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Adsorption of Globular Proteins on Locally Planar Surfaces: Models for the Effect of Excluded Surface Area and Aggregation of Adsorbed Protein on Adsorption Equilibria

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ABSTRACT Equilibrium statistical-thermodynamic models are presented for the surface adsorption of proteins modeled as regular convex hard particles. The adsorbed phase is treated as a two-dimensional fluid, and the chemical potential of adsorbed protein is obtained from scaled particle theory. Adsorption isotherms are calculated for nonassociating and self-associating adsorbing proteins. Area exclusion broadens adsorption isotherms relative to the Langmuir isotherm (negative cooperativity), whereas self-association steepens them (positive cooperativity). The calculated isotherm for adsorption of hard spheres using scaled particle theory for hard discs agrees well with that calculated from the hard disc virial expansion. As the cross section of the adsorbing protein in the plane of the surface becomes less discoidal, the apparent negative cooperativity manifested in the isotherm becomes more pronounced. The model is extended to the case of simultaneous adsorption of a tracer protein at low saturation and a competitor protein with a different size and/or shape at arbitrary fractional saturation. Area exclusion by competitor for tracer (and vice versa) is shown to substantially enhance the displacement of tracer by competitor and to qualitatively invalidate the standard interpretation of ligand competition experiments, according to which the fractional displacement of tracer by competitor is equal to the fractional saturation by competitor.

INTRODUCTION

Interactions between proteins and surfaces may be classified in terms of the specificity of the interaction. At one extreme are the many well-studied instances of very specific and selective interaction of proteins, such as antibodies and polypeptide hormones, with particular epitopes or receptors that are found on the surface of a cell or virus particle (for a general survey, see Conn, 1984, and succeeding volumes in the series). At the other extreme one finds relatively weak and nonspecific electrostatic or hydrophobic interactions leading to nonlocalized adsorption of proteins onto lipid bilayers (Sankaram and Marsh, 1993), other structural elements of the cell (Arnold and Pette, 1968; Lakatos and Minton, 1991), and the surfaces of synthetic polymeric materials (Andrade, 1985). In the present work our attention is directed to the latter class of interactions. Although less studied, these interactions are also important for several reasons. First, significant association of "soluble" proteins with membranes and other cellular structural elements can have major consequences for metabolic organization and control (Clegg, 1984). Surface adsorption may be in some cases linked to the catalytic properties of individual enzymes (Kurganov, 1985) and to the formation of multienzyme complexes (Minton, 1995). Finally, nonspecific interactions between proteins and the surfaces of synthetic polymers play an essential role in determining the biocompatibility of the synthetic material (Horbett, 1982).

The adsorption of small molecules from the gaseous state onto regular surfaces such as the planar faces of crystals has been extensively studied both experimentally and theoretically by physical chemists (Steele, 1974). The understanding of such adsorption phenomena has benefited greatly from the ability to prepare systems of extremely high purity and to measure equilibrium adsorption isotherms precisely over a very broad range of concentrations of the gaseous adsorbing species. Analogous measurements of the surface adsorption of soluble proteins have not yielded (and perhaps cannot yield) similarly extensive and precise data. Partly for this reason, the theoretical basis for the interpretation of such isotherms remains at a level that is quite crude relative to that applied to the analysis of the simpler inorganic systems.

Two classes of models for the nonspecific adsorption of biomolecules to surfaces have been formulated. The first class of models (Stankowski, 1983) is lattice models adapted from earlier treatments of gas adsorption (Hill, 1960). According to such models, there exist a large number of virtual sites on a surface, where a site represents not a specific binding site but rather a discrete increment of surface area. An adsorbed ligand is postulated to occupy a fixed number (≥ 1) of sites with a specified geometry. Combinatorics are employed to count the number of possible configurations of n ligands bound to m sites, and hence the chemical potential of the adsorbed ligand. As the number of sites occupied by an individual adsorbed ligand and the total number of sites become large, results approach the

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continuum limit. The second class of models (Stankowski, 1984; Tamm and Bartoldus, 1988) utilizes continuum theories of two-dimensional hard particle fluids (e.g., Andrews, 1976) to calculate directly the chemical potential of adsorbed ligand. In the present work we shall propose models of the second type for the adsorption of ligands that exclude surface area to each other, called "large" ligands by previous investigators. ("Large" ligands are distinguished from "small" ligands according to the following criterion: if the presence of ligand bound to a particular site or location on the surface prevents another ligand molecule from binding to any site or location on the surface other than the site to which it is bound, then the bound ligand is "large." Hence the appellation refers not to absolute size, but to the size of ligand relative to the spacing between sites or possible binding locations. According to this definition, if the binding potential is independent of lateral position on the surface, then any ligand of finite size must be treated as a large ligand.)

In the next section a model is presented for the adsorption of a single class of large ligands of various shapes. In the third section competition between two species of nonassociating large ligands is treated. In the fourth section a model is presented for the adsorption of a large ligand that can also undergo self-association on the surface. In the fifth section the model for adsorption of a single large ligand is extended to treat the case in which the ligand also excludes a significant fraction of volume in the supernatant solution and hence behaves nonideally in both three and two dimensions. The paper concludes with a summary and discussion of major findings.

ADSORPTION OF A SINGLE NONASSOCIATING LIGAND

The fundamental equilibrium relation governing adsorption of a single species of ligand is

$$\mu^{\mathrm{soln}} = \mu^{\mathrm{surf}}, \tag{1}$$

where μ^{soln} denotes the chemical potential of free ligand in solution and μ^{surf} the chemical potential of adsorbed ligand. For the present we shall assume that the ligand behaves ideally in solution; the effect of nonideal behavior of free ligand will be considered subsequently. Hence

$$\mu^{\mathrm{soln}} = \mu^{\mathrm{soln,o}} + RT \ln c \tag{2}$$

and

$$\mu^{\mathrm{surf}} = \mu^{\mathrm{surf,o}} + RT \ln \Phi + RT \ln \gamma(\Phi), \tag{3}$$

where $\mu^{\mathrm{x,o}}$ denotes the standard state chemical potential of the corresponding species, c the concentration of ligand in solution, Φ the fraction of surface area (or fraction of total sites) occupied by adsorbed ligand, and γ the activity coefficient of adsorbed ligand, which is a function of Φ. Equations 2 and 3 may be combined to yield

$$Kc = \Phi\, \gamma(\Phi), \tag{4}$$

where

$$K \equiv \exp[-(\mu^{\mathrm{surf,o}} - \mu^{\mathrm{soln,o}})/RT].$$

Because a small ligand is defined as one that prevents only those sites to which it is bound from binding additional ligand (see criterion for "large" ligand, above), the equilibrium adsorption of a monovalent nonassociating small ligand may be shown to obey the familiar Langmuir isotherm (Hill, 1960):

$$Kc = \frac{\Phi}{1 - \Phi}. \tag{5}$$

The effect of short-range (steric) repulsive interactions between protein molecules upon the chemical potential of proteins in solution is well described by models in which protein molecules are represented by equivalent hard convex particles of approximately molecular size and shape that exclude volume to each other (Zimmerman and Minton, 1993). We shall therefore employ hard particle models to investigate the effect of steric area-excluding interactions between adsorbed proteins on adsorption equilibria. It will be assumed that molecules adsorbed to a locally planar surface exclude volume to each other in two dimensions only, i.e., in a plane parallel to the plane of the surface.

The simplest two-dimensional representation of a space-filling ligand is the hard circular disk. For this model the activity coefficient may be represented by a power series in the fractional area occupancy of adsorbed ligand

$$\ln \gamma = \sum_{i=2}^{\infty} \lambda_i \Phi^{i-1} \tag{6}$$

where the interaction coefficients, calculated from the known 2-D hard disk virial coefficients (Ree and Hoover, 1964), are given to seventh order by $\lambda_2 = 4.0$, $\lambda_3 = 4.692$, $\lambda_4 = 5.685$, $\lambda_5 = 6.680$, $\lambda_6 = 7.642$, and $\lambda_7 = 8.512$. With these coefficients, the infinite series on the right-hand side of Eq. 6 converges within the first six terms for $\Phi \leq 0.5$. In Fig. 1 the dependence of Φ upon log Kc, calculated over the range of convergence of the truncated series using Eqs. 4 and 6, is plotted together with the Langmuir isotherm for comparison. The qualitative effect of area exclusion, evident at values of Φ exceeding ~0.05, is to lower the fractional surface occupancy of adsorbed ligand in equilibrium with a given concentration of free ligand.

Although the power series expansion for the activity coefficient of hard circular disks given above is thermodynamically exact, calculation of the value of interaction coefficients for three-body or higher-order interactions between particles of different sizes and/or shapes is prohibitively difficult. Approximate theories of hard particle fluids, and in particular the scaled particle theory (SPT)

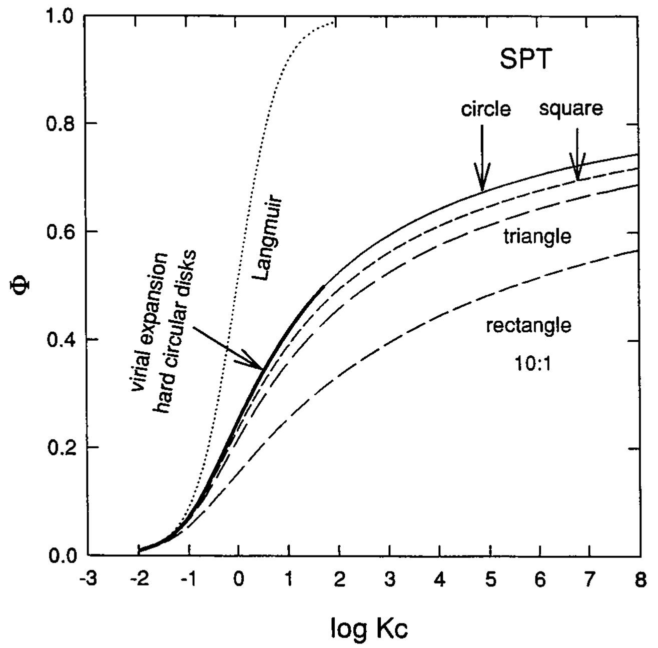

FIGURE 1 Adsorption isotherms calculated for various particle shapes, calculated using SPT Eq. 7, except "Langmuir" (Eq. 5) and "circle" (Eqs. 6 and 7).

originated by Reiss et al. (1959), have proved useful in providing a semiquantitative account of the behavior of more complex and/or more concentrated systems. The following expression for the activity coefficient of a single hard particle in a 2-D fluid of convex hard particles of arbitrary shape may be obtained from the SPT results of Boublik (1975):

$$\ln\gamma = -\ln(1-\Phi) - \epsilon - 1 + \frac{1}{1-\Phi} + \frac{\epsilon}{(1-\Phi)^2}, \qquad (7)$$

where ϵ is a shape parameter, the value of which is given for particles of various shapes in Table 1. Adsorption isotherms calculated using Eqs. 4 and 7 with values of ϵ for differently shaped particles are plotted in Fig. 1. The isotherm calculated for hard circular disks using SPT agrees quite well with that calculated using the exact virial expansion over the entire range of convergence of the virial expansion. Isotherms calculated for noncircular particles are even broader than those for circles, reflecting the fact that, of all convex particles, a circle has the minimum ratio of excluded area to particle area.

COMPETITION BETWEEN TWO SPECIES OF LARGE LIGAND

Let an adsorbent surface be equilibrated with solution containing free solute species A at a concentration c_A^o, chosen so that $K_A c_A^o \ll 1$. Under these conditions it follows from Eq. 4 that the equilibrium saturation of surface with A is given by

$$\Phi_A^o = K_A c_A^o. \qquad (8)$$

Let the surface be equilibrated with solution containing free A at concentration c_A^o and free solute B at arbitrary concentration c_B. To the extent that B competes with A for the surface, the fractional saturation of A will decline from Φ_A^o to Φ_A. If both A and B are small, monovalent ligands, and A does not self-associate, it may be shown that

$$\Phi_B = 1 - \frac{\Phi_A}{\Phi_A^o}, \qquad (9)$$

independently of the nature of the binding isotherm of B (Chatelier, 1987).

Next, consider an adsorbent surface in equilibrium with a solution containing two large ligands, A and B. The chemical potential of adsorbed ligands will be given by

$$\mu_A^{\text{surf}} = \mu_A^{\text{surf,o}} + RT\ln\Phi_A + RT\ln\gamma_A(\Phi_A, \Phi_B) \qquad (10a)$$

and

$$\mu_B^{\text{surf}} = \mu_B^{\text{surf,o}} + RT\ln\Phi_B + RT\ln\gamma_B(\Phi_A, \Phi_B), \qquad (10b)$$

where the dependence of activity coefficients on surface composition reflects interactions between adsorbed molecules. For a competition experiment in which the concentration of free ligand A is constrained to a value c_A^o such that $\Phi_A \ll 1$, the dependence of both γ_A and γ_B upon Φ_A

TABLE 1 Values of shape-dependent parameters defined in the text and in the Appendix

Shape/parameter	a	c	$\epsilon\ (\equiv c^2/4\pi a)$
Circle	π	2π	1
Regular polygon (n sides)	$n\tan(\pi/n)$	$2n\tan(\pi/n)$	$(n/\pi)\times\tan(\pi/n)$
Rectangle (axial ratio $g \geq 1$)	$4/g$	$4(1 + 1/g)$	$(g/\pi)\times(1 + 1/g)^2$
Ellipse (axial ratio $g \geq 1$)	π/g	$4\int_0^{\pi/2}\left(\frac{\sin^2\theta}{g^2} + \cos^2\theta\right)^{1/2} d\theta$	$\frac{4g}{\pi^2}\times\left[\int_0^{\pi/2}\left(\frac{\sin^2\theta}{g^2} + \cos^2\theta\right)^{1/2} d\theta\right]^2$

becomes negligible. In the absence of B, the equilibrium saturation of surface with A is given by Eq. 8. In the presence of an arbitrary concentration of B, the equilibrium saturation of surface with A is given by

$$\Phi_A = \frac{K_A c_A^o}{\gamma_A(\Phi_B)}. \tag{11}$$

Combination of Eqs. 2 and 11 yields

$$\frac{\Phi_A}{\Phi_A^o} = \frac{1}{\gamma_A(\Phi_B)}. \tag{12}$$

The activity coefficient of a single particle of species 2 in a 2-D fluid of particles of species 1 is calculated using SPT together with results obtained by Boublik (1975) in the Appendix. Letting A be species 2 and B species 1, combination of Eqs. 12 and A9 yields

$$\frac{\Phi_A}{\Phi_A^o} = (1 - \Phi_B) \times \Lambda, \tag{13}$$

where

$$\Lambda \equiv \exp\left\{-f_R\,\Phi_B\left[2\epsilon f_c \frac{1}{1 - \Phi_B} + f_a f_R\left(\frac{1 - (\epsilon - 1)\Phi_B}{(1 - \Phi_B)^2}\right)\right]\right\}.$$

ϵ is the shape factor defined in the Appendix for particles of species B, and the coefficients f_R, f_c, and f_a are functions, defined in the Appendix, of the relative sizes and shapes of particles of species A and B. It is evident that when the size of the particle of species A becomes infinitesimal relative to that of the size of the particle of species B, $f_R \rightarrow 0$, $\Lambda \rightarrow 1$, and the fractional saturation of the surface with B becomes equal to the fractional displacement of A. However, for f_R and $\Phi_B > 0$, $\Lambda < 1$, and

$$\Phi_B = 1 - \frac{\Phi_A}{\Lambda \Phi_A^o} < 1 - \frac{\Phi_A}{\Phi_A^o},$$

indicating that area exclusion arising from the finite sizes of tracer and unlabeled ligand may invalidate the conventional interpretation of competition experiments.

The dependence of Φ_A/Φ_A^o upon $K_B c_B$ is obtained by first calculating the value of Φ_B corresponding to a particular value of $K_B c_B$ using Eqs. 4 and 7, and then calculating Φ_A/Φ_A^o for that value of Φ_B using Eq. 13. In Fig. 2, *a* and *b*, the dependence of $1 - \Phi_A/\Phi_A^o$ upon $\log K_B c_B$ is plotted for three combinations of particle shapes: a tracer circle in a fluid of circles, a tracer ellipse in a fluid of circles, and a tracer circle in a fluid of ellipses. Also plotted for comparison is the calculated dependence of Φ_B on $\log K_B c_B$.

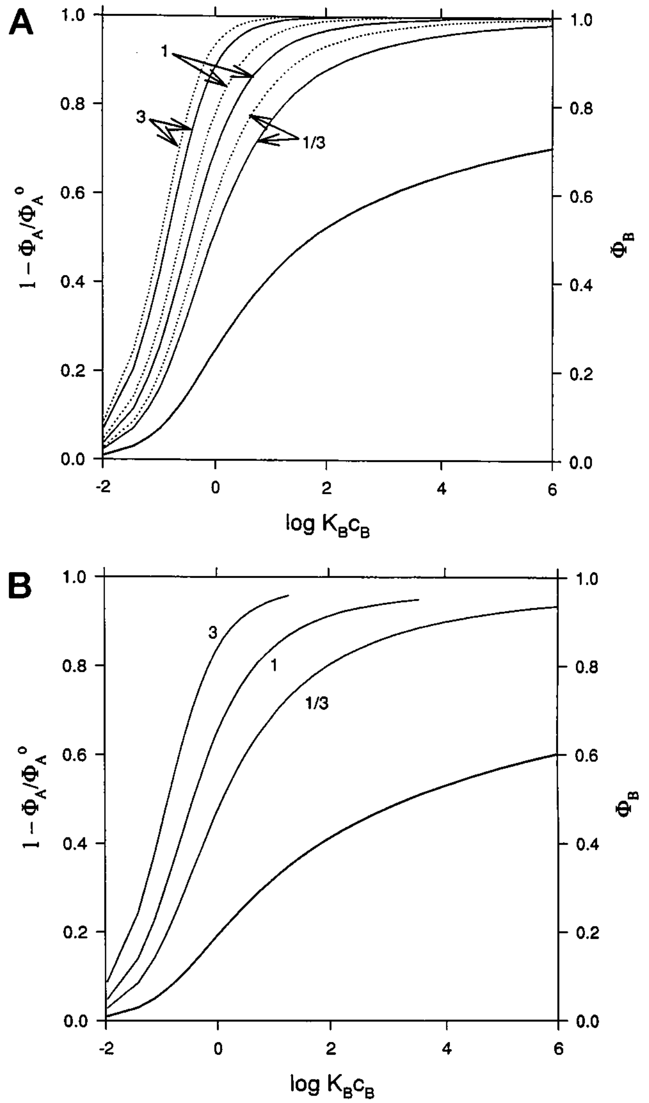

FIGURE 2 Fractional displacement of tracer (*left axis*) and fractional saturation of competitor ligand (*right axis*) plotted as a function of the normalized concentration of competitor ligand. Fractional displacement curves are plotted for tracer having an area 3, 1, or 1/3 times that of the competitor ligand. The heavy solid line is the fractional saturation of competitor ligand. (*a*) The tracer species is a circle (*solid lines*) or ellipse of axial ratio 5 (*dashed lines*), and the competitor ligand is a circle. (*b*) Tracer is described by a circle, and competitor ligand is an ellipse of axial ratio 5.

ADSORPTION OF A LARGE SELF-ASSOCIATING LIGAND

Consider a ligand that can adsorb to a surface, and when adsorbed can self-associate to form adsorbed z-mer. It will be assumed that the concentration of free ligand in solution is so low that the ligand behaves ideally in solution. The chemical potential of free (monomeric) ligand is then given by Eq. 2, and those of adsorbed monomer and adsorbed z-mer by

$$\mu_1^{\text{surf}} = \mu_1^{\text{surf,o}} + RT \ln \Phi_1 + RT \ln \gamma_1(\Phi_1, \Phi_z) \tag{14a}$$

$$\mu_z^{\text{surf}} = \mu_z^{\text{surf,o}} + RT \ln \Phi_z + RT \ln \gamma_z(\Phi_1, \Phi_z). \tag{14b}$$

Combination of Eqs. 2, 14, and the equilibrium conditions

$$\mu_1^{\text{soln}} = \mu_1^{\text{surf}}$$

$$z\,\mu_1^{\text{surf}} = \mu_z^{\text{surf}}$$

lead to the equilibrium relations

$$Kc = \Phi_1\,\gamma_1(\Phi_1, \Phi_z) \tag{15}$$

and

$$\Phi_z = zK_{1z}\frac{\gamma_1(\Phi_1, \Phi_z)^z}{\gamma_z(\Phi_1, \Phi_z)}\Phi_1^z. \tag{16}$$

The functional dependence of γ_1 and γ_z upon Φ_1 and Φ_z may be obtained for mixtures of hard circles from the 2-D multicomponent SPT theory of Lebowitz et al. (1965):

$$\ln\gamma_1 = -\ln(1-\Phi) + \frac{3\Phi_1 + \left[\frac{2}{f} + \frac{1}{f^2}\right]\Phi_z}{1-\Phi} + \frac{\Phi_1^2 + \frac{2\Phi_1\Phi_z}{f} + \frac{1}{f^2}\Phi_z^2}{(1-\Phi)^2} \tag{17a}$$

$$\ln\gamma_z = -\ln(1-\Phi) + \frac{3\Phi_z + (2f + f^2)\Phi_1}{1-\Phi} + \frac{\Phi_z^2 + 2f\Phi_1\Phi_z + f^2\Phi_1^2}{(1-\Phi)^2}, \tag{17b}$$

where $\Phi = \Phi_1 + \Phi_z$ and $f \equiv R_z/R_1$, where R_z and R_1 are the radii of the circles representing z-mer and monomer, respectively. Assuming that $f = z^{1/2}$ (i.e., area is conserved on self-association), the dependence of Φ upon log Kc, calculated using Eqs. 15–17, is plotted for $z = 4$ and various values of K_{14} in Fig. 3 *a*, and for various values of z with $K_{1z} = 1$ in Fig. 3 *b*. Self-association of adsorbed ligand leads to a steepening of the adsorption isotherm, and the steepness of the isotherm increases with the degree of self-association, as expected for a cooperative process. In the limit of large z, the behavior approaches that of a classical first-order phase transition, corresponding to the condensation of adsorbed ligand.

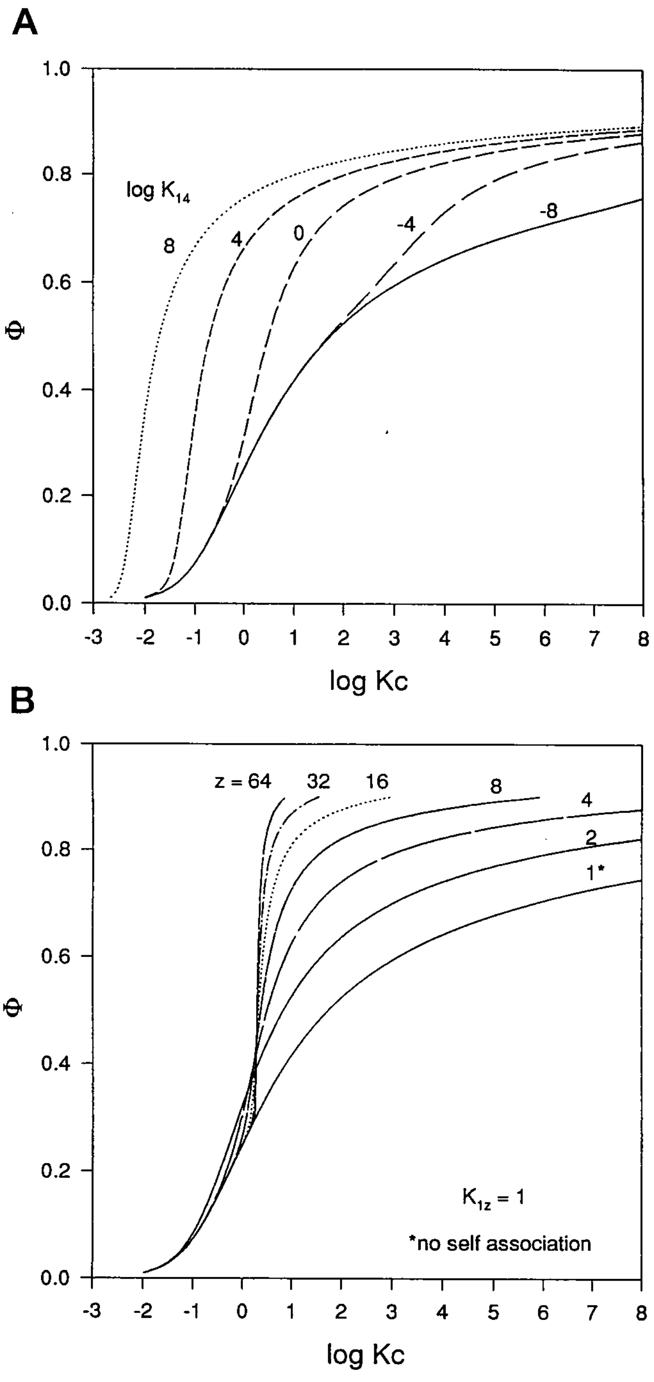

FIGURE 3 Adsorption isotherms for an area-excluding circular ligand undergoing self-association to form a circular z-mer. The heavy black curve represents the reference case of no self-association ($K_{1z} = 0$). (*a*) Dependence of isotherm on K_{14} for monomer-tetramer equilibrium. From left to right, curves were calculated for the following values of log K_{14}: 8, 4, 0, −4, and −8. (*b*) Dependence of isotherm upon the extent of self-association. From left to right, curves were calculated for $K_{1z} = 1$ and the following values of z: 64, 32, 16, 8, 4, and 2.

EFFECT OF EXCLUDED VOLUME IN THE SOLUTION PHASE

Up to this point we have considered only the exclusion of area by adsorbed ligand and have neglected the excluded volume of free ligand. This is an acceptable approximation so long as the intrinsic affinity of ligand for the adsorbent surface is so high that the concentration of ligand required to approach saturation of the surface remains small (i.e., occupying less than about 1% of the total volume of the solution). However, the results shown above clearly demonstrate that area exclusion of ligand on the surface causes extensive broadening of the adsorption isotherm; an increase in Kc of six or more orders in magnitude may be required to increase the fractional area occupancy of the surface from approximately 0.1 to 0.9 Φ_{max}. Unless the value of K is extremely large, it is likely that a complete experimental characterization of adsorption will require high concentrations of free ligand. Under such conditions the nonideal behavior of free ligand as well as adsorbed ligand must be considered. Hence Eq. 2 must be replaced by

$$\mu^{\mathrm{soln}} = \mu^{\mathrm{soln,o}} + RT \ln c + RT \ln \gamma_{\mathrm{soln}}(c), \tag{18}$$

where γ_{soln} denotes the activity coefficient of free ligand. Combination of Eqs. 1, 3, and 18 yields

$$K = \frac{\Phi\ \gamma(\Phi)}{c\ \gamma_{\mathrm{soln}}(c)}. \tag{19}$$

For didactic purposes we consider explicitly the simplest case of a ligand that may be modeled as a hard sphere in free solution and a hard circular disk when adsorbed; as seen in preceding sections, the effects of shape are second order. The 3-D hard sphere SPT theory of Reiss et al. (1959) leads to the relation

$$\ln \gamma_{\mathrm{soln}} = -\ln(1-\theta) + 7\left(\frac{\theta}{1-\theta}\right) + 7.5\left(\frac{\theta}{1-\theta}\right)^2 + 3\left(\frac{\theta}{1-\theta}\right)^3. \tag{20a}$$

Here θ denotes the fractional volume occupancy of solution by ligand, related to concentration by

$$\theta = v_{\mathrm{eff}} c, \tag{20b}$$

where v_{eff} denotes an effective ligand volume in units of reciprocal concentration. For the case of hard disks, Eq. 7 simplifies to

$$\ln \gamma = -\ln(1-\Phi) + 3\left(\frac{\Phi}{1-\Phi}\right) + \left(\frac{\Phi}{1-\Phi}\right)^2. \tag{21}$$

The dependence of Φ on c, obtained via numerical solution of Eqs. 19–21, is plotted in Fig. 4 for various values of log K. The calculation was performed using a value of v_{eff} (0.8 ml/g) that has been found to provide a reasonable description of the behavior of globular proteins in solutions of moderate ionic strength at pH values in the vicinity of the isoelectric point (Zimmerman and Minton, 1993). The results shown in Fig. 4 indicate that under these conditions, adsorption isotherms are significantly steepened when the concentration of free protein in solution exceeds about 30 g/l.

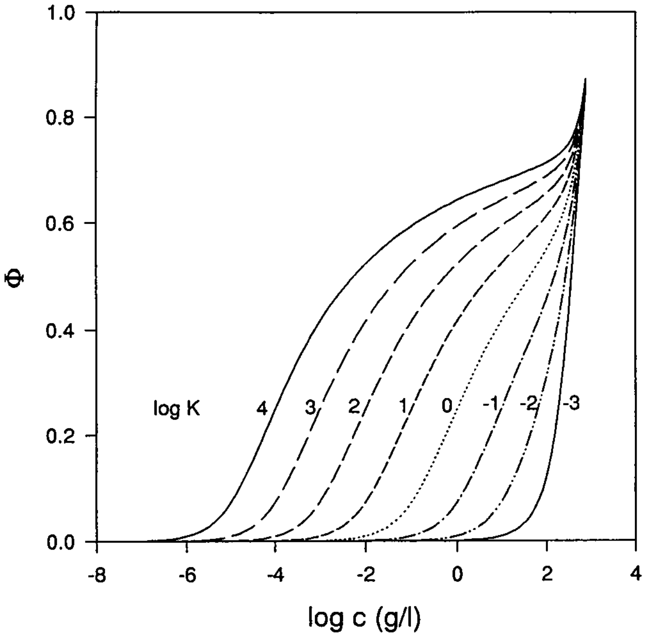

FIGURE 4 Dependence of fractional surface area occupancy upon free ligand concentration for a non-self-associating ligand that excludes volume in free solution as well as area on the surface. Curves were calculated assuming v_{eff} = 0.0008 l/g (Zimmerman and Minton, 1993) and the following values of log K, from left to right: 4, 3, 2, 1, 0, −1, −2, and −3.

DISCUSSION

Results obtained in the present work demonstrate that area exclusion by adsorbed proteins leads to a substantial broadening of the adsorption isotherm, and that attractive interactions between adsorbed ligands, here formally manifested as self-association, lead to a steepening of the adsorption isotherm. Qualitatively similar results have been obtained by previous investigators (Stankowski, 1984; Heimburg and Marsh, 1995). We believe that the present formulations are more rigorous and the results more accurate, at least within the context of the admittedly simplified geometric models for area exclusion employed by ourselves and the previous investigators.

Heimburg and Marsh (1995) employed a Van der Waals-type empirical description of a two-dimensional gas (Ross and Olivier, 1964) to obtain the following adsorption isotherm:

$$Kc = \frac{\Phi}{1-\Phi} \exp\left(\frac{\Phi}{1-\Phi} - 2a\Phi\right) \tag{22}$$

where a is a parametric "measure of the strength of interactions between ligand molecules." Assuming that the only interactions between adsorbed ligands are excluded area interactions, $a = 0$. The isotherm calculated from Eq. 22 with $a = 0$ is plotted in Fig. 5. It may be seen that the van der Waals isotherm seriously underestimates the effect of area exclusion.

Stankowski (1984) employed a simplification of the approximate theory of Andrews (1976) for a two-dimensional fluid of hard circular disks to calculate the following isotherm:

$$Kc = \frac{\Phi}{1-\Phi} \exp\left(\frac{3\Phi}{1-\dfrac{\Phi}{\Phi_{\mathrm{max}}}}\right), \tag{23}$$

where Φ_{max} denotes the maximum packing fraction of circular disks on a planar surface, 0.907. The isotherm calcu-

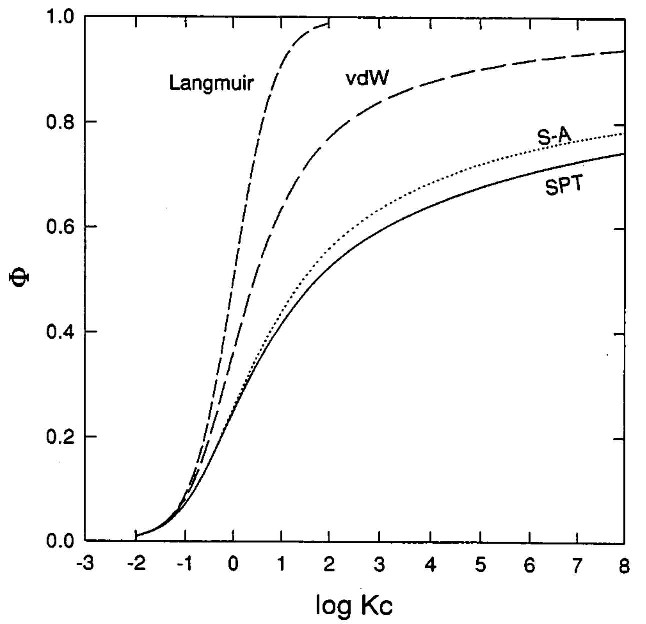

FIGURE 5 Dependence of fractional surface area occupancy calculated according to the Langmuir equation (Eq. 5) (*short dashed curve*), the van der Waals equation (Eq. 22) (*long dashed curve*), the Stankowski-Andrews equation (Eq. 23) (*dotted curve*), and Eq. 6 and the scaled particle equation (Eq. 7) for hard circular disks (*solid curve*).

lated using Eq. 23 is plotted in Fig. 5. The Stankowski-Andrews isotherm falls quite close to those calculated using the 2-D hard disk virial expansion and the 2-D SPT hard disk theory up to $\Phi \approx 0.3$, but seems to underestimate the effect of area exclusion at higher values of Φ. The Stankowski-Andrews formalism has not been generalized to treat noncircular particles or multicomponent systems.

In addition to elucidating factors affecting the adsorption isotherm of a single protein ligand, we have presented a new model for the effect of area exclusion on competitive surface binding of two protein species, one of which is a tracer present at low fractional saturation. The results presented in Fig. 3, *a* and *b*, demonstrate that if tracer and competitor exclude surface area (i.e., potential binding sites) to each other, the traditional mass-action interpretation of tracer displacement by competitor may lead to substantial overestimates of the fractional saturation of competitor as a function of the concentration of free competitor. Moreover, an uncritical investigator might erroneously conclude on the basis of a steep displacement isotherm that binding of competitor ligand to the surface is cooperative. However, it is possible to test for the presence of excluded area interactions between tracer and competitor in the following manner. The unlabeled parent of the tracer ligand should be used as the competitor. The binding of tracer may be measured as a function of the concentration of free tracer. Likewise, the displacement of tracer by increasing concentrations of competitor may be measured. Comparison of the isotherms obtained via direct measurement of tracer binding and by tracer displacement will indicate the presence or absence of significant excluded area effects, thereby enabling the investigator to ascertain whether the standard interpretation of displacement isotherms is justified in a particular experimental system.

Finally, it should be stressed that an adsorption isotherm that is broadened relative to the Languir isotherm is not necessarily an indicator of area exclusion or other repulsive interactions between bound ligands. It is well known that surface heterogeneity may lead to a broadening of the adsorption isotherm relative to the Langmuir isotherm, independent of interaction between adsorbed ligands (Steele, 1974). It is therefore impossible in principle for an investigator to discern whether a broadened isotherm is due to surface heterogeneity, repulsive interactions between bound ligands (including area exclusion), or a combination of both, on the basis of equilibrium adsorption data alone. In contrast, an adsorption isotherm that is steeper than the Langmuir isotherm may unequivocally be attributed to net attractive interactions between, and clustering of, bound ligand molecules.

APPENDIX: SPT CALCULATION OF THE ACTIVITY COEFFICIENT OF A SINGLE PARTICLE OF SPECIES 2 IN A 2-D FLUID OF SPECIES 1

Let each species of 2-D convex particle be characterized by a circumference C_i and an area A_i. These are related to a characteristic dimension of the particle R_i (such as the radius of a circle) by the dimensionless coefficients $c_i \equiv C_i/R_i$ and $a_i \equiv A_i/R_i^2$. Values of c and a for differently shaped particles are presented in Table 1. The co-area of particles 1 and 2, or the area excluded to the center of a particle of species 2 by a particle of species 1 (and vice versa), averaged over all relative orientations of the two particles, is given by (Boublik, 1975)

$$A_{ij} = a_i R_i^2 + a_j R_j^2 + c_i c_j R_i R_j / 2\pi. \qquad \text{(A1)}$$

The activity coefficient of a single hard particle of species 2 in a fluid of particles of species 1 is simply related to the excess work required to place the particle into the fluid:

$$\ln \gamma_2 = \beta W(R_2), \qquad \text{(A2)}$$

where $\beta \equiv 1/kT$, k denotes Boltzmann's constant and T the absolute temperature. This work, in turn, is related to the probability that no part of any particle of species 1 will occupy the element of area into which the particle of species 2 will be introduced, which is equal to the probability that the center of mass of no particle of species 1 will fall within the co-area of species 1 and 2:

$$\beta W(R_2) = \ln P_0(R_2). \qquad \text{(A3)}$$

Although W and P_0 depend upon variables in addition to the characteristic dimension of the particle of species 2, the reason for the notation declaring the explicit dependence upon R_2 will become evident in the following argument, based upon approximations introduced by Lebowitz et al. (1965).

SPT is based upon the idea that although it is not possible to precisely calculate $P_0(R_2)$ for a real particle with $R_2 \geq 0$, it is possible to calculate the value exactly for a virtual particle with $R_2 < 0$, because in this case the center of only one real particle at most can fall within the co-area of the real and virtual particles. Hence for $R_2 < 0$,

$$P_0(R_2) = 1 - P_1(R_2), \qquad \text{(A4)}$$

where $P_1(R_2)$ denotes the probability that a single particle of species 1 will be within the co-area of particles 1 and 2, given by

$$P_1(R_2) = \rho_1 A_{12}, \tag{A5}$$

and ρ_1 denotes the number density of particles of species 1 in the fluid. Combining Eqs. A1–A5, we obtain the exact result that for $R_2 < 0$,

$$\beta W(R_2) = \ln\left[1 - \rho_1(a_1R_1^2 + a_2R_2^2 + c_1c_2R_1R_2/2\pi)\right]. \tag{A6}$$

To obtain an approximate expression for $\beta W(R_2)$ with $R_2 \geq 0$, it is assumed that 1) $W(R_2)$ is continuous at $R_2 = 0$; 2) $\mathrm{d}W(R_2)/\mathrm{d}R_2$ is continuous at $R_2 = 0$; and 3) at sufficiently large R_2, the excess work of insertion of a particle of characteristic dimension R_2 asymptotically approaches pressure-area work, e.g.,

$$\lim_{R_2 \to \infty} W(R_2) = pA_2.$$

Combination of the three assumptions listed above yields the following power series expansion for $\beta W(R_2)$:

$$\beta W(R_2) = \beta W(R_2 = 0) + \left(\frac{\mathrm{d}\beta W(R_2)}{\mathrm{d}R_2}\right)_{R_2=0} R_2 + \beta p a_2 R_2^2. \tag{A7}$$

The first two terms may be evaluated using Eq. A6. The pressure is evaluated using the SPT equation of state for a fluid of particles of type 1 (Boublik, 1975):

$$\beta p = \rho_1\left[\frac{1 + (\epsilon - 1)\Phi}{(1 - \Phi)^2}\right], \tag{A8}$$

where $\epsilon = c_1^2/4\pi a_1$ and $\Phi = \rho_1 A_1$. The final result is

$$\ln \gamma_2 = -\ln(1 - \Phi) + 2\epsilon f_c f_R \frac{\Phi}{1 - \Phi} + f_a f_R^2 \Phi\left[\frac{1 + (\epsilon - 1)\Phi}{(1 - \Phi)^2}\right], \tag{A9}$$

where $f_c = c_2/c_1$, $f_a = a_2/a_1$, and $f_R = R_2/R_1$. It may be shown that for the case of a tracer circle in a fluid of circles, where $\epsilon = f_A = f_c = 1$, Eq. A9 reduces to an expression identical to that obtained from the 2-D multicomponent hard disk SPT theory of Lebowitz et al. (1965).

REFERENCES

Andrade, J. D. 1985. Surface and Interfacial Aspects of Biomedical Polymers, Vol. 2. Protein Adsorption. Plenum Press, New York.

Andrews, F. C. 1976. A simple approach to the equilibrium statistical mechanics of two-dimensional fluids. *J. Chem. Phys.* 64:1941–1947.

Arnold, H., and D. Pette. 1968. Binding of glycolytic enzymes to structure proteins of the muscle. *Eur. J. Biochem.* 6:163–171.

Boublik, T. 1975. Two-dimensional convex particle liquid. *Mol. Phys.* 29:421–428.

Chatelier, R. C. 1987. Analysis of competition binding assays: assessment of the range of validity of a commonly invoked assumption. *J. Recept. Res.* 7:871–884.

Clegg, J. S. 1984. Properties and metabolism of the aqueous cytoplasm and its boundaries. *Am. J. Physiol.* 246:R133–R151.

Conn, P. M. 1984. The Receptors, Vol. 1. Academic Press, Orlando, FL.

Heimburg, T., and D. Marsh. 1995. Protein surface distribution and protein-protein interactions in the binding of peripheral proteins to charged lipid membranes. *Biophys. J.* 68:536–546.

Hill, T. L. 1960. Chapter 7-1. Ideal lattice gas (Langmuir adsorption theory). *In* Introduction to Statistical Thermodynamics. Addison-Wesley, Reading, PA. 124–130.

Horbett, T. A. 1982. Protein adsorption on biomaterials. *In* Biomaterials: Interfacial Phenomena and Applications. S. L. Cooper and N. A. Peppas, editors. American Chemical Society, Washington, DC. 233–244.

Kurganov, B. I. 1985. Control of enzyme activity in reversibly adsorptive enzyme systems. *In* Organized Multienzyme Systems. G. R. Welch, editor. Academic Press, Orlando, FL. 241–270.

Lakatos, S., and A. P. Minton. 1991. Interactions between globular proteins and F-actin in isotonic saline solution. *J. Biol. Chem.* 266:18707–18713.

Lebowitz, J. L., E. Helfand, and E. Praestgaard. 1965. Scaled particle theory of fluid mixtures. *J. Chem. Phys.* 43:774–779.

Minton, A. P. 1995. Confinement as a determinant of macromolecular structure and reactivity. II. Effects of weakly attractive interactions between confined macrosolutes and confining structures. *Biophys. J.* 68:1311–1322.

Ree, F. H., and W. G. Hoover. 1964. Fifth and sixth virial coefficients for hard spheres. *J. Chem. Phys.* 40:939–950.

Reiss, H., H. L. Frisch, and J. L. Lebowitz. 1959. Statistical mechanics of rigid spheres. *J. Chem. Phys.* 31:369–388.

Ross, S., and J. P. Olivier. 1964. Chapter 1-5. Further descriptions of the dynamic equilibrium between the gas and the adsorbed phase. *In* On Physical Adsorption. Interscience, New York. 16–24.

Sankaram, M. B., and D. Marsh. 1993. Protein-lipid interactions with peripheral membrane proteins. *In* Protein-Lipid Interactions. A. Watts, editor. Elsevier, Amsterdam. 127–162.

Stankowski, S. 1983. Large-ligand adsorption to membranes. II. Disk-like ligands and shape-dependence at low saturation. *Biochim. Biophys. Acta.* 735:352–360.

Stankowski, S. 1984. Large-ligand adsorption to membranes. III. Cooperativity and general ligand shapes. *Biochim. Biophys. Acta.* 777:167–182.

Steele, W. A. 1974. The Interaction of Gases with Solid Surfaces. Pergamon, Oxford.

Tamm, L. K., and I. Bartoldus. 1988. Antibody binding to lipid model membranes. The large-ligand effect. *Biochemistry.* 27:7453–7458.

Zimmerman, S., and A. P. Minton. 1993. Macromolecular crowding: biochemical, biophysical, and physiological consequences. *Annu. Rev. Biophys. Biomol. Struct.* 22:27–65.