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22.3. Electrostatic interactions in proteins

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22.3.1. Introduction

Electrostatic interactions play a key role in determining the structure, stability, binding affinity, chemical properties, and hence the biological reactivity, of proteins and nucleic acids. Interactions where electrostatics play an important role include:

(1) Ligand/substrate association. Long-range electrostatic forces can considerably enhance association rates by facilitating translational and rotational diffusion or by reduction in the dimensionality of the diffusion space.

(2) Binding affinity. Tight specific binding is often a prerequisite for biological activity, and electrostatics make important contributions to desolvation and formation of chemically complementary interactions during binding.

(3) Modification of chemical and physical properties of functional groups such as cofactors (haems, metal ions *etc.*), alteration of the ionization energy (pK_a) of side chains and shifting of redox midpoints.

(4) The creation of potentials or fields in the active sites to stabilize functionally important charged or dipolar intermediates in processes such as catalysis.

In this chapter I will discuss, within the framework of classical electrostatics, how such effects can be modelled starting from the structural information provided by X-ray crystallography. Nevertheless, many of the concepts of classical electrostatics can be used in combination with molecular dynamics (MD), quantum mechanics (QM) and other computational methods to study a wider range of macromolecular properties, for example specific protein motions, the breaking or forming of bonds, determination of intrinsic pK_a's, determination of electronic energy levels *etc*.

The central aim in studying the electrostatic properties of macromolecules is to take the structural information provided by crystallography (typically the atomic coordinates, although *B*-factor information may also be of use) and obtain a realistic description of the electrostatic potential distribution $\varphi(\mathbf{r})$. The electrostatic potential distribution can then be used in a variety of ways: (i) graphical analysis may reveal deeper aspects of the structure and help identify functionally important regions or active sites; (ii) the potentials may be used to calculate energies and forces, which can then be used to calculate equilibrium or kinetic properties; and (iii) the electrostatic potentials may be used in conjunction with other computational methods such as QM and MD.

Three problems must be solved to obtain the electrostatic potential distribution. The first is to model the macromolecular charge distribution, usually by specifying the location and charge of all its atoms. Although the coordinates of the molecule are determined by crystallographic methods, the charge distribution is not. A number of atomic charge distributions have been developed for proteins and nucleic acids using quantum mechanical methods and/or parameterization to different experimental data. The second problem is that the positions of the water molecules and solvent ions are generally not known. (Water molecules and ions seen in even the best crystal structures usually constitute a small fraction of the total important in solvating the molecule. Moreover, the orientation of the crystallographic water molecules, crucial in determining the electrostatic potential, is rarely known.) Within the framework of classical electrostatics, inclusion of the *effect* of the solvating water molecules and ions is handled not by treating them explicitly, but implicitly in terms of an 'electrostatic response' to the field created by the molecular charge distribution. The third problem is that incorporation of the available structural information at atomic resolution results in a complicated spatial distribution of charge, dielectric response *etc*. Numerical methods for rapidly and accurately solving the electrostatic equations that determine the potential are therefore essential.

22.3.2. Theory

22.3.2.1. *The response of the system to electrostatic fields*

The response to the electrostatic field arising from the molecular charge distribution arises from three physical processes: electronic polarization, reorientation of permanent dipolar groups and redistribution of mobile ions in the solvent. Movement of ionized side chains, if significant, is sometimes viewed as part of the dielectric response of the protein, and sometimes explicitly as a conformational change of the molecule.

Electronic polarizability can be represented either by point inducible dipoles (Warshel & Åqvist, 1991) or by a dielectric constant. The latter approach relates the electrostatic polarization, $\mathbf{P}(\mathbf{r})$ (the mean dipole moment induced in some small volume V) to the Maxwell (total) field, $\mathbf{E}(\mathbf{r})$, and the local dielectric constant representing the response of that volume, $\varepsilon(\mathbf{r})$, according to

$$\mathbf{P}(\mathbf{r}) = [\varepsilon(\mathbf{r}) - 1]\mathbf{E}(\mathbf{r})/4\pi. \qquad (22.3.2.1)$$

The contribution of electronic polarizability to the dielectric constant of most organic material and water is fairly similar. It can be evaluated by high-frequency dielectric measurements or the refractive index, and it is in the range 2–2.5.

The reorientation of groups such as the peptide bond or surrounding water molecules which have large permanent dipoles is an important part of the overall response. This response too may be treated using a dielectric constant, *i.e.* using equation (22.3.2.1) with a larger value of the dielectric constant that incorporates the additional polarization from dipole reorientation. An alternative approach to equation (22.3.2.1) for treating the dipole reorientation contribution of water surrounding the macromolecules is the Langevin dipole model (Lee *et al.*, 1993; Warshel & Åqvist, 1991; Warshel & Russell, 1984). Four factors determine the degree of response from permanent dipoles: (i) the dipole-moment magnitude; (ii) the density of such groups in the protein or solvent; (iii) the freedom of such groups to reorient; and (iv) the degree of cooperativity between dipole motions. Thus, water has a high dielectric constant ($\varepsilon = 78.6$ at 25 °C). For electrostatic models based on dielectric theory, the experimental solvent dielectric constant, reflecting the contribution of electronic polarizability and dipole reorientation, is usually used. From consideration of the four factors that determine the dielectric response, macromolecules would be expected to have a much lower dielectric constant than the solvent. Indeed, theoretical studies of the dielectric behaviour of amorphous protein solids (Gilson & Honig, 1986; Nakamura *et al.*, 1988) and the interior of proteins in solution (Simonson & Brooks, 1996; Simonson & Perahia, 1995; Smith *et al.*, 1993), and experimental measurements (Takashima & Schwan, 1965) provide an estimate of $\varepsilon = 2.5$–4 for the contribution of dipolar groups to the protein dielectric.

The Langevin model can account for the saturation of the response at high fields that occurs if the dipoles become highly aligned with the field. The dielectric model can also be extended to incorporate saturation effects (Warwicker, 1994), although there is a compensating effect of electrostriction, which increases the local dipole density (Jayaram, Fine *et al.*, 1989). While the importance of saturation effects would vary from case to case, linear solvent dielectric models have proven sufficiently accurate for most protein applications to date.

Charge groups on molecules will attract solvent counter-ions and repel co-ions. The most common way of treating this charge rearrangement is *via* the Boltzmann model, where the net charge density of mobile ions is given by

$$\rho^m(\mathbf{r}) = \sum_i z_i e c_i^o \exp[-z_i e\varphi(\mathbf{r})/kT], \qquad (22.3.2.2)$$

where c_i^o is the bulk concentration of an ion of type i, valence z_i, and $\varphi(\mathbf{r})$ is the average potential (an approximation to the potential of mean force) at position $\mathbf{r}$. The Boltzmann approach neglects the effect of ion size and correlations between ion positions. Other models for the mobile-ion behaviour that account for these effects are integral equation models and MC models (Bacquet & Rossky, 1984; Murthy *et al.*, 1985; Olmsted *et al.*, 1989, 1991; Record *et al.*, 1990). These studies show that ion size and correlation effects do not compromise the Boltzmann model significantly for monovalent (1–1) salts at mid-range concentrations 0.001–0.5 *M*, and consequently it is widely used for modelling salt effects in proteins and nucleic acids.

22.3.2.2. *Dependence of the potential on the charge distribution*

The potential at a point in space, $\mathbf{r}$, arising from some charge density distribution $\rho(\mathbf{s})$ and some dipole density distribution $\mathbf{P}(\mathbf{s})$ (which includes polarization) is given by

$$\varphi(\mathbf{r}) = \int \rho(\mathbf{s})/(|\mathbf{s}-\mathbf{r}|) + \mathbf{P}(\mathbf{s})(\mathbf{s}-\mathbf{r})/(|\mathbf{s}-\mathbf{r}|^3)\, d\mathbf{s}. \qquad (22.3.2.3)$$

The total charge distribution is the sum of the explicit charge distribution on the molecule and that from the mobile solvent ion distribution, $\rho = \rho^e + \rho^m$. Substituting for the dielectric polarization using equation (22.3.2.1) and for the mobile ion charge distribution using equation (22.3.2.2), the potential may be expressed in terms of a partial differential equation, the Poisson–Boltzmann (PB) equation:

$$\nabla\varepsilon(\mathbf{r})\nabla\varphi(\mathbf{r}) + 4\pi\sum_i z_i e c_i^o \exp[-z_i e\varphi(\mathbf{r})/kT] + 4\pi\rho^e(\mathbf{r}) = 0, \qquad (22.3.2.4)$$

which relates the potential, molecular charge and dielectric distributions, $\varphi(\mathbf{r})$, $\rho^e(\mathbf{r})$ and $\varepsilon(\mathbf{r})$, respectively. Contributions to the polarizability from electrons, a molecule's permanent dipoles and solvent dipoles are incorporated into this model by using an appropriate value for the dielectric for each region of protein and solvent. Values for protein atomic charges, radii and dielectric constants suitable for use with the Poisson–Boltzmann equation are available in the literature (Jean-Charles *et al.*, 1990; Mohan *et al.*, 1992; Simonson & Brünger, 1994; Sitkoff *et al.*, 1994). For protein applications, the Boltzmann term in equation (22.3.2.4) is usually linearized to become $-8\pi\varphi(\mathbf{r})I/kT$ where I is the ionic strength, whereas for nucleic acids and molecules of similarly high charge density the full nonlinear equation is used.

22.3.2.3. *The concepts of screening, reaction potentials, solvation, dielectric, polarity and polarizability*

Application of a classical electrostatic view to macromolecular electrostatics involves a number of useful concepts that describe the physical behaviour. It should first be recognized that the potential at a particular charged atom i includes three physically distinct contributions. The first is the direct or Coulombic potential of j at i. The second is the potential at i generated by the polarization (of a molecule, water and ion atmosphere) induced by j. This is often referred to as the screening potential, since it opposes the direct Coulombic potential. The third arises from the polarization induced by i itself. This is often referred to as the reaction or self-potential, or if solvent is involved, as the solvation potential.

When using models that apply the concept of a dielectric constant (a measure of polarizability) to a macromolecule, it is important to distinguish between polarity and polarizability. Briefly, polarity may be thought of as describing the density of charged and dipolar groups in a particular region. Polarizability, by contrast, refers to the *potential* for reorganizing charges, orienting dipoles and inducing dipoles. Thus polarizability depends both on the polarity and the freedom of dipoles to reorganize in response to an applied electric field. When a protein is folding or undergoing a large conformational rearrangement, the peptide groups may be quite free to reorient. In the folded protein, these may become spatially organized so as to stabilize another charge or dipole, creating a region with high polarity, but with low polarizability, since there is much less ability to reorient the dipolar groups in response to a new charge or dipole without significant disruption of the structure. Thus, while there is still some discussion about the value and applicability of a protein dielectric constant, it is generally agreed that the interior of a macromolecule is a less polarizable environment compared to solvent. This difference in polarizability has a significant effect on the potential distribution.

Formally charged groups on proteins, particularly the longer side chains on the surface of proteins, Arg, Lys, and to a lesser extent Glu and Asp, have the ability to alter their conformation in response to electrostatic fields. In addition, information about fluctuations about their mean position may need to be included in calculating average properties. Three approaches to modelling protein formal charge movements can be taken. The first is to treat the motions within the dielectric response. In this approach, the protein may be viewed as having a dielectric higher than 2.5–4 in the regions of these charged groups, particularly at the surface, where the concentration and mobility of these groups may give an effective dielectric of 20 or more (Antosiewicz *et al.*, 1994; Simonson & Perahia, 1995; Smith *et al.*, 1993). A second approach is to model the effect of charge motions on the electrostatic quantity of interest explicitly, *e.g.* with MD simulations (Langsetmo *et al.*, 1991; Wendoloski & Matthew, 1989). This involves generating an ensemble of structures with different atomic charge distributions. The third approach is based on the fact that one is often interested in a specific biological process $A \rightarrow B$ in which one can evaluate the structure of the protein in states A and B (experimentally or by modelling), and any change in average charge positions is incorporated at the level of different average explicit charge distribution inputs for the calculation, modelling only the electronic, dipolar and salt contributions as the response.

The term 'effective' dielectric constant is sometimes used in the literature to describe the strength of interaction between two charges, q_1 and q_2. This is defined as the ratio of the observed or calculated interaction strength, U, to that expected between the same two charges in a vacuum:

$$\varepsilon^{\text{eff}} = [(q_1 q_2)/r_{12}]/U, \qquad (22.3.2.5)$$

where r_{12} is the distance between the charges. If the system were completely homogeneous in terms of its electrostatic response and involved no charge rearrangement then ε^{eff} would describe the dielectric constant of the medium containing the charges. This is generally never the case: the strength of interaction in a protein system is determined by the net contribution from protein, solvent and ions, so ε^{eff} does not give information about the dielectric property of any particular region of space. In fact, in the same system different charge–charge interactions will generally yield different values of ε^{eff}. Thus ε^{eff} is really no more than its definition – a measure of the strength of interaction – and it cannot be used directly to answer questions about the protein dielectric constant,

for example. Rather, it is one of the quantities that one aims to extract from theoretical models to compare with an experiment.

22.3.2.4. *Calculation of energies and forces*

Once the electrostatic potential distribution has been obtained, calculation of experimental properties usually requires evaluation of the electrostatic energy or force. For a linear system (where the dielectric and ionic responses are linear) the electrostatic free energy is given by

$$\Delta G^{\rm el} = 1/2 \sum_i \varphi_i q_i, \qquad (22.3.2.6)$$

where $\varphi_{\rm i}$ is the potential at an atom with charge q_i. The most common source of nonlinearity is the Boltzmann term in the PB equation (22.3.2.4) for highly charged molecules such as nucleic acids. The total electrostatic energy in this case is (Reiner & Radke, 1990; Sharp & Honig, 1990; Zhou, 1994)

$$\Delta G^{\rm el} = \int_V \{\rho^e \varphi - (\varepsilon E^2/8\pi) - kT \sum_i c_i^0 [\exp(-z_i e\varphi/kT) - 1]\}\, {\rm d}\mathbf{r}, \qquad (22.3.2.7)$$

where the integration is now over all space.

The general expression for the electrostatic force on a charge q is given by the gradient of the total free energy with respect to that charge's position,

$$\mathbf{f}_q = -\nabla_{\mathbf{r}q}(G^{\rm el}). \qquad (22.3.2.8)$$

If the movement of that charge does not affect the potential distribution due to the other charges and dipoles, then equation (22.3.2.8) can be evaluated using the 'test charge' approach, in which case the force depends only on the gradient of the potential or the field at the charge:

$$\mathbf{f} = q\mathbf{E}. \qquad (22.3.2.9)$$

However, in a system like a macromolecule in water, which has a non-homogeneous dielectric, forces arise between a charge and any dielectric boundary due to image charge (reaction potential) effects. A similar effect to the 'dielectric pressure' force arises from solvent-ion pressure at the solute–solvent boundary. This results in a force acting to increase the solvent exposure of charged and polar atoms. An expression for the force that includes these effects has been derived within the PB model (Gilson *et al.*, 1993):

$$\mathbf{f} = \rho^e \mathbf{E} - (1/2)E^2 \nabla\varepsilon - kT \sum_i c_i^0 [\exp(-z_i e\varphi/kT) - 1]\nabla A, \qquad (22.3.2.10)$$

where A is a function describing the accessibility to solvent ions, which is 0 inside the protein, and 1 in the solvent, and whose gradient is nonzero only at the solute–solvent surface. Similarly, in a two-dielectric model (solvent plus molecule) the gradient of ε is nonzero only at the molecular surface. The first term accounts for the force acting on a charge due to a field, as in equation (22.3.2.9), while the second and third terms account for the dielectric surface pressure and ionic atmosphere pressure terms respectively. Equation (22.3.2.10) has been used to combine the PB equation and molecular mechanics (Gilson *et al.*, 1995).

22.3.2.5. *Numerical methods*

A variety of numerical methods exist for calculating electrostatic potentials of macromolecules. These include numerical solution of self-consistent field electrostatic equations, which has been used in conjunction with the protein dipole–Langevin dipole method (Lee *et al.*, 1993). Numerical solution of the Poisson–Boltzmann equation requires the solution of a three-dimensional partial differential equation, which can be nonlinear. Many numerical techniques, some developed in engineering fields to solve differential equations, have been applied to the PB equation. These include finite-difference methods (Bruccoleri *et al.*, 1996; Gilson *et al.*, 1988; Nicholls & Honig, 1991; Warwicker & Watson, 1982), finite-element methods (Rashin, 1990; Yoon & Lenhoff, 1992; Zauhar & Morgan, 1985), multigridding (Holst & Saied, 1993; Oberoi & Allewell, 1993), conjugate-gradient methods (Davis & McCammon, 1989) and fast multipole methods (Bharadwaj *et al.*, 1994; Davis, 1994). Methods for treating the nonlinear PB equation include under-relaxation (Jayaram, Sharp & Honig, 1989) and powerful inexact Newton methods (Holst *et al.*, 1994). The nonlinear PB equation can also be solved *via* a self-consistent field approach, in which one calculates the potential using equation (22.3.2.5), then the mobile charge density is calculated using equation (22.3.2.3), and the procedure is repeated until convergence is reached (Pack & Klein, 1984; Pack *et al.*, 1986). The method allows one to include more elaborate models for the ion distribution, for example incorporating the finite size of the ions (Pack *et al.*, 1993). Approximate methods based on spherical approximations (Born-type models) have also been used (Schaeffer & Frommel, 1990; Still *et al.*, 1990). Considerable numerical progress has been made in finite methods, and accurate rapid algorithms are available. The reader is referred to the original references for numerical details.

22.3.3. Applications

An exhaustive list of applications of classical electrostatic modelling to macromolecules is beyond the scope of this chapter. Three general areas of application are discussed.

22.3.3.1. *Electrostatic potential distributions*

Graphical analysis of electrostatic potential distributions often reveals features about the structure that complement analysis of the atomic coordinates. For example, Fig. 22.3.3.1(*a*) shows the distribution of charged residues in the binding site of the proteolytic enzyme thrombin. Fig. 22.3.3.1(*b*) shows the resulting electrostatic potential distribution on the protein surface. The basic (positive) region in the fibrinogen binding site, which could be inferred from close inspection of the distribution of charged residues in Fig. 22.3.3.1(*a*), is clearly more apparent in the potential distribution. Fig. 22.3.3.1(*c*) shows the effect of increasing ionic strength on the potential distribution, shrinking the regions of strong potential. Fig. 22.3.3.1(*d*) is calculated assuming the same dielectric for the solvent and protein. The more uniform potential distribution compared to Fig. 22.3.3.1(*b*) shows the focusing effect that the low dielectric interior has on the field emanating from charges in active sites and other cleft regions.

22.3.3.2. *Charge-transfer equilibria*

Charge-transfer processes are important in protein catalysis, binding, conformational changes and many other functions. The primary examples are acid–base equilibria, electron transfer and ion binding, in which the transferred species is a proton, an electron or a salt ion, respectively. The theory of the dependence of these three equilibria within the classical electrostatic framework can be treated in an identical manner, and will be illustrated with acid–base equilibria. A titratable group will have an intrinsic ionization equilibrium, expressed in terms of a known intrinsic pK_a^0, where $pK_a^0 = -\log_{10}(K_a^0)$, K_a^0 is the dissociation constant for the reaction $H^+A = H^+ + A$ and A can be an acid or a base. The pK_a^0 is determined by all the quantum-chemical, electrostatic and environ-

references