Protein Charge[1]

A brief overview

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As protein charge is a fundamental property within biological systems, it is important to understand that in addition to charged amino acids and other charged protein substituents (e.g. sialic acid), there are at least three factors that contribute to protein charge:

1. **Shifts in pK_as of charged groups**

   pK_a shifts can be caused by changes in solvent accessibility, ion pair formation, the net charge on a protein and the proximity of other charged residues. The influence of pK_a shifts is considered in some computational programs such as PROPKA3. [2-5] However, the most significant short coming of this program is that only proton binding is considered. Charge estimates from amino acid composition and X-ray or NMR structure may be in serious error as these approaches only consider H^+ binding (e.g. Sednterp, PROPKA3) when it is known that proteins bind other ions, specifically anions. [6-8]

2. **Site-specific ion binding**

   Proteins bind ions through site-specific bonds which can be detected in X-ray crystallography or NMR structure. These site bound ions are fixed spatially and contribute to the net protein charge.

3. **Territorial ion binding due to polyelectrolyte effects**

   Territorial ion binding, a non-specific ion interaction, also contributes to the overall net charge of a protein. However, territorial bound ions cannot be detected in X-ray crystallography or NMR structure as they are not bound to a specific site on the protein [9-11] Instead, these ions are found diffusely bound to regions around the protein surface with high charge densities. [10, 12] When a highly charged region on a protein surface exceeds RT (2.5 kJ/mol), a counter-ion diffuses into that region to reduce the electric potential energy. [10, 13-15] A territorial bound ion can readily exchange with other ions in solution but, as soon as one ion diffuses away from the protein surface, another counter-ion immediately replaces it to maintain thermodynamic favorability in that region. [10, 11] Because territorial bound ions are found within close proximity to the protein surface or within the solvation layer, they will diffuse with the protein. [10, 13-15] The measured value for protein charge is a time-averaged value because proteins in solution are readily binding and releasing ions, giving rise to several distinct charged species. [10, 16] Protein charge must not be thought of as a component property but a system property, as it may depend on solvent composition, temperature and pH. [10]

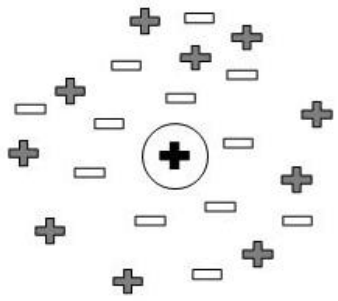

Figure 1 Cartoon of Debye-Hückel counterion cloud

In summary, the charge on a protein includes contributions from the formal protein charge, combined with the contributions from the territorial- and site-bound ions. Together, these contributions make up the net charge or "charge structure" of a protein. [11] In the presence of a charged structure, solvent ions form a counter ion cloud or a Debye-Hückel cloud around the protein. [17-19] That is, the nearest neighbors of a charged protein will most likely be ions of opposite charge. The formation of the Debye Hückel cloud is the solvents response to the presence of a charged protein. It is also important to note, that the ions that make up the Debye Hückel cloud are distinct from territorial bound ions.

Debye-Hückel ion cloud

The Debye-Hückel cloud acts as an electrostatic shield that decreases the electrophoretic mobility from what would be expected for a charged protein in the absence of shielding. Thus, the effective charge (Z_{eff}) is the formal charge of a protein reduced by the shielding effects of the Debye-Hückel counterion cloud. [19-21] In addition to the Debye-Hückel cloud, Z_{eff} does not adjust for the electrophoretic effect. There are two contributions that constitute the electrophoretic effect. One contribution is the distortion of the electric field lines in the presence of a non-conducting particle such as a protein. The other contribution is the momentum transfer from the counterion flow in the immediate vicinity of the particle. Both contributing effects were addressed by D.C. Henry, which he provided calculations for (Henry's functions) [16, 22, 23] As a consequence of the Debye-Hückel cloud and the electrophoretic effect, Z_{eff} cannot distinguish between bound ions (including territorial bound ions) and the Debye-Hückel cloud. Thus, Z_{eff} is a measurement of the protein charge reduced by solvent effects. [17, 23-25] The Debye-Hückel-Henry valence (Z_{DHH}) is considered the formal protein charge and is a more appropriate descriptor of protein valence as it accounts for electrostatic shielding and the electrophoretic effect. [8, 26] Using the Debye-Hückel-Henry model the value for Z_{eff} can be transformed into the Debye-Hückel-Henry charge, Z_{DHH} using equation 1:

$$Z_{DHH} = Z_{eff} \frac{1+\kappa a}{f(\kappa a)} \quad (1)$$

Where κ is the inverse Debye length and a is the sum of the Stokes radii of the protein and its Counterion. The denominator f (κa) is Henrys function and accounts for the the electrophoretic effect.

For clarification, the terms charge (q) and electrical valence are often used synonymously. The electrical valence is simply the unitless integral value acquired by dividing the charge on a molecule, Q (in Coulombs) by the elementary charge on a proton, Q_p (1.602 x 10-19 Coulombs), equation 2:

$$Z = \frac{Q}{Q_p} \quad (2)$$

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