTIC" THERMODYNAMIC PARAMETERS CHARACTERIZING ATTRACTIVE INTERACTION BETWEEN TRACE SPECIES AND BACKGROUND MOLECULES

It has been shown that the nonideal behavior of several proteins in highly concentrated and/or crowded solutions may be well described by simple structural models in which globular proteins and other macromolecules are represented by equivalent convex hard particles (see for example[10–12]). In what follows we shall present an extension of the equivalent hard particle model to treat the case of significant attractive nonspecific chemical interactions between test molecules and background molecules that can, depending upon their magnitude, attenuate, or even override excluded volume effects upon reaction equilibria.

To facilitate numerical computation, we shall make the following simplifying assumptions. In reversible dimerization Scheme 1, we shall represent the monomer A_1 by a sphere of radius r_1, and the dimer A_2 by a spherocylinder of cylindrical radius $r_2 = r_1$ and a cylinder length equal to L times the cylinder diameter. In order for the protein volume to be conserved upon dimerization, $L = 2/3$. A comparison between this equivalent hard particle model and a more detailed atomic model for the acid dimerization of α-chymotrypsin in shown in Figure 2. It may be seen that for the purpose of calculating volume excluded sterically to molecules of comparable size, the representation of molecular shape by simple convex particles is a reasonably accurate approximation. In addition, we represent the background species as another spherical particle of radius $r_B = r_1$. To calculate concentrations in molar units, we shall assume that monomer and crowder B have molar masses equal to that of α-chymotrypsin, 25,000. The specific excluded volume of all species is taken to be $\nu_{\mathrm{exc}} = 1$ cm^3/g.[3] It follows that $r_B = r_1 = 21.5$ Å, and the surface areas of spherical monomer (s_1) and spherocylindrical dimer (s_2) are, respectively, equal to 5755 and 9591 Å^2.

Using this structural model, the excluded volume contribution to the free energy of transfer of monomer and dimer from ideal to crowded solution may be estimated using the scaled particle theory of hard particle mixtures.[13–15] According to this theory, the negentropic work associated with the insertion of a single hard spherocylinder with cylindrical radius r_C and cylindrical axial ratio L into a suspension of

hard spheres of radius r_B that occupy a fraction ϕ of total solution volume is given by the following:

$$\ln\gamma_{\text{exvol},SC} = -\ln(1-\phi) + A_1 Q + A_2 Q^2 + A_3 Q^3 \quad (10a)$$

where

$$Q = \phi/(1-\phi) \quad (10b)$$

$$A_1 = R^3 + 3R^2 + 3R + 1.5L(R^2 + 2R + 1) \quad (10c)$$

$$A_2 = 3R^3 + 4.5R^2 + 4.5L(R^2 + R) \quad (10d)$$

$$A_3 = 3R^3 + 4.5LR^2 \quad (10e)$$

and $R = r_C/r_B$. Note that Eq. (10) also applies to insertion of a sphere of radius r_C in this fluid when $L = 0$.

The contribution of attractive interactions between crowder and test molecule to the free energy of transfer may be estimated by treating such interactions as formally equivalent to weak, unsaturable binding.[16] Let i-mer contain $n_{\text{site},i}$ sites for the binding of background molecule B, each of which can independently "bind," or attract, B according to the following scheme:

$$\text{site} + B \rightleftarrows \text{site}\cdot B$$

As an illustrative example of weak attractive intermolecular interactions describable as binding, we consider those between urea and an unfolded protein. At a fixed temperature, the dependence of the heat of binding of urea to unfolded ribonuclease A[17] may be well described by a simple independent binding site isotherm

$$Q = Q_{\max}\frac{K_A c_U}{1 + K_A c_U} \quad (11)$$

where K_A denotes the equilibrium association constant, and c_U the molar concentration of urea. The results obtained by Makhatadze and Privalov[17] at multiple temperatures could be accurately described by Eq. (11) with a temperature-dependent equilibrium association constant given by

$$\ln K_A(T) = \frac{\Delta G^o}{RT} = -\frac{\Delta H^o}{R}\frac{1}{T} + \frac{\Delta S^o}{R} \quad (12)$$

where R denotes the molar gas constant, T the absolute temperature, $\Delta H^o = -2000R$ and $\Delta S^o = -9.77R$. The calculated value of K_A is plotted as a function of temperature in Figure 3.

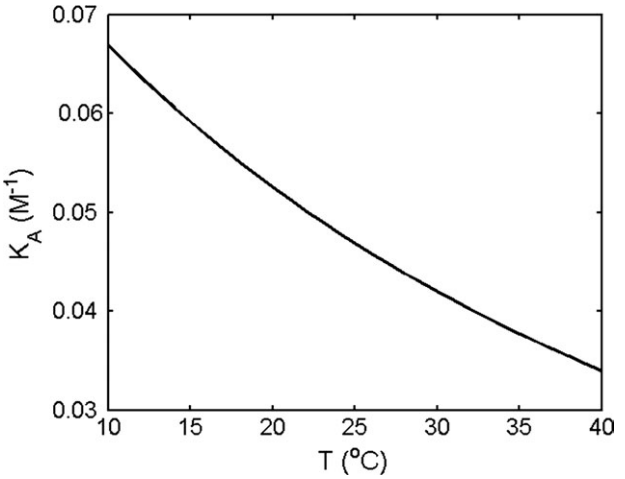

FIGURE 3 Temperature dependence of the equilibrium association constant for binding of urea to unfolded ribonuclease A, calculated as described in text.

In the example presented here, we shall assume that weak nonspecific attractive interactions between background species B and i-mer may be approximated by that between urea and unfolded ribonuclease, with the same temperature-dependent site binding constant: $K_{B,i}(T) = K_A(T)$. The free energy change associated with the binding of a nonideal ligand L to 1 mole of an ideal substrate S containing n identical and independent binding sites is given by[18]

$$\Delta G^0_{\text{bind}} = -nRT\ln(1 + K\gamma_L c_L) \quad (12)$$

where γ_L and c_L, respectively, denote the activity coefficient and molar concentration of ligand L. Equation (12) may be generalized to the case of a nonideal substrate[9]:

$$\Delta G^0_{\text{bind}} = -nRT\ln\left(1 + K\frac{\gamma_L\gamma_S}{\gamma_{LS}}c_L\right) \quad (13)$$

The free energy of binding B to nonideal i-mer is then given approximately by

$$\Delta G^0_{\text{bind},i} = -n_{\text{sites},i}RT\ln(1 + K^{(\text{app})}_{B,i}c_B) \quad (14)$$

where $K^{(\text{app})}_{B,i} = K_{B,i}\gamma_{\text{exvol},B}\gamma_{\text{exvol},i}/\gamma_{\text{exvol},B:i}$. As we have chosen B to have the same size and spherical shape as monomer, $\gamma_{\text{exvol},B} = \gamma_{\text{exvol},1}$ and $\gamma_{\text{exvol},B:1} = \gamma_{\text{exvol},2}$. We shall in addition approximate the shape of the complex of dimer and B by a spherocylinder with $r = r_1$ and a volume equal to three times that of monomer, such that $L = 4/3$. (Test calculations indicated that the final results are insensitive to the choice of shape). For the purpose of calculating the value of $K^{(\text{app})}_{B,i}$ as a function of T and φ, the activity coefficients of monomer,

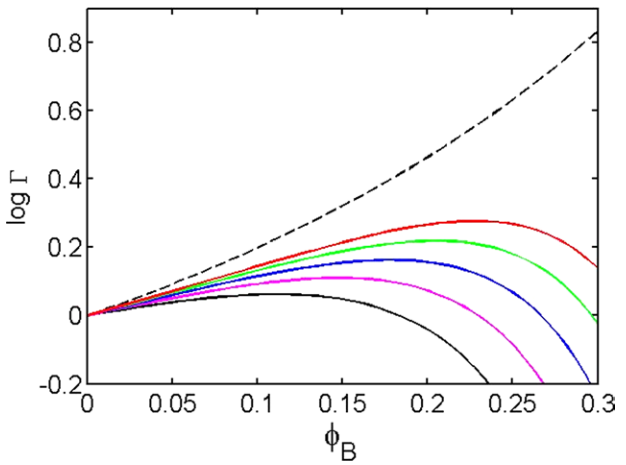

FIGURE 4 Dependence of the crowding factor for dimerization of trace molecules upon volume fraction of background species B and temperature, calculated as described in text. Dashed curve: calculated for pure excluded volume—no chemical interaction. Solid curves: red, green, blue, magenta, and black curves are calculated for $T = 40$, 30, 20, 10, and 0°C, respectively.

dimer, monomer:B, and dimer:B are estimated using Eq. (10). Since the number of virtual binding sites for B on i-mer is in general unknown, we make the reasonable approximation that $n_{\text{sites},i}$ is proportional to the surface area of i-mer, so Eq. (14) simplifies to

$$\ln\gamma_{\text{chem},i} = \Delta G^0_{\text{bind},i}/RT = -\alpha s_i \ln(1 + K^{(\text{app})}_{B,i} c_B) \qquad (15)$$

where α denotes a temperature-independent constant of proportionality that is equal for monomer and homodimer.

Given Eqs. (10), (15), and the simplified structural and thermodynamic models described above, we may use Eq. (9) to estimate the values of Γ_{exvol}, Γ_{chem}, and Γ for dimer formation as a function of φ_B and temperature. In Figure 4, the calculated dependence of ln Γ upon φ_B is plotted for a series of temperatures. For purposes of illustration, the value of α, which scales the magnitude of ln $\gamma_{\text{chem},i}$ was selected so that the temperature dependence of the crowding effect qualitatively resembles that reported for the heteroassociation of superoxide dismutase and catalase.[6]

CONCLUSIONS

The example presented above demonstrates that the general statistical-thermodynamic partitioning of total crowding effects into independent contributions from excluded volume (steric repulsion) and weakly attractive interactions can account for reports of temperature-dependent crowding effects[6] and crowding effects at fixed temperature that are substantially smaller, or of opposite sign, than predicted on the basis of excluded volume alone.[7,8,19] This is the result of competition between the tendency of nonspecific repulsive interactions to favor reactions that minimize surface exposure (e.g., protein folding and association) and the tendency of nonspecific attractive interactions to favor reactions that maximize surface exposure (e.g., protein unfolding and dissociation). Equation (15) suggests the intriguing but as yet experimentally unobserved possibility that in some systems (such as the numerical example presented here), the nonlinear dependence of γ_B upon φ_B may lead to an increasing fractional contribution of chemical interactions to the total crowding effect as φ_B increases. Under these conditions, steric effects would dominate at lower values of φ_B and higher temperatures, so that crowding agents would enhance protein association. However, at higher values of φ_B and lower temperatures, attractive interactions between trace reactants and crowder would dominate, and the crowding agent would inhibit selfassociation. The combination of the two effects is illustrated in Figure 4.

It is evident that future progress toward accurate interpretation of the observed effects of crowding in a particular medium will require that the free energy of transfer of reactants and products from dilute to the crowded medium be characterized as a function of temperature as well as the composition of the medium. Methods for doing so have been developed.[20,21] While preliminary studies aiming toward realization of this goal have been carried out,[22,23] much work remains to be done.

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REFERENCES

1. Hall, D.; Minton, A. P. Biochim Biophys Acta 2003, 1649, 127–139.
2. Zhou, H.-X.; Rivas, G.; Minton, A. P. Ann Rev Biophys 2008, 37, 375–397.
3. Zimmerman, S. B.; Minton, A. P. Ann Rev Biophys Biomol Struct 1993, 22, 27–65.
4. Minton, A. P. Methods Enzymol 1998, 295, 127–149.
5. Minton, A. P. Mol Cell Biochem 1983, 55, 119–140.
6. Jiao, M.; Li, H.-T.; Chen, J.; Minton, A. P.; Liang, Y. Biophys J 2010, 99, 914–923.
7. Phillip, Y.; Sherman, E.; Haran, G.; Schreiber, G. Biophys J 2009, 97, 875–885.
8. Miklos, A. C.; Sarkar, M.; Wang, Y.; Pielak, G. J. J Am Chem Soc 2011, 133, 7116–7120.
9. Minton, A. P. Biopolymers 1981, 20, 2093–2120.
10. Fernández, C.; Minton, A. P. Biophys J 2008, 96, 1992–1998.
11. Minton, A. P. J Pharm Sci 2007, 96, 3466–3469.
12. Minton, A. P. Biophys J 2008, 93, L57–L59.

13. Boublík, T. Mol Phys 1974, 27, 1415–1427.
14. Cotter, M. A. J Chem Phys 1977, 66, 1098–1106.
15. Lebowitz, J. L.; Helfand, E.; Praestgaard, E. J Chem Phys 1965, 43, 774–779.
16. Hill, T. L.; Chen, Y.-D. Biopolymers 1973, 12, 1285–1312.
17. Makhatadze, G. I.; Privalov, P. L. J Mol Biol 1992, 226, 491–505.
18. Peller, L. J Am Chem Soc 1959, 63, 1199–1206.
19. Minton, A. P. Biophys Chem 1995, 57, 65–70.
20. Fernández, C.; Minton, A. P. J Phys Chem B 2011, 115, 1289–1293.
21. Rivas, G.; Fernández, J. A.; Minton, A. P. Biochemistry 1999, 38, 9379–9388.
22. Fodeke, A. A.; Minton, A. P. J Phys Chem B 2010, 114, 10876–10880.
23. Fodeke, A. A.; Minton, A. P. J Phys Chem B 2011, 115, 11261–11268.

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