Salt Effects in Peptide Solutions: Theory and Simulation

Gail E. Marlow, John S. Perkyns, and B. Montgomery Pettitt*

Department of Chemistry, University of Houston, Houston, Texas 77204-5641

Received May 21, 1993 (Revised Manuscript Received September 7, 1993)

Contents

1. Introduction

The behavior of peptide or protein solutes in saline aqueous solution is a fundamental topic in physical chemistry. While ionic solutions have been long studied by electrochemistry,[1] the advent of improved experimental techniques has provided new insight into the structure and dynamics of aqueous solutions of electrolytes on a microscopic level. Both spectroscopic techniques[2] and neutron diffraction methods[3-6] have contributed to these advances. Theoretical studies have added to the interpretation of the experiments and often provided new insights. Ab initio electronic structure calculations have provided information on ion–water interactions[7-11] as well as improved classical potential functions.[12-14] This has encouraged the use of many body theory and computer simulations to study these systems.

While the primary importance of the study of molecules in aqueous electrolytes is widely appreciated, the topic also has wide-ranged applicability, perhaps most notably in the behavior of peptide molecules in solution. The addition of ions can strongly alter the thermodynamic and physical properties of peptides in solution.[15,16] This is experimentally evident in the process of salting peptides and proteins in and out of solution.[16] The often related process of denaturation of proteins[15,17,18] by changes in salt activity has also been studied extensively by experiment.

Hormones and mediators for various biological processes are known to often be peptides with lengths of less than 20 amino acid residues.[19] The thermally accessible conformations of these molecules in solution, and the broad distribution of these conformations, are central to understanding their activity. This information is especially important for the design of new drugs with increased potency, lifetime, or both.[20]

The conformation of peptides is sensitive to the composition of the solution environment.[2] The shapes available, bulk solubility, and thermodynamics of binding are strongly affected by relatively small changes in the activities of solvent and salt. Many peptides, especially short ones (less than 20 residues in length), often do not adopt a single stable conformation in solution as evidenced by NOE measurements in aqueous solution.[19]

There are several properties which characterize the behavior of water in the presence of ions. These can be separated into structural and dynamical properties. Structural properties include ion–water geometries, coordination numbers, and radial distribution functions. The radial distribution function is of particular interest, since from it all thermodynamic functions for the system can be derived.[21,22] Dynamical properties include diffusion coefficients, ion–ion and ion–water velocity correlation functions, relaxation times, and residence times, which in some cases have been linked to the hydration number.[23] Theoretical structural properties in condensed phase are obtainable from a variety of methods, including integral equations, Monte Carlo, and molecular dynamics. The theoretical dynamic characteristics for dense random systems, such as aqueous solutions, are available through molecular dynamics and its variants. In addition, other methods requiring phenomenological input such as those relying on stochastic dynamics (Langevin dynamics, Brownian dynamics, etc.) and generalized hydrodynamics are capable of yielding interesting insights into dynamics in condensed-phase systems. Connecting the microscopic structural and dynamical information from such theoretical methods with experimental observations and descriptions remains challenging.

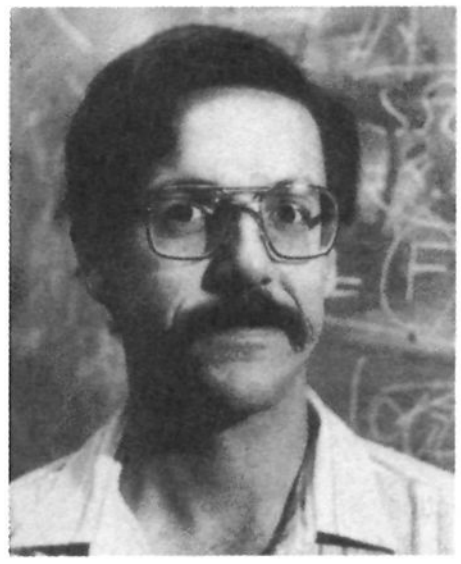
One of the older but more appealing descriptions of the effect of ions on water in solutions involves categorizing ions as either "structure making" or "structure breaking".[24,25] The water around an ion is viewed as having three domains, distinguished by the disruption in structure compared to that of bulk water. The innermost region consists of water molecules which are very strongly associated with the ion. These waters generally remain associated with the ion as it moves over distances several times its own diameter with the time scale for exchange depending on a number of characteristics of the ion. In the next domain further

0009-2665/93/0793-2503$12.00/0

Gail Marlow is a Keck Predoctoral fellow in the Chemistry Department at the University of Houston. She received her bachelors degree in Chemistry from the University of Houston. Her research interests are in the theory of the physical and biochemical behavior of peptides and proteins in saline solutions. She has published on the theory of nucleic acids and peptides in solution.

John Perkyns is a postdoctoral research associate at the University of Houston. He received his Ph.D. in Theoretical Chemistry from the University of British Columbia. There he pursued interests in liquid-state theory with Professor Gren Patey. Perkyns has published in the area of the theory of ionic solutions, both aqueous and nonaqueous.

from the center of the ion, the waters are still oriented by the ion but to a lesser extent and must bridge between the molecules in the inner shell and the outer bulk-like region. In the outermost region, the hydrogen-bonding characteristics of the water are close to that of bulk solution. An ion is classified as structure making or structure breaking depending on the relative significance and extent of the innermost and middle domains. In general, small ions are structure making, meaning they order the water more than bulk water. Large ions generally exert a structure-breaking effect through a disruption of the hydrogen bonding found in bulk water. An exception to this trend are the so-called "hydrophobic" ions such as the tetraalkylammonium ions. Although these are large ions, they tend to have a structure-making effect by increasing hydrogen bonding of water molecules at their surface. This effect is often viewed as similar to the hydrophobic effect. Because they provide both a microscopic view and a pathway to the calculation of standard thermodynamic quantities, molecular dynamics simulations and theory offer the opportunity to observe and quantify these effects for aqueous peptide solutions of different ions.



B. Montgomery Pettitt is a Professor of Chemistry and Computer Science at the University of Houston. He received all three of his degrees (B.S. in Chemistry, B.S. in Mathematics, and Ph.D. in Chemistry) from the University of Houston. He was a postdoctoral fellow at the University of Texas at Austin and an NIH Fellow at Harvard University. An Alfred P. Sloan fellowship was awarded to him in 1989. His research area is theoretical chemistry.

We will review literature concerning theoretical descriptions of saline solutions around peptides and the progress made in interpreting the molecular details of experiments involving peptide systems. Material relevant to analytical theories of solution effects on salt solutions and peptides has been included for perspective even though this area is less developed than that of computer simulations. In order to review the recent theoretical and simulation literature on peptides in saline solution, it will be necessary to limit our scope. As a result, we shall concentrate on those studies which demonstrated effects due to the explicit molecularity of the models involved. Our treatment of continuum methods will be more of an overview. Readers interested in such methods will find considerably more material on them in recent papers and reviews.[26-28] In addition, we will provide some background material on pure ionic solutions, thermodynamic and kinetic experiments of relevance, as well as the theoretical tools often used to consider these systems. It is our hope that this will help to place the subject matter in a proper context. It is not our intent, however, to provide a complete review of the experimental solution literature or of ionic solution theory in general.

We have chosen to start with a sketch of the methods used, both theoretical and computer experimental. Selected material on ionic solutions is then presented. Simulations and theory for peptides in such solutions are discussed. To aid in placing this work in context, a selected segment of the experimental literature concerning the effects of salt on protein and peptide physical (thermodynamic) properties is reviewed. We end with simulations of proteins in explicit saline environments. Nucleic acid–salt solutions will not be discussed even though there is an obviously strong overlap in places. Other relevant experiments will be included where comparisons with the mentioned theoretical tools can be made.

2. Theoretical Methods

Both simulations and approximate analytical methods have an important place in the theoretical study of peptides in saline solutions. Simulations of such

systems to date are done in high excess salt concentrations (≥1 M) to avoid intractable statistical problems. Still, in such studies, one would like many times (5–10) the number of ions currently used to improve the statistical averaging and accuracy. The problem rapidly becomes insurmountably difficult as concentrations are lowered by a few orders of magnitude.

The physiologically interesting isotonic saline solutions are about 0.15 M in salt. The composition varies widely with respect to the predominant monovalent cation depending on location. Nerves and muscles may have 10 times more K^+ than Na^+ whereas the situation is reversed in blood (or sea water) where Na^+ is 40 to 50 times the concentration of K^+.[29] We will generally take Cl^- to be the relevant anion.

Reliable simulations of peptides in <0.2 M saline solution are currently not economical. No simulations with mixed salts in excess of that required for electroneutrality have yet been reported on protein/peptide systems to our knowledge. However, approximate theoretical techniques have no such limitations in the dilute solution regime. Thus theory and computer experiments currently cover a complementary range of concentrations and phenomena. Below we briefly review the older methods such as the Born, Debye–Hückel (DH) and Poisson–Boltzmann (PB) methods. This is followed by progress made in more current areas of modern solution theory. Then a brief outline of simulation methods is given. An extensive review of certain aspects of modern structural theories of the liquid state may be found in ref 30. The reader interested in more details on simulation methodology is referred to the extensive literature available.[31–34] For a useful work which clearly ties experiment to theory, ref 35 is recommended.

2.1. Early Methods

The earliest method for predicting the free energy of transfer of an ion from the gas phase to aqueous solution is due to Born.[36] The process considered was that of creating a charge in an ion-sized low-dielectric cavity surrounded by a high-dielectric (aqueous) continuum medium. The result of integrating the potential change along a path from zero to full charge is

$$\Delta G^{\text{Born}} = \left(\frac{q^2}{2a}\right)\left(\frac{1}{\epsilon_{\text{high}}} - \frac{1}{\epsilon_{\text{low}}}\right) \tag{1}$$

where q is the charge on the ion and a is the radius of the low-dielectric cavity. This equation gives many of the gross features of ionic solvation thermodynamics, including the roughly quadratic dependence of the free energy on charge (for a given size atomic cavity). Such a general result has been confirmed by both experiment and more exact theory.[37,38]

To obtain results on finite concentrations Debye and Hückel (DH) used the Poisson–Boltzmann equation in the limit of low salt concentration. Their result for the effective interaction potential between any pair of ions predicted screening by the intervening ion atmosphere is

$$w = \frac{q_1 q_2}{\epsilon r}\exp(-\kappa r) \tag{2}$$

where r is the interionic distance and κ is the usual DH inverse length parameter.

In the 1940s Mayer established this as a true limiting law and showed that it was the lowest order term in an infinite series representation of the effective interactions in ionic solution.[39] It is possible to show the universality of this limiting law for both continuum and molecular solvents.[40]

Poisson–Boltzmann (PB) methods were also used by Kirkwood to explain the titration curves of small zwitterionic peptides and organic acids represented as either spheres or ellipsoids of low dielectric.[41–43] This was extended to protein titration curves in the 1950s[44,45] with some (marginal) success. More recently numerical solutions to the PB equation for arbitrary shapes have become routine.[46,47] Those calculations have established the importance of using a realistically shaped low-dielectric cavity to improve agreement with experiment.[46] Such methods are, however, limited by a lack of ability to be systematically corrected to arbitrary precision. Details dependent on phenomena such as explicit salt or solvent associations or the size and shape fluctuations of the peptide/protein solute itself are clearly lost.

Starting from the partition function, rather than an ansatz differential equation, Mayer was able to define a hierarchy of approximations for solution properties based on interactions and the n-body distribution functions that characterize the system.[48] Recently, the resulting equations are most often cast in the form of integral or integro-differential equations for the density distributions or correlations in the systems.[30,49] Below, we briefly review recent progress with these methods, moving toward theories capable of describing peptides in saline solutions.

Integral equation methods have as their fundamental goal the calculation of the n-body distributions. For practical reasons, however, they have most often concentrated on systems where only pair interactions (real and effective) are considered, and for which the pair distribution function,[49]

$$g(12) = \frac{\rho^{-2}N(N-1)\int \exp[\sum_{i<j} -\beta u(ij)]\, dX_3 dX_4 \ldots dX_N}{\int \exp[\sum_{i<j} -\beta u(ij)]\, dX_1 dX_2 \ldots dX_N} \tag{3}$$

is of fundamental importance. Here $\beta = (k_B T)^{-1}$, k_B is the Boltzmann constant, T the absolute temperature, $\rho = N/V$ is the number density, V the volume of the system, and $g(12) = g(X_1,X_2)$ where X_n denotes the complete set of configurational variables for particle n. Distribution functions obtained from integral equation theories can, in principle, yield all thermodynamic equilibrium properties for the solution of interest.

Since the integral in eq 3 is analytically intractable, other techniques must be used. Simulation methods which give an approximate sampling of the intergrand, and therefore an approximation to the integrals, will be covered later. Another, somewhat more indirect method is often used involving the Ornstein–Zernike integral equation[49]

$$h(12) = c(12) + \frac{\rho}{8\pi^2}\int h(13)c(32)\, dX_3 \tag{4}$$

(or some closely related expression) in terms of $h(12)$

$= g(12) - 1$, the total pair correlation function, and $c(12)$, the direct correlation function. Equation 4 can be viewed as a defining relation for $c(12)$. Alternatively, $c(12)$ can be defined as the appropriate functional derivative of the partition function and eq 4 readily derived.[49]

A second expression relating $c(12)$ and $h(12)$ is now needed to close the system of equations. A formally exact closure can be written as

$$c(12) = h(12) - \ln\{g(12)\} - \beta u(12) + b(12) \quad (5)$$

but $b(12)$, the so called bridge function, is not easily expressed in terms of $u(12)$ the pair potential, $h(12)$ and $c(12)$. The formal inexactness of all integral equation approaches can be seen as arising from approximating $b(12)$. The simplest and most commonly used approximations are the hypernetted-chain (HNC) approximation, which sets $b(12) = 0$ to give

$$c(12) = h(12) - \ln\{g(12)\} - \beta u(12) \quad (6)$$

and the Percus–Yevick (PY) approximation, which further linearizes eq 6 with respect to $h(12) - c(12)$,

$$c(12) = \{1 - \exp[\beta u(12)]\}g(12) \quad (7)$$

Unfortunately, none of these expressions has a perturbative derivation. The terms that are omitted are done so without proper justification (other than convenience), and the results from such theories must be validated *post facto*, most often by comparison with a simulation employing the same Hamiltonian.

Much work has gone into the search for a better closure relation. The HNC and PY closures have been amended,[50,51] and other physically based closures have been proposed.[52] Phenomenological functional forms for $b(12)$ which force thermodynamic consistency or provide results for a given system which match simulation correlation functions have been suggested.[53–57] Unfortunately, the most physically appealing or formally reasonable ideas often give less satisfactory results, and phenomenological approaches tend to be only useful for a narrow range of models.

The application of integral equation theories to finite concentration salt solutions, possibly including peptide molecules, requires the extension of eqs 4–7 to multicomponent systems, where any given component may be comprised of many sites, or may include angular dependent potentials and correlation functions. While these extensions may present computational difficulties, the fundamental obstacle in obtaining meaningful results using any integral equation technique is simply the essential inexactness of all presently known formulations, and thus the quality of all results must be evaluated carefully. Since many of the properties that the correct distribution functions for ionic systems should exhibit can be gleaned from analysis of asymptotic behavior arising from the inclusion of a Coulomb potential, many shortcomings of available theories can be easily demonstrated.[53–69]

The extension of simple liquid theories to models of peptide molecules in the presence of aqueous electrolyte solutions is the main topic of discussion here, so it is with the point of view of usefulness to this task that presently existing theories will be evaluated. As yet, no completely satisfactory approach exists, but recent advances indicate that such systems are within reach.[70,71] We begin by looking at results from the most developed approaches to simple aqueous electrolyte systems.

2.2. Long-Ranged Forces in Integral Equations

In order to study saline solutions with these methods, the problem of the divergence of the integrals arising from using eqs 4–7 with a Coulombic potential must be dealt with. The subtle screening and the specific associations that develop in the ion–solvent atmosphere around a peptide or protein depend intimately on the balance and cancellation of the Coulomb forces. The screening involves not only dielectric shielding but Debye screening and higher order phenomena.

Perhaps the simplest extension of liquid theory to electrolytes is the so-called primitive model: a neutral system of charged particles with the Coulomb interaction potential, and a short-ranged hard-sphere repulsion to prevent catastrophic overlap. For such a system the Percus–Yevick or HNC closure must be amended so the direct correlation function exhibits the correct asymptotic behavior, $c(12) \rightarrow -\beta u(12)$, as the interparticle separation r_{12} gets large. The Percus–Yevick equation is consistently modified by the form suggested by Allnatt[72]

$$c_s(r_{12}) = \exp[-\beta u_s(r_{12})](1 + \tau(r_{12}) + Q(r_{12})) - \tau(r_{12}) - Q(r_{12}) - 1 \quad (8)$$

where, if we define $\phi(r_{12}) = -\beta q_1 q_2 / r_{12}$ with q_1 the charge on ion 1, we then can write $-\beta u_s(r_{12}) = -\beta u(r_{12}) + \phi(r_{12})$, $c_s(r_{12}) = c(r_{12}) + \phi(r_{12})$, $\tau(r_{12}) = h(r_{12}) - c_s(r_{12})$, and we have assumed all functions to be dependent only on r_{12}. We have also used the chain sum of $\phi(r_{12})$ on ρ, $Q(r_{12}) = \mathcal{C}[\phi(r_{12})|\rho]$ as defined in ref 73. It is in this term, which contains both the original bare divergence, $\phi(r_{12})$, as well as the infinite series of terms which give rise to convergence (and Debye–Hückel behavior) that the renormalization or resummation occurs. Careful, direct numerical subtraction of the divergences has also been used in various numerical schemes, especially in the context of angular expansion integral equations[30] (see below).

This modification applied to the HNC closure appears to be a better choice than the Allnatt modified P–Y closure,[74] although over certain regions of 1–1 electrolyte density,[75] and over larger density regions for ions with higher charges,[52] unphysical short-range interionic distributions are obtained with the modified HNC equation.

We will not review the analytically tractable mean spherical approximation (MSA)-like closures[76] due to their generally poor qualitative behavior. The MSA methods do find use in a variety of applications, however.[35] For peptide and protein systems, comparisons of the average positions of ions near given sites on such large molecules are experimentally interesting, and the use of hard sphere models (apart from being unphysical) will in general cause the distance of closest approach of ions to each other, and to other species, to be chosen rather than determined. For this reason parameterized potentials, such as the Lennard–Jones 6–12 potential, are usually used. There is however a general problem which is unavoidable in the use of soft spheres in continuum solvent models. Because the Coulomb contribution to the direct force between two

ions is input as $q_1q_2/\epsilon r_{12}$ rather than q_1q_2/r_{12}, with ϵ being the dielectric constant of the continuum, the distance of closest approach can be significantly underestimated for cation–anion interactions and overestimated with like-charge interactions.[70,71] Also, when the structural stability of proteins is of crucial importance, it is unlikely that useful results with salts can be obtained without explicit solvent molecules to form the kind of highly structured regions known to occur in the interfacial region around both biological molecules[77] and large nonbiological "structures" such as those used to model surfaces or macroions.[78,79]

2.3. Angular-Dependent Molecular Integral Equation Theories

The inclusion of explicit solvent necessitates the introduction of a dipolar species and with it an angular dependent molecular potential and distribution functions. If a single molecular center is maintained in order to carry over important formalisms from simple fluids, it becomes necessary to expand the angular distributions in an angular basis set.[30] Of great utility are the rotational invariants $\Phi_{\mu\nu}^{mnl}(\underline{\Omega}_1,\underline{\Omega}_2,\underline{\hat{r}}_{12})$ of Blum and Torruella,[80-82] which are an orthogonal basis set spanning the space of Euler angles of particles 1 and 2 and of the interparticle vector, $\underline{\Omega}_1$, $\underline{\Omega}_2$, and $\underline{\hat{r}}_{12}$, respectively. All pair functions can be written as expansions in this basis set, for example,

$$h(12) = \sum_{\substack{mn \\ \mu\nu}} h_{\mu\nu}^{mnl}(r_{12})\Phi_{\mu\nu}^{mnl}(\underline{\Omega}_1,\underline{\Omega}_2,\underline{\hat{r}}_{12}) \qquad (9)$$

Because of their many useful mathematical properties,[83] particularly under Fourier transformation, it is possible to write the equations of interest in terms of the radially dependent coefficient vectors only, albeit with complicated combinatorial scalar coefficients.[80,84] The basis set is infinite, so approximations in terms of algebraically closed subsets must be used. While the HNC theory was the theory of choice for the primitive model of electrolyte solutions, the difficulty in taking the logarithm of an angular expansion (see eq 6) when applying any useful water-like model necessitated the use of further simplifications such as the MSA[76] and the truncated log expansions, the linearized HNC (LHNC)[85] and quadratic HNC (QHNC).[86]

The important work of Fries and Patey[84] showed a surprisingly simple solution to this formal problem. By taking the derivative of the HNC equation with respect to the interparticle distance and then integrating the result, the log term is replaced by the integral over sums of products of r_{12}-dependent coefficients, so more accurate large basis set solutions to the HNC theory for more complicated models became possible. With the further use of bridge functions from a related short-range spherical solvent model, the RHNC theory[50] has been used to demonstrate many important results, and the work of Patey and others[66,67,79] has shown to be one of the most satisfactory water–electrolyte theories to date. The extension of Kirkwood–Buff (KB) theory[87] to electrolytes[88] created a convenient route to thermodynamic quantities using distribution functions.

Unfortunately, while single center theories extend easily in a formal sense to amino acid systems, the practical problems inherent in the far from spherical symmetries involved are essentially computationally insurmountable. The number of basis functions used to obtain a clear structural picture of water, itself of close to spherical symmetry, is in the hundreds.[66] The number needed to accurately represent the correlations in a system containing water, a few ions and a single relatively small molecule such as a monopeptide would quickly lose the vast computational advantage over a simulation of the same system. Fortunately, the link to thermodynamics via KB theory is completely general and useful for all distribution function theories, and the results of many quantities serve as a test of the quality of results from other more adaptable approaches.

2.4. Site–Site Molecular Theories

Theories based on spherically symmetric atomic sites or interaction site models are, by contrast, not explicitly dependent in any computationally restricting way on the symmetry of the conformation in which the sites are associated. Each (usually atom based) site–site interaction is taken to be dependent on site separation only. Further, they are more easily related to results of scattering or diffraction experiments than single-center models.

However, well-known formal difficulties arise in the juxtaposition of bonding and nonbonding potentials, or inter- and intramolecular forces.[89] However, to the extent that the entire electronic Born–Oppenheimer surface may not be relevant to the structural or thermodynamic questions at hand, one can choose a model which reasonably characterizes the important features of the system without referencing the troublesome areas. One method is to simply add restricted models of bonding and nonbonding potentials and solve with the same equations as for simple liquids.[90-94] Potentials must be judiciously chosen to permit exactly the correct number of atoms (stoichiometry), on average, to be found at the desired bonding positions. For water this presents the problem of having to "tune" the functional forms of the potentials with somewhat artificial characteristics in order to achieve the bent shape of the molecule—a symptom which can apparently only be alleviated when three body forces are included in the model.

One example of this approach is the so-called central-force model[90,92] which gives results that are only of high precision when good approximations for the missing bridge functions are included. Precision of various integral equations aside, the accuracy of this model is less satisfactory than the popular molecular mechanics functions in common use.[95] This approach does have definite advantages with integral equations, however, such as a predictive and presumably reasonable dielectric constant. Also, since bonds are neither rigid nor permanent, the concept of pH can be examined from a statistical mechanical point of view.[96] Once again, however, the extension to peptide systems is difficult, primarily because the creation and adjustment of the potential functions necessary to consider a large molecule of unspecified symmetry would be a difficult problem.

The most easily utilized class of theoretical approach is that where the Ornstein–Zernike equation is extended to a site–site form, such as the reference interaction site

model (RISM) equation,[97] where the relationship between the site–site pair correlation functions $h_{ij}(r)$ and direct correlation functions $c_{ij}(r)$ can be thought of as defined by the RISM equation, which can be written in fourier space in matrix form as

$$\rho\tilde{\mathbf{h}}\rho = \tilde{\omega}\tilde{\mathbf{c}}\tilde{\omega} + \tilde{\omega}\tilde{\mathbf{c}}\rho\tilde{\mathbf{h}}\rho \quad (10)$$

The (i,j) element of the matrix $\tilde{\mathbf{h}}$ is given by,

$$\tilde{h}_{ij}(k) = 4\pi \int_0^\infty r^2 h_{ij}(r) j_0(kr)\, dr \quad (11)$$

where $\tilde{\mathbf{c}}$ is constructed in a similar way and $j_0(kr)$ is the zeroth order spherical Bessel function $\sin(kr)/kr$. The intramolecular correlation matrix $\tilde{\omega}$ is usually taken to have a form related to completely rigid molecules. In this case the distance between atoms and therefore Boltzmann distribution function for the intramolecular pairs is a δ function in real space whose elements in Fourier space are $\tilde{\omega}_{ij}(k) = x_i\rho_T j_0(kd_{ij})$ where d_{ij} is the (positive, fixed) distance between site i and site j within the same molecule, and when $d_{ij} = 0$, $\tilde{\omega}_{ij}(k) = 1$. Both the density matrix ρ and ω contain a scalar ρ_T, the total *molecular* number density. In the case of ρ the scalar density is multiplied by a diagonal matrix whose (i,i)th term is the mole fraction, x_i, of the molecule in which the ith site is situated. These matrices can easily be extended to represent any mixture of single- and multisite molecules by adding one to the dimension of each matrix for each additional type of site, the only difference being that $\tilde{\omega}_{ij}(k) = 0$ if site i and site j are in different species. Formally the approach has a set of terms in the density expansion which are unphysical.[63,68,69,98]

This formalism was extended (XRISM) to polar systems by renormalization[99] but is not without its drawbacks which have been examined by several authors.[63,65,68-71] Nevertheless, the similarity to simulation results in the short-range structure of distribution functions for many model systems,[64] and the reasonably accurate energetics obtainable[37,38,58] have stimulated a fair-sized body of work. The diagrammatic inconsistencies underlying the equations have been examined and similar theories developed which are on the same (correct) diagrammatic level as the better known simple fluid HNC and PY theories,[98,100] but unfortunately the results are significantly inferior to the RISM/XRISM results, presumably due to some fortuitous cancellation of errors in the latter.

One unacceptable problem with XRISM results for polar salt solutions is the trivial (incorrect) dielectric constant which they predict. While this has been shown to be easily corrected for pure solvents and for salt solutions in the limit of infinite dilution by a scaling of the Coulombic potential term,[69] this scaling method cannot be extended to finite concentration salts because the various expression for the dielectric constant are inconsistent with each other. Recently, however, a way of correcting the XRISM theory by the addition of an expression which partially corrects for the missing correlations (via a bridge function) has been introduced[70,71] which yields both consistent dielectric properties and does not appreciably change the reasonably good short range structure of the XRISM site–site distribution functions. This theory (DRISM) yields reasonable energetics and osmotic pressures for 1–1 salts up to concentrations of a few molar. This theory has also yielded insights on the common ion effect[101] and the molecular origins of solubility limits.[102]

2.5. Intramolecular Problems: Peptide Systems

The only integral equation applications to date to systems of peptides and nucleic acids in explicit water (i.e. noncontinuum) have not included explicit salt ions, but such work is underway currently. Related simulation studies have primarily concentrated on intermolecular distribution functions and their associated thermodynamics in solution. All the intermolecular theories presented above dealt with pair distributions of atoms or molecules. In order to deal with intramolecular degrees of freedom, especially the thermally malleable torsions, the entire n-body distribution function for the atoms of the solute must be approximated or dealt with in some manner.[103]

Biologically interesting molecular systems are often intrinsically complicated. The effective interactions between even two nonreactive rare gas atoms change remarkably in condensed fluid phases such as the liquid state. For highly associated liquids such as water, this change is even more pronounced. In condensed phases for complex solutes one must consider the interaction energies as mediated by the solvent.[89] These effective interactions depend on the free energy of the solvent as well as the configuration of the solute and are called potentials of mean force. The potential of mean force, $W(r)$, gives the free energy or reversible work necessary to take a particle or set of particles from an infinite separation to a given distance, r. The intramolecular PMF for the entire molecule is represented by $W(1_m)$ where 1_m is the entire set of coordinates for the molecule.

One of the most familiar, manifest effects of these solvent-mediated potentials is the reduction of interaction energy of two charges separated by a large distance in solvent.[104] As discussed above, this macroscopic dielectric screening is related to several structural features of the intervening solvent.[105,106] There are other microscopic consequences for conformations as well as the intermolecular interactions. These typically manifest themselves in specific (and nonspecific) associations and solvophobic effects.

Given the pair correlations between solvent and an n-body solute from eqs 6 and 10, it can be demonstrated[107] that the free energy change on solvation is simply

$$\frac{-\beta}{N}\Delta A = \rho\sum_{ij}\int c_{ij}(r) + \frac{1}{2}h_{ij}(r)^2\, dr - \frac{1}{2}\mathbf{c} * \mathbf{X} * \mathbf{c} \quad (12)$$

where $\mathbf{X}$ is the total solvent density pair correlation function which consists of the sum of the inter- and intramolecular pair correlations of the solvent. In fact it may be shown that this equation without the $h_{ij}(r)^2$ term is equivalent to a gaussian field theory[107] of the free energy change. This equation requires the use of a new solution of eqs 6 and 10 for each molecular geometry to be considered which can be cumbersome for large solutes or for tagging more than one degree of freedom. We can approximate the n-body distribution via a superposition approximation at the pair level in the spirit of the Kirkwood approximation to the triplet distribution function.

The intramolecular PMF for the entire molecule, represented by $W(1_m)$ may be obtained within a

superposition approximation[108,109] as

$$W(1_m) = \sum_{\alpha\gamma} \Delta W_{\alpha\gamma}(r_{\alpha\gamma}) + E(1_m) \qquad (13)$$

where 1_m is the entire set of coordinates for the molecule and $E(1_m)$ is the intramolecular potential (molecular mechanics) energy. $\Delta W_{\alpha\gamma}(r_{\alpha\gamma})$ is frequently called the cavity potential for sites or atoms α and γ at a distance $r_{\alpha\gamma}$ at infinite dilution. $W(1_m)$ can, thus, be interpreted as the coordinate dependent Helmholtz free energy for the molecule in solvent environment. The superposition approximation takes the solvent modification as acting only upon distant pairs of free atoms and then superimposes them to build a molecule in solvent.

Knowing $\Delta W_{\alpha\gamma}(r)$ from the solution to eq 13, a matrix table may be constructed consisting of all different nonbonded pair combinations of atom types α and γ on a distance grid. An effective potential surface for the peptide in aqueous solution is then constructed by adding the solvent cavity potential to an existing vacuum molecular mechanics potential surface. Once the free energy function is assembled, we may optimize the geometry of the system with respect to the free energy. This technique is referred to as free energy molecular mechanics. It, like all molecular mechanics approaches, assumes that the degrees of freedom of interest can be partially decoupled from other degrees of freedom by placing the rest of the molecule at its local minimum. The difference is that in the case of free energy molecular mechanics, a solvent probability distribution modifies the local energetic surface corresponding to the solvation shells of the atoms involved.

Stochastic dynamics in the form of Langevin dynamics may be used to model the kinetic effects on such a free energy surface. Langevin dynamics on a potential of mean force energy surface has recently been used to model the effects of aqueous solvent on the structure and dynamics of the alanine dipeptide.[110] Using such a method has many inherent approximations. The most severe approximations are: (1) not using a frequency-dependent friction and (2) approximating the solute n-point internal distribution function by superposition of pair free energy terms. Approximation 1 gives a flat (white noise) force–force autocorrelation function which means one can drive (and damp) the system at some frequencies not necessarily accessible to the physical system we wish to model. In making the second approximation, one assumes that the solvent-mediated nonbonded interactions between atoms in the solute may be represented by the interaction between the same isolated atoms in a polar molecular solvent at infinite dilution.

Obviously, for molecules with a well-defined interior, such as globular proteins, this is not appropriate, but it appears to be a good approximation for a solvent-exposed system like di- or tripeptides and other small organics.[111] Clearly, the potential of mean force is a thermodynamic, equilibrium property. Thus, many individual short-time events are averaged out of the resulting solvation picture utilizing a potential of mean force and Langevin dynamics while preserving the correct long-time, equilibrium result. The applicability of the potential of mean force surface thus assumes a Born–Oppenheimer-like separation of time scales between rapid internal and solvent motions *versus* the slower transitions that are being studied. It is likely that the present scheme will be a useful approximation for many problems including other small molecules and peptides.

2.6. Molecular Dynamics Computer Experiments

Computer experiments, while computationally tedious, provide one of the most accepted routes to connect the microscopic model with the macroscopic behavior. We will concentrate on molecular dynamics rather than Monte Carlo techniques due to the latter's far less frequent use with peptide/protein systems.[33]

With recent advances in the techniques of computer simulations, it is now possible to perform molecular dynamics simulations in each of the six main ensembles: *NVE*, *NVT*, *NPH*, *NPT*, μVL, and μVT (N = number of particles, V = volume, E = total energy, T = temperature, P = pressure, H = enthalpy content, μ = chemical potential, and L = Hill energy) and for many other systems where couplings to nonequilibrium baths or representations of electronic degrees of freedom are possible. This has been accomplished in large part by the extended system approach first proposed by Andersen,[112] where extra "extension" variables are introduced which couple the physical system to either a heat, pressure, chemical potential, or other forms of a surrounding bath.

We present the equations of motion which describe the simplest system to simulate: one with a constant number of particles, at a constant volume and with a constant total energy. While the use of extended system dynamics is having an important impact on this area, it is beyond our present scope. Surveys of the area of computer simulation methods exist[31-34] and should be referred to by the serious worker in this area.

In most common molecular dynamics simulations, a system of N atoms is described by the generalized coordinates and momenta of the atoms (or sites) $\{q,p\}$, and a classical Hamiltonian, $\mathcal{H}$, composed of kinetic, $\mathcal{K}$, and potential, $\mathcal{V}$, contributions

$$\mathcal{H} = \mathcal{K}(\{p\}) + \mathcal{V}(\{q\}) = \frac{1}{2}\sum_i \frac{p_i p_i}{m_i} + \mathcal{V}(\{q\}) \qquad (14)$$

where m_i, q_i, and p_i are the mass, position, and momentum of atom i, respectively. From this Hamiltonian the Newtonian equations of motion are readily derived.

$$m_i \ddot{q}_i = -\frac{\partial \mathcal{V}(\{q\})}{\partial q_i} \qquad (15)$$

where $\ddot{q}_i$ is the acceleration of particle i. In addition to the Hamiltonian or total energy, the conserved quantities are the total linear momentum and the total angular momentum. In practice the total angular momentum is not conserved if periodic boundary conditions are used; however, the time average of the components of the total angular momentum should be zero[31] for cubic or rectilinear samples. This is not the case for more exotic boundary conditions based on rotational groups rather than simple space groups.[113]

In order to handle the problem of long-ranged forces in a simulation, a number of approaches have been proposed. The use of interaction truncation methods is commonly used to model the expected reduction and therefore importance of interatomic forces at a large

distance.[114] This approximation results in far less costly equations of motion to integrate. For dipolar systems this seems to be a good qualitative approximation and leads to few artifacts. For ionic systems and systems involving charged peptides or protein and ions even the qualitative behavior can be drastically affected by such truncations.[115,116] To alleviate this, one method commonly used is that of lattice sums or Ewald sums.[31]

The Ewald potential[117] for a system of N atoms each carrying a charge, q, in a box of length L, surrounded by a medium of dielectric, ϵ', is given by[118]

$$V(r,\epsilon') = \sum_{1\le i<j\le N} q_i q_j \psi(r_{ij}) + \frac{\xi}{2}\sum_{i=1}^{N} q_i^2 + \frac{2\pi}{(2\epsilon'+1)L^3}\left|\sum_{i=1}^{N} q_i \mathbf{r}_i\right|^2 \quad (16)$$

where

$$\psi(r) = \sum_{|\mathbf{n}|=0}^{\infty} \frac{\operatorname{erfc}(\kappa|\mathbf{r}+\mathbf{n}L|)}{|\mathbf{r}+\mathbf{n}L|} + \frac{1}{\pi L}\sum_{|\mathbf{n}|\neq 0}^{\infty} \frac{1}{|\mathbf{n}|^2}\exp\left(\frac{2\pi i}{L}\mathbf{n}\cdot\mathbf{r} - \frac{\pi^2|\mathbf{n}|^2}{\kappa^2 L^2}\right) \quad (17)$$

with

$$\xi = \sum_{|\mathbf{n}|\neq 0}^{\infty}\left[\frac{\operatorname{erfc}(\kappa|\mathbf{n}L|)}{|\mathbf{n}L|} + \frac{1}{\pi L|\mathbf{n}|^2}\exp\left(-\frac{\pi^2|\mathbf{n}|^2}{\kappa^2 L^2}\right)\right] - \frac{2\kappa}{\sqrt{\pi}} \quad (18)$$

and

$$\operatorname{erfc}(\mathbf{x}) = 1 - \frac{2}{\sqrt{\pi}}\int_0^x \exp(-t^2)\,dt \quad (19)$$

The sum over the lattice vectors, $\mathbf{n}_x$, $\mathbf{n}_y$, and $\mathbf{n}_z$ is performed for all $|\mathbf{n}| \le$ a small integer usually between 4 and 10 which produces a roughly spherical arrangement of images layered around the central box. The value of κ which produces converged results depends on the number of lattice vectors taken. In addition, there is the choice of the dielectric response of the medium which surrounds the set of images which is specified in ϵ'. Common choices of ϵ' are the dielectric constant of the system (if known), unity, or ∞ (so called "tin foil" boundary conditions).

Ewald sums are certainly not artifact free, but they do have the advantage of producing a smooth force at all distances.[31,116] Other reaction field-based methods also exist and may be used (with some care) for these systems. This is an active area of current research.

3. Results

We now review the results relevant to the focus of this article. For background we have chosen to include a brief survey of salt water results. This, we hope, will give the reader a basis for comparison of the behavior of salt and solvent with and without the presence of peptides and proteins.

3.1. Integral Equations Applied to Water–Electrolyte Systems

3.1.1. Continuum Solvents

The early works utilizing integral equations applied to realistic models of a 1–1 electrolyte such as the alkali halides[119,120] produced important insights into the relation between the interionic correlations and thermodynamic properties such as the osmotic pressure and mean activity coefficients. These early calculations used primitive or decorated primitive models. Thus, only a simple dielectric was used to account for solvent effects. The only explicit effect due to solvation shell overlaps were included phenomenologically via the so-called Gurney cosphere overlap terms in the input potential surfaces.[120,121] In these results no oscillations in the resultant potentials of mean force are seen until the density of ions is sufficient to produce mild density waves in the system. This will be contrasted with molecular theories below.

While the results utilizing the HNC integral equations with a continuum solvent dielectric for monatomic ions are reasonable up to around 1 M in concentration,[121] there is a formal problem associated with the neglect of the dielectric constant on the interior of the solute.[122] When the size of the solute becomes large enough so that it is reasonable to speak of an interior and a solvent-exposed exterior, this becomes a serious problem affecting the computed properties. This is clearly the case for a macromolecule such as a protein. The extent to which this matters for smaller solutes depends on the accuracy of the desired results (see below).

3.1.2. Molecular Solvents

Molecular solvents produce qualitatively different interionic correlations. This is confirmed using both angular expansion methods,[66,67,123] and site–site techniques[58] for a model system of 1–1 electrolytes resembling the alkali halides in explicit water. The observed interionic correlations, even at infinite dilution,[58,67] contain oscillations related to the structure of surrounding and intervening water molecules. The trends observed in a related series of alkali halide potentials of mean force and the relative sizes of the solvent-separated free energy minima and contact minima are reasonable. Selected results have been qualitatively confirmed by simulations employing umbrella sampling[124–126] or other free energy techniques.[127,128] That such nontrivial correlations exist and are related to solvent structure is now widely accepted. The magnitudes, however, are still in question.

Sensitivity to both solvent and ion model parameters has been demonstrated.[129] Ionic mobilities have been calculated for these systems using a variety of model potential of mean force results.[130,131] In particular in the case of certain like ion pairs such as Cl^-–Cl^-, it was found that the concentration dependence of the distinct diffusion coefficients could not be fit with oscillations as large as those predicted by simulation and integral equation theories for certain nonpolarizable ion–water potential models.

This observed sensitivity to models is both exciting and frustrating for the workers in this field. On one hand the sensitivity means that it may be possible to extend these techniques to larger systems such as peptides and proteins and obtain results that match experiment and thus have predictive value. Conversely, such sensitivity may be pathological and mean that the current level of mean field models in common use can never be trusted to give more than qualitative agreement

with experiment, especially when used on large intrinsically complicated solutes.

3.2. Simulations of Water–Electrolyte Systems

The earliest simulation studies of ionic solutions were, much as the early theoretical results given above, performed in a dielectric continuum model of water (no explicit solvent molecules) and many excellent reviews (such as ref 132) to that literature are available. These primitive models will not be discussed further.

3.2.1. Structure

While Monte Carlo and molecular dynamics computer simulations have been employed in many informative studies of ions in explicit water[12,133–136] most of these have involved isolated ions in water. Structural correlations obtained from these studies have allowed the determination of interaction energies between ions and water. Such techniques are useful in testing and tuning parameters against experiment[35] (given the caveats mentioned above).

Molecular dynamics (MD) simulation has been used to investigate the structure-making and structure-breaking capability of various ions. A number of early attempts were made on various ionic systems, testing different technological (methodological) approaches.[137] One very early attempt quantified the negative hydration effect for a model system.[138] While some concentrated solutions have been studied early on,[137,139,140] most simulations were conducted in a manner to approximate infinite dilution.

The advent of improved parameters and methods, as compared to those available for use in the earliest explicit water studies, have allowed ionic simulations to consider subtle chemical effects in a periodic series. A series of MD calculations for systems composed of $[M^+]_{aq}$ and $[X^-]_{aq}$ where M^+ was Li^+, Na^+, and K^+ and X^- was F^- and Cl^- investigated the mobility and hydration of ions in solution.[23,141] The systems were composed of either 64 or 125 water molecules and one $[M^+]$ or $[X^-]$ ion. Infinite dilution was approximated by not allowing the ion to interact with its images via the usual periodic boundary conditions. The water molecules were treated as rigid bodies. All electrostatic interactions were calculated using the Ewald procedure[142,143] (see above). As previously mentioned, this method avoids the problems associated with the use of electrostatic cutoffs.[132,144–146] Structural data obtained included radial distribution functions, coordination numbers, and ion–water geometries. Individual radial distribution functions for ion–water oxygen and ion–water hydrogen were analyzed in terms of an idealized model proposed previously.[3–6] Simple ion size based trends reflected the available experimental data.

The radial distribution functions, $g(r)$, for the cations were characterized by a large first peak in $g(r)$ for cation–water oxygen. The height of this ion–water peak decreased in size from Li^+ to Na^+ to K^+. This result was also noted in the integral equation studies mentioned above.[58,66,67,123] This same trend was followed by the second peak (solvation shell) as well. It is worth noting that the cation–water oxygen $g(r)$ fell to nearly zero after the first peak for both Li^+ and Na^+, indicating that the first solvation shell was very tightly bound to the ion. This minimum was much shallower for K^+. This is consistent with the picture that smaller ions are better structure makers. For the anions, the main peak in the $g(r)$ for anion–water hydrogen was of approximately the same height. Two closely spaced peaks were interpreted as representing two nonequivalent hydrogen atoms, supporting a configuration where one water hydrogen points, on average, nearly directly at the ion and the other points toward the bulk. The coordination number, defined as the mean number of water molecules in the first coordination sphere, was roughly octahedral for the smaller ions Li^+, Na^+, and F^-. The coordination number for the larger ions, K^+ and Cl^-, was somewhat larger, as might be expected,[12] but has been shown to vary with method and model.[38,58] Some experimental data has suggested an octahedral first shell for Cl^- in concentrated solutions, however.[147]

The relationship between the radial distribution function of cation–water oxygen and that of cation–water hydrogen has been used to derive information about the strength with which the first solvation shell is held.[12,148] For instance, a firmly held solvation shell is indicated when the coordination number, found using the usual integral of $g_{cat-O}(r)$ up to the first minimum, has a value equal to half the number of hydrogens within the first peak of $g_{cat-H}(r)$. Such a stoichiometric relationship has been observed for the sodium and lithium ions. If, however, the first peak of $g_{cat-H}(r)$ integrates to more than twice that of $g_{cat-O}(r)$, as has been observed for the potassium ion, appreciable penetration by the bulk solvent is indicated. Other studies[141] have indicated that some penetration from the bulk occurs even for the smallest ions.

While most simulation studies have dealt with monovalent ions, some divalent ions have been investigated. The development of new potential surfaces for Ca^{2+}/H_2O and Ni^{2+}/H_2O has allowed the calculation of individual radial distribution functions, $g_{CaO}(r)$ and $g_{CaH}(r)$, for these systems.[141] It was found that the water around Ca^{2+} is more structured than water around Li^+. This is likely due to the fact that the larger radius is more than compensated for by the higher charge on the Ca^{2+}. The first peaks in the partial radial distribution functions, $g_{NiO}(r)$ and $g_{NiH}(r)$, are higher and narrower than in the calcium case, indicating a more stable solvation shell. It has been asserted that this could be expected on the grounds of ligand field stabilization and, more simply, electrostatics, due to the deeper well in the Ni^{2+}/H_2O potential. While the comparisons with experiment for these systems are not perfect, such models may be useful as "working models" as the techniques are refined. Microsecond exchange times for inner sphere water molecules make the equilibration of simulations with divalent cations problematic and thus aspects of the results obtained so far are suspect. This is especially unfortunate given the role that certain divalents play in peptide and protein systems.[149]

The structure of water around ions, represented by ion–solvent correlation functions, is more readily obtained from experiments than are the ion–ion correlations. This is primarily a concentration/detection problem for the diffraction experiments. Nonetheless information on all the partial contributions to the experimentally observed structure factor are available for some ionic solutions.[3–6]

Below we will contrast this behavior with that seen in the three-component peptide–salt–water systems. Some sharp differences will be found in comparing radial distribution functions and coordination numbers. The effects of other strongly polar molecules on the properties of salt water is profound. The effect of salt water on such molecules is found to be equally striking.

3.2.2. Simulated Dynamics of Ionic Solutions

Dynamical data obtainable from MD simulations includes self-diffusion coefficients and velocity and orientational correlation functions. Aspects of these functions can be obtained through various spectroscopic techniques. The relationship between experimentally observed features and computationally derived correlation functions has been investigated and reviewed elsewhere.[148,150,151]

The self-diffusion constant is related to the temperature and viscosity of the solution and the effective radius of the molecule or ion (see ref 21). A clear trend of increasing self-diffusion coefficient with increasing ion size has been simulated[23] in accordance with what is observed experimentally.[152] Unfortunately, in studies involving only one ion, sufficient statistics are difficult to obtain, and the results employing interaction truncation schemes have been shown to provide an underestimate of the actual number for a particular model.[153] In order to avoid these difficulties, a method has been proposed[23] in which the diffusive behavior of the ion is explored in terms of a solution to a Langevin equation[49] where the frictional coefficient is represented by the result for hydrodynamic friction on an oscillating sphere in a medium with nonzero viscosity.[154] While some problems with this method exist,[23] the statistical difficulties are avoided.

In addition, this view creates an explicit connection between the hydration of the ion and its mobility in solution.[131] For instance, the relatively low value previously found for Li^+ [23] is consistent with the effective radius of the ion being larger than that of the bare ion. It appears that strongly hydrated ions, such as Li^+, behave, loosely, as "solventbergs",[23] diffusing over many solvent diameters with a virtually intact first coordination shell. This further supports the evidence that small ions tend to behave as structure makers.

While self-diffusion constants provide a link between the hydration and mobility of an ion, microscopic details of the dynamic environment of the ion are gained through orientational and velocity correlation functions.[130,131] These functions are also related to experimentally obtained NMR spectra.[150] Both the velocity and orientational correlation functions for the alkali halides have been calculated[23,148] and have proven to be similar to those of pure water. This compares favorably with experimental data for alkali halide solutions.[2,155,156] One interesting feature[23] is that the larger ions, such as K^+, F^-, and Cl^-, exhibited contributions from two distinct low frequencies. The smaller ions, Li^+ and Na^+, however, showed a single (damped) higher frequency, interpreted as the rattling of the ion in a "cage" of solvent molecules. The frequency found in such systems, assigned as the Einstein frequency, is characteristic of both the solute and its interactions with the solvent. This interpretation appears to have been complicated by the use of interaction truncation schemes in the early simulations. It was recently demonstrated that in the limit of infinite cutoff distance no cage frequency exists for the monovalent ions.[153]

Another property important in the study of the dynamic behavior of ions in aqueous solution is the residence time, τ_R, defined as the mean time a particular water molecule resides in the first solvation sphere of an ion. This quantity can be measured for paramagnetic ions by NMR. Because of the diamagnetic nature of solutions of alkali and halide ions, direct spectroscopic measurement is not practical at this time;[157] however, values have been inferred.[158] Residence times are readily calculated during a simulation of large monovalent monoatomic ions,[23] and the relevance of residence times to the concept of hydration is well established. Clearly, for the divalent and transition metal ions where the interactions are less clearly purely electrostatic (i.e. some covalent character exists), residence times are not easily observed in current simulations given that one needs to simulate for many times the mean exchange time in order to have reliable statistics.

The concept of hydration has been extensively explored. It has been asserted that hydration can be viewed as "persisting coordination".[23] Following the definition put forth by Bockris and Reddy,[1] this includes water molecules which are associated with the ion for times of length that allow the waters to participate in the diffusive motion of the ion. Consistent with smaller ions being better structure makers, residence times decrease with increasing ion size. Evidence suggests that temperature is also a factor. As previously mentioned, these results were incorporated into a model which relates ionic mobility to fluid viscosity, thereby linking structural data such as coordination number with ionic mobility, a dynamical quantity.[23]

The effects of temperature[23,141] and pressure[141] on hydration number have also been studied by simulation. It has been found that the first solvation shell around Cl^- is relatively insensitive to pressure, with the predominant structural change as pressure increases occurring in the second shell, as determined by the shift in location and increase in height of the second peak in $g_{ClO}(r)$. While these results can be experimentally obtained,[159] the experimental systems studied so far have been at significantly higher concentration where effects of counterions are not negligible. The coordination shell around Ca^{2+} was even less sensitive to pressure and temperature than that of Cl^-.

3.3. Integral Equation Results on Aqueous Peptide Systems

With integral equation techniques in hand it is possible to consider the effects of solvent environment on conformations of a given system of interest in a qualitative way with little computational effort. The effects of an aqueous environment on the soft conformational degrees of freedom, such as torsional motions, can be quite profound. As an example we consider the solvent modified surface for the alanine dipeptide, *N*-methylalanylacetamide. This molecule has been the subject of a number of solvation studies in the past.[160–163] Using the solvent contribution to the free energy and a molecular mechanics potential, the angles ϕ and ψ were fixed and the other degrees of freedom were allowed to relax to the energy minimum (geometry

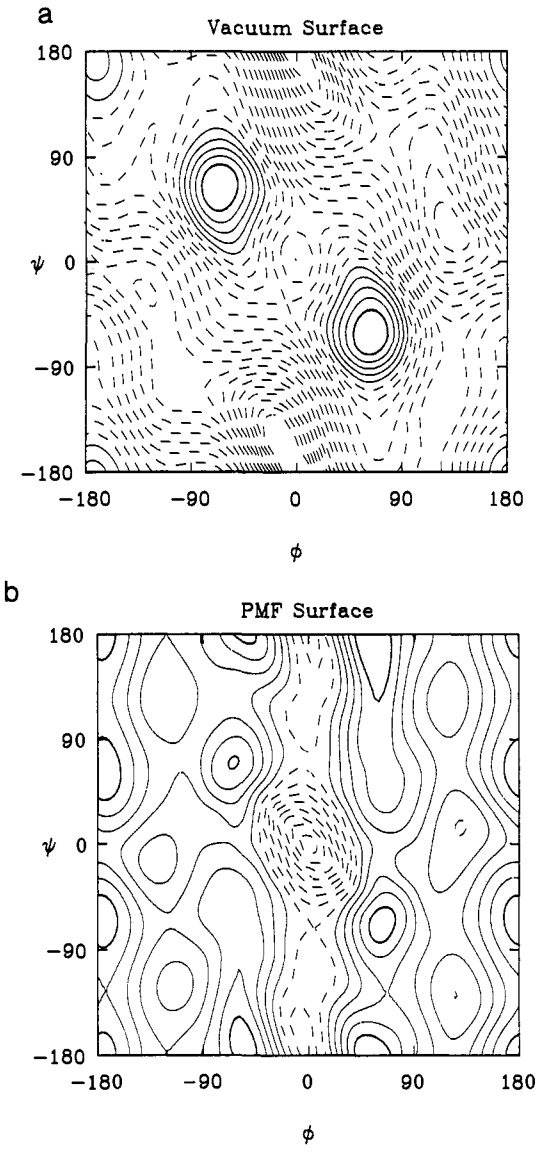


Figure 1. Comparison of the adiabatic vacuum potential surface (a) and the solvated free energy surface or potential of mean force for the ϕ–ψ dihedral angles in the alanine dipeptide in water calculated from a superposition approximation (b). Lowest contour is bold. Highest contours are dashed. Contours are spaced by 1 kcal/m.

optimized). Then the angles were changed, and the procedure was repeated until the entire torsional angle free energy surface was mapped out.

Many of the barriers between torsional states were found to be lowered, and several other low-energy wells not seen in the vacuum surface appeared. A comparison between an energy surface for the angles ϕ and ψ and the free energy surface is given in Figure 1. Considering the populations of energy states, it is obvious that many new conformations are accessible and stabilized in solution that were not even apparent in vacuum. The difference between the intramolecular potential energy and the intramolecular free energy in solution is often striking.

Studies on tripeptides,[164] and even decapeptides, are now in progress. This method should be reasonable up to around 20 or so amino acid residues. Beyond that point most polypeptides begin to have recognizable stable secondary structure. As such they begin to exclude salt and solvent from the interior, and the

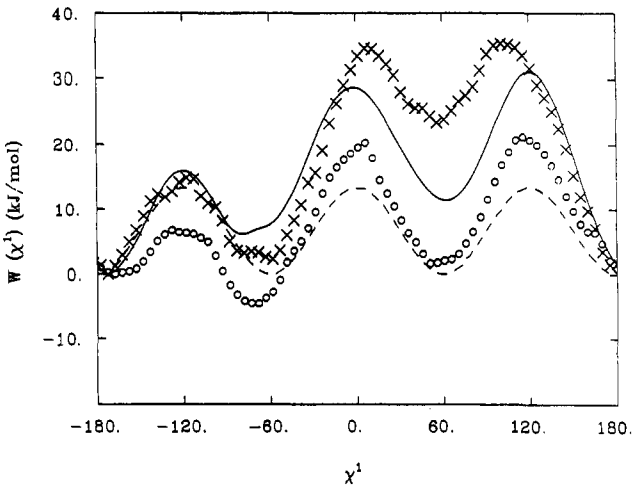


Figure 2. The calculated PMFs for the DPDPE χ^1 dihedral of tyrosine in aqueous (circles) and saline (crosses) solution, Note that the relative stability of the g(−) and t conformations are reversed in the presence of salt. The solid line is the corresponding adiabatic surface for a dielectric continuum of 80. The dashed curve is the underlying dihedral potential (taken from ref 181).

superposition approximation breaks down. In such case the nonpairwise free energy surface, eq 12, must be used with all of its attendant computational complexities.

As we have demonstrated above the packing and screening effects can be profound in a solvent-like water. Even more phenomena are possible in salt solution. In the following section, in the case of the DPDPE peptide molecule, via simulation, we show in Figure 2 that not only is there a difference for the rotameric free energy surface in changing from vacuum to water but that an equally striking change occurs with salt addition. Such studies often require 10^6–10^9 more computation time than an equivalent integral equation study. Currently, methods that seek to eliminate explicit salt and solvent molecules by use of the effective interactions via a potential of mean force are limited to solute molecules where most atoms are directly exposed to solvent[109] (i.e. the limitations of the superposition approximation). For small peptides and extended biopolymers, this does not appear to be a severe limitation.[111] In any case, for larger complexes explicit simulation of the aqueous environment is still the method of choice.

3.4. Simulations of Peptide Systems in Salt Water

As previously mentioned, understanding the behavior of salts in aqueous solution has profound importance in biological applications.[29,149] In recent years, molecular biological and biotechnological techniques have grown to make modification of the structure of proteins a realistic experimental endeavor. Information on molecular structure and behavior that can be gained from a computer simulation can potentially provide clues about which modifications in structure might produce the desired activity. Furthermore, the ability to accurately calculate thermodynamic quantities such as free energies of binding and free energies of solvation can lead to information vital to the elucidation of the role of salt and solvation in the stability and solubility of macromolecules.[128] Calculation of free energies of binding to within kilojoule/mole accuracy may lead to

the possible use of theory to design or alter protein structures from first principles.[165] Few computer simulations have yet reached this point, however. Excellent reviews cover many of the methodological topics (for instance ref 128 and references therein).

The two predominant effects of salt on peptides and proteins in solution are the altering of solubility, which can cause a precipitate or crystal to form, and the altering of conformational stability, which can cause a denaturing of the native conformation. These two effects are clearly coupled and interdependent in a number of systems. For over a century, the trends have been qualitatively observed.[166] The nature and mechanism of the underlying processes, however, are still not well understood at the microscopic level.

It is widely appreciated that differences in cation or anion charges as well as their relative sizes and polarizabilities have important consequences on the solvation of ions by water and their subsequent interactions with solvated peptides and proteins.[1,167-169] These differences are expressed in the lyotropic, or Hofmeister, series.[166,170] The effects of ions are thus ordered according to their ability to denature (or stabilize) a protein or to increase (or decrease) the solubility of a peptide in solution. Possible explanations for these processes fall into two broad categories: specific association of ions with the peptide and ordering of the solvent around the peptide by the ions. Many experiments have been done to try to determine which of these processes is the driving force in any particular situation (for a review see ref 171), but much is still unknown.

The inclusion of salt in molecular dynamics simulations of biological systems presents certain technical difficulties.[172,173] While simulations of DNA with salt are possible,[174-176] they present a diverse range of phenomena unique to polyelectrolytes, and we will concentrate on the addition of salt to the simulation of proteins and peptides. Some of the first studies including ions and explicit water with a biological molecule involved the simulation of proteins in their observed crystalline environment.[165,177] Although salt was primarily present in these studies in order to achieve electroneutrality, and the main focus was to compare structural data obtained using MD to experimental X-ray data, some investigation of the behavior of the ions and their interaction with the protein was conducted.

Recently, molecular dynamics has begun to be used to look directly at the origins of the Hofmeister series. While previous simulations of peptides in explicit aqueous solution used methyl blocking groups to avoid technical problems associated with the long-ranged forces in a salt atmosphere,[160] a zwitterionic bis-(penicillamine) derivative of sequence Tyr-c[D-Pen-Gly-Phe-D-Pen], or DPDPE, was more recently simulated in 1.0 M sodium chloride.[178] In this study, the explicitly represented system contained 52 peptide atoms, 411 water molecules, 9 sodium ions, and 9 chloride ions. Two simulations of 200 ps each were performed to demonstrate insensitivity to initial conditions, especially the placement of salt ions. The electrostatic interactions were calculated using the Ewald procedure.[142,143] As discussed above, this method avoids the problems associated with the use of electrostatic cutoffs (for further discussion on the differences between this procedure and the use of cutoffs, see refs 144 and 153). Because this enkephalin had previously been simulated in water in the absence of salt,[179] the study afforded the opportunity to observe average structural and dynamic changes due to the presence of salt.

An effect that is expected[109] is that the radial correlation (distribution) functions of salt and solvent around the peptide do not have the same structure as those around a simple solute. Rather than a sharp rise at "contact" one sees a gradual slope with subdued structures. In fact, $g(r)$ between solute and the other solution components does not approach unity for most atomic sites until after a distance greater than the radius of gyration. This is a physical or spacial screening effect. The density of solvent and salt should not approach that of bulk until the greatest distance from that site to the surface of the molecule is exceeded.[34]

Some surprising spatial effects were, however, observed. An inner sphere complex was found to form between two to three chloride ions and the N-terminus of the peptide. In addition, several chlorides were found to be simultaneously associated with multiple amide hydrogens. This led to some close contact between like charged chloride ions. These contacts were made additionally favorable by the presence of bridging water molecules between them. This type of structure was known from the ion pairing literature.[124,125,127,129] The cations were not observed to associate directly with the peptide and retained their individual octahedral coordination in what could be described as an outersphere complex.

These effects have several structure consequences. The ion association modified the position of the N-terminus relative to its neighboring amide hydrogens. This encouraged multiple hydrogen bonding to the chlorides. Binding of the ions decreased the end-to-end distance and subtly altered the appropriate dihedrals in order to facilitate the formation of a transient intramolecular hydrogen bond. This effect had not been observed in the corresponding simulation of DPDPE in aqueous solution.

The associations observed above are in good thermodynamic agreement and in rough structural agreement with the mechanism proposed by Timasheff and Arakawa[180] (see the next section). It had been inferred form thermodynamic stability constants that chloride was the least preferentially excluded of the anions studied and that sodium was the most preferentially excluded. The direct interaction between the peptide and the chloride ions and the absence of association between sodium and the peptide in the simulation reflects this trend. This agreement aids in the elucidation of the molecular effects underlying the trends observed and categorized by the Hofmeister series.

The diffusion constants for the salt in the presence of DPDPE were found to vary widely (more than a factor of 10) for ions which were essentially bound and ions free in solution (away from the peptide). Overall, the diffusion of ions in such concentrated peptide solutions was found to be smaller in magnitude that that of pure solutions.[144,178]

Further study of DPDPE provided information on the effects of salt on the rotations of its side chains.[181]

The potentials of mean force (PMFs), or relative conformational free energies, for rotation about the χ^1 aromatic side-chain dihedrals were investigated in aqueous and saline solution. It was found that the associations between the chloride ions and the peptide, described above, led to an alteration in the energies between gauche − (g−), gauche + (g+), and trans (t) conformations as well as the barriers between them. These differences can be seen for tyrosine in Figure 2. The relative stability of the g− and t conformations are reversed in the presence of salt. The stability of the g+ conformation is also affected. In aqueous solution, the g+ conformation is unfavorable by 6.1 kJ/mol. In the presence of salt, however, this increases to 23.4 kJ/mol. This indicates that the t conformation is favored by the binding of chloride ions to the N-terminus. Analysis of the average salt configuration suggested that the interaction of the chloride ions with the N-terminus was disrupted in the g− and g+ conformations, but optimal for the t conformation. The side chains are known to be associated with the pharmacophore, and their conformation is believed to be crucial for its δ-opioid activity.[181]

The difference in solvation and structure of DPDPE in the presence of a different anion has recently been demonstrated.[182] Here, DPDPE was studied in 1.0 M sodium acetate solution. The details of the method were similar to those in previous studies of this peptide.[178,179] During the simulation several acetate ions associated closely with the N-terminus of the peptide and several NH groups of the peptide backbone. Subtle conformational changes in the peptide backbone accompanied these associations. In comparison to the DPDPE simulations involving NaCl,[178] the number of acetates bound to the peptide is reduced, consistent with the experimental observation that acetate ions have a larger preferential exclusion than chloride ions.[183] In addition, sodium ion binding to the backbone carbonyl groups near the negatively charged C terminus occurred. While the extent of this binding was small, no interaction between the sodium ions and the peptide had been observed during the DPDPE–sodium chloride simulation. Still, the limited association is in accordance with the degree of preferential exclusion expected for sodium ions. The final configurations of DPDPE in water,[179] DPDPE in sodium chloride solution,[178] and DPDPE in sodium acetate solution[182] are shown in Figure 3. For comparison of the instantaneous salt distributions, we present Figure 4. The difference in clustering is immediately apparent. While such individual configurations provide a tempting and even compelling basis for comparison, it is only the averages over many such configurations which should be associated with likelihood or probability.

Such simulations provide a wealth of detailed information. However, it is somewhat dangerous to make too many direct comparisons with the experimental effects of salts on the solubility and stability of proteins as described by the Hofmeister series (see below) with the results on this particular peptide system.[182] While charged residues most often reside near or on the surface of globular proteins, such binding sites do not often occupy such a high percentage of the available surface area as they do in the case of the example peptide DPDPE. Considering the uncertainties in the interaction model employed in this and all such studies, the quantitative magnitudes of these effects are still open to question.

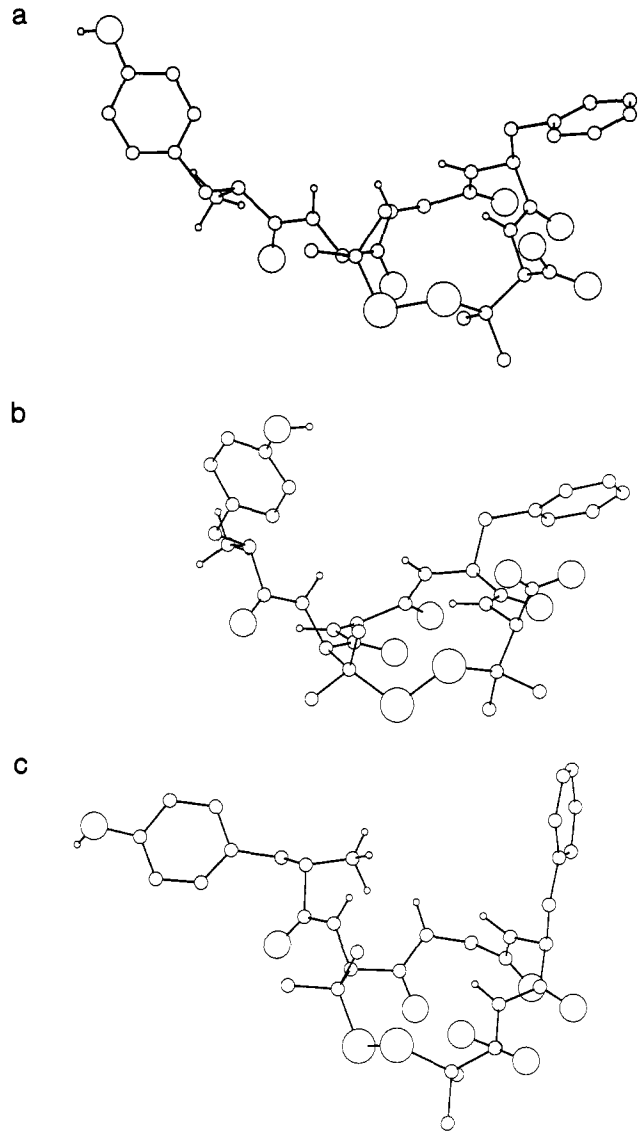


Figure 3. Final configuration of DPDPE in (a) aqueous, (b), saline, and (c) sodium acetate solution. Water and ions have been removed for clarity. Note that the disulfide bridge is in the same configuration in the three media.

Similarly, the size of DPDPE compared to globular proteins must be considered. All the backbone NH groups of DPDPE have a significant average exposure to solvent whereas, in most globular proteins, these groups are involved in the maintenance of secondary structure. With respect to this structural aspect DPDPE seems closer to being a denatured protein. The weaker binding (larger preferential exclusion) seen could then explain why chloride and acetate ions stabilize the native form of proteins. On the other hand the disulfide bond in DPDPE strongly restricts the available conformational space just as secondary structure restricts β-bends and short loops exposed on the surface of proteins. This argues for DPDPE as a reasonable small molecule model compound of proteins in so far as salt effects are concerned. Considerably more work has to be done in order to separate the various effects before one can fully understand the complex processes at work in these systems.

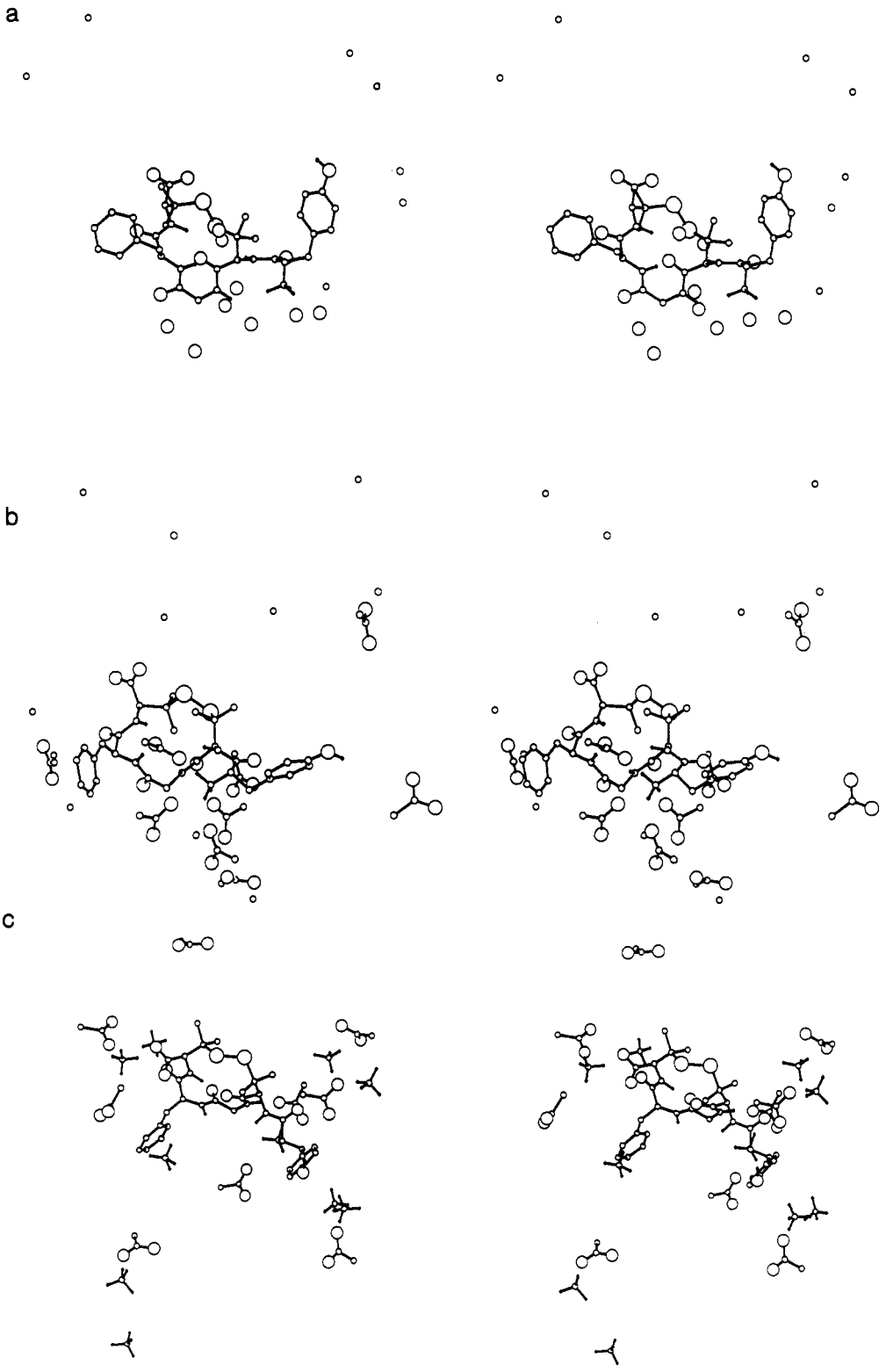


Figure 4. Instantaneous salt configurations about DPDPE in (a) sodium chloride (small nonbonded atoms Na^+, nonbonded larger circles Cl^-), (b) sodium acetate, and (c) ammonium acetate solution.

3.5. Experiments on Protein Systems in Salt Water

Primarily because of their size and complexity of structure, proteins in saline solution present special theoretical and experimental challenges. Here we consider some of the experimentally observed effects of salt on the solution behavior of proteins. The current overlaps with theory and computer experiments as well as the distance between certain observations and current simulation efforts will then be more obvious.

The most striking effects are related to solubility and conformational stability. It was suggested some time ago that solubility changes in protein systems are governed by processes involving ionic association and conformational stability by the reordering of solvent.[184,185] Such pictures may be valid as limiting behaviors or indeed as limiting laws. Since different

conformations can have different free energies of solvation, solubility and conformational stability must be intimately linked, and the behavior of many systems is, therefore, the result of an interplay of various mechanisms.

A number of studies have been performed to try to determine which of the limiting processes were dominant. A substantial effort focused on the effects of dilute aqueous solutions of several salts on the thermodynamic (structural) stability of a series of collagens.[186-188] A reversible folding–unfolding transformation was induced in these experiments by gradually increasing the temperature. The change in melting temperature, T_m, or temperature where the supposed structural transition occurs, was observed as a function of salt concentration. As commonly defined, T_m represents the temperature of the midpoint of the temperature range over which unfolding (or folding) occurs. Equivalently at T_m, the helical and random coil conformations are equally probable and therefore, $\Delta G = 0$ at that point. Thus

$$T_m = \Delta H/\Delta S \tag{20}$$

for a two state transition. It was found that increasing the salt concentration moved T_m to increasingly lower temperatures. As observed, the change in T_m was nearly linear with concentration of salt.

The difference in T_m with the addition of $CaCl_2$ has been investigated for various proteins. It seems from those studies that $CaCl_2$ has a destabilizing effect not unlike an increase in temperature on these species suggesting that the effects of salts such as $CaCl_2$ involve the solvent structure itself independent of the conformational details of the proteins involved.[189]

The effects of other salts on the thermal transition temperature have also been investigated.[189] Some salts, such as KSCN and $CaCl_2$, appear to destabilize the folded or ordered form and others, such as KH_2PO_4 and $(NH4)_2SO_4$, stabilize the ordered form. Simple alkali halide salts such as KCl and NaCl were found to be essentially inert in this respect. The apparent linear relation with concentration has been fit to

$$T_m = T_m^{(o)} + K_m C_s \tag{21}$$

where $T_m^{(o)}$ and T_m are the melting temperatures of the protein in water and in aqueous solution of salt of concentration C_s, respectively. K_m, the slope, varies with chemical composition of the salt and has been ascribed to be a measure of its ability to perturb conformational stability.

Such results have been rationalized on the basis that the stability of native protein structure is affected by the salts through a competitive reorganization of the water. This type of reorganization has been postulated to be a necessary factor for the stabilization of the collagen type helix.[188,189] In terms of free energy, the major effect of an ion in a protein salt–water mix is seen then to increase or decrease the free energy of transfer of residues from the inside of the protein to the solvent environment. This class of effects were seen to apply to a wide range of systems which involve a net transfer of residues from an unsolvated environment to solution.[190] This is supported by the observation that the effect of a given concentration of salt is nearly independent of the concentration of protein over a physiologically relevant thermodynamic state range.[186]

Data on thermal transitions can be correlated with information from other experiments.[190] An interesting comparison involves the effects of increased salt concentration on the activity coefficients of a number of nonpolar nonelectrolytes, such as benzene and polar nonelectrolytes like adenine.[191] As salting out agents, both anions and cations closely follow the Hofmeister series in order of effectiveness. Since the Hofmeister series holds not only for proteins but for an assortment of other chemical species, it has been taken as support for the fact that the predominant effect of ions occurs through a competitive reorganization of the water.[190]

The picture given that the dominant effect of salts on protein molecules is via a solvent perturbation mechanism has been challenged.[184,192,193] While previous results[186-188] had been seen as support for a solvent disruption mechanism, they could also be explained by the direct binding of ions to the amino acid residues of the protein.[192] The apparently simple linear relation above then seems to be an approximation to a case involving binding. In support of this idea, Flory and Weaver showed that the rate at which the native form could be repopulated in solutions of various salts could be essentially quantitatively explained by the mechanism proposed for the same process in the absence of salt.[194] Thus a consistent, quantitative description of the effects of salts could be obtained for both the thermal transition temperature and the reversion kinetics by employing a direct binding process for melting and the Flory–Weaver mechanism for the reversion kinetics.

The approach to this problem by Jencks used the determination of the activity coefficient for a small blocked (nonzwitterionic) tetrapeptide, acetyltetraglycine ethyl ester (ATGEE), in the presence of various salt solutions.[192] In this system both the denaturation and solubility of the peptide could largely be accounted for by the effects of the salts on the amide groups which become exposed during these processes. A combination of two effects was proposed: a general salting out effect described by a nonspecific "internal pressure" effect and a direct interaction between large anions and the amide dipole. This latter effect correlates well with the association seen in the DPDPE–NaCl simulations described above. The association of chloride ions with the amide hydrogens fits the model proposed nearly 25 years before.

It has been proposed that certain ions might act by both association and solvent reorganization simultaneously, with one mechanism or the other dominant with different proteins or different ions or at different stages of denaturation.[184] There was the suggestion that cations and anions acted by different mechanisms. Experiments were interpreted as indicating that in addition to direct ion–peptide interactions, ions act by increasing the ability of water to denature proteins.[185] Presumably this would be caused by a significant alteration of the liquid structure of water, accompanied by a concomitant release of potential hydrogen-bonding sites, or via the interaction of water with ions in such a way as to modify the hydrogen-bonding ability of water. Thus two classes of salts exist: those that operate

directly on proteins and others that assert their influence on the protein through the solvent.

A simple solvation shell picture has been offered[180,195] to codify these results. It has been proposed that solubility and stability are governed by a balance between specific associations and solvent reordering effects.

A considerable body of experimental results related to this problem exists in the form of crystallization conditions.[180] Interactions between proteins and various substances which lead to stabilization or precipitation are then investigated somewhat indirectly. Stabilization and precipitation appear to be accompanied by preferential exclusion of the cosolvent (salt) from the solvent volume immediately surrounding the protein. Thus, the protein is preferentially solvated. This effect leads to an increase in the chemical potential (change in free energy) of both components making both unfolding or solubilization less favorable.

This effect is quantified in terms of a salting out constant, K_s, related to the change of the chemical potential of the protein by the salt, in both the precipitate and in solution. This sort of thermodynamic interaction parameter may be used to construct a phase diagram (isotherm) for any given solution, which gives the limiting value of protein solubility.

These effects on protein solubility for a series of salts[180] are indeed complicated. The solubility of a protein is increased at low salt concentration (<1.0 M), that is, salting in occurs. Solubility of the protein decreases with increasing salt concentration, leading eventually to a salting out effect. Thus the salting in action of various salts appears primarily due to nonspecific electrostatic effects generally thought of in terms of Debye–Hückel effects; salting out is more complicated. It is widely appreciated that the order of effectiveness of the salts as precipitants indeed does follow the Hofmeister series.

The salting out effect may be viewed as two competing mechanisms with contributions from nonspecific preferential exclusion and specific binding of the ligand to the protein. Possible sources of preferential exclusion and the ability to more accurately describe this phenomena are a current aim of computer simulations and analytical theories. The example of DPDPE in various salts (see above) shows this, at the microscopic level, to be a consequence of a delicate energetic balance when viewed from the pair energy distribution functions for the system.

Timasheff has suggested these effects be separated into two categories: those which are independent of the protein and those influenced by the chemical nature of the protein.[180,196] An interesting combination of microscopic and macroscopic concepts have been invoked to explain this separation. The category which is independent of the protein is governed by interactions with the solvent and includes steric exclusion and alteration of the surface tension (surface free energy). Steric exclusion arises from the size and packing of the cosolvent relative to water which discourages penetration beyond the initial contact with the protein. This type of preferential exclusion effect is the same as the typical preferential hydration seen in water–poly(ethylene glycol) (PEG) systems.[197] Surface tension effects are ascribed to arise due to the increase in the surface tension of water by the cosolvent. This would then lead to a depletion of the cosolvent from the volume immediately surrounding the protein.

Whether this type of macroscopic analogy holds for a microscopic system is not clear. Here, the area of contact between two molecular species is viewed as a macroscopic (continuum) interface, with a corresponding interfacial surface tension. The mechanism invoked by this picture suggests that the interfacial tension is changed by the presence of ions. Viewed thermodynamically as a Gibbs adsorption isotherm,[198] this results in a deficiency of ions in the surface layer. Such a preferential exclusion has been used to interpret data for a variety of cosolvents and additives: sugars[198,199] many salts,[200,201] and amino acids.[202] For each of these cases, addition of the substance increases the surface tension of water.

The second category from above seeks to describe those effects which depend explicitly on the specific chemical nature of a particular protein. Presumably the microscopic details should then involve a net effective attractive or repulsive interaction between the cosolvent and the protein. In the case of MPD (2-methyl-2,4-pentanediol), a known precipitator of aqueously soluble globular proteins, the alkyl diol appears effectively repelled by charged groups on the protein surface; that is, hydration is more favorable than solvation by the diol.[203] This category also includes compounds which are often described as solvophobic such as glycerol. Compounds such as these play off the hydrophobic effect, making contact between the nonpolar regions of the protein and the solvent less thermodynamically favorable.[196,204]

The thermodynamically based picture that emerges shows that the effect of preferential exclusion leads to salting out, while conformational stabilization relies on a balance between exclusion from and binding to the native and denatured states, respectively.[196] This sort of thermodynamic analysis is useful to demonstrate the fundamental difference between specific site binding and preferential interactions. It demonstrates that both stabilization and denaturation are controlled by microscopic correlations leading to the observed preferential interactions. Such correlations are a primary result of analytic theory and simulation. Understanding the energetics and mechanisms underlying such correlations is a worthy goal of such studies.

3.6. Simulations of Protein Systems in Salt Water

While single configuration calculations of the solvent around a significant macromolecule are possible[205] with the site–site (XRISM or DRISM) style of calculations, no studies yet exist which take into account the correlations induced by the significant structural fluctuations found in proteins. Simulations thus provide a convenient route to examine a microscopic model of these flexible systems in a nontrivial environment.

One such study has been performed on a pancreatic trypsin inhibitor (PTI) molecule, a postively charged protein at neutral pH.[177] The simulation of a full crystallographic unit cell of PTI, involving 4 protein molecules, 522 water molecules, and 24 chloride ions, limited the study to a trajectory (40 ps) that is short by today's standards. A range of self-diffusion constants

was observed for the chloride. These were dependent on the degree of interaction between the chloride ions and the protein. It was also observed that the PTI seemed to stabilize a number of water molecules in the first solvation shell. As mentioned above, simulations of DPDPE also exhibit a range of self-diffusion constants.

An excellent test of simulation data on proteins or protein crystals is provided by comparison to experimental X-ray data. In the PTI crystal simulation, root-mean-square (RMS) positional fluctuations for the protein atoms, averaged over each residue, showed satisfactory agreement with fluctuations obtained from a set of X-ray (Debye–Waller) temperature factors.[206,207] In addition, simulated inter- and intramolecular hydrogen bonds were compared to those inferred from the experimental data. Most of those known in the crystal form were observed in the simulation. It is less clear how much of this agreement was due to the accuracy of the simulation versus the precision obtained. It has been demonstrated that the use of interaction cutoffs in these early simulations restricted the motions available to the protein model.[115]

The chloride ions in the PTI crystal study were analyzed in terms of their diffusion constants. The hydration shell of the chloride appeared to be quite stable. This was attributed to stabilizing effects exerted by the surrounding protein. Interestingly, it was found that 40% of the chloride ions did not exhibit hydrogen bonding to the protein due to their own tightly bound first solvation shell. As previously mentioned, the role of salt in the solubility and stability of proteins is thought to be due to direct association of the ions with the protein, ordering/disordering of the solvent around the protein, or an interplay of both. Information from simulation studies may play a part in the understanding of this phenomenon.

Another molecular dynamics study of a protein in its crystalline environment involved the simulation of the behavior of a serine protease, *Streptomyces griseus* protease A (SGPA).[165] All atoms of two serine protease molecules were included. The solvent of crystallization was also explicitly represented, including water and salt ions. The simulation included two of the four asymmetric units present in the SGPA crystal unit cell studied. At the pH of the study, the protein had a net charge of +5. The crystal was grown in 1 M sodium dihydrogen phosphate. Again to mimic the experimental conditions, and given the charge of +5 on the protein, 26 dihydrogen phosphates and 16 sodiums were included to produce an electrically neutral system. The addition of 1429 water molecules defined a system with 9427 atoms for the simulation. Similar to the previously mentioned PTI study, the length of the simulation was restricted to 60 ps. A large cutoff distance of 15 Å was used in an attempt to avoid severe damage to the significant long-range protein–ion interactions. A switching function was used to avoid discontinuities in energy and force at the cutoff.

Agreement with the experimental X-ray data was checked on several levels, including overall RMS deviations between whole molecules, their individual secondary structures, and individual residues. For the structure averaged over the two molecules during the period from 16 to 60 ps, the RMS deviation from the experimental structure was 1.17 Å for all heavy, non-hydrogen atoms and 1.09 Å for C^{α} atoms. The RMS deviations were somewhat higher for individual molecules. It was noted that the averages were still continuing to increase at the end of the run, pointing to the need for simulations of more sizable length. More recently it has been demonstrated that in aqueous environment the RMS deviations often do not converge for times less than 100 ps.[208]

The analysis of the interaction of protein with solvent and ions in the SGPA simulation was centered on choosing those residues that had large RMS deviations from the experimental structure and analyzing the interaction of these residues with the solvent. The energy of the residues was broken down into the following components: interresidue interactions and interactions between the residues and their aqueous environments, counterions, and the other protein. Some direct interaction between residues on the protein and ions was observed. In some cases, the ions pulled the residues into solution. For other residues, interactions between certain residues and the protein became more favorable when the residue moved out of solution. It was noted that significant interaction energies between charged residues and counterions account, in part, for the ability of these residues to extend into solution. These electrostatic interaction energies are a vital component in the analysis of hydrogen-bonding interactions that occur during a simulation. As previously discussed, both of these factors may influence the effects of salt on the behavior of biological macromolecules in solution. It is clear, therefore, that this type of energetic analysis may be indispensible in the elucidation of the Hofmeister series.

4. Concluding Remarks

In this review we have attempted to give the reader an appreciation for several aspects involved in the calculation of the properties of peptide molecules in aqueous saline solution. Salt effects can manifest themselves in a number of ways macroscopically, and we have attempted to show how the thermodynamics can be correlated with microscopic distribution functions of atoms in solution. Much has been learned about the microscopic structures responsible for certain classes of solution phenomena; much remains to be uncovered for others. In the case of intramolecular (protein folding) problems, the coupling between the intrinsic intramolecular surface and the associations and screening in solution are apparent in the coupling of shape and solubility. When there are soft (low-energy barrier) torsional degrees of freedom, the shape of the peptide molecule may be highly dependent on the solvent environment.[103]

Efficient inspection of potentials of mean force both within and between molecules is currently a topic of research in statistical mechanics.[34,209] For simpler systems both machine simulations[210–212] and analytical methods[103,209,213,214] are bearing fruit. The very structure in the potential of mean force that reflects the solvent ordering is what complicates their calculation, yet several relatively simple approximate methods are available for easy use[213–216] in evaluating these effects for small peptide chains. The information from these sorts of structural tools may be used to gain insight

into the free energy of binding and the solubility of proteins by applying the methods described in this review.

Acknowledgments. The authors wish to thank the Robert A. Welch Foundation, the Alfred P. Sloan Foundation, and the National Institutes of Health for partial support. The SDSC and NSF provided computational support for some of the reported data not yet published. G.E.M. thanks the W. M. Keck Center for Computational Biology for a Computational Biology Fellowship. In addition, the authors would like to thank Dr. Paul E. Smith for many years of stimulating conversations covering methods, chemistry, and biological implications.

References

(1) Bockris, J. O.; Reddy, A. K. N. *Modern Electrochemistry*; Plenum Press: New York, 1973; Vol. 1.
(2) Franks, E. *Water–A Comprehensive Treatise*; Plenum Press: New York, 1973; Vol. 3.
(3) Soper, A. K.; Neilson, G. W.; Enderby, J. E.; Howe, R. A. *J. Phys. Chem.* **1977**, *10*, 1793.
(4) Enderby, J. E.; Neilson, G. W. *Adv. Phys.* **1980**, *29*, 323.
(5) Enderby, J. E.; Neilson, G. W. *Rep. Prog. Phys.* **1981**, *44*, 593.
(6) Newsome, J. R.; Neilson, G. W.; Enderby, J. E. *J. Phys. Chem.* **1980**, *133*, 1.
(7) Kistenmacher, H.; Popkie, H.; Clementi, E. *J. Chem. Phys.* **1973**, *58*, 5627.
(8) Corongiu, C.; Migliore, M.; Clementi, E. *J. Chem. Phys.* **1989**, *89*, 4629.
(9) Foresman, J.; Brooks, C. *J. Chem. Phys.* **1987**, *87*, 5892.
(10) Bene, J. D.; Frisch, M.; Raghavachari, K.; Pople, J.; von Schleyer, P. *J. Phys. Chem.* **1983**, *87*, 73.
(11) Zhao, X.; Gonzalez-Lafont, A.; Truhlar, D.; Steckler, R. *J. Chem. Phys.* **1991**, *94*, 5544.
(12) Chandrasekhar, J.; Spellmeyer, D. C.; Jorgensen, W. L. *J. Am. Chem. Soc.* **1984**, *106*, 903.
(13) Dang, L. *J. Chem. Phys.* **1992**, *96*, 6970.
(14) Dang, L.; Rice, J.; Caldwell, J.; Kollman, P. *J. Am. Chem. Soc.* **1991**, *113*, 2481.
(15) Hipple, P. H. V.; Schleich, T. *Acc. Chem. Res.* **1969**, *2*, 257.
(16) Robinson, D. R.; Jencks, W. P. *J. Am. Chem. Soc.* **1965**, *87*, 2470.
(17) Franks, F.; Eagland, D. *CRC Crit. Rev. Biochem.* **1975**, *3*, 165.
(18) Flanagan, M. A.; Ackers, G. K.; Matthew, J. B.; Hanania, G. I. H.; Gurd, F. R. N. *Biochemistry* **1981**, *20*, 4739.
(19) Hruby, V. J. *Design of Conformationally Constrained Cyclic Peptides With High Delta and Mu Opioid Receptor Specificities. Opioid Peptides: Medicinal Chemistry*; NIDA Research Monograph: Rockville, MD, 1986; Vol. 69.
(20) O'Connor, S.; Smith, P. E.; Al-Obeidi, F.; Pettitt, B. M. *J. Med. Chem.* **1992**, *35*, 2870.
(21) McQuarrie, D. A. *Statistical Mechanics*; Harper Collins: New York, 1976.
(22) Hill, T. L. *J. Chem. Phys.* **1959**, *30*, 93.
(23) Impey, R. W.; Madden, P. A.; McDonald, I. R. *J. Chem. Phys.* **1983**, *87*, 5071.
(24) Frank, H. S.; Evans, M. *J. Chem. Phys.* **1945**, *13*, 507.
(25) Gurney, R. W. *Ionic Processes in Solution*; McGraw-Hill: New York, 1953.
(26) Warshel, A.; Russell, S. *Q. Rev. Biophys.* **1984**, *17*, 283.
(27) Honig, B. *Theor. Biochem. Mol. Biophys.* **1991**, *2*, 63.
(28) Rashin, A. *J. Phys. Chem.* **1989**, *93*, 4664.
(29) Giese, A. C. *Cell Physiology*; W. B. Saunders Co.: Philadelphia, PA, 1973.
(30) Gray, C.; Gubbins, K. *Theory of Molecular Fluids*; Oxford Science Publications, Clarendon Press: Oxford, 1984.
(31) Allen, M.; Tildesley, D. *Computer Simulation of Liquids*; Oxford Science Publications: Oxford, 1987.
(32) Haile, J. *Molecular Dynamics Simulation: Elementary Methods*; Wiley Interscience: New York, 1992.
(33) McCammon, J.; Harvey, S. *Dynamics of Proteins and Nucleic Acids*; Cambridge Press: Cambridge, 1987.
(34) Brooks, C.; Karplus, M.; Pettitt, B. *Proteins: A theoretical perspective of structure, dynamics and thermodynamics, in Advances in Chemical Physics*; Wiley: New York, 1988; Vol. 71.
(35) Egelstaff, P. *An Introduction to the Liquid State*, 2nd ed.; Oxford Science Publications, Clarendon Press: Oxford, 1992.
(36) Born, M. *Z. Phys.* **1920**, *1*, 221.
(37) Yu, H.-A.; Karplus, M. *J. Chem. Phys.* **1988**, *89*, 2366.
(38) Yu, H.-A.; Roux, B.; Karplus, M. *J. Chem.* Phys. **1990**, *92*, 5020.
(39) Mayer, J. E. *J. Chem. Phys.* **1950**, *18*, 1426.
(40) Stell, G.; Patey, G. N.; Høye, J. S. *Adv. Chem. Phys.* **1981**, *38*, 183.
(41) Kirkwood, J. G. *J. Chem. Phys.* **1934**, *2*, 351.
(42) Kirkwood, J. G.; Westheimer, F. H. *J. Chem. Phys.* **1938**, *6*, 506.
(43) Westheimer, F. H.; Kirkwood, J. G. *J. Chem. Phys.* **1938**, *6*, 513.
(44) Tanford, C.; Kirkwood, J. G. *J. Am. Chem. Soc.* **1957**, *79*, 5333.
(45) Tanford, C. *J. Am. Chem. Soc.* **1957**, *79*, 5340.
(46) Honig, B. Environmental Effects on Electrostatic Interaction. In *Theoretical Biochemistry & Molecular Biophysics, Volume 2: Proteins*; Beveridge, D. L., Lavery, R., Eds.; Adenine Press: Schenectady, NY, 1991; p 63.
(47) Davis, M. E.; McCammon, J. A. *J. Comput. Chem.* **1989**, *10*, 386.
(48) Mayer, J. E.; Mayer, M. G. *Statistical Mechanics*; John Wiley and Sons, Inc.: New York, 1940.
(49) Hansen, J. P.; McDonald, I. R. *Theory of Simple Liquids*, 2nd ed.; Academic Press Inc.: London, 1986.
(50) Lado, F. *Phys. Rev. A* **1973**, *8*, 2548.
(51) Verlet, L. *Physica* **1964**, *30*, 95.
(52) Rossky, P. J.; Dudowicz, J. B.; Tembe, B. L.; Friedman, H. L. *J. Chem. Phys.* **1980**, *73*, 3372.
(53) Rowlinson, J. S. *Mol. Phys.* **1965**, *9*, 217.
(54) Rosenfeld, Y.; Aschroft, N. W. *Phys. Rev. A* **1979**, *20*, 1208.
(55) Rogers, F. J.; Young, D. A. *Phys. Rev. A* **1984**, *30*, 999.
(56) Zerah, G.; Hansen, J.-P. *J. Chem. Phys.* **1986**, *84*, 2336.
(57) Valachy, V.; Pohar, C.; Haymet, A. D. J. *J. Chem. Phys.* **1988**, *88*, 2066.
(58) Pettitt, B. M.; Rossky, P. J. *J. Chem. Phys.* **1986**, *84*, 5836.
(59) Chandler, D. *Faraday Discuss. Chem. Soc.* **1978**, *66*, 74.
(60) Sullivan, D. E.; Gray, C. G. *Mol. Phys.* **1981**, *42*, 443.
(61) Rasaiah, J. S.; Isbister, D. J.; Stell, G. *J. Chem. Phys.* **1981**, *75*, 4707.
(62) Rasaiah, J. C. *J. Chem. Phys.* **1982**, *77*, 5710.
(63) Høye, J. S.; Stell, G. *J. Chem. Phys.* **1976**, *65*, 18.
(64) Rossky, P. J.; Pettitt, B. M.; Stell, G. *Mol. Phys.* **1983**, *50*, 1263.
(65) Hummer, G.; Soumpasis, D. M. *Mol. Phys.* **1992**, *75*, 633.
(66) Kusalik, P. G.; Patey, G. N. *J. Chem. Phys.* **1988**, *88*, 7715.
(67) Kusalik, P. G.; Patey, G. N. *J. Chem. Phys.* **1988**, *89*, 5843.
(68) Chandler, D. *J. Chem. Phys.* **1977**, *67*, 1113.
(69) Cummings, P. T.; Stell, G. *Mol. Phys.* **1981**, *44*, 529.
(70) Perkyns, J. S.; Pettitt, B. M. *Chem. Phys. Lett.* **1992**, *190*, 626.
(71) Perkyns, J. S.; Pettitt, B. M. *J. Chem. Phys.* **1992**, *97*, 7656.
(72) Allnatt, A. R. *Mol. Phys.* **1964**, *8*, 533.
(73) Rossky, P. J.; Dale, W. D. T. *J. Chem. Phys.* **1980**, *73*, 2457.
(74) Pettitt, F. H. B. M.; Rossky, P. J. *J. Chem. Phys.* **1982**, *77*, 509.
(75) Friedman, H. L.; Larsen, B. *J. Chem. Phys.* **1979**, *70*, 92.
(76) Wertheim, M. S. *J. Chem. Phys.* **1971**, *55*, 4291.
(77) Lounnas, V.; Pettitt, B. M.; Findsen, L.; Subramanian, S. *J. Phys. Chem.* **1992**, *96*, 7157.
(78) Lee, C. Y.; McCammon, A. J.; Rossky, P. J. *J. Chem. Phys.* **1984**, *80*, 4448.
(79) Torrie, G. M.; Kusalik, P. G.; Patey, G. N. *J. Chem. Phys.* **1988**, *88*, 7826.
(80) Blum, L.; Torruella, A. J. *J. Chem. Phys.* **1971**, *56*, 303.
(81) Blum, L. *J. Chem. Phys.* **1972**, *57*, 1862.
(82) Blum, L. *J. Chem. Phys.* **1973**, *58*, 3295.
(83) Gray, C. G.; Gubbins, K. E. *Theory of molecular fluids*; Clarendon Press: Oxford, 1984; Vol. 1.
(84) Fries, P. H.; Patey, G. N. *J. Chem. Phys.* **1985**, *82*, 429.
(85) Patey, G. N. *Mol. Phys.* **1977**, *34*, 427.
(86) Patey, G. N. *Mol. Phys.* **1978**, *35*, 1413.
(87) Kirkwood, J. G.; Buff, F. P. *J. Chem. Phys.* **1951**, *19*, 774.
(88) Kusalik, P. G.; Patey, G. N. *J. Chem. Phys.* **1987**, *86*, 5110.
(89) Chandler, D. Equilibrium Theory of Polyatomic Fluids. In *THE LIQUID STATE OF MATTER: Fluids, Simple and Complex*; Montroll, E. W., Lebowitz, J. L., Eds.; North Holland Pub. Co.: Amsterdam, 1982; p 275.
(90) Lemberg, H. L.; Stillinger, F. H. *J. Chem. Phys.* **1975**, *62*, 1976.
(91) Stillinger, F. H. *Isr. J. Chem.* **1975**, *14*, 130.
(92) Lemberg, H. L.; Stillinger, F. H. *Mol. Phys.* **1976**, *32*, 353.
(93) Stillinger, F. H.; Rahman, A. *J. Chem. Phys.* **1978**, *68*, 666.
(94) Ichiye, T.; Haymet, A. D. J. *J. Chem. Phys.* **1988**, *89*, 4315.
(95) Jorgensen, W. L.; Chandrasekhar, J.; Madura, J. D.; Impey, R. W.; Klein, M. L. *J. Chem. Phys.* **1983**, *79*, 926.
(96) Haymet, A. D. J. Personal communication.
(97) Chandler, D.; Andersen, H. C. *J. Chem. Phys.* **1972**, *57*, 1930.
(98) Chandler, D.; Silbey, R.; Ladyani, B. M. *Mol. Phys.* **1982**, *46*, 1335.
(99) Hirata, F.; Rossky, P. J. *Chem. Phys. Lett.* **1981**, *83*, 329.
(100) Rossky, P. J.; Chiles, R. A. *Mol. Phys.* **1984**, *51*, 661.
(101) Valdeavella, C. V.; Perkyns, J. S.; Pettitt, B. M. Unpublished results.
(102) Perkyns, J. S.; Pettitt, B. M. Unpublished results.
(103) Pratt, L. R.; Hsu, C. S.; Chandler, D. *J. Chem. Phys.* **1978**, *68*, 4202.
(104) Böttcher, C. J. F. *Theory of Electric Polarisation*; Elsevier Pub. Co.: Amsterdam, 1952.
(105) Hoye, J. S.; Stell, G. *J. Chem. Phys.* **1976**, *65*, 18.
(106) Chandler, D. *J. Chem. Phys.* **1977**, *67*, 1113.
(107) Chandler, D.; Singh, Y.; Richardson, D. *J. Chem. Phys.* **1984**, *81*, 1975.
(108) Lau, W. F.; Pettitt, B. M. *Biopolymers* **1987**, *26*, 1817.
(109) Pettitt, B. M.; Karplus, M. *Chem. Phys. Lett.* **1985**, *121*, 194.
(110) Smith, P.; Pettitt, B.; Karplus, M. *J. Phys. Chem.* **1993**, *97*, 6907.
(111) Yu, H.-A.; Pettitt, B.; Karplus, M. *J. Am. Chem. Soc.* **1991**, *113*, 2425.

(112) Andersen, H. C. *J. Chem. Phys.* **1980**, *72*, 2384–2393.
(113) Çağin, T.; Holder, M.; Pettitt, B. *J. Comput. Chem.* **1991**, *12*, 627.
(114) Brooks, C.; Pettitt, B.; Karplus, M. *J. Chem. Phys.* **1985**, *83*, 5897.
(115) Loncharich, R. J.; Brooks, B. R. *Proteins* **1989**, *6*, 32.
(116) Smith, P.; Pettitt, B. *J. Chem. Phys.* **1991**, *95*, 8430.
(117) Ewald, P. *Ann. Phys.* **1921**, *64*, 253–287.
(118) de Leeuw, S. W.; Perram, J. W.; Smith, E. R. *Proc. R. Soc. London A* **1980**, *373*, 27–56.
(119) Rasaiah, J.; Friedman, H. *J. Chem. Phys.* **1968**, *48*, 2742.
(120) Ramanathan, P.; Friedman, H. *J. Chem. Phys.* **1971**, *54*, 1086.
(121) Friedman, H. *Ann. Rev. Phys. Chem.* **1981**, *32*, 179.
(122) Klapper, I.; Hagstrom, R.; Fine, R.; Sharp, K.; Honig, B. *Proteins: Struct. Funct. Gene.* **1986**, *1*, 46.
(123) Kusalik, P. G.; Patey, G. N. *J. Chem. Phys.* **1983**, *79*, 4468.
(124) Dang, L.; Pettitt, B. *J. Am. Chem.* **1987**, *109*, 5531.
(125) Dang, L.; Pettitt, B. *J. Phys. Chem.* **1990**, *94*, 4303.
(126) Berkowitz, M.; Karim, O.; McCammon, J.; Rossky, P. *Chem. Phys. Lett.* **1984**, *105*, 577.
(127) Buckner, J.; Jorgensen, W. *J. Am. Chem. Soc.* **1987**, *111*, 2507.
(128) Straatsma, T.; McCammon, J. A. *Annu. Rev. Phys. Chem.* **1992**, *43*, 407.
(129) Dang, L.; Pettitt, B.; Rossky, P. *J. Chem. Phys.* **1992**, *96*, 4046.
(130) Friedman, H. *Faraday Discuss. Chem. Soc.* **1988**, *85*, 1.
(131) Wu, Y.; Koch, W.; Zhong, E.; Friedman, H. *J. Phys. Chem.* **1988**, *92*, 1692.
(132) Valleau, J. P.; Torrie, G. M. Statistical Mechanics. In *A. Modern Theoretical Chemistry*; Berne, B. J., Ed.; Plenum: New York, 1976; p 169.
(133) Beveridge, D. L.; Mezei, M.; Swaminathan, S.; Harrison, S. W. *Computer Modeling of Matter*; Lykos, P. G., Ed.; American Chemical Society: Washington, D.C., 1978.
(134) Jorgensen, W. L.; Gao, J. *J. Phys. Chem.* **1986**, *90*, 2174.
(135) Alagona, G.; Ghio, C.; Kollman, P. *J. Am. Chem. Soc.* **1986**, *108*, 189.
(136) Buckner, J. K.; Jorgensen, W. L. *J. Am. Chem. Soc.* **1989**, *111*, 2507.
(137) Bopp, P.; Dietz, W.; Heinzinger, K. *Z. Naturforsch. A* **1979**, *34*, 1424.
(138) Geiger, A. *Ber. Bunsenges Phys. Chem.* **1981**, *85*, 52.
(139) Szasz, G. I.; Heinzinger, K.; Riede, W. O. *Z. Naturforsch. A* **1981**, *36*, 1067.
(140) Szasz, G. I.; Heinzinger, K.; Palinkas, G. *Chem. Phys. Lett.* **1981**, *72*, 194.
(141) Bounds, D. G. *Mol. Phys.* **1985**, *54*, 1335.
(142) Ewald, P. *Ann. Phys.* **1921**, *64*, 253.
(143) Leeuw, S. W. D.; Perram, J. W.; Smith, E. R. *Proc. R. Soc. London A* **1980**, *373*, 27.
(144) Smith, P. E.; Pettitt, B. M. *J. Chem. Phys.* **1991**, *95*, 8430.
(145) Card, D.; Valleau, J. *J. Chem. Phys.* **1970**, *52*, 6232.
(146) Brooks, C. L.; Pettitt, B. M.; Karplus, M. *J. Chem. Phys.* **1985**, *83*, 5897.
(147) Neilson, G. W.; Enderby, J. E. *Proc. R. Soc. London A* **1983**, *390*, 353.
(148) Madden, P. A.; Impey, R. W. *Ann. N.Y. Acad. Sci.* **1986**, *482*, 91.
(149) Stryer, L. *Biochemistry*; Freeman: New York, 1999.
(150) Gordon, R. *Adv. Magn. Reson.* **1968**, *3*, 1.
(151) Impey, R. W.; Madden, P. A.; McDonald, I. R. *Mol. Phys.* **1982**, *46*, 513.
(152) Robinson, R. A.; Stokes, R. H. *Electrolyte Solutions*; Butterworths: London, 1955.
(153) Buono, G. S. D.; Cohen, T. S.; Rossky, P. J. *J. Mol. Liquids* Submitted.
(154) Landau, L. D.; Lifshitz, E. M. *Fluid Mechanics*; Pergamon Press: Oxford, 1963.
(155) Walrafen, G. E. *Water–A Comprehensive Treatis*; Plenum Press: New York, 1972; Vol. 1.
(156) Desnoyers, J. E.; Jolicoeur, C. *Comprehensive Treatise in Electrochemistry*; Plenum Press: New York, 1983; Vol. 5.
(157) Hertz, H. G. *Water–A Comprehensive Treatise*, Plenum Press: New York, 1973; Vol. 3.
(158) Hung, J. P.; Freidman, H. L. *Prog. Inorg. Chem.* **1983**, *30*, 359.
(159) Neilson, G. W. *Chem. Phys. Lett.* **1979**, *68*, 247.
(160) Rossky, P. J.; Karplus, M. *J. Am. Chem. Soc.* **1979**, *101*, 1913.
(161) Rossky, P. J.; Karplus, M.; Rahman, A. *Biopolymers* **1979**, *18*, 825.
(162) Karplus, M.; Rossky, P. J. *ACS Symp. Ser.* **1980**, *127*, 23.
(163) Rossky, P. J.; Karplus, M. *Ann. N.Y. Acad. Sci.* **1981**, *367*, 151.
(164) Ramé, G.; Lau, W.; Pettitt, B. *Int. J. Peptide Protein Res.* **1990**, *35*, 315.
(165) Avbelj, F.; Moult, J.; Kitson, D. H.; James, M. N. G.; Hagler, A. T. *Biochemistry* **1990**, *29*, 8658.
(166) Hofmeister, F. *Arch, Exp. Pathol. Phar.* **1888**, *24*, 247.
(167) Rasaiah, J. C.; Friedman, H. L. *J. Chem. Phys.* **1968**, *48*, 2742.
(168) Rasaiah, J. C. *J. Chem. Phys.* **1972**, *56*, 3071.
(169) Wood, R. H.; Wicker, R. K.; Kreis, R. W. *J. Phys. Chem.* **1971**, *75*, 2313.
(170) Collins, K. D.; Washabaugh, M. L. *Q. Rev. Biophys.* **1985**, *18*, 323.
(171) Marlow, G. E.; Pettitt, B. M., 1993. *Adv. Comput. Biol.* In press.
(172) Mohan, V. M.; Smith, P. E.; Pettitt, B. M. *J. Am. Chem. Soc.* Submitted.
(173) Dang, L.; Kollman, P. *J. Am. Chem. Soc.* **1990**, *112*, 503.
(174) Lavery, R.; Sklenar, H. *J. Biomol. Struct.* **1989**, *6*, 655.
(175) Langley, D.; Doyle, T.; Beveridge, D. *J. Am. Chem. Soc.* **1991**, *113*, 4395.
(176) Swaminathan, S.; Ravishankar, G.; Beveridge, D. *J. Am. Chem. Soc.* **1991**, *113*, 5027.
(177) Berendsen, H. J. C.; van Gunsteren, W. F.; Zwinderman, H. R. J.; Guertsen, R. G. *Ann. N.Y. Acad. Sci.* **1986**, *482*, 269.
(178) Smith, P. E.; Pettitt, B. M. *J. Am. Chem. Soc.* **1991**, *113*, 6029.
(179) Smith, P. E.; Dang, L. X.; Pettitt, B. M. *J. Am. Chem. Soc.* **1991**, *113*, 67.
(180) Timasheff, S. N.; Arakawa, T. *J. Cryst. Growth* **1988**, *90*, 39.
(181) Smith, P. E.; Pettitt, B. M. *Biopolymers* **1992**, *32*, 1623.
(182) Smith, P. E.; Marlow, G. E.; Pettitt, B. M., 1993. *J. Am. Chem. Soc.* In press.
(183) Timasheff, S. N.; Arakawa, T. Stabilization of Protein Structure by Solvents. In *Protein Structure: A Practical Approach*, Creighton, T. E., Ed.; IRL Press: London, 1989; p 331.
(184) Bello, J. *Biochemistry* **1963**, *2*, 276.
(185) Bello, J.; Haas, D.; Bello, H. *Biochemistry* **1966**, *5*, 2539.
(186) von Hipple, P.; Wong, K. *Biochemistry* **1962**, *1*, 664.
(187) von Hipple, P.; Wong, K. *Biochemistry* **1963**, *2*, 1387.
(188) von Hipple, P.; Wong, K. *Biochemistry* **1963**, *2*, 1399.
(189) von Hipple, P.; Wong, K. *Nature* **1964**, *145*, 557.
(190) von Hipple, P.; Schleich, T. *Acc. Chem. Res.* **1969**, *2*, 257.
(191) Long, F.; McDevitt, W. *Chem. Rev.* **1952**, *51*, 119.
(192) Mandelkern, L.; Stewart, W. *Biochemistry* **1964**, *3*, 1135.
(193) Robinson, D.; Jencks, W. *J. Am. Chem. Soc.* **1965**, *87*, 2470.
(194) Flory, P.; Weaver, E. *J. Am. Chem. Soc.* **1960**, *82*, 4518.
(195) Arakawa, T.; Timasheff, S. *Biochemistry* **1987**, *26*, 5147.
(196) Timasheff, S. *Curr. Op. Struct. Biol.* **1992**, *1*, 35.
(197) Arakawa, T.; Timasheff, S. *Biochemistry* **1985**, *24*, 6756.
(198) Lee, J.; Timasheff, S. *J. Biol. Chem.* **1981**, *256*, 7193.
(199) Arakawa, T.; Timasheff, S. *Biochemistry* **1982**, *21*, 6536.
(200) Arakawa, T.; Timasheff, S. *Biochemistry* **1982**, *21*, 6545.
(201) Arakawa, T.; Timasheff, S. *Biochemistry* **1984**, *23*, 5912.
(202) Arakawa, T.; Timasheff, S. *Arch. Biochem. Biophys.* **1983**, *224*, 169.
(203) Pittz, E.; Timasheff, S. *Biochemistry* **1978**, *17*, 615.
(204) Gekko, K.; Timasheff, S. *Biochemistry* **1981**, *20*, 4667.
(205) Ramé, G. Ph.D. Disertation, University of Houston, Houston TX, 1992.
(206) Walter, J.; Huber, R. *J. Mol. Biol.* **1983**, *167*, 911.
(207) Wlodawer, A.; Walter, J.; Huber, R.; Sjolin, L. *J. Mol. Biol.* **1984**, *180*, 301.
(208) Findsen, L.; Subramanian, S.; Lounnas, V.; Pettitt, B. M. Time Scales and Fluctuations of Protein Dynamics: Metmyoglobin in Aqueous Solution. In *Interaction Energies*; Buckingham, A. D., Legon, A., Roberts, S., Eds.; Chapman & Hall: London, 1993.
(209) Pettitt, B. M.; Karplus, M.; Rossky, P. J. *J. Phys. Chem.* **1986**, *90*, 6335.
(210) Postma, J. P. M.; Berendsen, H. J. C.; Haak, J. R. *Faraday Discuss. Chem. Soc.* **1982**, *17*, 55.
(211) Mon, K. K.; Griffiths, R. B. *Phys. Rev. A* **1985**, *31*, 956.
(212) Alagoma, G.; Ghio, C.; Kollman, P. A. *J. Am. Chem. Soc.* **1985**, *107*, 2229.
(213) Schweizer, K. S.; Curro, J. G. *Phys. Rev. Lett.* **1987**, *58*, 246.
(214) Nichols, A. L., III; Chandler, D.; Singh, Y.; Richardson, D. M. *J. Chem. Phys.* **1984**, *81*, 5109.
(215) Pierotti, R. A. *Chem. Rev.* **1976**, *76*, 717.
(216) Lowdin, L. *RISMGR*. QCPE 305, Quantum Chemistry Program Exchange, Indiana University, Bloomington, IN 47401.