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Effects of excluded surface area and adsorbate clustering on surface adsorption of proteins
I. Equilibrium models[☆]

Allen P. Minton*

Section on Physical Biochemistry, Laboratory of Biochemistry and Genetics, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892-0830, USA

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Abstract

Statistical–thermodynamic models for the equilibrium adsorption of proteins onto homogeneous, locally planar surfaces are presented. An extension of earlier work [R.C. Chatelier, A.P. Minton, Biophys. J. 71 (1996) 2367], the models presented here allow for the formation of a broadly heterogeneous population of adsorbate clusters in addition to excluded volume interactions between all adsorbate species. Calculations are carried out for three simple models for the structure of adsorbate, illustrating similarities and differences in the equilibrium properties of maximally compact clusters, minimally compact clusters and isomerizing clusters. Depending upon the strength of attractive interactions between adsorbate molecules, the resulting equilibrium isotherms may exhibit negative cooperativity, positive cooperativity, essentially no apparent cooperativity, or a mixture of positive cooperativity at low surface density and negative cooperativity at high surface density of adsorbate. The condition of apparent lack of cooperativity, which might naively be interpreted as evidence of a lack of interaction between adsorbate molecules, actually conceals a balance between attractive and repulsive interactions and extensive clustering of adsorbate. © Published by Elsevier Science B.V.

Keywords: Protein adsorption; Excluded volume; Protein assembly

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[☆] This work is dedicated to Prof. Henryk Eisenberg in appreciation of his lifelong contributions to the field of physical biochemistry, his inspiring mentorship during the author's formative years as a young scientist (1968–70) and his continued friendship.

* Tel.: +1-301-496-3604; fax: +1-301-402-0240.
E-mail address: minton@helix.nih.gov (A.P. Minton).

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1. Introduction

Experimental studies of the adsorption of several globular proteins to molecularly ‘flat’ surfaces often reveal an equilibrium (or steady-state) dependence of the amount of protein adsorbed upon the concentration of protein in the supernatant solution that is describable to within experimental precision by an ideal or Langmuir adsorption isotherm (see, e.g. [2–5]). Such a relationship is predicted in the absence of any interaction — either attractive or repulsive — between adsorbed protein molecules. However, it has been observed that the surface density of adsorbed protein in the limit of high supernate protein concentration may correspond closely to that of a close-packed monolayer of molecules having the dimensions of the native protein [6]. It is well-established that steric repulsion between adsorbate molecules (surface area exclusion) leads to substantial enhancement of the chemical potential of adsorbate and very large decreases in both the equilibrium affinity and net rate of adsorption at surface densities well below close-packing [1,6–9]. It follows that in the absence of compensating attractive interactions between adsorbate molecules [10], it would seem to be impossible to attain surface densities approaching close packing. However, simple two-state models of adsorbate self-association [1,6] are not capable of generating the types of adsorption isotherms observed experimentally. The purpose of the present work is to explore adsorption models that provide for the formation of highly heterogeneous populations of aggregated adsorbate (adsorbate clusters).

2. Methods

2.1. Thermodynamic relations

The following development is a generalization of the treatment previously presented by Chatelier and Minton [1] and Minton [6]. The system we wish to model consists of a monomeric protein in solution that may reversibly adsorb onto a locally planar surface. The adsorbed protein may reversibly self-associate to form oligomeric adsorbate clusters of arbitrary size and shape (within certain limits to be described subsequently). While attempting to maintain as much thermodynamic generality as possible, in the interests of calculational tractability we shall make a few simplifying assumptions.

It is stipulated that the protein behaves ideally in solution, i.e.

$$\mu^{\mathrm{soln}} = \mu^{\mathrm{o,soln}} + RT\ln c \tag{1}$$

where $\mu^{\mathrm{o,soln}}$ and c denote, respectively, the standard state chemical potential and concentration of protein in solution.

Adsorbed protein may exist as a variety of species, distinguished by conformation and degree of oligomerization. The chemical potential of an adsorbed species i is given by

$$\mu_i^{\mathrm{surf}} = \mu_i^{\mathrm{o,surf}} + RT\ln\rho_i + RT\ln\gamma_i(\{\rho\}) \tag{2}$$

where ρ_i and γ_i are, respectively, the surface number density and activity coefficient of species i. The notation $\gamma_i(\{\rho\})$ is used to make explicit the dependence of the activity coefficient of each adsorbed species upon the number densities of all adsorbed species.

It is stipulated that adsorbed monomer exists in a single conformation, denoted as species 1. Multiple conformations of adsorbed monomer can be treated within the context of the present formalism [6], but are neglected here as adding mathematical complexity without contributing significantly to an understanding of the role of adsorbate clustering in protein adsorption.

Adsorbed monomer may self-associate reversibly to form oligomers denoted by species $i > 1$, where the degree of oligomerization of species i is denoted by n_i.

At equilibrium

$$\mu^{\mathrm{soln}} = \mu_1^{\mathrm{surf}} \tag{3}$$

and

$$n_i\mu_1^{\mathrm{surf}} = \mu_i^{\mathrm{surf}} \tag{4}$$

It follows from Eqs. (3) and (4) that we may define the equilibrium constants

$$K_1^{\mathrm{ads}} \equiv \exp(-\Delta G_1^{\mathrm{o,ads}}/RT) \equiv \frac{\rho_1\ \gamma_1(\{\rho\})}{c} \tag{5}$$

and

$$K_{1i}^{\mathrm{surf}} \equiv \exp\left[-\Delta G_{1i}^{\mathrm{o,surf}}/RT\right] = \frac{\gamma_i(\{\rho\})\ \rho_i}{[\gamma_1(\{\rho\})\rho_1]^{n_i}} \tag{6}$$

where

$$\Delta G_1^{\mathrm{o,ads}} = \mu_1^{\mathrm{o,surf}} - \mu^{\mathrm{o,soln}} \tag{7}$$

is the standard state free energy change associated with the ideal adsorption of an isolated monomer (i.e. $\gamma_1 = 1$) and

$$\Delta G_{1i}^{\mathrm{o,surf}} = \mu_i^{\mathrm{o,surf}} - n_i\mu_1^{\mathrm{o,surf}} \tag{8}$$

is the standard state free energy change associated with the formation of an isolated adsorbate oligomer of species i from n_i isolated adsorbate monomers (i.e. $\gamma_1 = \gamma_i = 1$). It follows from Eqs. (5) and (6) that

$$\rho_i = K_{1i}^{\mathrm{surf}} \frac{c^{*\,n_i}}{\gamma_i(\{\rho\})} \tag{9}$$

where $c^* \equiv K_1 c$ is the concentration of free (soluble) protein scaled to the intrinsic adsorption affinity of that protein.

Let the number of adsorbed species be n_{ads}. In order to calculate $\{\rho\}$ as a function of c^*, it is necessary to specify K_{1i}^{surf} and $\gamma_i(\{\rho\})$ for all i (1 to n_{ads}). Then one must solve a set of n_{ads} equations of the form of Eq. (9), which are linked by the dependence of each γ_i on all ρ. The required specifications depend upon particular structural models, several of which are described below.

2.2. Calculation of activity coefficients

In the present work, as in previous treatments [6,9], activity coefficients of individual adsorbate species will be calculated as a function of adsorbate composition using the two-dimensional scaled particle theory of mixtures of convex hard particles [11]:

$$\ln\gamma_i(\{\rho\}) = -\ln(1-\langle\rho a\rangle) + \frac{a_i\langle\rho\rangle + s_i\langle\rho s\rangle/(2\pi)}{1-\langle\rho a\rangle} + \frac{a_i}{4\pi}\left[\frac{\langle\rho s\rangle}{1-\langle\rho a\rangle}\right]^2 \tag{10}$$

where a_i and s_i, respectively, denote the area and circumference of the 'footprint' (projection on the plane of the surface) of adsorbed species i, and $\langle\rho\rangle \equiv \Sigma\rho_j$, $\langle\rho a\rangle \equiv \Sigma\rho_j a_j$, and $\langle\rho s\rangle \equiv \Sigma\rho_j s_j$. The representation of proteins as hard particles for the purpose of quantitative estimation of excluded surface area or volume has been abundantly justified elsewhere [1,12].

2.3. Numerical calculation of equilibrium adsorption isotherms

For a given structural model the geometric parameters a_i and s_i appearing in Eq. (10) are evaluated as described below. Values for intermolecular contact energies are then specified, from which the various K_{1i} are evaluated as described below. The dependence of $\{\rho\}$ on c is then calculated via the following strategy. Eq. (9) is rewritten in logarithmic form:

$$\ln\rho_i = \ln K_{1i} - \ln\gamma_i(\{\rho\}) + n_i \ln c^* \tag{11}$$

The calculation is started with a value of ln c^* (ln $c^{*(1)}$) so small that adsorption is essentially ideal, i.e. ln $\gamma_i \ll$ ln K_{1i} for all i. It is assumed initially that ln $\gamma_i = 0$, and Eqs. (10) and (11) are solved recursively to self-consistency, yielding $\rho_i^{(1)}$ and $\gamma_i^{(1)}$ for all i. The value of ln c^* is then incremented to a greater value ln $c^{*(2)}$, and Eqs. (10) and (11) are again solved recursively, starting with initial values of ln $\gamma_i =$ ln $\gamma_i^{(1)}$, to obtain $\rho_i^{(2)}$ and $\gamma_i^{(2)}$ and for all i. The value of ln c^* is again incremented, and the cycle repeated. In general, for each value of ln $c^{*(j)}$, Eqs. (10) and (11) are solved recursively to self-consistency starting with initial values of $\ln \gamma_i = \ln \gamma_i^{(j-1)}$. As c^* (and the ρ_i) become larger, the values of γ_i become increasingly sensitive to small changes in $\{\rho\}$, and the recursive calculation becomes increasingly less stable and prone to oscillation or divergence. Buffering measures are employed to diminish the instability, but ultimately the calculation fails to converge at an upper limit of c^* corresponding to a surface density of adsorbate approaching the steric packing limit. However, enough of the isotherm is successfully calculated for significant conclusions to be drawn regarding its shape and amplitude, as will be shown in the results section. All calculations were performed using MATLAB (MathWorks, Inc., Natick, MA), and the MATLAB scripts used are available upon request from the author.

3. Structural models for oligomers

In order to estimate the effect of excluded surface area on adsorption we are required to adopt highly simplified and physically unrealistic geometric models for cluster footprints. It is therefore necessary to perform equivalent calculations using a variety of structural models in order to determine what — if any — features of the final results are insensitive to the choice of structural model, and hence are more likely to remain approximately correct for more complex and realistic models of cluster structure. It is, however, uniformly assumed that all protein molecules in an equilibrium cluster are in contact with the surface, i.e. a cluster is only one protein molecule ‘tall’. We shall also assume that the standard state free energy change $\Delta G_{1i}^{\mathrm{o,surf}}$ may be expressed approximately as the sum of the free energies of individual intermolecular contacts within the cluster:

$$\Delta G_{1i}^{\mathrm{o,surf}} = \sum_j n_{\mathrm{c},i}^{(j)} U_{\mathrm{c}}^{(j)} \qquad (12)$$

where $U_{\mathrm{c}}^{(j)}$ denotes the free energy of an intermolecular contact of type j, and $n_{\mathrm{c},i}^{(j)}$ denotes the number of contacts of type j in the cluster of species i.

Simulations of adsorption equilibria have been carried out for the three models described below in order of increasing complexity.

3.1. Circular (maximally compact) cluster model

All cluster footprints are represented as circles. Let the species denoted by i contain $n_i = i$ protomers. Then

$$a_i = i a_1 \qquad (13)$$

and

$$s_i = 2\sqrt{i \pi a_1} \qquad (14)$$

There is only one type of intermolecular contact, with energy U_{c}. The number of intermolecular contacts in cluster species i is calculated using the following empirical expression:

$$n_{c,i} = 3.777\{\exp[-(i-1)/2.508] - 1\} + 2.5(i-1) \qquad (15)$$

This relation was obtained by a process of placing circles representing protomers on a hexagonal lattice in such a manner as to maximize the number of inter-circle contacts in a cluster of given size. The value of $n_{\mathrm{c},i}$ calculated as a function of i using Eq. (14) has a maximum error of less than 0.4 and is believed to be as accurate as warranted by the approximate nature of the present model. In the present work our calculations are limited to systems containing a maximum of 20 species of cluster ($i = 1$–20).

3.2. Linear cluster (minimally compact) model

This model was selected as the opposite extreme of the circular cluster model within the family of models that may be treated using the scaled particle theory of hard convex particle fluids. A square monomer forms linear arrays with $n_i = i$. Then

$$a_i = i a_1 \qquad (16)$$

and

$$s_i = 2(i+1)\sqrt{a_1} \tag{17}$$

There is only one type of intermolecular contact, with energy U_c, and

$$n_{c,i} = i - 1 \tag{18}$$

In the present work our calculations are limited to systems containing a maximum of 20 species of cluster ($i = 1$–20).

3.3. Rectangular cluster model with two types of interactions

This is the simplest model that allows for variability in shape among clusters of the same size (stoichiometry). We shall postulate that a monomeric footprint, square in shape, has a directional axis so that one may refer to two opposing faces as ‘ends’ and the other two faces as ‘sides’. We shall permit monomers to assemble side-to-side and end-to-end, to form rectangular clusters characterized by the dimensions $n_{1,i}$ and $n_{2,i}$, respectively, where $n_{2,i} \geq n_{1,i}$. Then

$$a_i = (n_{1,i} \times n_{2,i})a_1 \tag{19}$$

and

$$s_i = 2(n_{1,i} + n_{2,i})\sqrt{a_1} \tag{20}$$

The numbers of side-to-side contacts (type 1) and end-to-end contacts (type 2) are, respectively,

$$n_{c,i}^{(1)} = n_{2,i}(n_{1,i} - 1) \tag{21}$$

and

$$n_{c,i}^{(2)} = n_{1,i}(n_{2,i} - 1) \tag{22}$$

In the present work our calculations are limited to the set of 27 rectangular clusters that may be constructed from up to 16 protomers: $1 \times 1 \ldots 16$ (16 species), $2 \times 2 \ldots 8$ (seven species), $3 \times 3 \ldots 5$ (three species), and 4×4 (one species).

4. Results

Results are presented in terms of the four equilibrium adsorption parameters defined below. The total fraction of surface area occupied by adsorbed protein is given by

$$\phi = \sum_i \rho_i a_i \tag{23}$$

Since protein is assumed to be adsorbed as a monolayer, φ is proportional to the amount of protein adsorbed. The shape of the adsorption isotherm may be described by the empirical parameter known as the ‘Hill n’ [13], defined as

$$n(\varphi^*) \equiv \left[\frac{d\log[\varphi/(1-\varphi)]}{d\log c^*}\right]_{\varphi=\varphi^*} \tag{24}$$

The Hill n may be interpreted as follows. A value of $n(\varphi)$ that is less than 1, equal to 1, or greater than one indicates that the affinity of additional free protein for remaining vacant surface is respectively reduced, unaffected, or enhanced by the presence of adsorbate already present at surface occupancy φ. When n is equal to unity independent of the value of φ, adsorption is described by the ideal, or Langmuir, adsorption isotherm [14]

$$c^* = \frac{\varphi}{1-\varphi} \tag{25}$$

The weight-average degree of polymerization or cluster size is given by

$$P_w = \sum_i \rho_i n_i^2 / \sum_i \rho_i n_i \tag{26}$$

For the rectangular cluster model, we may also calculate a measure of average particle aniso-

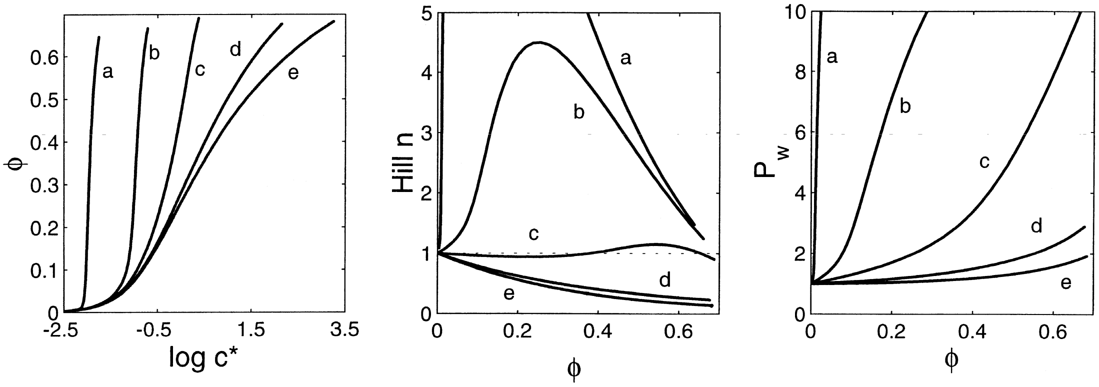

Fig. 1. Equilibrium results obtained from the circular cluster model. The three plots indicate fractional surface coverage φ as a function of normalized soluble ligand concentration, the cooperativity parameter n as a function of φ, and the weight-average cluster size P_W as a function of φ. Curves a–e represent calculations carried out using $U_C/RT = -2, -1, -0.2, 1$ and 2, respectively.

metricity, the weight-average axial ratio, defined as

$$R_w = \frac{\sum_i \rho_i n_i (n_{2,i}/n_{1,i})}{\sum_i \rho_i n_i} \tag{27}$$

4.1. Circular cluster model

Results obtained for various values of U_c are shown in Fig. 1. Salient features are as follows. In the limit of large positive values of U_c, the adsorption isotherm asymptotically approaches that calculated for non-interacting hard disks [1], and is, as expected, highly negatively cooperative ($n < 1$ and $dn/d\varphi < 0$ for all $\varphi > 0$). As the value of U_c becomes more negative, and attraction between adsorbed protein particles becomes significant, the isotherm becomes steeper, and n increases monotonically at constant φ. At a value of $U_c \sim -0.2\ RT$, the value of n becomes approximately 1 (± 0.1) over the entire range $0 \leq \varphi \leq 0.7$. Although the corresponding isotherm would be experimentally indistinguishable from the ideal

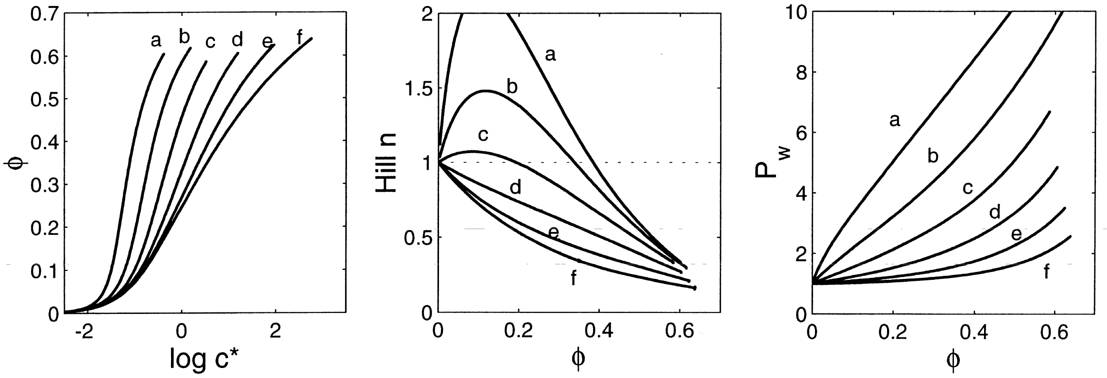

Fig. 2. Equilibrium results obtained from the linear cluster model. Plots as in Fig. 1. Curves a–f represent calculations carried out using $U_C/RT = -3, -2, -1, 0, 1$ and 2, respectively.

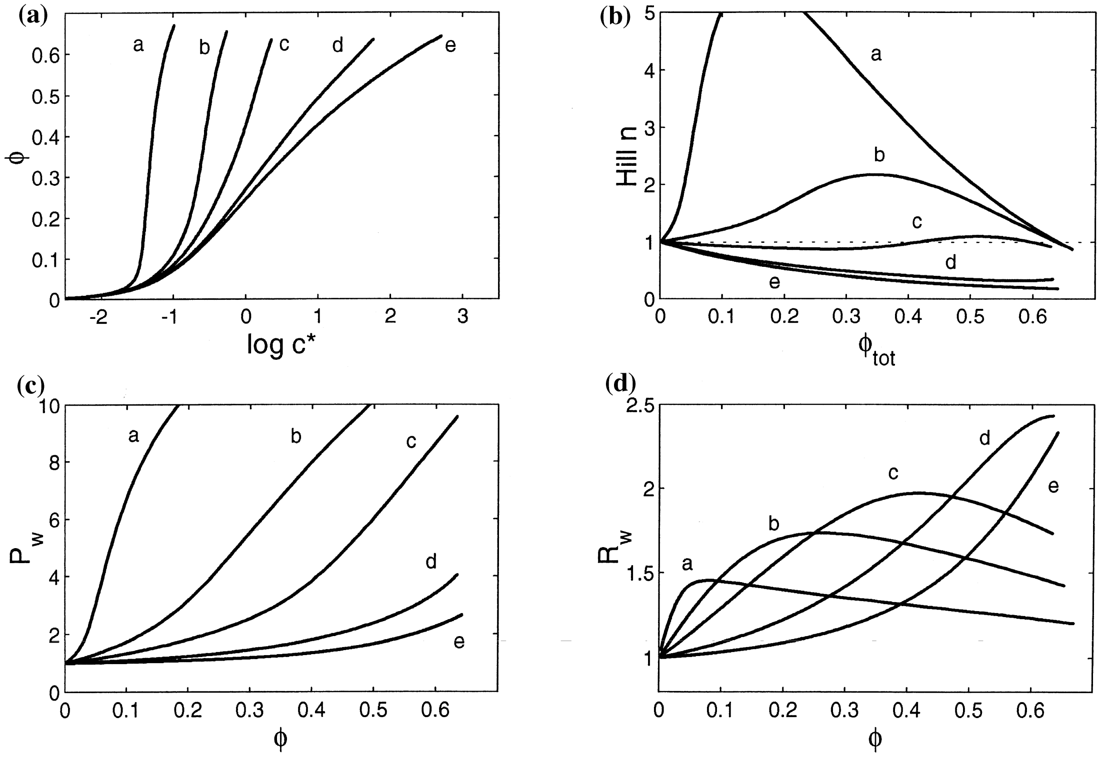

Fig. 3. Equilibrium results obtained from the rectangular cluster model with equal end-to-end and side-to-side interactions. The first three plots are as in Figs. 1 and 2, and the fourth plot indicates weight-average axial ratio R_W as a function of φ. Curves a–e represent calculations carried out using $U_C^{(1)}/RT = U_C^{(2)}/RT = -2, -1, -0.3, 1$ and 2, respectively.

isotherm over this range (we shall refer to such an isotherm as a 'pseudo-Langmuir' isotherm), extensive clustering is present under these conditions, with a weight-average cluster size exceeding 10 at $\varphi > 0.6$. At more negative values of U_c, the adsorption isotherm steepens markedly, and asymptotically approaches the phase-transition-like behavior exhibited by a system containing only two significant states of adsorbate: monomer and 20-mer (cf. Fig. 3 of Chatelier and Minton [1]).

4.2. Linear cluster model

Results obtained for various values of U_c are shown in Fig. 2. Salient features are as follows. In the limit of large positive values of U_c, the adsorption isotherm asymptotically approaches that calculated for non-interacting hard squares [1], and, as expected, is highly negatively cooperative ($n < 1$ and $dn/d\varphi < 0$ for all $\varphi > 0$). Similar to the circular cluster model, as the value of U_c becomes more negative, the isotherm steepens and the value of n increases monotonically at constant φ. However, unlike the circular cluster model, the linear cluster model does not exhibit a regime of intermediate interaction in which attractive and repulsive interactions between adsorbate particles roughly cancel each other over a range of φ, leading to a pseudo-Langmuir isotherm. This is because the onset of net repulsive behavior ($n < 1$) occurs at a lower value of φ due to greater area exclusion by linear particles than by circular particles of equal area. [It should be pointed out that the present linear cluster model does not take into account the possibility of an isotropic-nematic phase transition in a two-dimensional fluid of sufficiently anisometric particles. Such a transition would lead to a reduction in volume exclusion (and a thermodynamically favorable increase in translational entropy), albeit at the cost of a thermodynamically unfavorable reduction in the orientational entropy of anisometric clusters. Such transitions in linearly aggregating systems have been explored theoretically in three dimensions [15, 16].

4.3. Rectangular cluster model

Results obtained for isotropic interactions ($U_c^{(1)} = U_c^{(2)} = U_c$) are presented in Fig. 3. These results are qualitatively similar to those obtained for the circular cluster model, including the existence of a pseudo-Langmuir isotherm at $U_c \sim -0.3\ RT$ that conceals the presence of extensive adsorbate clustering. One additional feature exhibited by this model is the variability of the weight-average axial ratio. For any given value of U_c, R_w exhibits a maximum as φ increases, which is a reflection of area exclusion favoring more isometric (in the present instance, more square-like) clusters at higher fractional surface occupancy. The increase of the maximum value of R_w with more positive values of U_c is an artifact arising from the lower average cluster size: the smallest possible cluster in our model (dimer) has a 'large' axial ratio of two.

Results obtained for anisotropic interactions ($U_c^{(2)} \geq U_c^{(1)}$) are presented in Fig. 4. The combinations of $U_c^{(1)}$ and $U_c^{(2)}$ selected for illustrative calculation were chosen to explore a gradual transition between totally isotropic association (curve *g*, cf. curves *a* of Figs. 1 and 3), and totally linear association (curves *j* and *k*, cf. curves *a* of Fig. 2).

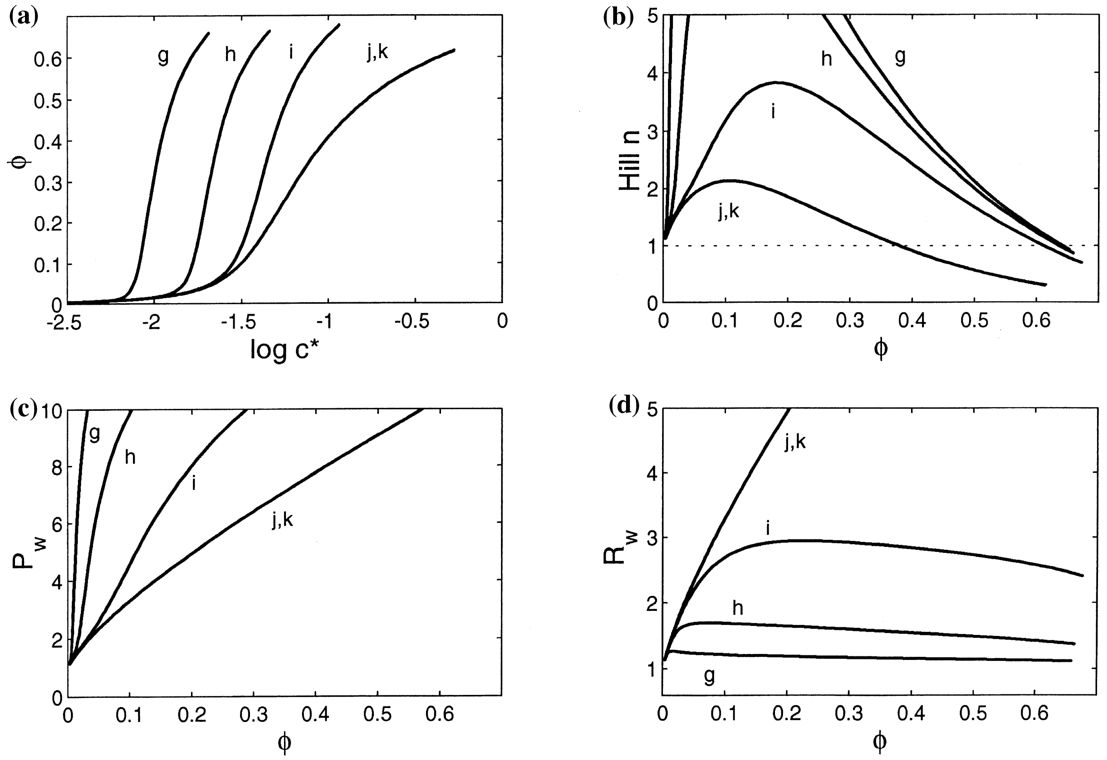

Fig. 4. Equilibrium results obtained from the rectangular cluster model with constant end-to-end interactions and variable side-to-side interactions. Plots as in Fig. 3. Curves g–k represent calculations carried out using $U_C^{(2)}/RT = -3$, and $U_C^{(1)}/RT = -3, -2, -1, 1$ and 3, respectively.

Curves *i* represent an interesting and possibly unique intermediate case in which the weight-average axial ratio becomes large — approximately 3 — at relatively low fractional surface occupancy, and remains large even at high fractional surface occupancy and high weight-average cluster size.

5. Discussion

Although the models presented here are simplistic, they demonstrate unambiguously that by modulating the extent of attractive interaction between adsorbate molecules and the concomitant extent of adsorbate clustering, it is possible to generate equilibrium adsorption isotherms with a broad variety of shapes characteristic of negative cooperativity, independent site (Langmuir-type) behavior, positive cooperativity, or a combination of positive cooperativity at low fractional surface occupancy and negative cooperativity at high fractional surface occupancy. Of particular interest is the apparent Langmuir-like behavior exhibited by the circular and rectangular models for small attractive contact energies. The conventional interpretation of a Langmuir-like isotherm is that it reflects a lack of interaction between adsorbed molecules. However, in the present case this behavior reflects a balance of attractive and repulsive interactions, and masks the presence of extensive adsorbate clustering. Although clustering of adsorbed protein has been proposed for a few specific proteins under special conditions, e.g. [17–19], the frequent and widely-spread occurrence of Langmuir-type or positively cooperative adsorption isotherms in the experimental literature, e.g. [2–5,20,21], suggests that clustering of adsorbate may be a more common or general feature of protein adsorption than recognized heretofore.

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