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Electrostatic Chameleons in Biological Systems

Mikael Lund*

Department of Theoretical Chemistry, Lund University, POB 124, SE-22100 Lund, Sweden

Received July 22, 2010; E-mail: mikael.lund@teokem.lu.se

Abstract: Due to large equilibrium fluctuations of protons at physiological pH, the orthophosphate ion as well as the imidazole group on histidine substantially regulate their charge upon approaching charged interfaces. This implies that these—and comparable—ions function as electostatic "proximity switches" when interacting with lipid membranes, DNA, proteins, etc. Using straightforward statistical thermodynamics as well as mesoscopic computer simulations we quantify the charge regulation mechanism and argue that it is important in a range of biological as well as technical processes.

The protonation state of solvated acidic and basic compounds is subject to equilibrium fluctuations due to proton exchange with the surrounding medium. For the orthophosphate ion and the imidazole group on histidine we show that these ions, at neutral pH, effectively behave as "electrostatic chameleons" when approaching charged interfaces. That is, due to proton fluctuations their ionization state changes with distance to other charged molecules, thereby regulating electrostatic interactions. This has important implications for biological function as well as for technical applications, including drug delivery.

Ever since the very introduction of the pH concept by S. P. L. Sørensen in 1909, proton equilibria in chemical and biological systems have been extensively scrutinized.[1] A major reason for this interest is that proton binding sites can be regarded as electrostatic switches that, chiefly via pH but also in other ways, can be turned on and off. Due to the long ranged nature of electrostatic interactions, the particular charge distribution of a molecule has a significant impact on its physicochemical properties. In a biological setting, the function of proteins is intimately connected with the charge state of titratable amino acid side chains and a wealth of studies address stoichiometric acid dissociation constants, $K_a^* = K_a/\Gamma$, in proteins.[1] The thermodynamic equilibrium constant, K_a, is frequently corrected with the activity coefficient fraction, Γ, of the participating species. This adjustment takes into account the excess free energy change of incorporating the (otherwise isolated) amino acid in a complex chemical environment. Consequently, to determine the average charge state of any given protein residue we need "only" to obtain the activity coefficient of the protonated and deprotonated forms in the protein. From the definition of the equilibrium constant we see that

$$\mathrm{pH} = \mathrm{p}K_a - \log\frac{[\mathrm{HA}]}{[\mathrm{A}^-]} - \log\frac{\gamma_{\mathrm{HA}}}{\gamma_{\mathrm{A}^-}} \qquad (1)$$

and it follows that when $\mathrm{pH} = \mathrm{p}K_a$ the ratio between the protonated (HA) and deprotonated (A^-) form is determined *solely* by the activity coefficients, γ. Any perturbation of the local electrostatic environment therefore leads to a change in the mean protonation state. For an unperturbed acidic group or site i the activity coefficients approach unity and the average charge number, $\langle z_i\rangle$, is simply minus one-half. Over time, protons migrate to and from the site, giving rise to equilibrium fluctuations around the mean charge.[1c,2] In the canonical ensemble the thermodynamic charge average can be written as

$$\langle z_i\rangle_{NVT} = \frac{\sum z_i \exp[-\beta e z_i \varphi_i]}{\sum \exp[-\beta e z_i \varphi_i]} \qquad (2)$$

where the sums run over all of configurational space (all possible molecular coordinates, orientations, and protonation states), e is the elementary unit charge, $\beta = 1/kT$ is the inverse thermal energy, and ϕ_i is the external electric potential on the site i. Disturbing $\langle z_i\rangle$ with a small potential change, one immediately retrieves the fluctuation term,

$$c_i = -\frac{\partial\langle z_i\rangle}{\beta e \partial\varphi_i} = \langle z_i^2\rangle - \langle z_i\rangle^2 \qquad (3)$$

where c_i can be regarded as an electric capacitance. The capacitance can be related to the pH[2c,3] titration curve via $c_I = -\partial z_i/\partial\mathrm{pH}/\ln(10)$. Assuming that the probability, $P(z)$, of finding a certain charge, z, follows a normal distribution, the capacitance conveniently defines the variance or width of the Gaussian around the mean charge, $\langle z_i\rangle$. The above considerations have some interesting consequences, namely that (a) the fluctuation, c_i, around the mean value can be obtained from the derivative of the site titration curve; (b) charge can be induced in a site upon exposure to an electric potential; (c) each site, i, can be regarded as an electric capacitor with a capacitance, c_i; and (d) the site capacitance, and the ability to respond to an external potential, peaks when $\mathrm{pH} = \mathrm{p}K_{a,i}$. Experimental evidence of charge induction or *charge regulation* between entire protein molecules has been elegantly presented by Timasheff et al.[2b]

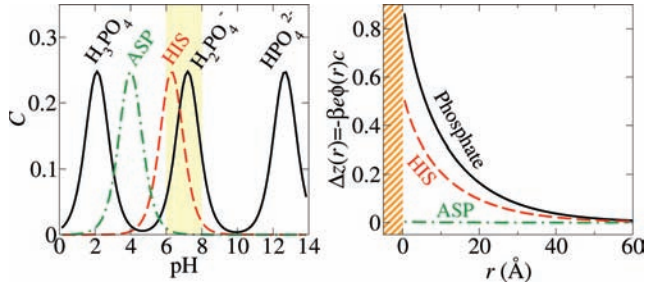

Figure 1. Left: Capacitance of phosphate, aspartic acid, and the imidazole side chain of histidine. Biological pH window illustrated by yellow area. Right: Induced charge, Δz_i, of the three ions as they approach a negatively charged, planar surface located at $r = 0$. The distance dependent potential from the surface, $\phi(r)$, is calculated using the nonlinearized Gouy−Chapman theory[4] with a surface charge density of 180 Å^2 per charge and a 50 mM 1:1 salt concentration at pH 7.

In a biological environment, where pH does not usually deviate significantly from neutral, high capacitances can be expected only for titratable groups with pK_a values close to 7. Two such examples are the imidazole side chain of histidine (pK_a = 6.0−6.5) and the orthophosphate ion (pK_{a2} = 7.2). The high capacitances (Figure 1, left) imply that when these groups approach other charged objects—proteins, lipid membranes, DNA, etc.—their charge changes with $\Delta z_i(r) = -\beta e \phi_i(r) c_i$ according to eq 3. Since the perturbing electric potential decays with the distance, r, from the alien object, the proton induction mechanism may function as a *proximity switch*. Examples now follow.

Near Hydrophilic Interfaces. Figure 1, right, shows the charge induction in three biologically relevant ions when approaching a planar, charged surface in an aqueous salt solution. It is clear that ions with capacitance peaks around neutral pH (phosphate, histidine) are strongly influenced by the interface, while the side chain of aspartic acid remains unchanged (pK_a = 4). At contact, phosphate picks up almost one extra positive charge while the imidazole group on histidine increases its average charge by one-half. This serves to lower the free energy of interaction with the charged surface.

While the vast majority of known proteins have capacitance peaks at low and high pH[2c] (due to a predominance of acidic and basic residues), fewer have peaks at neutral pH. Still, nature has produced a number of *histidine rich* molecules such as hisactophilin, HRP II, histatin saliva proteins, and other antimicrobal proteins.[5] As the histidine content can be as high as 30%, these proteins are consequently cationic and tend to function by interacting with anionic phospholipid membranes. We have previously shown how charge regulation, triggered by minor intracellular pH changes, can induce attractive interactions between hisactophilin and a charged membrane.[6] With their higher degree of structural flexibility, histatins—a family of short, unstructured peptides present in saliva[5b,7]—are expected to exhibit even stronger charge regulation behavior as several histidines may simultaneously border the surface. While this study suggests that such a biochemical regulation mechanism is indeed feasible (Figure 1), it has, to the best of our knowledge, not before been considered.

Near Hydrophobic Interfaces. In the previous section it was shown that the electric potential from a charged surface causes charge induction in titratable ions. Conversely, using a classic image charge argument, the absolute ionic charge is expected to *decrease* when approaching or entering nonpolar regions so as to minimize reaction field interactions caused by desolvation. A solvated charge z approaching a nonpolar, planar interface experiences a repulsive potential due to interfacial desolvation,

$$\beta e \varphi^{rf}(r,z) = \frac{l_B z}{4r} \cdot \left(\frac{\varepsilon_{oil} - \varepsilon_{water}}{\varepsilon_{oil} + \varepsilon_{water}} \right) \tag{4}$$

Here l_B = 7 Å is the Bjerrum length for water at 300 K, r is the distance from the interface, and ε_{water} and ε_{oil} are the relative dielectric constants of water and the nonpolar medium ("oil"). Assuming that the unperturbed fluctuating charge follows a normal distribution we write the intrinsic charge probability in terms of the mean charge and the capacitance (i.e., variance) as

$$P(z) = \frac{1}{\sqrt{2\pi c}} \exp\left(\frac{-(z - \langle z \rangle)^2}{2c} \right) \tag{5}$$

The instantaneous interaction energy of the ion with the surface is simply the potential times the charge, and we then write the Helmholtz free energy of interaction as

$$\beta A(r) = -\ln Q_{NVT} = -\ln \int_{-\infty}^{\infty} P(z) \exp[-\beta e \varphi(r,z) \cdot z] dz \tag{6}$$

where we have Boltzmann weighted over every imaginable charge state according to its coupled interaction with the nonpolar surface. While the integration boundaries of the above integral are clearly unphysical, the Gaussian function quickly approaches zero when $|z - \langle z \rangle|$ becomes large relative to c.

Figure 2 shows the free energy contribution from charge fluctuations for a phosphate ion near an air/water interface at various pH and separations. As expected, the effect of charge regulation peaks when pH equals pK_a and gets stronger as more and more protons are released; this is because the reaction field is stronger for highly charged ions. It is interesting to note that, at neutral pH, charge fluctuations act to lower the free energy by several kT and extend up to 4 Å into the solution. In reality hydrophobic interfaces are however slightly more complex since water molecules may orient and create an oscillating potential.[8] Nevertheless, the mean field interaction with titratable ions can still be considered using the framework presented here.

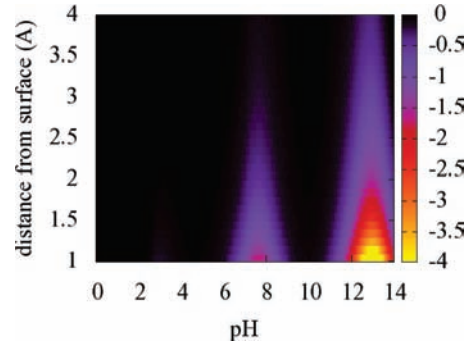

Figure 2. Contribution to the free energy (color scale, units of kT) from charge regulation for phosphate near an air/water interface (i.e., the difference in $\beta A(r)$ for a fluctuation charge distribution and a fixed, mean charge $P(z)$ as a Dirac delta function).

It should be noted that while we here examine fluctuations on a per-site basis, it is slightly more complicated to predict induction in sites with titratable neighbors, as in the case of protein molecules. The reason is that the charge states are coupled, meaning that induction in one site will affect nearby sites that again will induce charge in the first site and so on. While this cascade can be conveniently studied by numerical simulation (see following) it can also be solved in a Gaussian ansatz by considering all possible charge states,

$$Q_{NVT} = \int \ldots \int \prod_i^N P_i(z_i) \exp(-\beta u_i/2) dz_1 \ldots dz_N \tag{7}$$

where N is the number of charged sites and u_i is the ith site interaction energy with the rest of the system. From this partition function, Q, all thermodynamic functions follow, including average charges and pK_a shifts in proteins, for example. The latter could be evaluated by calculating perturbed charge averages at various pH.

In Protein Binding Pockets. Lastly we investigate the phosphate binding protein (PBP1) of *M. Tuberculosis*. A puzzling feature of this molecule is that the electric potential in the phosphate binding cleft is negative.[9] According to the framework presented here, the binding free energy is lowered by spontaneous proton uptake by phosphate. To quantify this proton induction we use continuum

electrostatic Metropolis Monte Carlo simulations (Figure 3, left) to study the charge state of phosphate in the binding site of PBP1 relative to a bulk salt solution. As shown in Figure 3, right, the protonation state of bound phosphate is constantly minus one in the pH interval 6−7, while for the unbound form the charge rapidly drops to −1.8 at pH 7.5. The constant charge of the former is a direct effect of the negative cleft potential that makes it unfavorable to introduce anions into the binding site. While charge regulation is not responsible for the entire binding free energy—hydrogen bonding seems important[9]—it reduces the electrostatic repulsion. This is the case even for intermediate separations where H-bonding is impossible and proton fluctuations may thus act to lower the free energy barrier upon approaching the binding site.

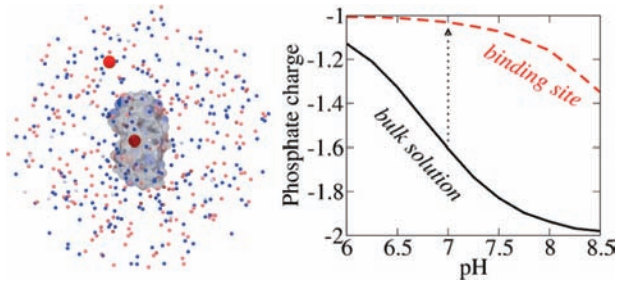

Figure 3. Left: Snapshot of continuum electrostatic Monte Carlo simulations of *M. Tuberculosis* PBP1 (central) in an aqueous solution of 100 mM 1:1 salt (small spheres) and two phosphate ions (larger, red spheres)—one free in solution and one bound in the binding site. Right: Protonation state of the two phosphate ions (free and bound to PBP1) as a function of pH. See Supporting Information for details.

In summary, straightforward statistical thermodynamics leads to an easy to use scheme for predicting charge regulation in titratable sites under the influence of nearby charged macromolecules; the main concepts and relations are listed in Table 1. At physiological conditions (pH close to seven) the orthophosphate ion and the imidazole side chain of histidine display *maximum* susceptibilities for charge regulation; i.e. their mean charges are subject to large fluctuations. Is this a mere coincidence? Unlikely. The presented framework indeed suggests that distance dependent charge induction can be utilized in biochemical mechanisms by letting small ions and molecules act as electrostatic chameleons with properties specific to their location. More technically, drug molecules could be equipped with functional groups that transform from charged to neutral upon migration through hydrophobic interfaces. This will address the well-known challenge of delivering water-soluble pharmaceuticals across lipid membranes.

Table 1. Key Concepts of Charge Regulation[a]

Property	Expression
The charge capacitance, c, quantifies how easily the charge state is perturbed and is related to the pH titration curve.	$c = -\frac{1}{\ln 10}\frac{d\langle z\rangle}{d\mathrm{pH}} = \langle z^2\rangle - \langle z\rangle^2$
The intrinsic probability, P, of finding a site with charge z is well described by a normal distribution.	$P(z) = \frac{1}{\sqrt{2\pi c}}\exp\left(\frac{-(z-\langle z\rangle)^2}{2c}\right)$
Charge induction, Δz, due to an external electric potential, ϕ_{ext}.	$\Delta z = -\beta e \phi_{ext} c$
Free energy of interaction, A, between a site and an external potential[2c].	$\beta A \approx \langle z\rangle \beta e \phi_{ext} - \frac{c}{2}\left(\beta e \phi_{ext}\right)^2$
Free energy in external potential using a charge probability function $P(z)$.	$\beta A = -\ln \int_{-\infty}^{\infty} P(z)\exp(-\beta e z \phi_{ext})dz$

[a] $\beta = 1/kT$ is the inverse thermal energy and the angled brackets denote thermodynamic averages.

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Supporting Information Available: Monte Carlo simulation details are available in the Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org.

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