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Protein Adsorption at Charged Surfaces: The Role of Electrostatic Interactions and Interfacial Charge Regulation

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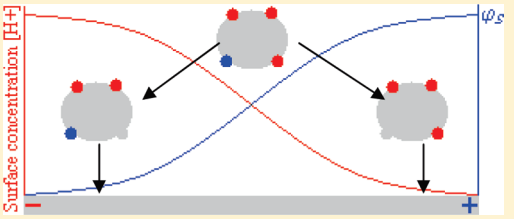
ABSTRACT: The understanding of protein adsorption at charged surfaces is important for a wide range of scientific disciplines including surface engineering, separation sciences and pharmaceutical sciences. Compared to chemical entities having a permanent charge, the adsorption of small ampholytes and proteins is more complicated as the pH near a charged surface can be significantly different from the value in bulk solution. In this work, we have developed a phenomenological adsorption model which takes into account the combined role of interfacial ion distribution, interfacial charge regulation of amino acids in the proximity of the surface, electroneutrality, and mass balance. The model is straightforward to apply to a given set of experimental conditions as most model parameters are obtained from bulk properties and therefore easy to estimate or are directly measurable. The model provides a detailed understanding of the importance of surface charge on adsorption and in particular of how changes in surface charge, concentration, and surface area may affect adsorption behavior. The model is successfully used to explain the experimental adsorption behavior of the two model proteins lysozyme and α-lactalbumin. It is demonstrated that it is possible to predict the pH and surface charge dependent adsorption behavior from experimental or theoretical estimates of a preferred orientation of a protein at a solid charged interface.



INTRODUCTION

The adsorption of proteins and peptides to charged surfaces is of great importance in many areas of science and engineering, examples include food science, surface engineering and the pharmaceutical sciences. In the field of pharmaceutical sciences, the emergence of proteins and peptides as powerful therapeutics has led to a renewed interest in protein adsorption in connection to e.g. chromatography[1] and drug delivery systems; including nanocarriers,[2−5] liposomes,[6] chitosan films[7] and polymeric systems.[8] Understanding of protein adsorption is vital to the development of efficient analytical methods and advanced drug delivery systems and there is therefore a need for physicochemical adsorption models for interpreting results and ultimately for accurate predictions.

A central part of the problem is the properties of the electrolyte solution near the charged interface and the interfacial distribution of ions. Several models have been considered to describe adsorption of charged species at charged interfaces based on the Poisson−Boltzmann equation. The simplified case corresponding to adsorption of spheres with an evenly distributed net-charge has been treated,[9−15] and other approaches include structural features and orientation of the macromolecule.[16−18] An important difference is the fact that the later approach introduces some anisotropy in the charge distribution on the protein, as it has long been recognized that protein adsorption at charged interfaces cannot be satisfactorily explained by the overall net-charge,[19−21] due to the asymmetrical distribution of groups of different charge on the protein surface.[19,20] The region of the protein which is in contact with the surface material upon adsorption does not necessarily bear the same charge sign as the overall protein and a symmetrical spherical model becomes misleading for such proteins. Another important point to consider is the *charge regulation* mechanism, which is the phenomenon dictating that the charge of the ionizable groups on a molecule is not constant, but is affected by the local electrostatic environment.[13,22−24] Specifically, the solution of the Poisson−Boltzmann equation at a charged interface results in an uneven distribution of ions near the interface, resulting in a pH at the surface which differs from that in bulk solution.[13] Therefore, ionizable groups may have a different charge at the surface than in bulk phase and thus have a different electrostatic affinity for a charged surface than expected from the bulk properties.[13] It should also be considered that adsorption of charged species in itself will affect the potential and charge at the interface, thus altering the surface pH and the electrostatic attraction of the specie. This is illustrated experimentally by the change of ζ-potential following protein adsorption on particles[25] and can be considered in mathematical models by introduction of electroneutrality conditions at the interface.[26,27]

In this work, we have developed a numerical model which takes into account the combined role of interfacial ion distribution, charge regulation of amino acids in the proximity of the surface, electroneutrality and mass balance. The model is directly applicable to small ampholytes and can be applied to proteins by

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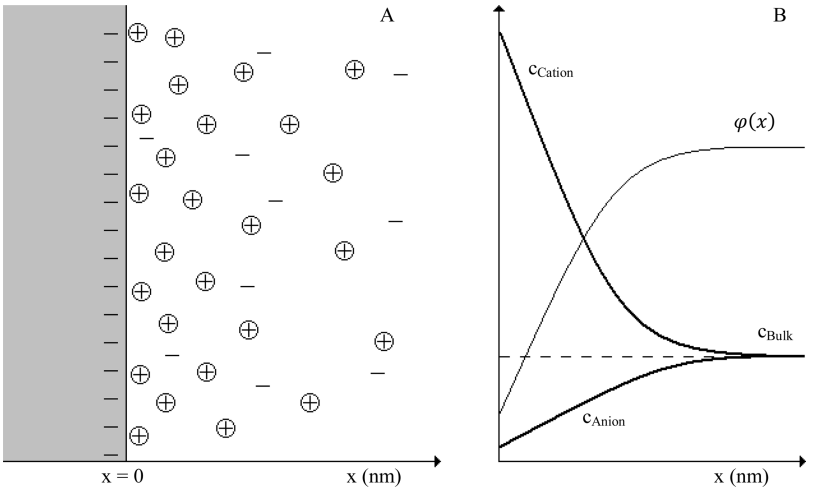

Figure 1. (A) Distribution of ions in an electrolyte solution at a charged interface. (B) Concentration profiles of anions and cations near a negatively charged surface, an effect of the ion distribution is the potential profile, the potential at the interface ($x = 0$) is φ_s and at greater distances the potential is $\varphi(x)$.

assuming a specific orientation of the protein at the interface and that electrostatic interactions are dominated by interfacial amino acids as shown in the present work to be valid in electrolyte solutions having an ionic strength in the mM range or higher. The free energy of adsorption is split into two contributions composed of a charge independent part ($\Delta G^0_{Ads}(Chem)$) and a term describing electrostatic interactions at the interface ($\Delta G^0_{Ads}(EL)$). The electrostatic interaction, which is an important force in protein adsorption, is readily obtainable from classical theory and knowledge of the spatial distribution of charges in the system. Furthermore, the electrostatic contribution to the free energy of adsorption is the most likely to change when studying surfaces with differing surface charge densities. The chemical free energy, $\Delta G^0_{Ads}(Chem)$, is assigned a value in the simulation and can be regarded as a phenomenological fitting parameter. The model provides a detailed understanding of the importance of surface charge on adsorption and in particular of how changes in surface charge, acid dissociation constants, concentration, surface area and ionic strength may affect adsorption behavior. The model was applied to interpret the adsorption of the two model proteins lysozyme and α-lactalbumin, which is experimentally known to have very different adsorption behavior at a solid surface and the apparent counterintuitive behavior of α-lactalbumin is explained by the model. From a practical point of view the model is straightforward to apply as all input parameters are easily obtainable.

THEORY

Consider the interface between a charged surface S and an electrolyte solution E. The charges that are associated with the surface, e.g., covalently bound to the surface will here be termed *surface charges* and should be distinguished from adsorbed charges or electrolyte particles that are near the surface of the interface. The number of *surface charges* per area of the surface is defined by the surface charge density σ_s (C/m^2) with the subscript S designating surface. By the electroneutrality condition it is given that σ_s must be counter-balanced by a charge density of opposite sign in the solution near the interface

$$-\sigma_s = \sigma_E \tag{1}$$

However, while the charges on the surface (σ_s) are considered to be evenly distributed on a flat plane the charges of the electrolyte (σ_E) are distributed in a layer from the charged surface out into the solution, as seen in Figure 1. The layer is formed by the random movement of particles and is termed the diffusive layer, its length is the Debye length.

The difference in charge distributed through the layer gives rise to a potential profile which is described by the Poisson−Boltzmann equation. From the Poisson−Boltzmann equation an expression for the charge density (σ_s) of the electrolyte can be found, in this case derived for a 1:1 electrolyte, such as for example NaCl.[28,29]

$$\sigma_E = -\sqrt{8RT\varepsilon_0\varepsilon c_E \sinh\left(\frac{F\varphi_s}{2RT}\right)} \tag{2}$$

Where R is the gas constant, T is the absolute temperature, ε is the relative permittivity of the solution, ε_0 the permittivity of a vacuum, c_E is the bulk concentration of electrolyte, F is Faradays number and φ_s is the surface potential. From the Poisson−Boltzmann equation the relationship between the potential at any distance x from the interface and the surface potential can be derived.[28,29]

$$\varphi(x) = \frac{4RT}{F}\tanh^{-1}\left(e^{\left(-F\sqrt{\frac{2c_E}{RT\varepsilon_0\varepsilon}}x\right)}\tanh\left(\frac{F}{4RT}\varphi_s\right)\right) \tag{3}$$

For a charged surface of known σ_s (obtained for example from ζ-potential measurement or calculation) the potential at the surface (φ_s) can be calculated from eqs 1 and 2. Finally, the potential at any distance from the surface is obtained from eq 3. A charged compound, which adsorbs to the surface, can be introduced to the system is illustrated in Figure 1. If the adsorbant compound has a much lower concentration times charge value than the electrolyte, as is usually the case in buffered peptide and protein solutions, the compound can be neglected in the Poisson−Boltzmann distribution in the diffuse layer, and eqs 2 and 3 are still valid. However, as the concentration of the adsorbant can be high at the interface its contribution to the charge density at the surface must be taken into account. In the following, σ_{Ads} is the charge density brought into the diffuse layer by adsorption. The sign of σ_{Ads} depends on the net charge of the adsorbing compound and can have great influence on the charge

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distribution and potential profile in the diffuse layer. For example, the adsorption of a positively charged compound at the interface in Figure 1 could completely match the negative surface charge. This would neutralize the surface charge and eliminate the diffuse layer. The contribution of adsorption to charge density (σ_{Ads}) is found from the charge of the adsorbed particle (z) Faradays constant (F), the maximum coverage of the surface by the adsorbant (Γ_{Max}), and the fractional coverage (θ):

$$\sigma_{Ads} = zF\Gamma_{\max}\theta \tag{4}$$

zF is the charge of 1 mol of adsorbant molecules, $\Gamma_{Max}\theta$ is the number of adsorbant moles per surface area at equilibrium, and the result of multiplication of the two terms is charges per area. By the electroneutrality condition, the sum of the charges of the adsorbed species and the electrolyte in the diffuse layer must equal, with opposite sign, the *surface charge*. Therefore, the surface charge densities are related as

$$-\sigma_s = \sigma_E + \sigma_{Ads} \tag{5}$$

Introducing eq 2 and 4 into eq 5 gives the final relation between the charge density of the surface (σ_s) and the surface potential (φ_s)

$$-\sigma_s = \sigma_E + \sigma_{Ads} = -\sqrt{8RT\varepsilon_0\varepsilon c_E \sinh\left(\frac{F\varphi_s}{2RT}\right)} + zF\Gamma_{\max}\theta \tag{6}$$

From these equations it is possible to calculate the surface potential (eq 6) and the potential profile in the solution (eq 3) if the adsorption parameters, Γ_{Max} and θ, and the charge of the adsorbed molecule are known.

Adsorption Isotherm. As a model for adsorption the Langmuir isotherm is chosen, but other adsorption isotherms could also be used. For an adsorption equilibrium between a peptide or protein (P) and a surface (S), P + S $\rightleftarrows$ PS, the isotherm takes the form

$$\theta = \frac{\beta_{PS}a_P}{1+\beta_{PS}a_P} \tag{7}$$

where θ is the fractional coverage, a_P is the activity of the adsorbant protein and β_{PS} is the equilibrium constant of the adsorption equilibrium. Taking the activity of the surface as unity, the equilibrium constant, β_{PS}, takes the form

$$\beta_{PS} = \frac{a_{PS}}{a_P} = \exp\left(\frac{-\Delta\bar{G}^0_{Ads}}{RT}\right) \tag{8}$$

where $\Delta\bar{G}^0_{Ads}$ is the standard electrochemical free energy of adsorption which is defined by both a chemical and an electrical free energy contribution[30]

$$\Delta\bar{G}^0_{Ads} = \Delta G^0_{Ads}(Chem) + \Delta G^0_{Ads}(EL) \tag{9}$$

where $\Delta\bar{G}^0_{Ads}$ is the overall standard free energy of adsorption that one would ideally measure in an experiment. The electrical contribution is given by

$$\Delta G^0_{Ads}(EL) = z_{Ads}F\varphi(x) \tag{10}$$

with $\varphi(x)$ being the distance-dependent potential experienced by the charge z_{Ads}. If the charge of the adsorbant is considered to be located on the surface ($x = 0$) the relevant potential is φ_s, and the Langmuir isotherm can be written in the following surface potential dependent form, by inserting eqs 8−10 into eq 7

$$\theta = a_P \cdot \frac{\exp\left(-\frac{\Delta G^0_{Ads}(Chem)}{RT}\right)\cdot\exp\left(-\frac{z_{Ads}F\varphi_s}{RT}\right)}{1 + a_P\cdot\exp\left(-\frac{\Delta G^0_{Ads}(Chem)}{RT}\right)\cdot\exp\left(-\frac{z_{Ads}F\varphi_s}{RT}\right)} \tag{11}$$

where a_P is the bulk activity of the adsorbing compound and φ_s is the potential at the surface relative to the bulk solution. The $\Delta G^0_{Ads}(Chem)$ term is the potential-independent part of the standard free energy of adsorption. The value of the overall standard free energy of adsorption ($\Delta\bar{G}^0_{Ads}$) is thus found from eqs 9 and 10. The activity of the adsorbant a_P is defined by $a_P = \gamma_p c_p/c^0$. Because the standard state of the adsorbant (c^0) is 1 M and because at low concentrations the activity coefficient (γ_p) is approximately 1, the activity (a_P) can be replaced by the concentration (c_p) for most practical purposes. Further, c_p is the equilibrium bulk concentration of the adsorbant, which is coupled to the initial concentration (c_{Init}) by mass balance. The mass balance condition can be expressed as

$$c_P = \frac{c_{Init}V - \Gamma_{\max}\theta A}{V} \tag{12}$$

where V is the volume of the solution. If the concentration of the adsorbant is high, the adsorbed amount will constitute a negligible part and the mass balance can be ignored. It should be noted that the introduction of eq 12 into eq 11 results in a quadratic equation, resulting in two solutions of which only one is physically meaningful.

Choice of Amino Acids to Consider in Protein Adsorption. It is assumed that the protein obtains a preferred orientation when adsorbed at the interface. It is further assumed that the preferred orientation is fixed in the simulations presented, but it is possible to change the orientation as a function of other model parameters if necessary. It is observed from eq 3 that the potential is a declining function with distance to the surface and the contribution of a charged group on an adsorbed protein to the adsorption isotherm in eq 11 will therefore be largest for amino acids close to the surface. For an adsorbed point charge, $z_{Ads} = z$ if it is assumed that all charges are found directly at the surface. However, for proteins such an assumption cannot be made as the size of a protein is comparable to the Debye layer. Therefore, only amino acids close to the plane of adsorption are considered when evaluating the electrostatic attraction in eq 11 and the net-charge of these groups are termed z_{Ads}. Further, these amino acids are assumed to be at distance $x = 0$ from the surface such that the relevant potential to consider is the surface potential φ_s and eq 11 is then suitable.

Surface pH and Charge Regulation. According to Gouy−Chapman theory the concentration (c_i) of a given ion at any distance from the surface is given from its bulk concentration (c_i^*) according to:[28]

$$c_i = c_i^* \exp\left(\frac{-z_iF\varphi(x)}{RT}\right) \tag{13}$$

This means that, for example, the distribution of the H^+ ion in solution will be altered by the presence of a charged surface. The pH at the surface is defined as pH_S and the potential at the surface as φ_s since the value at the interface will be of primary interest. By taking the negative base 10 logarithm on both sides of eq 13, one

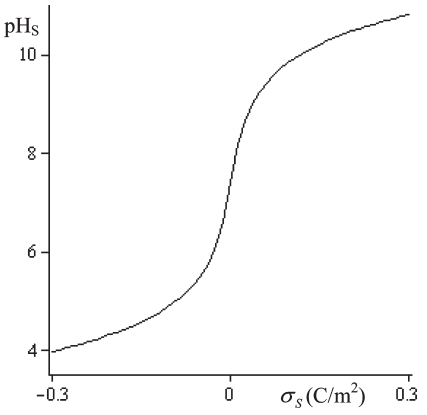

Figure 2. Relation between surface charge density and pH at the surface calculated from eqs 1, 2 and 14. Bulk phase pH is 7.4 and c_E = 10 mM.

can obtain the relation between pH and potential. For the surface potential and surface pH the relation then takes the form

$$pH_s = pH_{Bulk} + 0.434\frac{F\varphi_s}{RT} \quad (14)$$

It should be noted that pH is calculated as the negative base 10 logarithm of the proton concentration; the activity coefficient of the proton is thus assumed to be 1. From eq 14 it is apparent that if the surface potential (φ_s) has a negative value the pH at the surface will be lower than the bulk pH, while the opposite is true for a positive potential. The influence of a charged surface on the local pH at the surface can be computed through eqs 1, 2 and 14 (Figure 2).

The charge state of ampholytes is determined by the pH in the surrounding solution, however, pH values can differ noticeably between the bulk solution and close to a charged surface (Figure 2). As a distinction is made between amino acids at the surface and the other amino acids residues on the protein which are approximately under bulk conditions, the charges must be calculated separately. The net charge of the amino acids at the interface can be calculated from their respective pK_a values

$$z_{Ads} = \sum_i^k \frac{10^{pKa_i}}{10^{pHs} + 10^{pKa_i}} - \sum_j^l \frac{10^{pHs}}{10^{pHs} + 10^{pKa_j}} \quad (15)$$

where pH_s is the surface pH, pK_{ai} is the pK_a value of the N-terminus and the side chains of arginine, histidine and lysine of the adsorbed amino acids. The pK_a value of the C-terminus and aspartate, glutamate, cysteine and tyrosine amino acid side chains is given by pK_{aj}. The corresponding equation for the net charge of the adsorbed protein sums the amino acids under bulk conditions (bulk pH) and then adds the charge of amino acids present at the interface:

$$z = \left(\sum_i^m \frac{10^{pKa_i}}{10^{pH} + 10^{pKa_i}} - \sum_j^n \frac{10^{pH}}{10^{pH} + 10^{pKa_j}}\right) + z_{Ads} \quad (16)$$

To summarize, the net-charge of the adsorbed molecule, z, is used in the calculation of the adsorbed surface charge density (σ_{Ads}) encountered in eq 4, and the charge of the interfacial groups, z_{Ads}, determine the contribution of electrostatic interactions to the potential-dependent adsorption isotherm in eq 11. It

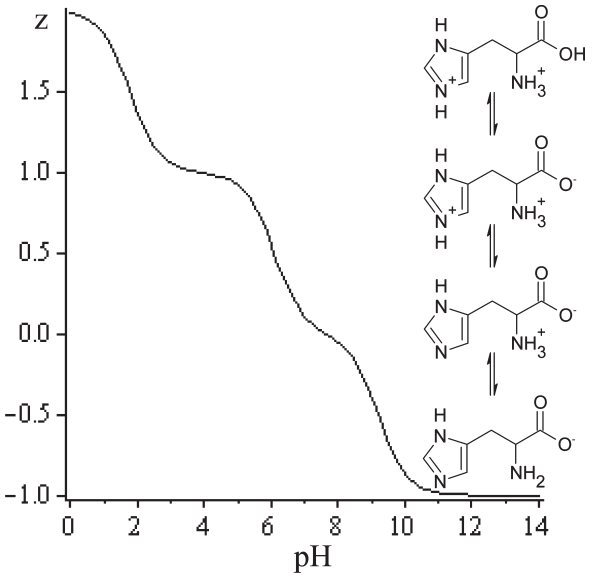

Figure 3. Ionization of histidine at different pH values. Calculated from eq 16 with z_{Ads} = 0, pK_{ai} values 6.10 and 9.18 and pK_{aj} value 1.77.

should be noted that the present model relies on a continuum description of ionic concentrations, pH and potentials in which point charges are assumed. This is an approximation which may be expected to be less valid when considering very small length scales (comparable to the size of a charged atom), whereas it should be an adequate description for the relatively large length scales considered in the present work.

The model was solved numerically using a computer program written in Maple ver. 11. The program is available from the authors upon request. Briefly, eq 14 is introduced to 15 which is then introduced into 11. Introducing mass balance to the equation system is achieved by introducing 12 to 11, this produces a quadratic equation of which only one solution is meaningful, this choice is done consistently within the algorithm. At last eq 11 is introduced to 6 and solved, with iterative numerical approximation techniques, for the surface potential using the surface charge density as a variable. Equations such as 3 and 11 can then be solved by substitution of the computed surface potential values.

RESULTS AND DISCUSSION

Adsorption of a Model Amino Acid. For clarity, we first solve the model for the amino acid histidine, and then continue to proteins. Histidine is chosen because of its ampholytic nature, the effect of which is the ability to switch between positive and negative charge at a physiological relevant pH (Figure 3), which is especially interesting when investigating interfacial charge regulating effects. The model parameters used are summarized in Table 1.

In Table 1 the maximum surface coverage is based on a value approximating a full monolayer of a molecule of the given size, the volume and area represents a 1 mL sample with 1 cm^2 adsorption area and the relative permittivity is the commonly recognized value for an aqueous phase.

By comparing Figure 2 and Figure 3, it is obvious that the charge of the surface material can have a large effect on the local pH at the surface and thus on the net charge of histidine at the surface. As adsorption is expected to be strongly influenced by

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Table 1. Model Parameters for Histidine, Lysozyme, and α-Lactalbumin[a]

parameter	Γ_{Max}[b]	$\Delta G^0_{Ads}(Chem)$[c]	V[d]	A[e]	c_E[f]	pH_{Bulk}[g]	ε[h]	T[i]
unit	mol/m^2	J/mol	m^3	m^2	mM	-	-	K
histidine	3×10^{-6}	−5000	1×10^{-6}	1×10^{-4}	10	7.4	78	298
lysozyme	1×10^{-7}	−5000	1×10^{-6}	1×10^{-4}	10	7.4	78	298
α-lactalbumin	1×10^{-7}	−5000	1×10^{-6}	1×10^{-4}	10	7.4	78	298

[a] These are the parameters used in the simulations presented in this paper, unless otherwise stated in the presented data. [b] Maximum surface coverage. [c] Chemical free energy of adsorption. [d] Volume of solution. [e] Surface area for adsorption. [f] Concentration of 1:1 electrolyte. [g] Bulk pH. [h] Relative permittivity of aqueous phase. [i] Temperature.

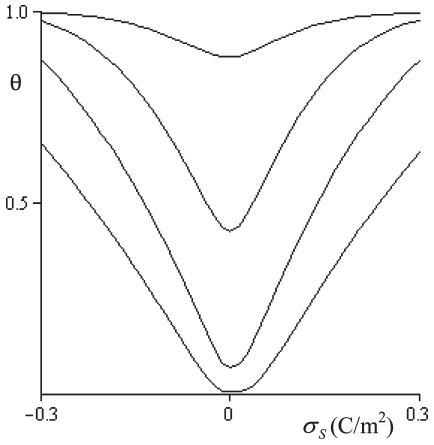

Figure 4. Histidine adsorption isotherm as a function of surface charge density for different concentrations of histidine, from bottom to top: [1, 10, 100, 1000] μM. See Table 1 for further model parameters.

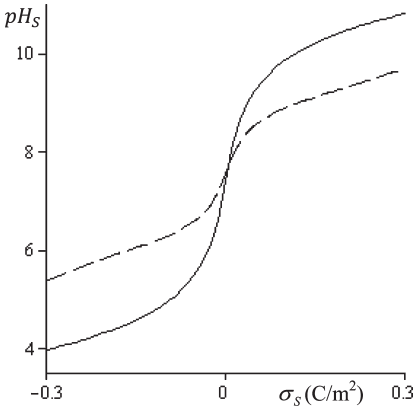

Figure 5. Surface pH as a function of surface charge density in the absence (—) and presence (---) of 100 μM histidine at bulk pH 7.4. See Table 1 for additional details.

electrostatic interactions, the charge regulating effect therefore needs to be taken into account. Consider for example a histidine containing electrolyte solution at pH 7.4 in contact with a surface for which histidine has adsorption affinity $\Delta G^0_{Ads}(Chem)$ independent of the charge. The surface charge can be varied, analogous to a polarizable electrode or a series of surfaces with different surface charges but comparable $\Delta G^0_{Ads}(Chem)$ values, such as e.g. polystyrene particles or liposomes. The results of the model for histidine under such circumstances are presented in Figure 4 where the adsorption is shown to be stronger when the *surface charge* (σ_s) is shifted in any direction from zero.

If σ_s is zero there is no contribution to adsorption from electrostatics. However, if the surface is negatively charged, σ_s is negative and the pH near the surface will be lowered according to Figure 2. In effect, histidine will be protonated, leading to an electrostatic attraction between histidine and the surface. If, however, the surface is positively charged the pH at the surface will increase due to repulsion of the H^+ ions and histidine will be more negatively charged near the surface as compared to the bulk solution. This will produce additional electrostatic attraction between histidine and the surface. Thus, near the pI value of an adsorbing ampholytic compound the addition of charge, of any sign, to the surface can be expected to increase the attractive ionic interactions between adsorbant and surface. The fact that the charge of ionizable groups is affected by their local electrostatic environment is termed *charge regulation*. One example includes the effect of surface pH, mentioned above, which is of importance in protein adsorption[13,22,31] and protein−protein interactions.[31,32] It should also be noted that pK_a values may be affected by the local concentration of ions, adsorption to interfaces,[33,34] nearby amino acids[35] and surface charge. These effects are not considered in the present treatment, but may easily be included.

Another contributing factor to the interaction between a surface and a charged compound is the effect of the presence of the adsorbant itself at the interface. This is illustrated in Figure 5 by comparing the pH distribution with or without adsorption of histidine.

From Figure 5 it is observed that at negative σ_s the surface pH is higher when histidine is present. This is explained from Figure 4 that shows strong adsorption at negatively charged surfaces and from Figure 3 which shows that the pH_S is such that histidine is positively charged at the surface. The adsorption of positive charges at the surface results in a positive σ_{Ads}, compensating the negative σ_s and leading to a surface potential (φ_s) closer to zero, and therefore the surface pH (14) will be closer to pH_{Bulk} (H^+ is electrostatically repelled from the surface by adsorbed cationic histidine). At positive σ_s, pH_S is observed to be lower when histidine is present. In this situation, histidine is negatively charged at the surface and its presence neutralizes the surface charge so that H^+ is repelled to a smaller extend from the surface and the pH is lowered. In general, the adsorption of charged compounds at the interface changes the charge distribution at the interface through changing the surface potential, as described by eqs 4 and 6. At low pH where histidine is positively charged the effects are even more pronounced (Figure 6). At this pH, histidine only adsorbs strongly onto negatively charged surfaces.

At low concentrations of the adsorbant and in systems with large surface to volume ratios, the amount of adsorbed species may represent a significant percentage of the total amount of adsorbant. In practice, this is often relevant for adsorbing protein and peptide solutions, and it can be incorporated in the solution of the model. In Figure 7, results are compared for solving the model with or without considering the mass balance of eq 12.

As can be seen from Figure 7, the adsorption isotherm can be considerably overestimated when the concentration/surface area ratio

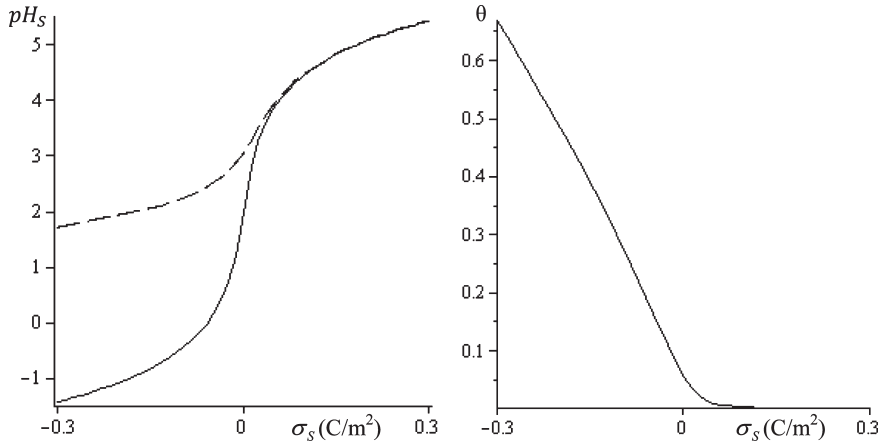

Figure 6. (Left) Surface pH as a function of surface charge density in the absence (—) and presence (---) of 100 μM histidine at bulk pH 2.0. (Right) Corresponding adsorption isotherm of histidine at pH 2.0.

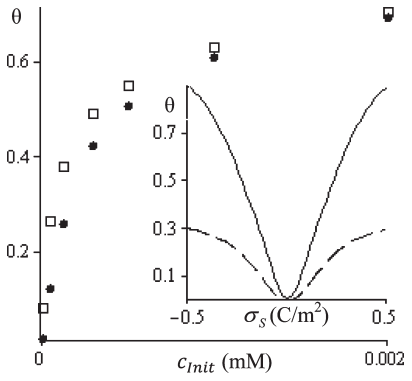

Figure 7. Adsorption isotherms at $\sigma_s = -0.1$ C/m^2 with different initial concentrations of histidine: (●) with mass balance equation; (□) Without mass balance equation. Inset shows the effect of surface charge on two concentrations of histidine: (—) without extensive depletion, c = 1 μM; (---) with extensive depletion, c = 0.1 μM.

is low, especially at surface charge densities far from zero. Solutions to the Poisson−Boltzmann equation, as represented in this paper, thus provide a good model for adsorption of single amino acids or peptides, which are small enough that their charged groups can be considered to be at the interface. Proteins and larger peptides, however, should be treated differently when evaluated under this theory.

Protein Adsorption. The most important difference in the present model between a simple compound like histidine and a protein is the size. For example, the protein lysozyme consists of 129 amino acids with 32 ionizable groups distributed in the spatial dimensions of the macromolecule; $(3.0 \times 3.0 \times 4.5)$ nm. If adsorption induced conformational changes are disregarded, an amino acid residue on the adsorbed protein furthest from the interface would then be 3−4.5 nm away from the surface and thus be subjected to a very different potential resulting in a pH several units from that experienced by the amino acids closest to the surface. Furthermore, the electrostatic interaction with the surface of a charged amino acid at such a distance would be much smaller than for a charged group in the proximity of the surface. It may thus be misleading to represent a protein by a single net charge value in electrostatic calculations. Consequently, the description of protein adsorption should be based on considerations of both orientation at the interface, pK_a values and the interfacial environment (pH and surface charge).

Both experimental[1,36−39] and theoretical[18,40−44] studies suggest that proteins typically obtain a preferred orientation and structure when adsorbed at a specific interface, though at low surface coverage the orientation may be more random.[36,37] Considering the surface charge dependency, a study[45] on the adsorption of serum albumin, lysozyme, ribonuclease A, superoxide dismutase, myoglobin, α-lactalbumin and, in more details,[46] cytochrome C, found that the orientation distribution was largely unaffected by the surface charge. In another study of lysozyme, experimental factors like ionic strength and pH was also shown not to affect the adsorption orientation considerably.[1] Consequently, it is assumed in the following that the orientation of a given protein is relatively unchanged with the experimental factors, including ionic strength, pH, and charge of the surface. The chemical nature of the surface is also assumed not to change, and therefore the chemical contribution to the adsorption free energy is kept at a constant value for a specific protein at a specific surface, independent of experimental factors. On the basis of previous studies these assumptions are valid in most cases, but should be carefully evaluated for unknown systems.

In order to use the model, a specific protein orientation thus needs to be considered. Fortunately, within the past few years, combinations of molecular modeling and new experimental techniques have made this possible.[38,43] From this knowledge, one could use the approximate distances of all the charged groups to the interface and the potential at that distance to calculate the electrostatic interaction energy through eq 10. However, when considering charges at distances $x > 0$, the potential profile, $\varphi(x)$, in the diffusion layer must be considered and the effect on the relative permittivity by the presence of a protein layer, becomes important. By taking into account the relative permittivity of a protein,[47] the relative contribution to the $\Delta G^0_{Ads}(EL)$ term of a single charge at any distance from the surface is calculated in Figure 8 for different surface charges and electrolyte concentrations. It is clear that, particularly with increasing surface charge and electrolyte concentration, the contribution of amino acids not at the interface is relatively small. In favor of simplicity and computing speed, it is therefore considered a good first approximation to evaluate only the contribution of charges at the interface.

On the basis of the discussion above, amino acids at the adsorbing face in the preferred orientation are selected for evaluation of the electrostatic interaction. The two proteins

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lysozyme and α-lactalbumin are structurally related and are about the same size, but they have very different adsorption profiles on charged surfaces. Further, adsorption orientation data are available for these two proteins together with titration studies of their amino acids residues. For hen egg white lysozyme the residues Lys1, Glu7, Arg14, and Arg128 has been suggested, from molecular dynamics studies, to be present at the adsorbing face, when adsorbed to a mica surface,[43] which is consistent with results using site-directed mutagenesis of human lysozyme adsorbed at hydroxyapatite surfaces.[48] For α-lactalbumin the amino acids at the adsorbed plane was selected from bovine α-lactalbumin adsorption data on polystyrene, investigated with proton exchange NMR.[38] The study identified a region of adsorbed α-lactalbumin that was in contact with the surface and contained 4 ionizable amino acids, Lys79, Asp82, Asp88, and Lys98.[38] In Figure 9 the relation between pH and charge is shown for both the interfacial amino acids alone and for the entire protein (inserts). Where possible, the pK_a-values were found in the literature for lysozyme[49] and α-lactalbumin.[50] Arginine because of its high pK_a value was not measured in the literature and was assigned a value of 13 in both lysozyme and α-lactalbumin, lysine was not measured in the case of α-lactalbumin and was assigned a value of 10. Further, the pK_a values for his107, asp82, and asp87 was not obtained in the α-lactalbumin study and therefore his107 was assigned a value of 6 and the two aspartate groups a value of 3. These assigned values are within expectations of such groups in proteins.

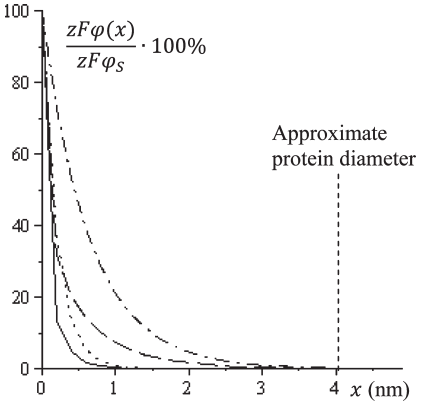

Figure 8. Theoretical relative contribution to the electrostatic interaction of a single charge as a function of distance to the surface, at ionic strength 100 mM and surface potential 0.3 V (line) and close to 0 V (dots). Ionic strength 10 mM and surface potential 0.3 V (dash) and close to 0 V (dash dot).

As the Langmuir isotherm in eq 11 is strongly influenced by electrostatic interactions, the net charge of the amino acids at the interface (z_{Ads}) is anticipated to play a decisive role in protein adsorption to a charged interface. As mentioned, the pH in the vicinity of the charged surface can be different from the bulk pH depending on the surface potential. Therefore, the surface pH, calculated from eq 14, is used in calculating the charge state of the amino acids on the adsorbing face of the preferred orientation (eq 15), while the bulk pH is used for the rest of the ionizable groups on the protein (eq 16). As a first step it is therefore required to evaluate the surface potential (φ_s).

The usual approach for simulations at interfaces is to evaluate the ion distribution at the interface from the surface potential (φ_s) through the Poisson−Boltzmann equation independent of the charge of the adsorbed species. However, simulations of small charged species at liquid−liquid interfaces suggest great influence on the surface potential and potential profile from adsorption,[27] which is further supported by the findings for histidine at a solid−liquid surface in this study. Furthermore, experimental data for ζ-potentials, which is closely related to the surface potential, show considerable changes upon adsorption of charged proteins.[25] The simulation results for adsorption of lysozyme and α-lactalbumin and the influence on surface potential is presented in Figure 10. It is found that the theoretical influence of protein adsorption on the surface potential (φ_s) may be quite significant, as observed from the deviation of φ_s in the absence and presence of the proteins.

At the selected pH of 7.4, lysozyme has an overall charge of +6.8 while the net charge of the amino acids at the interface in the assumed orientation has a net charge of +2 (Figure 9). Even when considering the pH change at the charged surface, which can be considerable (Figure 4 and Figure 5), the surface amino acid net charge does not change much because it is effectively positively charged in this region (Figure 9). Because of the electrostatic repulsion lysozyme is, at the given simulation $\Delta G^0_{Ads}(Chem)$, not adsorbed at surface charge densities roughly above zero. Therefore, the surface potential is the same with or without lysozyme in the solution at $\sigma_s > 0$. At $\sigma_s = 0$ and below, the adsorption of the charged protein considerably changes the surface potential (φ_s), an effect that is more pronounced at higher concentrations because of the concentration dependence of the adsorption isotherm. The change in surface potential, from more negative, at c[lysozyme] = 0, to less negative or even slightly

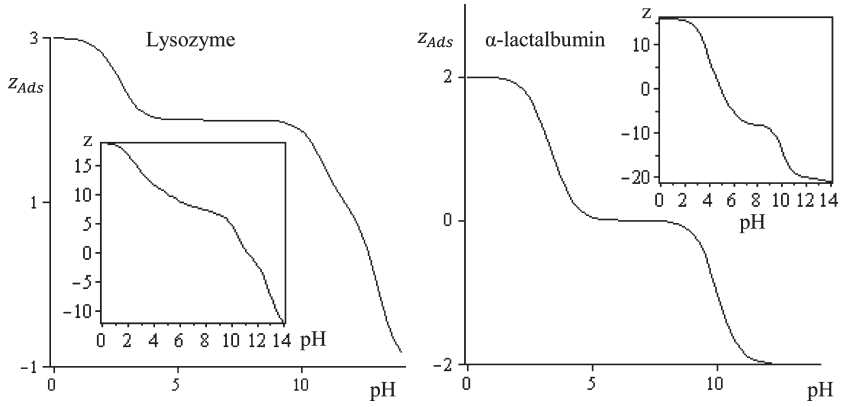

Figure 9. Charge of the proteins lysozyme and α-lactalbumin with pH for the amino acids considered at the interface and for the overall protein (insets).

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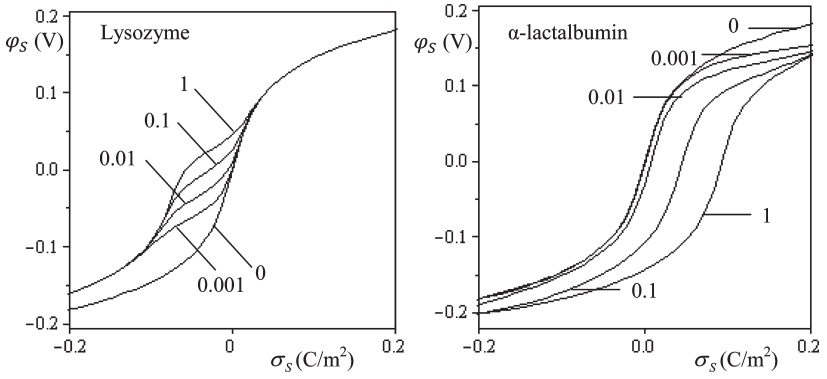

Figure 10. Effect of adsorption of lysozyme and α-lactalbumin on the surface potential. Surface potential as a function of the surface charge density at different concentrations [0, 0.001, 0.01, 0.1, 1] mM. See Table 1 for details about modeling parameters.

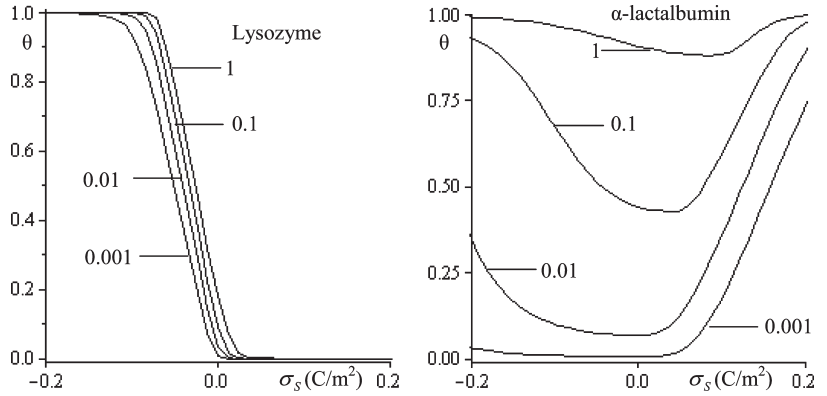

Figure 11. Adsorption isotherms as a function of the surface charge density at different concentrations [0.001, 0.01, 0.1, 1] mM. See Table 1 for model parameters.

positive with the adsorption of positive lysozyme has two effects: (1) the pH at the surface is raised compared to the situation without adsorption. This leads to a lower net charge of the lysozyme, thereby decreasing the electrostatic attraction to the surface and counteracting further adsorption. (2) The adsorption-induced neutralization of the surface potential (φ_s) also directly decreases the electrostatic interaction through eq 11. As σ_s is brought to even lower values the interaction between the interface amino acids and the surface are strong enough that adsorption maximum is reached at all concentrations resulting in the same value of the surface potential.

The second model protein is α-lactalbumin which at pH 7.4 has a net charge of −10.9, calculated from measured pK_a values. In contrast, the amino acids at the adsorbed face of the protein in the preferred orientation are approximately electro-neutral, consisting of two basic and two acidic groups.[38] It is observed that at all surface charge densities (σ_s), the surface potential (φ_s) is consistently lower when the protein is present, although most pronounced at high concentrations. This results from the fact that the protein adsorbs at all σ_s values because of the charge regulation of the interfacial amino acids, while the overall charge of the protein is negative, thereby lowering the surface potential. Considering only the overall net charge it might have been predicted that the protein should adsorb exclusively at the neutral and positively charged surfaces, even when taking into account the shift in pH values near the surface. This is significantly different from what will be expected when considering specifically the amino acids at the surface in the preferred orientation, and illustrates that the overall net charge is not necessarily a good predictor for protein adsorption, even when only considering electrostatic interactions, as has also been pointed out previously.[19−21,42]

Overall, the surface potential is significantly altered by adsorption of the charged species, and a failure to take this into account leads to an overestimate of the surface charge density in the regions with adsorption, which again influence factors such as the surface pH and electrostatic interactions.

From the complete numerical solution of the equation system the Langmuir adsorption isotherm (11) is obtained. The resulting relationship between surface charge density and adsorption isotherm is plotted in Figure 11.

The results show a strong dependence of charge sign for lysozyme at all concentrations. The adsorption isotherm raises quickly to the maximum when moving toward more negative surfaces, while no adsorption is observed at positive potentials. These results are in good agreement with experimental observations of lysozyme at charged interfaces.[51−54] The adsorption profiles of α-lactalbumin are more diverse both in respect to surface charges and initial concentrations. In general, due to charge regulation, adsorption increases with increasing charge. However, by looking only at the profile for [0.001] mM, the adsorption isotherm is close to zero except for the most positive values, where it sharply increases. In theory, an experiment in this concentration region might conclude that the adsorption of α-lactalbumin was governed by the net charge of the protein. At higher concentrations the results show a characteristic U-shaped profile, which in these simulations points at charge regulation of the ampholytic protein at the interface. The results for adsorp-

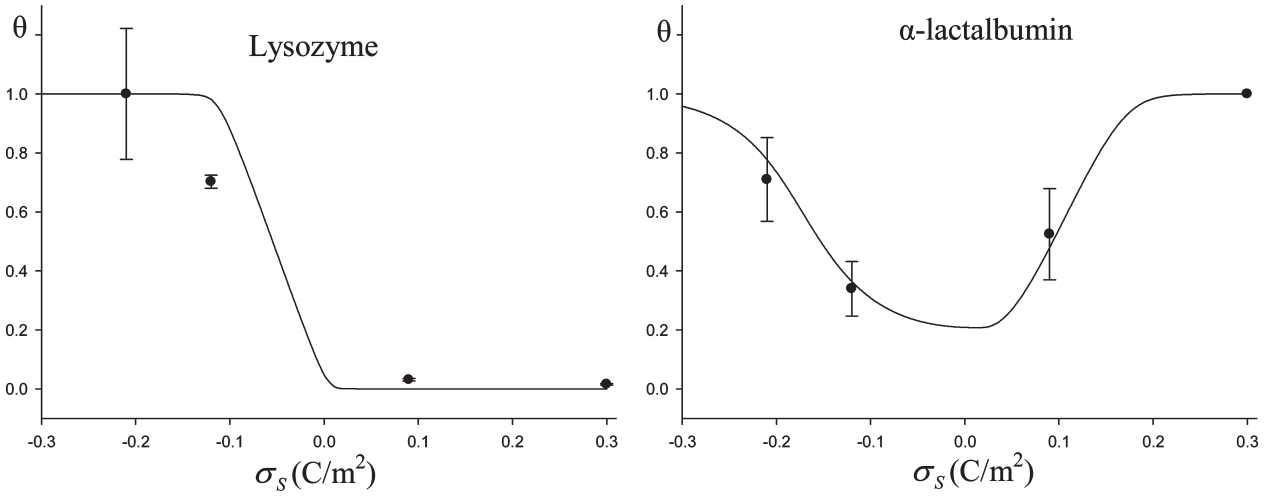

Figure 12. Comparison of experimental data[51] (dots) and simulation results (lines) for lysozyme and α-lactalbumin. Model parameters are found in the text.

tion of α-lactalbumin on comparable solid surfaces with shifting surface charge are in good agreement with experiments.[51,54]

It is possible to directly compare the simulation data to experimental data when information on the concentrations of all charged compounds including the protein, the volume of the sample, the surface area of adsorption, the surface charge density and pH is available. Further, data of the orientation of the adsorbed protein must be available, and the pK_a values of the charged amino acids. These requirements were met in the study by Pasche et al[51] on the adsorption of lysozyme, α-lactalbumin and myoglobin onto solid surfaces of varying degrees of surface charge density estimated to vary from −0.21 to +0.3 (C/m^2). The simulation was adjusted to the experimental settings[51] by assigning the model parameters as follows: T = 298 K, ε = 78, pH_{Bulk} = 7.4, $V = 1.6 \times 10^{-7}$ m^3, and $A = 9.6 \times 10^{-5}$ m^2. The protein concentration was c_{Init} = 0.035 mol m^{-3}, the electrolyte, which in the experiment was a 10 mM HEPES buffer was simulated as a 10 mM 1:1 electrolyte in the model, c_E = 10 mol m^{-3}, and Γ_{Max} was assigned the value of the highest observed amount of protein adsorbed for each protein in the experiment, $\Gamma_{Max}(Lysozyme) = 1.54 \times 10^{-7}$ mol·m^{-2} and $\Gamma_{Max}(\alpha\text{-}lactalbumin) = 8.10 \times 10^{-8}$ mol·m^{-2}. The chemical free energy parameter which was set at $\Delta G^0_{Ads}(Chem) = -5000$ J·mol^{-1} for α-lactalbumin and $\Delta G^0_{Ads}(Chem) = -5000$ J·mol^{-1} for lysozyme.

The excellent agreement between the simulation and the experimental data (Figure 12), suggest that the fundamental assumptions represented in the model are valid, at least within the present experimental systems. That is, the adsorption behavior of lysozyme and α-lactalbumin at charged solid surfaces is accountable for by means of electrostatic interactions and charge regulation at the interface. Considering the complexity and diversity of protein adsorption, the discussed assumptions will probably not be valid in all cases. Deviations from the simulation results might then be an indicator of shifts in the preferred orientation, random orientation, or proliferation of more than one preferred orientation under given conditions.

CONCLUSION

A model for the adsorption of ampholyte molecules at charged solid interfaces is derived from the Gouy−Chapman theory of the electrical double layer, a potential dependent adsorption isotherm and taking into account the effect of charge regulation. For small ampholytic molecules, such as histidine, the model is directly applicable by assuming that the net-charge of the molecule is found at a distance x = 0 from the surface. However, larger molecules such as proteins may contain structural features such that some charged groups are found directly at the surface while others may be several nanometers away. In such cases, the model is solvable if it is assumed that the protein obtains a preferred orientation at the interface and that electrostatic interactions are dominated by interfacial amino acids as shown to be valid in electrolyte solutions having an ionic strength of a few mM or more. In this way it was demonstrated that the complicated adsorption behavior of lysozyme and α-lactalbumin at surfaces of different charge could be explained on the basis of charge regulation of the amino acid residues at the adsorbing face of the protein. The model includes several experimentally controllable parameters and thus provides a link between knowledge of protein orientation and adsorption under different experimental conditions, e.g., pH, ionic strength, surface charge. It is therefore straightforward to use the model in the interpretation of experimental adsorption data. Once the model has been validated for a given set of experimental conditions, it may be used to extend the experimental data to surfaces having different charge and area, proteins with altered amino acid sequences as well as to conditions corresponding to different pH and ionic strength of the solution.

It is relatively straightforward to extend the numerical model to include surfaces with a pH dependent surface charge. This will for example be relevant for the understanding of protein ion chromatography and protein adsorption in fused silica capillaries used for capillary electrophoresis. Finally, the model may be extended to two phase systems for describing protein interactions and adsorption at interfaces between immiscible electrolyte solutions (ITIES), emulsions, liposomes and cell membranes.

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