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Fractional statistics description applied to protein adsorption: Effects of excluded surface area on adsorption equilibria

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ABSTRACT

Adsorption of large molecules blocking more than one adsorption center on a lattice is described as a fractional statistics problem, based on Haldane's statistics. Excluded surface area is characterized by a statistical exclusion parameter, g, which relates to the molecular size and lattice geometry. The theoretical formalism reproduces the classical Langmuir equation (one excluded state limit), and gives a framework and compact equations to consistently describe the adsorption thermodynamics of structurally diverse proteins ranging from simple species to large ligands.

1. Introduction

Protein adsorption to solid surfaces is a common but very important event that has stimulated a huge research interest in various areas including medicine, pharmaceutical and analytical sciences, biotechnology, cell biology or biophysics [1–16]. Although the adsorption of proteins is a simple phenomenon at first glance, this behavior needs further elucidation in function of many factors that affect it [17]. The understanding of these factors is imperative to improve our ability to design biocompatible materials and biotechnological devices.

Due to the large size and different shapes of these adsorbing particles, the interactions between the adsorbed proteins on the surface are nontrivial and can be strongly influenced by (i) the entropic contribution to the thermodynamic potential arising from the spatial structure of the molecules in the adsorbed state, and (ii) conformational changes after adsorption [17–23].

Recent contributions on equilibrium and kinetics of proteins adsorbed on solid surfaces have focused on the relevance of accounting for the structure and entropic effects of the adsorbed species [17–19,23]. For example, when blood plasma solutions of albumin, immunoglobulin-G (IgG) and fibrinogen (Fgn) are in contact with a polystyrene surface, the initial adsorption is dominated by the smaller protein (albumin), which is also at larger concentrations in the bulk, to be later replaced by the larger proteins like IgG and Fgn [4,5].

Other studies have suggested the possibility that proteins may adsorb in more than a single conformation, and that the probability of adsorbing in a given conformation may vary with the surface density of adsorbed protein [17,20,23–27]. In Refs. [24,25], the adsorption of single- and two-domain antifreeze proteins onto an ice crystal was studied. The authors derived equations to describe the two adsorbed states of the protein: state I, with the protein adsorbed perpendicular to the surface on single sites; and state II, with the protein lying parallel to the surface and occupying 2 adjacent sites. The phenomenon has also been observed in experiments. In this line, the adsorption of creatine phosphokinase (CPK) onto hydrophilic (silicon wafers and amino-terminated surfaces) and hydrophobic (Polystyrene, PS, coated wafers) substrates has been investigated [26,27]. This study led to a model, where the adsorption of CPK takes place in four stages: (i) a diffusive one, where all the arriving biomolecules are immediately adsorbed; (ii) the arriving biomolecules might stick on the latter one and afterward diffuse to the free sites on the substrate, followed by conformational changes, (iii) formation of a monolayer and (iv) continuous and irreversible adsorption. A multilayer system might be formed, as well as aggregation processes might play a role at this stage.

The observations described above demonstrate that the solution conditions and the protein–surface interactions have to be considered for the proper understanding and description of the adsorption process. The adsorption of small molecules from the gaseous state onto regular surfaces such as the planar faces of crystals has been extensively studied by physical chemists [28,29]. 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 basis for the

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interpretation of such isotherms remains at a level that is quite crude relative to that applied to the analysis of the simpler inorganic systems.

From a theoretical point of view, two classes of models for the adsorption of proteins to surfaces have been formulated. The first class of models [29–31] utilizes continuum theories of two-dimensional hard particle fluids to calculate the chemical potential of the adsorbed species. The second class of models [32] corresponds to lattice models derived from earlier treatments of gas adsorption [33]. Most of these theoretical developments rely upon the crude assumption of spherically symmetric admolecules (each molecule of adsorbate occupies a single adsorption site and one adsorption state is excluded when one particle is adsorbed). As a consequence of these limitations, there exists a lack of research concerning the probable mechanism of protein adsorption and the configuration of the protein in the adsorbed state.

In this context, the main purpose of the present work is to apply the fractional statistical theory of adsorption (FSTA) [34,35] to study the effects of excluded surface area on the adsorption of proteins of different sizes and shapes. The formalism of FSTA have been recently developed on the basis of a generalization of the Pauli's exclusion principle proposed by Haldane [36] and Wu [37]. The appealing feature of Haldane's statistics is that a system of interacting particles confined in a finite region of the space can be characterized by an 'statistical exclusion parameter' g, accounting for the number of states that are excluded from the states spectrum when a particle is added to the system.

The paper is organized as follows: FSTA is presented in Section 2. In order to test the applicability of the proposed model, Section 3 is devoted to the analysis of the adsorption of proteins of arbitrary size and shape. Finally, the conclusions are given in Section 4.

2. Fractional statistical theory of adsorption of polyatomics

In this section, we summarize the basis of the FSTA description [34,35], which allows to describe the configurational entropy through a single function (parameter), namely the statistical exclusion, g, accounting for the configuration of the molecules in the adsorbed state. In this approximation, the interaction of one isolated molecule with a solid surface confined in a fixed volume is represented by an adsorption field having a total number G of local minima in the space of coordinates necessary to define the adsorption configuration. Thus, G is the number of equilibrium states of a single molecule at infinitely low density. In general, more than one state out of G are prevented from occupation upon adsorption of a molecule. Furthermore, because of possible concurrent exclusion of states by two or more molecules, the number of states excluded per molecule, $g(N)$, being a measure of the 'statistical' interactions, depends in general on the number of molecules N within the volume. From the definition of the number of states available for a Nth molecule after $(N-1)$ ones are already in the volume V, $d_N = G - \sum_{N'=1}^{N-1} g(N') = G - G_0(N)$, which is a generalization of the one recently established by Haldane [36], the generalized configurational factor, $W(N) = (d_N + N - 1)!/[N!(d_N - 1)!]$, can be calculated. Consequently, the Helmholtz free energy function can be expressed as $\beta F(N,T,V) = -\ln W(N) + N \ln q_i + \beta N \epsilon_o$, q_i being the partition function from the internal degrees of freedom of a single molecule in the adsorbed state. The general form for the chemical potential of noninteracting adsorbed polyatomics is obtained from $\beta\mu = \left(\frac{\partial F}{\partial N}\right)_{T,V}$, as:

$$\beta\mu = \ln\left[\frac{n\left[1-\widetilde{G}_0(n)+n\right]^{\left(\widetilde{G}_0'-1\right)}}{\left[1-\widetilde{G}_0(n)\right]^{\widetilde{G}_0'}}\right] - \ln K, \tag{1}$$

where $n = N/G$ is the density (n finite as $N, G \to \infty$), $\widetilde{G}_0(n) \equiv \lim_{N,G\to\infty} G_0(N)/G$, $\widetilde{G}_0' \equiv d\widetilde{G}_0/dn$ and $K = q_i \exp(-\beta\epsilon_o)$.

It is worth noticing that the Eq. (1) has well known approximate isotherms as limiting cases. Namely, for constant adsorption energy per particle $\epsilon_o = const.$, and spherically symmetric adsorbates (or single-site occupation in the lattice fashion of the adsorption field) which exclude only one state (one minimum), $\tilde{G}_0(n) = n$, $\tilde{G}_0'(n) = 1$, then Eq. (1) reduces to the Langmuir isotherm

$$Kp = \frac{\theta}{(1-\theta)}, \tag{2}$$

where $p \propto \exp(\beta\mu)$, $\theta = n = N/M$ is the surface coverage (fraction of occupied sites) and we have identified the total number of states G with the total number of adsorption sites M.

On the other hand, if the exclusion parameter is constant, $g(n) = g$, $\tilde{G}_0(n) = gn$ and $\tilde{G}_0'(n) = g$, a particular isotherm function arises from Eq. (1)

$$Kp = \frac{n[1-n(g-1)]^{g-1}}{[1-ng]^g}. \tag{3}$$

The parameter g in the last equation have a precise physical meaning, can be obtained from adsorption experiments and is related directly to the spatial configuration of a polyatomic molecule in the adsorbed state. Alternatively, Eq. (3) can be used by assuming some approach to calculate g as a function of the model's parameters. Thus, given shape and size of adsorbate, the adsorption isotherm it is straightforwardly obtained.

For molecules made out of k identical units (each of which can occupy an adsorption site in a lattice), a simple approximation for g can be obtained, assuming independence of adsorption sites. Under this consideration, $g = mk$, where $m(\equiv m(c,k))$ is the number of distinguishable configurations of the molecule per lattice site (at zero density) and depends on the lattice/molecule geometry. In addition, $G = mM$ and $n = N/G = N/(mM) = kN/(gM) = \theta/g$. For instance, straight k-mers adsorbed "in registry' on sites of a square lattice would correspond to $m = 2, g = 2k$ and $n = \theta/(2k)$. On the other hand, if $g = k$ ($m = 1$) and $n = \theta/k$, Eq. (3) reduces to the exact adsorption isotherm of noninteracting chains (k-mers) adsorbed flat on a one-dimensional lattice [38]. This is already a simple example of the underlaying relationship between the statistical exclusion parameter g and the spatial configuration of the admolecule.

The proposed formalism allows us to deal very simply with a whole variety of configuration of the adsorbed molecule that can occur. In the next section, analysis of lattice-gas simulations has been carried out in order to bear the significance of the parameter g in terms of the adsorbate/surface geometry.

3. Applications: adsorption of proteins of arbitrary size and shape

Before starting the study, and in order to analyze adsorption from liquid solutions, it is convenient to write the theoretical isotherms given in Section 2 in a more appropriate form. In this framework, Eq. (3) adopts the form

$$\ln(Kc) = \ln\theta + (g-1)\ln[g-\theta(g-1)] - g\ln(g-\theta g), \tag{4}$$

where $n = \theta/g$ and the pressure p has been replaced by the concentration of the solute in the liquid c.

Figure 1 shows the adsorption isotherms calculated using Eq. (4) with values of g for various differently shaped particles (see Figure 2): $(a \times a)$-squares, $k = 4, m = 1$ and $g = 4$; $(a \times \sqrt{2}a)$-triangles, $k = 3, m = 4$ and $g = 12$; and $(a \times 10a)$-rectangles, $k = 22, m = 2$ and $g = 44$. The curves from top to bottom correspond to increasing values of g. This effect can be explained as follows. Rectangles

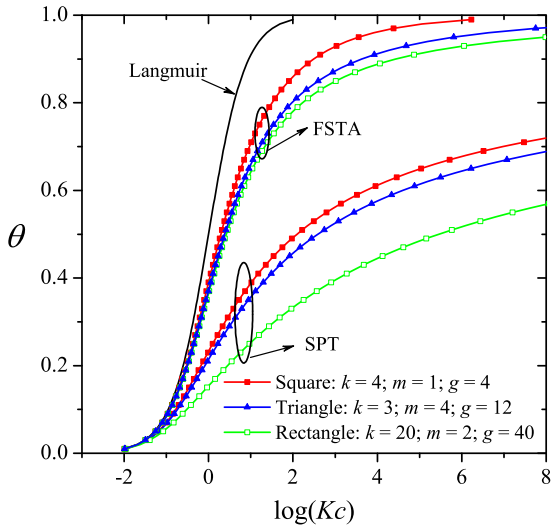

Figure 1. Adsorption isotherms obtained for various particle shapes as indicated. The curves were calculated using FSTA [Eq. (4)] and SPT [Eq. (5)]. Langmuir case corresponds to Eq. (4) with $g = 1$.

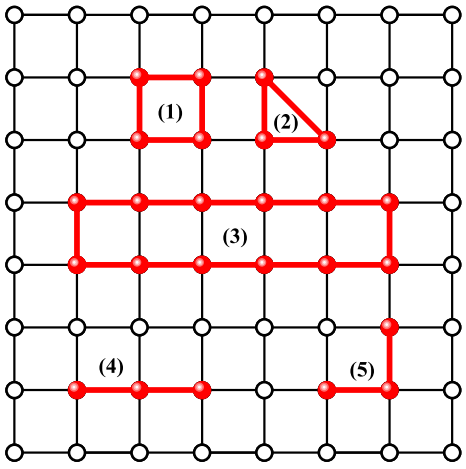

Figure 2. Examples of large ligands adsorbed on square lattices. (1): $(a \times a)$-square (a being the lattice constant); (2): $(a \times \sqrt{2}a)$-triangle; (3): $(a \times 5a)$-rectangle; (4): straight trimer and (5): bent trimer.

($g = 44$) exclude more states than triangles ($g = 12$), and triangles exclude more states than squares ($g = 4$). Consequently, the slope of the *square-shaped* isotherm is more pronounced than the slope of the *triangle-shaped* isotherm, and the slope of the *triangle-shaped* isotherm is more pronounced than the slope of the *rectangle-shaped* isotherm.

On the other hand, it is interesting to note that, in the limit $g = 1$, FSTA provides the Langmuir isotherm. In this case, the number of excluded states by an adsorbed molecule is the minimum. Consequently, the capacity of adsorption is maximum and the corresponding adsorption isotherm constitutes the upper limit of the theoretical isotherms. Thus, the statistical exclusion parameter g is a measure of the capacity of the molecule to be adsorbed and results in being highly sensitive to the spatial configuration of the adparticles. This represents evidence of the physical and experimental significance of g.

Figure 1 also includes theoretical results obtained from scaled particle theory (SPT). This theory, originated by Reiss et al. [39], has shown to be useful in providing a semiquantitative account of the behavior of two-dimensional (continuum) fluids of hard particles. In this framework, the adsorption isotherm for convex hard particles can be written as [40,41]

$$\ln(Kc) = \ln\theta - \ln(1-\theta) - \epsilon - 1 + \frac{1}{1-\theta} + \frac{\epsilon}{(1-\theta)^2}, \tag{5}$$

where ϵ is a parameter which depends on the shape of the adsorbate. In the case of squares, triangles and rectangles, ϵ takes the values $(4/\pi)\tan(\pi/4)$, $(3/\pi)\tan(\pi/3)$ and $(r/\pi)(1 + 1/r)$ (being r is the axial ratio, $r = 10$ in the present study), respectively [29]. The corresponding adsorption isotherms are shown in Figure 1. As in the case of FSTA data, the slope of the *square-shaped* isotherm is more pronounced than slope of the *triangle-shaped* isotherm, and the slope of the *triangle-shaped* isotherm is more pronounced than slope of the *rectangle-shaped* isotherm.

Even though the SPT has been developed in the continuum, the good qualitative agreement between FSTA and SPT (1) demonstrates the importance of considering the effect of excluded surface area on the adsorption of large ligands [29], (2) reinforces the validity of the results obtained from FSTA and (3) shows that FSTA model is a good one, which explicitly includes the effect of excluded surface area on the adsorption isotherm.

In addition to the comparison shown in Figure 1, FSTA results were validated by simulation experiments. The system chosen for the comparison was a lattice-gas of trimers. This system is the simplest case of a polyatomic adsorbate allowing for two possible adsorption states, each one with a different value for the exclusion parameter (see Figure 2). As the size of the adsorbate increases, the relaxation time increases very fast with the density and the system gets trapped in some small set of nearby states. Consequently, MC simulations at high density are very time consuming and may produce artifacts related to non-accurate equilibrium states.

The adsorption process was simulated through a grand canonical ensemble method [42]. In adsorption–desorption equilibrium there are two elementary ways to perform a change of the system state, namely, adsorbing one molecule onto the surface (adding one molecule into the adsorbed phase volume M) and desorbing one molecule from the adsorbed phase (removing one molecule from the volume M). The algorithm to carry out an elementary Monte Carlo Step (MCS) is the following:

(1) Given a square lattice of M adsorption sites, set the value of temperature T and concentration c.
(2) One of over the m possible configurations for the ad-particle is randomly selected.
(3) Choose randomly one configuration of sites on the lattice with the form selected in step (2) and generate a random number $\xi \in [0, 1]$.
(4)
(a) If the sites selected in step (3) are empty, then adsorb a molecule if $\xi < W_{ads}$, being W_{ads} the adsorption probability [43],

$$W_{ads} = \min\{1, c\exp(-\Delta E/k_B T)\}, \tag{6}$$

where $\Delta E = E_f - E_i$ is the difference between the energies of the final and initial states.
(b)If the sites selected in step (3) are occupied by atoms belonging to the same molecule, then desorb the molecule if $\xi < W_{des}$, being W_{des} the desorption probability [43],

$$W_{des} = \min\left\{1, \frac{1}{c}\exp(-\Delta E/k_B T)\right\}. \tag{7}$$

(5) Repeat from step (2) M times.

The approximation to thermodynamical equilibrium is monitored through the fluctuations in the number of adsorbed particles; this is usually reached in 10^7 MCS. After that, the adsorption isotherm, or mean coverage as a function of the concentration $[\theta(c)]$, is obtained as a simple average:

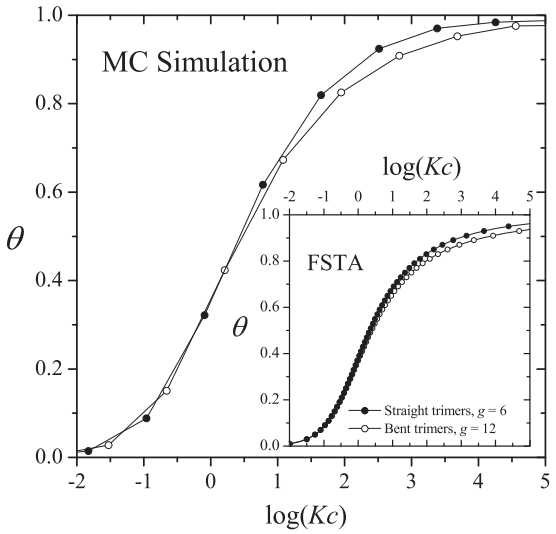

Figure 3. Simulation and FSTA (inset) adsorption isotherms for straight and bent trimers adsorbed on square lattices. The symbology is indicated in the figure.

$$\theta(c) = \frac{k\langle N\rangle}{M}, \tag{8}$$

where N is the mean number of adsorbed particles, and $\langle \ldots \rangle$ indicates the time average over the Monte Carlo simulation runs (in the present case, 10^7 MCS have been used). In addition, the computational simulations have been developed for square lattices of 120×120 sites.

All calculations were carried out using the parallel cluster BACO of Universidad Nacional de San Luis, Argentina. This facility, located at Instituto de Física Aplicada, Universidad Nacional de San Luis-CONICET, San Luis, Argentina, consists of 50 CPUs each with an Intel Core i7 processor running at 2.93 GHz and 512 MB of RAM per core.

Following the procedure described above, simulation adsorption isotherms corresponding to straight (solid circles) and bent (open circles) trimers were obtained. The results are shown in Figure 3. As it can be observed, the slope of the *straight-shaped* isotherm is more pronounced than the slope of the *bent-shaped* isotherm. This behavior can be easily understood in terms of FSTA. In fact, $k = 3, m = 2$ and $g = 6$ for straight trimers adsorbed on square lattices and $k = 3, m = 4$ and $g = 12$ for bent trimers deposited on a substrate with a square structure. Then, bent trimers ($g = 12$) exclude more states than straight trimers ($g = 6$) and consequently, the surface coverage increases more rapidly for straight trimers than for bent trimers. See inset of Figure 3, where FSTA adsorption isotherms for straight and bent trimers are plotted.

The analysis in Figure 3 indicates that the behavior of the adsorption isotherms obtained from FSTA follows qualitatively the behavior observed by Monte Carlo simulations. This finding reinforces the robustness of the theory introduced here.

4. Conclusions

The statistical thermodynamics of adsorbate clusters of arbitrary size and shape has been studied using FSTA. In this theory, site exclusion is explicitly considered through a statistical exclusion parameter, g, which allows to handle the complexity of the entropy of adsorbed polyatomics traced to the adsorbate's configuration and interactions, and thus, the spatial mode of adsorption (or the configuration of the molecule in the adsorbed state) can be characterized by the parameter g.

Comparisons with previous theoretical calculations and MC simulations validate the applicability of FSTA to study the effects of excluded surface area and adsorbate clustering on adsorption of proteins. The results obtained indicate that small values of g are associated with 'compact' molecules (small excluded area), while large values of g are associated with 'extended molecules' (large excluded area). From another perspective, for a given chemical potential, the equilibrium coverage increases (decreases) as g is decreased (increased).

In summary, FSTA gives a framework and compact equations to consistently describe the adsorption thermodynamics of structurally diverse proteins ranging from simple species to large ligands (that occupy more than one site when adsorbed on a lattice).

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