A Modified Poisson−Boltzmann Model Including Charge Regulation for the Adsorption of Ionizable Polyelectrolytes to Charged Interfaces, Applied to Lysozyme Adsorption on Silica

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The equilibrium adsorption of polyelectrolytes with multiple types of ionizable groups is described using a modified Poisson−Boltzmann equation including charge regulation of both the polymer and the interface. A one-dimensional mean-field model is used in which the electrostatic potential is assumed constant in the lateral direction parallel to the surface. The electrostatic potential and ionization degrees of the different ionizable groups are calculated as function of the distance from the surface after which the electric and chemical contributions to the free energy are obtained. The various interactions between small ions, surface and polyelectrolyte are self-consistently considered in the model, such as the increase in charge of polyelectrolyte and surface upon adsorption as well as the displacement of small ions and the decrease of permittivity. These interactions may lead to complex dependencies of the adsorbed amount of polyelectrolyte on pH, ionic strength, and properties of the polymer (volume, permittivity, number, and type of ionizable groups) and of the surface (number of ionizable groups, p*K*, Stern capacity). For the adsorption of lysozyme on silica, the model qualitatively describes the gradual increase of adsorbed amount with pH up to a maximum value at pH_c, which is below the iso-electric point, as well as the sharp decrease of adsorbed amount beyond pH_c. With increasing ionic strength the adsorbed amount decreases (for $pH > pH_c$), and pH_c shifts to lower values.

Introduction

Polyelectrolyte adsorption to charged interfaces is a fundamental problem throughout the natural sciences and in many technological applications.[1−6] In this paper, the adsorption on a charged planar interface is described of a polyelectrolyte class with multiple types of ionizable groups, namely, protein molecules. We include in the model the fact that the ionization degree of the amino acids depends on the local pH, which is a function of bulk pH, ionic strength, and the proximity to other molecules and nearby surfaces. The effect of the presence of other molecules and surfaces on the charge is called charge regulation. Charge regulation not only influences the actual charge on the molecules and the surface but also results in a chemical contribution to the free energy, besides an electrical contribution due to the formation of the double layer.[7−14] This implies that the driving force for polyelectrolyte adsorption is not only counterion release but an increase in ionization degree of the surface groups and amino acids as well. Only when surface and polyelectrolyte have a fixed charge (are "quenched") is the chemical contribution to the free energy zero, and models that only include the electric contribution suffice.

Besides these electrostatic effects we must include in the free energy analysis entropic and energetic contributions from van der Waals dispersion forces, hydrophobic interactions, and structural rearrangements (e.g., protein unfolding[4]). However, in this work we focus on the electrostatic and chemical aspects of polyelectrolyte adsorption to charged interfaces, forces that may to a large extent govern the adsorption behavior.[5,15]

Two lines of models are used to describe polyelectrolyte adsorption on charged interfaces, dependent on whether internal conformations of the polyelectrolyte molecule are considered in detail. For flexible polyelectrolyte molecules, conformations are included and polyelectrolyte adsorption is modeled using one-dimensional self-consistent mean-field theories by a combination of the Edwards and the Poisson−Boltzmann (PB) equation. The equations can be solved in the ground state dominance approximation[16,17] or by the use of a lattice formalism.[10,18] Scaling theories are presented in refs 11 and 19. The ionizable character of weak polyelectrolytes has been discussed,[10,11,16−18] but always for polyelectrolyte with a single type of ionizable group. The effects related to the exclusion of small ions and the reduction in permittivity of the adsorption layer are not implemented in all cases.

For more rigid polyelectrolytes (DNA, globular protein), much work has been done for spherical and cylindrical molecules with a fixed and homogeneous surface charge. For instance, the electrostatic interaction between a homogeneously charged spherical protein molecule and a planar charged interface has been calculated, either for a constant electrostatic charge at the two interfaces,[20] for a constant electrostatic potential,[21] or for one material having a constant charge and the other a constant potential.[22] In refs 23−25, a fixed charge on the surface and the protein molecule is assumed as well, while models of different degrees of detail are used to model the adsorption free energy as function of the tertiary structure and orientation of the protein molecule. Johnson et al.[26]

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considered high coverage, by assuming that the spherical protein molecules pack in an ordered array on the surface. A fixed charge on surface and particle is assumed, and the adsorption free energy is minimized to obtain the equilibrium adsorption of protein. Oberholzer et al.[27] consider protein−protein and protein−surface interactions simultaneously, by superimposing the electrostatic potential for the case of a spherical particle 1 interacting with the surface with that for a particle 2 interacting with the surface and use a 2D random sequential adsorption simulation to describe irreversible protein adsorption. They use a constant surface charge for the protein particle, a constant electrostatic potential at the adsorbent surface, and calculate the fractional coverage as function of the Debye length, charge, and potential. May et al.[28] calculate the adsorption free energy of a spherical protein molecule with a fixed surface charge adsorbing onto a planar mixed lipid membrane. The influence of coverage on the adsorption free energy is considered by the use of a Wigner−Seitz cell geometry in which virtual cylinders are placed perpendicular to the surface with a single protein molecule at the centerline of each cell. Because the lipid molecules are assumed to be mobile within the interface, those charged oppositely to the protein tend to cluster in the contact region with the protein molecule. A similar exercise for the binding of DNA to lipid membranes is presented by Harries et al.,[29] who calculate the equilibrium spacing of DNA (equilibrium coverage) by minimization of the adsorption free energy. Also Ubbink and Khokhlov[30] use such an approach to calculate the spacing of polyelectrolytes adsorbing onto an oppositely charged surface (both of fixed charge).

Charge regulation during hetero-interaction of ionizable planar interfaces was discussed by Parsegian and Gingell[31] and quantified for the electrostatic adsorption of protein by Ståhlberg and Jönsson,[2] who include it in a model for the interaction free energy of two plane-parallel opposite and dissimilar surfaces. Ståhlberg and Jönsson[2] use the Debye−Hückel approximation, linearize the charge regulation boundary condition and analyze the interaction free energy as function of separation. Fleck and Netz[32] describe the interaction of a single DNA molecule (fixed line charge) with a mobile and charge regulating lipid membrane and calculate the distribution of charged surface groups in the membrane. Bremer et al.[33] include charge regulation in a model in which a repulsive protein homo-interaction component is added to an attractive protein-surface component (based on a geometry somewhat similar to Figure 4 in ref 27) and the Deryagin approximation is used to describe the adsorption of immunoglobulin on (amine-functionalized) silica.

The present work uses a one-dimensional mean-field theory but does not use the Edwards equation. Instead, a structureless (or gel-like) protein adsorption layer of a certain thickness is assumed with the protein homogeneously distributed across this layer.[34] We include all six types of ionizable amino acid residues and solve the PB equation and the free energy expressions in the direction perpendicular to the surface. The mean-field approximation is useful when the spacing between the charges of different polyelectrolyte molecules is small compared to the Debye length. In that case the diffuse ions clouds around molecules overlap, which leads to an approximately constant electrostatic potential (independent of location within a plane). This may be a good approximation at high polyelectrolyte coverage and/or at low ionic strength. In that case the mean-field model contains the relevant elements of charge regulation and the competition between the different charged species (surface, polymer, small ions), while it is much less formidable to solve than corresponding descriptions that include the structure and structural changes of adsorbed molecules in detail. Because such detailed models including the regulation of the various charges, the interactions at high protein coverage, and the competition with the small ions do not yet exist, we believe that despite its approximate nature the model is useful to study the various forces involved during protein adsorption, especially related to the regulation of the charge of protein and surface.

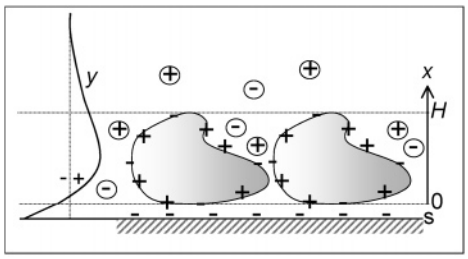

Figure 1. Representation of the model for adsorption of (net positive) protein molecules on a (negatively) charged surface. *x* is the place coordinate, *y* the dimensionless electrostatic potential.

In the model, it is assumed that adsorbing molecules do not have a preferred orientation (for instance with a positive patch oriented toward a negative surface). For a homogeneously charged polyelectrolyte, this should be a valid assumption, but for a polymer with a dipole (such as protein molecules with positive and negative patches) preferential orientation is certainly likely. Preferential orientation can be included in a modified version of the model, for instance, by displacing polymer charges of the same sign as the surface outward and placing those of opposite sign closer to the surface.[34]

By using the model, we are able to construct equilibrium adsorption−pH curves as a function of ionic strength and other parameters. We apply the method to the adsorption of (succinylated) lysozyme on silica, but obviously the theory can be applied as well to other types of protein molecules and other weakly charged polyelectrolytes (with one or several types of ionizable groups) and to other surfaces, such as self-assembled thiol monolayers, charged lipid membranes,[15,32] or the outermost surface of multilayers.[5]

Theory

The one-dimensional mean-field model is schematically represented in Figure 1. In the adsorption layer ($0 < x < H$) adjacent to the surface the (ionizable) amino acids of adsorbed protein molecules are assumed to be homogeneously distributed in the lateral direction. The (fractional) coverage, or adsorbed amount, denoted by ϕ, is the fraction of the volume of the adsorption layer taken up by protein and not available to solvent and small ions. With increasing protein adsorption, the number density of amino acids in the adsorption layer increases, and the volume available to small ions becomes smaller because the adsorbed protein molecules block out part of the volume (namely, by a factor ϕ).

A flat distribution over the adsorption layer is assumed (block profile) both for the number concentration of ionizable polymer groups as for the volume fraction of polymer, ϕ. This simple approximation should be valid for coil-like molecules adsorbing homogeneously throughout a layer of a certain thickness and also for globular molecules when they adsorb in random orientations. For spherical molecules, the block profile for the density of ionizable groups is valid when the ionizable groups are homogeneously distributed over the spherical surface and/or when the molecules adsorb in random orientations. It must be noted that, for these adsorbing spherical molecules, the

volume fraction ϕ is not constant but follows a $\phi \propto \sqrt{2\lambda-\lambda^2}$ dependence ($\lambda = x/r$ with x the distance from the surface and r the radius of the sphere). Though such a dependence of ϕ on x can be readily implemented, ϕ will be assumed constant over the adsorption layer to keep the calculation results as general as possible.

In the present version of the model only adsorption until monolayer coverage is described, when the adsorption layer has become completely filled with protein ($\phi = 1$). However, it is possible to construct a modified model that includes adsorption beyond a monolayer, by considering a second layer adjacent to it in equilibrium with the solution phase and the primary adsorption layer.

From $x > H$, small ions are diffusely distributed into the solution phase. The ionization degree changes over the adsorption layer as well as the electrostatic potential y, which may change sign somewhere within the adsorption layer (see Figure 4, which will be discussed later). We start describing the uncovered surface and initially neglect the Stern layer at the surface. We assume that all small ions are monovalent. The present theory is valid for a sufficiently dilute protein solution such that the diffuse ion clouds around protein molecules in solution do not overlap, while the diffuse layer that extends from the adsorption layer is not truncated by the ion clouds around the protein molecules in solution.

Uncovered Oxide Plane Surface. For an isolated plane surface ($x = 0$), the electrostatic potential gradient at the surface (dy/dx) can be obtained from integrating the PB equation once, resulting in

$$\frac{1}{\kappa}\left|\frac{dy}{dx}\right| = \sqrt{2(\cosh y_0 - 1)} \tag{1}$$

where y is the dimensionless electrostatic potential ($=e\psi/kT$), x is the place coordinate, ψ is the dimensional electrostatic potential, e is the electronic charge, k is the Boltzmann constant, and T is the temperature (the subscript "0" refers to the surface). κ is the inverse of the Debye length, which for a monovalent salt solution is given by

$$\kappa = \sqrt{\frac{2e^2 n_\infty}{\epsilon_0 \epsilon^w kT}} \tag{2}$$

where n_∞ is the ionic strength (in m^{-3}, $n_\infty = N_{av}c_\infty$, c_∞ in mol/m^3 = mM, N_{av} Avogadro's number), ϵ_0 is the permittivity of vacuum (8.8514×10^{-12} F/m), and ϵ^w is the relative permittivity of water ($\epsilon^w = 78$) (κ is based on the permittivity of water throughout this paper).

At the surface, the ionization degree of the surface groups, α_0 (a number between 0 and 1), and dy/dx are related by Gauss' law:

$$\alpha_0 = \frac{\epsilon_0 \epsilon^w kT}{Ne^2}\left|\frac{dy}{dx}\right| \tag{3}$$

where N is the number density of ionizable surface groups (m^{-2}). In eq 3, it is assumed that the surface contains one class of ionizable groups only. For an oxide surface described by a Langmuir adsorption isotherm (1 − pK model), the degree of charge α^* is given by[35]

$$\alpha^* = p - \frac{1}{1 + 10^{pK-pH}e^{-y_0}} = p - \frac{1}{1 + e^{y_N - y_0}} \tag{4}$$

with $p = {}^1/_2$ for all common oxides except silica, for which $p = 0$; the Nernst potential (y_N) is given by

$$y_N = \ln 10\ (pK - pH) \tag{5}$$

Here we focus on silica and use the ionization degree α_0 instead of the degree of charge α^*, which then results in

$$\alpha_0 = \frac{1}{1 + 10^{pK-pH}e^{-y_0}} = \frac{1}{1 + e^{y_N - y_0}}. \tag{6}$$

Equations 1, 3, and 6 can be jointly solved, after which the electrostatic free energy of the system can be obtained, which is a summation of electric work and chemical work. Within the PB framework, the electric work has contributions from the field energy[28,29]

$$\frac{\epsilon kT}{2e^2}\int_V (\nabla y)^2\, dV \tag{7}$$

and the entropy of the (i types of) small (monovalent, and fully dissociated) ions,[28,29]

$$-T\Delta S = \int_V \sum_i \left(n_i \ln\frac{n_i}{n_{i,\infty}} - n_i + n_{i,\infty} \right) dV = 2n_\infty \int_V (y \sinh y + 1 - \cosh y)\, dV \tag{8}$$

which must both be evaluated over all volumes, V. Note that in this report all free energy terms are scaled with kT and expressed per unit surface area (or per protein particle in eqs 17−19).

Equations 7 and 8 suffice when both the surface and adsorbing polymer have fixed charge. However, when the charge regulates during adsorption, a chemical contribution must be added, which per ionizable group is given by[7]

$$-z\int_0^\alpha y'\, d\alpha' \tag{9}$$

where y' is the dimensionless electrostatic potential at the location of the ionizable charge, related to α via an ionization isotherm such as eq 6, and z is the charge sign. Instead of using these three equations, it is mathematically more convenient to write the electrostatic free energy as a summation of a surface term (an integration over all charges of protein and surface), F^S, and a diffuse term (which leads to an integration over all volumes), F^D, resulting in[8,9,12−14]

$$F = \int_S dS\{\sum_i N_i \ln(1 - \alpha_i)\} - n_\infty \int_V dV\left\{ 2(\cosh y - 1) + \frac{1}{\kappa^2}(\nabla y)^2 \right\} \tag{10}$$

where S denotes an integration over all surfaces (S) on which there is a concentration N_i of i types of ionizable groups (m^{-2}; be they anionic or cationic). In eq 10, a Langmuir adsorption isotherm such as eq 6 has been assumed.

For an isolated planar surface, the diffuse term is given by (ref 36, p 81)

$$F^{D,\infty} = -\frac{8n_\infty}{\kappa}\left(\cosh\frac{y_0}{2} - 1\right) = -16\frac{n_\infty}{\kappa}\sinh^2\left(\frac{y_0}{4}\right) \tag{11}$$

TABLE 1: Number of Ionizable Amino Acids per Protein Molecule, Intrinsic PK Values,[49] and Isoelectric Point (pI)

			p*K*	lysozyme	succinylated lysozyme
R	Arg	+	12	11	11
H	His	+	6.5	1	1
K	Lys	+	10	6	0
D	Asp	−	4.4	7	16
E	Glu	−	4.4	2	2
Y	Tyr	−	10	3	0
pI				10.7	4.7

while in case of a single type of ionizable group, the surface term ($F^{S,\infty}$) is

$$F^{S,\infty} = N \ln(1 - \alpha_0) \tag{12}$$

The free energy per ionizable site "ln(1 − α)" was already (implicitly) given by Payens[37] and Brenner and McQuarrie[38] and explicitly by Chan and Mitchell,[7] Stigter and Dill,[39] and Borisov et al.[11]

Protein in Solution. To obtain the free energy of an isolated protein molecule in solution, we can assume a homogeneous distribution of charges over its surface as well as a spherical diffuse layer around the globular particle and solve the PB equation in spherical coordinates with boundary conditions $y = 0$ at $r = \infty$ and

$$\frac{dy}{dr} = -\frac{Z\lambda_B}{R^2} \tag{13}$$

at the particle surface where $r = R$ with R the particle radius, λ_B the Bjerrum length, $\lambda_B = e^2/(4\pi\epsilon kT)$, and Z the surface charge per molecule, which can be obtained from[40]

$$Z = Z_+ - \sum_i \frac{q_i}{1 + 10^{pK_i - pH} e^{-y_p}} \tag{14}$$

with Z_+ the maximum positive charge (obtained at very low pH), Σ_i a summation over the six types of amino acids that are ionizable (both cationic and anionic), q_i the number per protein molecule of each of these types (the positive *R*, *H*, and *K* residues and the negative *D*, *E*, and *Y* residues), see Table 1, and y_p the electrostatic potential at the particle surface. pH is defined in bulk solution, and the intrinsic p*K* values used in eq 14 describe the affinity of a proton to the amino acid and are not influenced by the local electrostatic environment (in contrast to apparent p*K* values).[41]

Completely equivalent to eq 14 is the expression

$$Z = \sum_i q_i z_i \alpha_i \tag{15}$$

with z_i the charge sign of the amino acid residues (+1 for cationic, −1 for anionic residues) and with the ionization degrees α_i given by the general equation for the ionization degree of an acid or base group

$$\alpha_i = \frac{1}{1 + 10^{z_i(pH - pK_i)} e^{-z_i y_p}} = \frac{1}{1 + e^{z_i(y_p - y_{N,i})}} \tag{16}$$

with the Nernst potential y_N given by eq 5. Equations 15 and 16 are more convenient than eq 14 because the ionization degrees α_i are also required in the expressions for the electrostatic contribution to the free energy of an ionizable protein molecule

$$F^{p,\infty} = F^D + \sum_i q_i \ln(1 - \alpha_i) \tag{17}$$

in which the diffuse contribution F^D per (spherical) protein molecule is given by

$$F^D = -4\pi n_\infty \int_R^\infty \left\{ 2(\cosh y - 1) + \frac{1}{\kappa^2}\left(\frac{dy}{dr}\right)^2 \right\} r^2 \, dr \tag{18}$$

Because of the spherical symmetry the field energy within the molecule is zero, and F^D needs only be evaluated for the aqueous phase around the molecule. For the Debye−Hückel limit of the PB equation, eq 17 simplifies to

$$F^{p,\infty} = -\frac{1}{2} Z y_{p,\infty} + \sum_i q_i \ln(1 - \alpha_{i,\infty}) \tag{19}$$

where "p,∞" refers to a protein molecule in solution. For an isolated spherical particle, surface charge Z and potential $y_{p,\infty}$ are related by[40]

$$Z = \frac{\kappa}{\lambda_B} R^2 \left(1 + \frac{1}{\kappa R}\right) y_{p,\infty}. \tag{20}$$

The above set of equations can be used to calculate the electrostatic contribution to the free energy of a protein molecule in solution, $F^{p,\infty}$, based on a spherical double layer around the protein molecule.

Protein-Covered Surface. As mentioned, we assume that the (ionizable) amino acids of the adsorbed protein molecules are distributed evenly over the surface, throughout a layer of thickness *H*. This is somewhat similar to the approach of Norde and Lyklema[34] where the charge density is homogeneously distributed within certain layers parallel to the surface as well.

The adsorption layer ($0 < x < H$) is described by the Poisson equation in the direction perpendicular to the surface, x, with both the fixed and free ions included in the calculation. The free ion concentration is given by the Boltzmann relation, and the fixed ion concentration is given by eq 15, resulting in the modified PB equation

$$\epsilon^{eff} \frac{d^2 y}{dx^2} = \kappa^2 \left((1 - \phi) \sinh y - \frac{\phi}{2 n_\infty \upsilon} \sum_i z_i q_i \alpha_i \right) \tag{21}$$

where υ is the volume of a protein molecule, and ϵ^{eff} is an effective relative permittivity of the mixed water/protein phase. Assuming a linear dependence on ϕ,[18] ϵ^{eff} is given by

$$\epsilon^{eff} = 1 - \phi(1 - \epsilon^p/\epsilon^w) \tag{22}$$

with ϵ^w and ϵ^p as the relative permittivity of water and of the protein phase, respectively.

At the oxide surface (partly covered by protein), eq 3 is used with ϵ^{eff} added before ϵ^w. At $x = H$, eq 1 can be used (with 0 replaced by H, and y_0 replaced by y_H) to relate y_H to the potential gradient (dy/dx) at the solution side of the adsorption layer. However, the potential gradient just within the adsorption layer is a factor $1/\epsilon^{eff}$ higher. Thus, eq 1 must be replaced by

$$\frac{\epsilon^{eff}}{\kappa} \left| \frac{dy}{dx} \right|_H = \sqrt{2(\cosh y_H - 1)}. \tag{23}$$

with $dy/dx|_H$ as the potential gradient at $x = H$, within the adsorption layer. (In ref 42, continuity of dy/dx at the adsorption layer−diffuse layer interface was erroneously assumed. How-

ever, the predicted adsorbed amount is influenced only very marginally, ~1% or less).

Having obtained the electrostatic potential profile from the surface (at $x = 0$) to the boundary of the adsorption layer ($x = H$) and into the solution ($x \rightarrow \infty$), we can proceed by calculating the electrostatic free energy, which contains the surface term for the oxide surface (given by eq 12), the diffuse term for the solution phase (given by eq 11) but with y_0 replaced by y_H, and a third term that is obtained from integrating over the adsorption layer:

$$F = -n_\infty \int_0^H \left(2(1-\phi)(\cosh y - 1) + \frac{\epsilon^{\mathrm{eff}}}{\kappa^2}\left(\frac{\mathrm{d}y}{\mathrm{d}x}\right)^2 - \frac{\phi}{n_\infty \upsilon}\sum_i q_i \ln(1-\alpha_i) \right) \mathrm{d}x \quad (24)$$

The summation of these three terms gives the electrostatic contribution to the free energy per unit surface area of the protein covered surface, F^{pcs}.

Minimization of Adsorption Free Energy. At equilibrium the chemical potential of protein molecules in solution equals that of the protein molecules adsorbed. For a dilute solution in which the double layer overlap of the molecules in solution is weak (osmotic pressure is zero), the chemical potential of the molecules in solution is given by

$$\mu^{\mathrm{p},\infty} = \ln \phi^{\mathrm{p},\infty} + F^{\mathrm{p},\infty} \quad (25)$$

with the electrostatic free energy per molecule given by eq 19. For the adsorbed protein molecules, the chemical potential is given by

$$\mu^{\mathrm{p,ads}} = \ln \phi + \frac{\upsilon}{H}\frac{\mathrm{d}F^{\mathrm{pcs}}}{\mathrm{d}\phi} + \Delta\mu^{\mathrm{ads}} = \ln \phi + \frac{1}{H}\int_0^H \left(\sum_i q_i \ln(1-\alpha_i) + 2n_\infty \upsilon(\cosh y - 1) + \frac{n_\infty \upsilon}{\kappa^2}\left(1 - \frac{\epsilon^{\mathrm{p}}}{\epsilon^{\mathrm{w}}}\right)\left(\frac{\mathrm{d}y}{\mathrm{d}x}\right)^2 \right)\mathrm{d}x + \Delta\mu^{\mathrm{ads}} \quad (26)$$

where ϕ is the volume fraction of protein in the adsorption layer and $\Delta\mu^{\mathrm{ads}}$ is the difference in standard state chemical potential between a molecule adsorbed and in solution, or simply the (nonelectrostatic) adsorption energy, a term that can be used to contain the nonelectrostatic forces discussed in the Introduction. Equations 25 and 26 assume an ideal entropic contribution, $\ln \phi$. The first term in the integral in eq 26 is a chemical component due to the ionization of the amino acid residues, the second term is due to ion exclusion by the protein, and the third term is due to polarization.[43,44]

Instead of setting $\mu^{\mathrm{p},\infty} = \mu^{\mathrm{p,ads}}$ at equilibrium, we can also minimize the free energy function

$$\int_0^\phi (\mu^{\mathrm{p,ads}} - \mu^{\mathrm{p},\infty})\mathrm{d}\phi = \phi(\ln \phi - 1) + \frac{\upsilon}{H}F^{\mathrm{pcs}} + (\Delta\mu^{\mathrm{ads}} - \mu^{\mathrm{p},\infty})\phi \quad (27)$$

valid when the concentration of protein in solution is not influenced by the adsorption process, and thus $\phi^{\mathrm{p},\infty}$, $F^{\mathrm{p},\infty}$, and $\mu^{\mathrm{p},\infty}$ are independent of the degree of adsorption, ϕ (grand canonical approach). We have used both methods, and they give exactly identical results.

Because $\Delta\mu^{\mathrm{ads}}$ is independent of adsorption degree, it can be used to describe forces (energies) that relate to the interaction of the protein with the surface. We can also add to eq 27 a term such as $\chi^{\mathrm{pp}}\phi^2$ (leading to an additional term $2\chi^{\mathrm{pp}}\phi$ in eq 26) that describes nonelectrostatic (e.g., hydrophobic) interactions between mutual, adsorbed, protein molecules.

When a flexible polyelectrolyte chain adsorbs to a surface where it is confined to an adsorption layer of thickness, H, the conformational free energy increases approximately according to $\propto N(l_k/H)^2$ (N kuhn segments of length l_k, θ-solvent, see, for example, refs 11 and 45). In the present work we will not consider such contributions and assume that the reduction in conformational entropy, by limiting the polymer to a layer of thickness H, is small: the polymer is sufficiently stiff and/or sufficiently retains its globular character.

Stern Layer Capacity. To include a Stern layer for the oxide surface in the theory just discussed, y_0 in eqs 4 and 6 must be replaced by y_s, the potential at the surface, where protons adsorb/desorb. Additionally, we need

$$C^* = \frac{z_s \alpha_0}{y_s - y_0} \quad (28)$$

with z_s the charge sign of the surface (when α_0 is defined as a positive number; $z_s = -1$ for silica), while

$$C^* = C_1 \frac{kT}{e^2 N} \quad (29)$$

where C_1 is the Stern capacity (F/m^2). Equations 12 and 24 for the surface free energy must be supplemented by the term[14]

$$F^{\mathrm{S},*} = -\frac{N\alpha_0^2}{2C^*} \quad (30)$$

to reflect the presence of a Stern layer. Specific adsorption of small ions to the surface (except for protons) is not included in the above theory.

Results and Discussion

Table 1 summarizes the number of ionizable amino acids per protein molecule for lysozyme and succinylated lysozyme (*R*, *H*, and *K* are positively charged; *D*, *E*, and *Y* are negatively charged). We assume in Table 1 and the model calculations that in succinylation of lysozyme, the six lysine and three tyrosine residues are converted into succinyl groups (with a carboxylic acid end group) with an intrinsic p*K* value equal to that of the Asp residues.

For the two protein molecules, van der Veen et al.[46] present titration data at $c_\infty = 50$ mM. The data are reproduced in Figure 2 together with a model calculation based on the protein composition of Table 1 and an electrical double layer model for a spherical particle with a homogeneous surface charge (Debye−Hückel low-potential limit) given by combination of eqs 15, 16, and 20. This approach is completely analogous to that by Tanford et al.[47] if we replace their ϵ by $4\pi\epsilon$ (conversion of cgs into SI units) and their $2wZ$ by y_p. Rewriting results in the familiar form[47,48]

$$\mathrm{pH} - {}^{10}\log\frac{\alpha_i}{1-\alpha_i} = \mathrm{pK}_i - 0.434\frac{\lambda_B Z}{R(1+\kappa R)} \quad (31)$$

The agreement between data and model is quite satisfactory

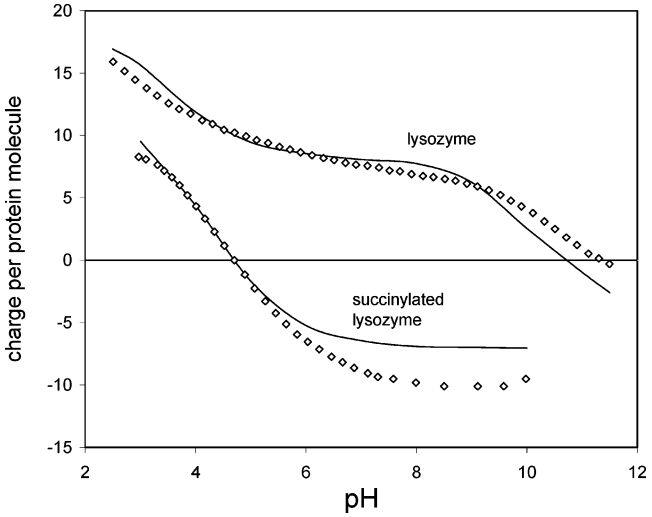

Figure 2. Titration data[46] and model (based on low-potential limit for spherical particle) for (succinylated) lysozyme (radius $R = 2$ nm, $c_\infty = 50$ mM).

over a wide range of pH values that supports the use of the regulation model presented in the Theory section.

The protein concentration in the experiments by van der Veen et al.[46] is 0.1 g/L, which translates into a volume fraction of $\phi^{p,\infty} \sim 1.5 \times 10^{-4}$ and an average separation of the molecules in solution (for a radius of 2 nm) of ~70 nm, which results in negligible double layer overlap down to an ionic strength of ~0.3 mM. Thus, under these conditions, the assumptions made in the Theory section are valid.

The silica surface is described as an acid by a $1 - pK$ model with $pK = 7.5$, a density of ionizable groups $N = 8$ nm^{-2}, and a Stern capacity (C_1) of 2.7 F/m^2 (ref 35).

Van der Veen et al.[46] used reflectometry to obtain the equilibrium adsorbed amount of (succinylated) lysozyme on silica as function of pH and ionic strength (the adsorbed amount after 10^3 s in the flow cell is assumed to be the equilibrium adsorption). Figure 3 reproduces the experimental results (a small modification here is that all data points are presented instead of the average per pH point). The data will be discussed further on in conjunction with results of the theoretical calculations.

In the model we use $H = 3$ nm for the thickness of the adsorption layer and assume that each protein molecule covers 3×4 nm^2 surface area (hence, volume of protein $\upsilon = 36$ nm^3). These numbers are comparable to the dimensions of lysozyme ($4.5 \times 3.0 \times 3.0$ nm).[50] With a molar mass of 14.3 kg/mol, the adsorbed amount for a full monolayer is then $\Gamma_{mL} = 2.0$ mg/m^2. Results are presented in terms of the coverage ϕ, which is unity at full coverage (full monolayer). This implies that 2ϕ equals the adsorbed amount Γ in mg/m^2. For the protein, we use a relative permittivity of $\epsilon^p = 20$ ($\epsilon^w = 78$).

In the calculation, the modified PB equation (eq 21) is discretized and together with the boundary conditions (eqs 1 and 3) and additional relations (eqs 16 and 28) solved using a Newton routine. Subsequently, the free energy given by eq 24 is calculated from numerical integration, to which we add the components given by eqs 11, 12, and 30 to obtain the total electrostatic free energy, F^{pcs}. The equilibrium adsorption is found from numerically evaluating at which ϕ the free energy of eq 27 is at a minimum. Alternatively, we solve the PB equation as described above, in conjunction with the equality eq 25 = eq 26.

Figure 4 shows for pH 7 and pH 9 results for the electrostatic potential profile y over the adsorption layer ($0 < x < H$) and

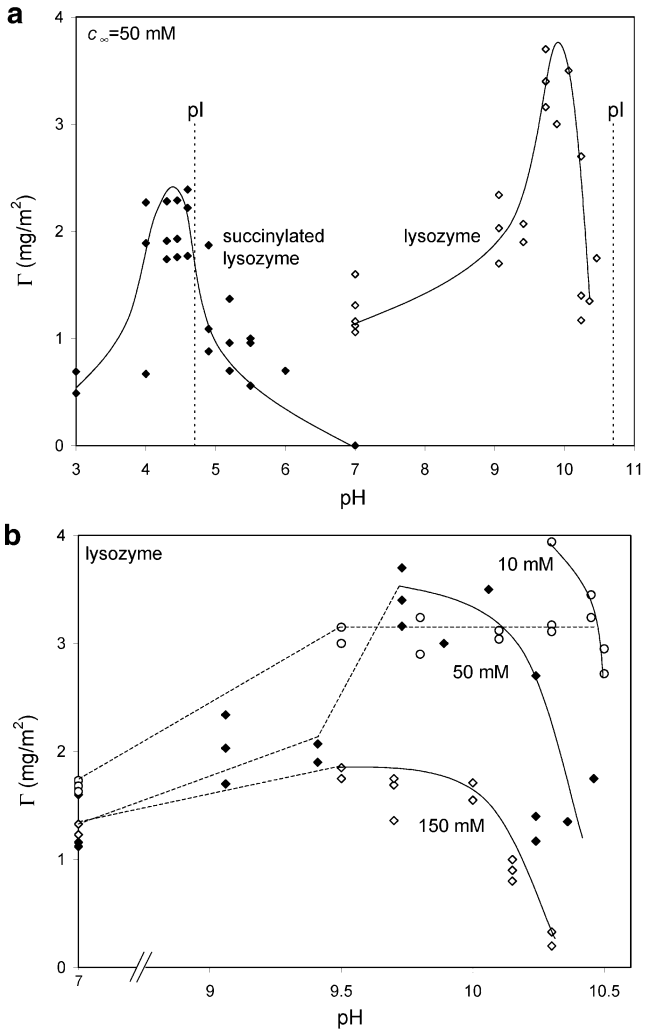

Figure 3. Experimental data[46] of (succinylated) lysozyme adsorption on silica. (a) 50 mM salt. (b) Lysozyme adsorption at different ionic strengths. Lines serve to guide the eye.

into the solution phase ($x > H$) for different values of ϕ (irrespective of the value of ϕ_{eq} at which the free energy is at a minimum). With increasing adsorption, the potential in the adsorption layer becomes less negative and above a certain adsorption the charge will reverse. Charge reversal occurs at a lower adsorption for pH 7 than for pH 9, because at pH 7 silica has a less negative charge and the protein has a higher positive charge. At pH 9 silica is strongly negatively charged and will not increase its negative charge further when protein adsorbs. Actually, because of the lower permittivity of the protein-filled layer, the silica surface charge decreases somewhat (becomes less negative). However, at pH 7 the silica is more weakly charged and the charge responds more strongly to a change in y (higher regulation capacity[51]). In this case, the surface ionization degree increases during protein adsorption from $\alpha_0 = 0.016$ ($\phi = 0$) to 0.036 ($\phi = 0.8$), an increase by more than a factor of 2. In all situations the charge on the protein molecules strongly increases during adsorption. Surface and polyelectrolyte titrate each other.[10]

A complication in mean-field models is the description of the diffuse layer around molecules in solution. Indeed, in calculations based on the theory as outlined earlier applied to lysozyme adsorbing to silica, at sufficiently low pH the adsorbed amount starts to increase with lowering the pH. This is not in line with experimental observations and is not expected from the model: with decreasing pH the protein molecules repel each

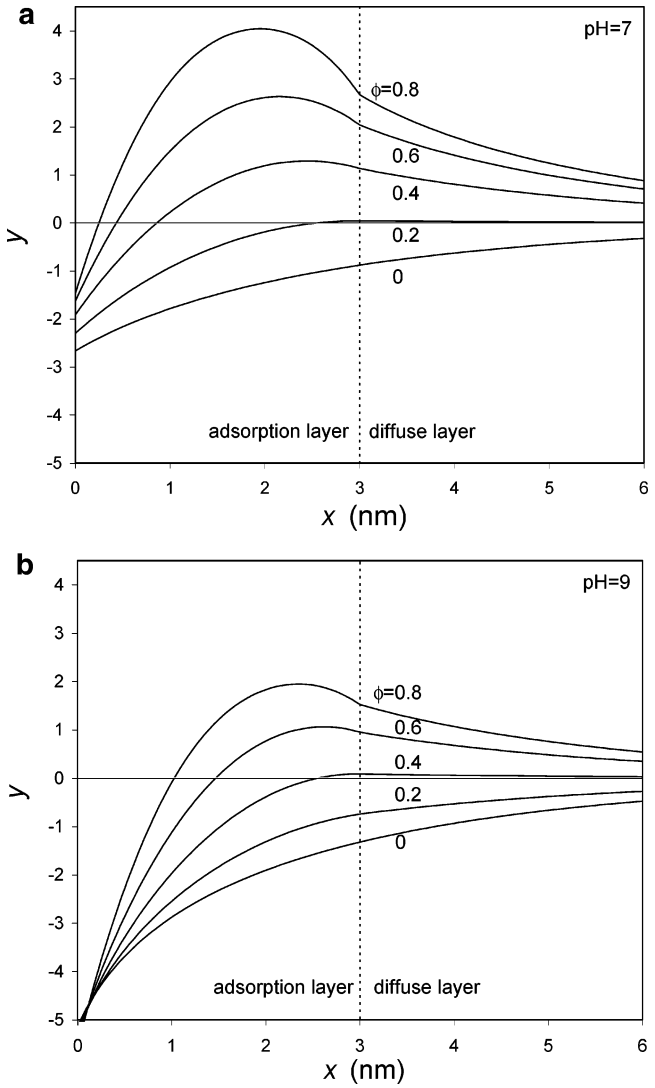

Figure 4. Electrostatic potential in the adsorption layer ($0 < x < H$), and in the diffuse layer extending from H, for lysozyme adsorption on silica for different values of the protein volume fraction ϕ ($c_\infty = 10$ mM, $H = 3$ nm). (a) pH 7. (b) pH 9.

other more while the surface becomes more neutral and attraction with the surface decreases. What is expected is a gradual decline of adsorbed amount when the pH is decreased. The calculation result is due to the fact that for a low coverage the diffuse environment around an adsorbed (globular) protein molecule is not properly accounted for. In a one-dimensional mean-field model, small ions around adsorbing molecules are smeared out in planes parallel to the surface, with the result that at a low coverage the electrostatic potential in the adsorption layer, y, is almost zero for an uncharged surface. Consequently, the free energy of an adsorbed molecule will be much lower than in bulk solution (when $y = 0$ the electric work is zero, while the chemical work is much more negative), and the model predicts adsorption. In fact, a charged particle is always repelled from a neutral surface because its double layer is deformed (the neutral plane can be envisioned as the midplane between two equal, thus repelling, particles). Under these conditions of low coverage (or high ionic strength), the one-dimensional model fails and one would like to use a more detailed 2D-model in which the protein molecules at the surface are considered as globules and the PB equation is accurately solved in the space between the molecules.[28] Such a model can be based on the equations already presented in this report, but the PB equation

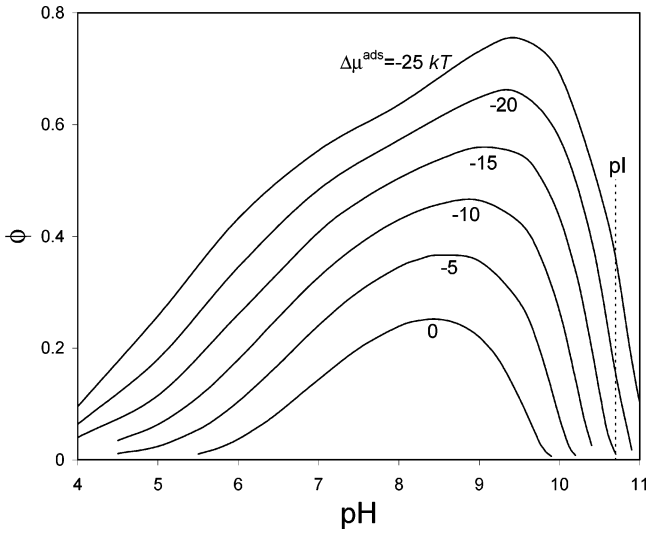

Figure 5. Influence of adsorption energy $\Delta\mu^{ads}$ on the adsorption of lysozyme on silica ($c_\infty = 10$ mM; 2ϕ is adsorbed amount Γ in mg/m^2; $y_{p,\infty} = 0$).

and free energy expressions must be solved for the more complicated geometry of an array of globules adsorbed to a plane (with regulation boundary conditions on all interfaces). Interestingly, however, the problem of increasing adsorption with decreasing pH can be circumvented in mean-field models by assuming a mean field for the polyelectrolyte molecules in solution as well, leading for a low enough polymer concentration to $y_{p,\infty} = 0$.[10] When assuming $y_{p,\infty} = 0$ there is no erroneous electrostatic free energy decrease upon protein adsorption; therefore, $y_{p,\infty} = 0$ is assumed in the calculations for Figures 5−7.

In Figure 5, results are presented for the equilibrium adsorbed amount ϕ_{eq} of lysozyme in 10 mM monovalent salt and for different values of the adsorption energy, $\Delta\mu^{ads}$. The model predictions are in qualitative agreement with the experimental data[46] in several respects (compare with the curves for lysozyme in Figure 3): (1) the adsorbed amount has a maximum at a certain pH, denoted as pH_c, (2) the adsorption curve is skewed to the right, with a rather prolonged tail for $pH < pH_c$ and a much sharper decrease of adsorbed amount at $pH > pH_c$, and (3) pH_c is below the iso-electric point, pI, of the protein molecule. An important difference between model and experiment is the actual adsorbed amount: in the experiment, the coverage at $9.6 < pH < 10$ is beyond a fully packed monolayer while in the calculation such a high coverage is not considered. Furthermore, the model predicts the maximum at a pH that is too low, while the predicted dependence of ϕ on pH is too weak around the maximum.

Figure 5 shows different curves for the adsorbed amount ϕ as function of pH and $\Delta\mu^{ads}$. As function of $\Delta\mu^{ads}$, the maximum coverage varies between $\phi \sim 0.25$ ($\Delta\mu^{ads} = 0kT$) and $\phi \sim 0.75$ ($\Delta\mu^{ads} = -25kT$). It can also be observed that the pH at maximum adsorption, pH_c, shifts to higher pH with increasing magnitude of $\Delta\mu^{ads}$, from $pH_c \sim 8.5$ at $\Delta\mu^{ads} = 0$ to $pH_c \sim 9.5$ at $\Delta\mu^{ads} = -25kT$. In the experiment, pH_c is ∼10.3 for 10 mM salt, and the adsorbed amount is more than a monolayer ($\phi > 1$). These two elements tentatively suggest that an appropriate value for $\Delta\mu^{ads}$ would rather be $\sim -25kT$ than $0kT$.

Let us discuss the theoretical results in somewhat more detail. At low pH (<5) silica has a very low charge and does not exert a significant electrostatic attraction on the protein molecules. The protein molecules are highly charged, and when adsorbed they repel each other strongly. As a consequence, adsorption is

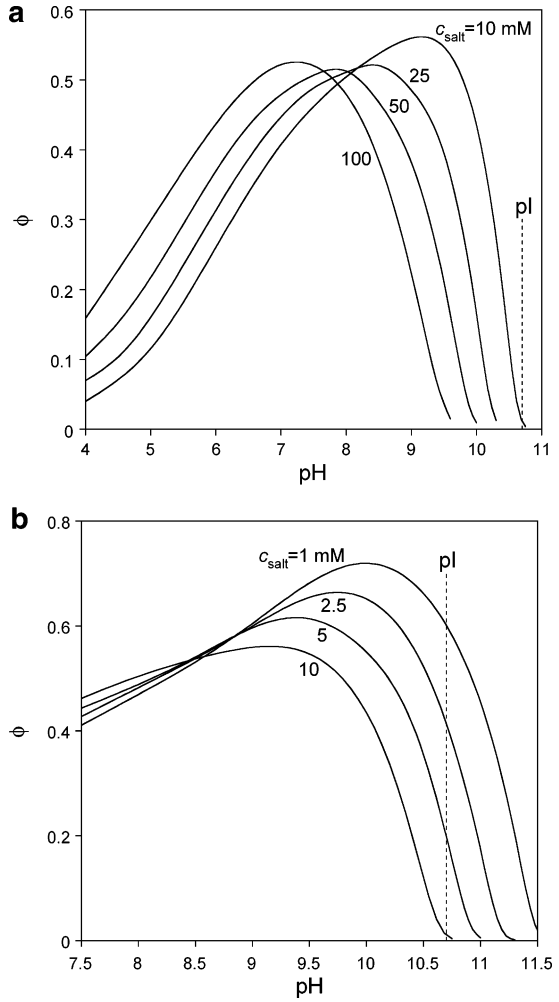

Figure 6. Influence of ionic strength c_∞ on the adsorption of lysozyme on silica ($\Delta\mu^{ads} = -15\ kT$; $y_{p,\infty} = 0$).

low if $\Delta\mu^{ads}$ is zero. The repulsion between adsorbed protein molecules is due to the energetic penalty of having to find counterions to compensate the protein charge—possible counterions being either the limited number of silica surface sites or the small ions in the intermediate space between the molecules. With increasing pH, the silica charges up, the protein molecules discharge, repel each other less, and it becomes more favorable for protein to adsorb (the protein molecules require less counterions, while more counterions become available in the form of silica surface sites).

Beyond pH_c the protein molecules must increasingly compete with the small ions in compensating the surface charge. At a certain pH the charge on the protein molecules, though still positive, becomes too low to compensate for the displacement of the small ions from the surface, which requires electric work. Consequently, protein adsorption starts to decrease sharply even though the protein molecules still have a charge opposite to that of the surface. Ultimately the adsorption becomes almost zero at a certain pH (pH_0), which may be below the iso-electric point (pI). Interestingly, pH_c and pH_0 shift to lower values with increasing ionic strength, see Figure 6a, due to the fact that at a higher ionic strength more counterions must be displaced outward when a molecule adsorbs, requiring electric work. According to Vermeer et al.,[10] "the higher the electrolyte concentration, the sooner salt ion adsorption takes over in the charge compensation process". With some precaution we can state that the experimental data (Figure 3b) support the predicted influence of ionic strength: both in the calculation (Figure 6a) and in the experiment (Figure 3b) the pH at which adsorption is at a maximum (pH_c) shifts to lower values with increasing ionic strength (in the experiment from ~10.3 (10 mM) to ~9.7 (50 mM) to ~9.5 (150 mM)). Also, beyond pH_c the adsorbed amount decreases with increasing ionic strength both in the experiment (Figure 3b) and in the calculation (Figure 6a).

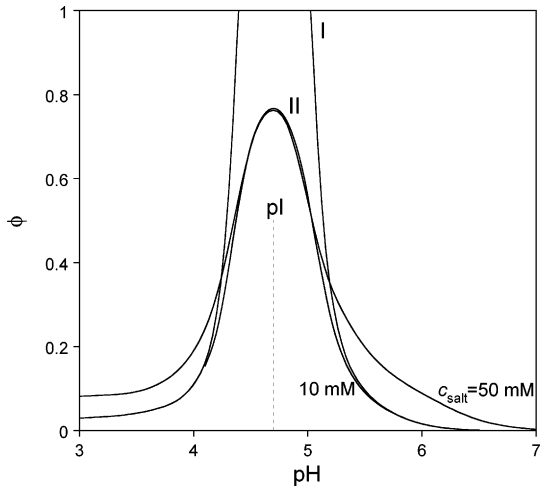

Figure 7. Adsorption of succinylated lysozyme on silica ($\Delta\mu^{ads} = -10\ kT$; $y_{p,\infty} = 0$). I, ideal entropy; II, Langmuir correction.

Having identified that protein molecules compete with the small ions that form the diffuse part of the electrical double layer, it is interesting to investigate the influence of a further reduction in the ionic strength to values below the experimental range (c_∞ < 10 mM). Figure 6b shows that the model predicts that around pI the adsorption increases with decreasing ionic strength and that for $c_\infty = 5$ mM and lower, adsorption can be expected even for pH > pI, where the protein in solution has the same charge sign as the surface. During adsorption the highly (negatively) charged surface induces charge reversal of the amphoteric protein molecules because the pH at the protein surface shifts from a value above pI (when protein is in solution) to below pI near the negative silica surface (where the local pH is lower than in solution).[2,12,31,40] The system accepts the energetic penalty of charge reversal because at low ionic strength the alternative, which is surface charge compensation using the small ions, is very expensive as well. When only small ions would be available to compensate the surface charge, very high electrostatic potentials and potential gradients are required.

Model calculations for succinylated lysozyme are presented in Figure 7. In line with the experiments (Figure 3a), we calculate maximum adsorption around the iso-electric point (pI = 4.7) with the adsorbed amount decreasing to zero at pH 7 and to a low value at pH 3. The model predicts adsorption beyond a monolayer in a small region around pI. This is due to the fact that the surface is rather weakly charged and, hence, the electrostatic protein−surface interaction weak. As a result, the adsorbed amount is determined by a balance between $\Delta\mu^{ads}$ and the electrostatic repulsion between protein molecules. Around pI the protein is sufficiently weakly charged that $\Delta\mu^{ads}$ is enough to result in adsorption beyond monolayer coverage. Beyond that pH range, the lateral repulsion between adsorbed protein molecules (which increases with |pH−pI| and with coverage ϕ) becomes too high, and the adsorbed amount rapidly decreases.

To maximize the adsorption to below $\phi = 1$ in the model, we corrected the ideal gas entropy term (ln ϕ in the chemical potential) for a high adsorption using a Langmuir isotherm,

resulting for the chemical potential of adsorbed protein molecules in an additional term, $-\ln(1-\phi)$, while we must replace $\phi(\ln\phi - 1)$ by $\phi\ln\phi + (1-\phi)\ln(1-\phi)$ in the free energy of eq 27 (ref 28). Now, we find a "bell-shaped" curve not unlike the trend in the experimental data.

Conclusions

The influence of pH and ionic strength on the equilibrium adsorption of lysozyme on silica can be described in a first approximation by a one-dimensional mean-field model including charge regulation, in which the adsorbed protein molecules are assumed to be homogeneously distributed across a layer of predefined thickness adjacent to the sorbent surface. A mean-field model can in principle only be used when the assumption of a close spacing of polyelectrolyte on the surface (relative to the Debye length) is valid. However, by setting the electrostatic potential of polyelectrolyte molecules in solution to zero, the model can be applied to conditions of low coverage as well.

For lysozyme adsorption on silica, the model is capable of describing the experimentally observed increase of adsorbed amount with pH, until a pH value (pH_c) below the iso-electric point of the protein (pI = 10.7), as well as the sharp decrease in adsorbed amount beyond pH_c. The model also correctly predicts that with increasing ionic strength (1) pH_c shifts to lower pH values and (2) the adsorbed amount decreases (for pH beyond pH_c). The model suggests that at ionic strengths of 5 mM and lower significant adsorption occurs for pH > pI where the strongly charged surface reverses the effective charge of the amphoteric protein molecules. For succinylated lysozyme, including a Langmuir correction for the entropy of adsorbed molecules results in a bell-shaped adsorption curve not unlike the trend in the experimental data.

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