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