The Effect of the Neglect of Electronic Polarization in Peptide Folding Simulations

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The effect of the neglect of electronic polarization, on the relative stability of different conformations of a model peptide in a nonpolar environment, has been investigated. Configurations generated in molecular dynamics simulations of polyalanine (ACE-ALA_{15}-NH_2) in cyclohexane were analyzed in terms of a nonmutual polarization model. The work clearly demonstrates that the stability of conformations which have an enhanced electric field, such as is generated by the formation of a helix dipole, can be significantly underestimated by the neglect of the effects of electronic polarization in weakly polar or nonpolar solvents.

Introduction

As with any condensed phase, a system consisting of a protein immersed in solvent will show a dielectric response. This is because the electron cloud surrounding each atom within a given molecule will reorganize in response to the presence of an electric field resulting in the induction of a dipole and an energetically favorable interaction between the electric field and the molecule. The primary effect of this dielectric response and the generation of induced dipoles is 2-fold. First, it leads to the modification, in general to the reduction, of the Coulomb interaction between charges. Second, it gives a contribution to the free energy of solvation due to favorable interaction of charges or dipoles with the polarizable medium (dielectric solvation). However, in most empirical force fields that are used in molecular dynamics simulations of biomolecular systems[1−4] (proteins, lipids, and nucleic acids) the effects of electronic polarization are not explicitly treated. This is primarily for two reasons. First, to explicitly model the effect of electronic polarization is nontrivial. Polarization effects are not pairwise additive and to account for polarization effects explicitly a self-consistent solution must be found, often by iteration.[5−9] This makes the interaction expensive to calculate. Second, many of the effects of electronic polarization can be effectively modeled implicitly. The average effects of polarization on the bulk properties of materials can be modeled by an appropriate allocation of (fixed) partial charges on the particles simulated. These particles (interaction sites) normally correspond to the centers of atoms which make up the molecules in the system but may also represent groups of atoms or alternatively lone pair electrons. An example of this is the increase in the effective dipole in models of water intended to reproduce the properties of the liquid as compared to models designed for vacuum calculations.[10]

Other effects of electronic polarization are simply ignored in atomic simulations based on empirical force fields with fixed charges. In particular, the local effects of groups with a high charge density are ignored despite electronic polarization having been shown, for example, to play a significant role in the stabilization of chloride ions in proteins.[11]

In this study, we concentrate on collective polarization effects due to the spatial arrangement of charges or dipoles. Specifically, the spatial arrangement of NH and CO groups within the backbone of a peptide can lead to the creation of a helix dipole. Helices are stabilized significantly by charged groups that interact favorably with the helix dipole.[12] In a similar manner, a helix will be stabilized in a dielectric medium due to the interaction of the helix dipole with the dipoles induced within the medium. The question is: Is the contribution of electronic polarizability to the stability of specific conformational states, such as helices, significant?

Two aspects must be considered (i) the relative stabilization of different conformational states and (ii) the relative contribution of electronic polarizability to the overall dielectric response of the medium. For simulations performed in a medium with a high dielectric constant, such as water, it is clear that the relative contribution of electronic polarizability will be small. The dielectric response of water is dominated by the reorientation of individual water molecules, which have a large dipole, and the dielectric properties of water can be very well reproduced using fixed charge models such as SPC/E.[13,14] This is not, however, true for less polar solvents. The dielectric properties of methanol[15] and trifluroethanol,[16] for example, are not well reproduced by models that do not include polarization explicitly. In the case of nonpolar solvents such as cyclohexane that are typically modeled using only Lennard-Jones potentials there is no dielectric response of the system. Peptide and protein systems are, nevertheless, increasingly being studied in nonpolar or weakly polar environments,[17] for example, to simulate the folding of small peptides in solvents such as methanol,[18] for comparison with experimental studies, or to simulate the behavior of peptides in membrane environments.[19] In such cases, the neglect of electronic polarization may significantly effect the outcome of the simulations by altering the relative stability of different conformational states.

To quantify the magnitude of the error that is made by the neglect of electronic polarization on the relative stability of different backbone conformations of a peptide in a weakly polar or nonpolar solvent we have simulated an uncharged model peptide (ACE-ALA_{15}-NH_2) in cyclohexane and estimated the

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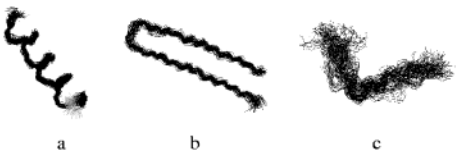

Figure 1. Overlays of conformations used to estimate the contribution from the electronic polarization of a nonpolar solvent to the stability of a particular conformation of a model peptide (ACE-ALA_{15}-NH_2). The configurations were sampled every 5 ps between 0.5 and 1 ns from separate simulations of the peptide in cyclohexane starting from (a) an idealized α-helix, (b) an idealized *β*-hairpin, and (c) an extended structure.

electrostatic interaction between the peptide and the solvent due to electronic polarizability induced in the solvent by the electric field of the peptide for different conformations.

MD Simulations

Three simulations of the peptide ACE-ALA_{15}-NH_2 in cyclohexane were performed in order to generate configurations representing different conformational states, an α-helix, a *β*-hairpin, and an extended structure. The initial (idealized) structure for each of the simulations was generated using the program WHATIF.[20] Each peptide was placed at the center of a cubic periodic box with the minimum distance between the solute and the wall of the box being 0.8 nm. Each box was then filled with an equilibrated configuration of cyclohexane and the solvent molecules that overlapped with the peptide were removed. The total charge on the peptide was zero. The GROMOS96 43A1 force field[3] was used to describe both the peptide and the cyclohexane. This force field uses a united atom approach to model the CH_2 groups of cyclohexane with the hydrogens incorporated into the carbon to which they are attached. Before starting the molecular dynamics simulations, each system was energy minimized using a steepest descent algorithm. A 50 ps simulation at 300 K was then performed with position restraints on the peptide to relax the solvent. After this a 50 ps simulation was performed without position restraints to obtain the starting configuration for the simulations used for analysis. In all simulations, the temperature was maintained close to the intended value (300 K) by weak coupling to an external temperature bath[21] with a coupling constant of 0.1 ps. The LINCS[22] algorithm was used to constrain bond lengths within the peptide and cyclohexane. The time step was 2 fs. A twin-range cutoff of 0.8/1.4 nm was used for the nonbonded interactions with interaction within the short-range cutoff updated every step and within the long-range cutoff every 10 steps together with the pairlist. Initial velocities were generated from a Maxwellian distribution at 300K. All simulations were performed using the GROMACS software package.[23,24] A 1 ns simulation of each system was used to generate representative configurations for analysis. For each of the three geometries (α-helix, *β*-hairpin, and extended chain), configurations were saved for analysis every 5 ps in the period $0.5 < t < 1$ ns yielding 100 configurations in each case. Convergence was tested by performing longer simulations of the α-helical, *β*-hairpin, and extended structure. The α-helical and *β*-hairpin structure was well-converged. The conformations of the extended structure varied with time but these variations do not affect the conclusions of the work. Overlays of the configurations used in the analysis are shown in Figure 1.

Electronic Polarization

A nonmutual electronic polarization model[11,25,26] was used to estimate the magnitude of the interaction between the peptide solute (ACE-ALA_{15}-NH_2) and the nonpolar solvent (cyclohexane) resulting from the electronic polarization within the solvent due to the electric field of the solute. In this model, two classes of particles are assumed to exist: *polarizing* and *polarizable* particles. *Polarizing* particles are able to polarize other particles but are themselves not polarizable. *Polarizable* particles can be polarized but do not induce polarization in other particles. In this way, the dielectric solvation energy is approximated, but the Coulomb interactions are not modified.

For the system studied, all point charges within the peptide were assumed to be *polarizing* particles and all particles within the solvent (each CH_2 united atom group) were assumed to be *polarizable*. Our aim is to obtain, to a first approximation, the magnitude of the interaction between the solute and the charge-induced dipole within the solvent. The induced dipole moment $\mathbf{p}_i$ on a *polarizable* particle *i* with isotropic polarizability α_i was calculated using the expression

$$\mathbf{p}_i = \alpha_i \mathbf{E}_i \tag{1}$$

where $\mathbf{E}_i$ is the local electrostatic field acting on the particle *i*. $\mathbf{E}_i$ is given by

$$\mathbf{E}_i = f\sum_j \frac{q_j}{r_{ij}^3}\mathbf{r}_{ij} \tag{2}$$

where $\mathbf{r}_{ij} = r_i - r_j$ is the Cartesian vector from atom *j* of the solute (*polarizing* particle) to atom *i* in the solvent (*polarizable* particle), r_{ij} is the norm of that vector, q_j is the partial charge on atom *j* (in the solute), $f = 1/(4\pi\epsilon_o)$, and ϵ_o is the permittivity of vacuum. To account for the electrostatic energy U_i due to the induced electronic polarization effect, for each polarizable particle *i* two terms are taken into account: The interaction $U_{\text{perm-ind}}$ between the permanent and the induced dipoles and the energy U_{pol} required to distort the electron cloud of the polarizable particles (see ref 27)

$$U = U_{\text{perm-ind}} + U_{\text{pol}} \tag{3}$$

with

$$U_{\text{perm-ind}} = -\mathbf{p}_i \cdot \mathbf{E}_i \tag{4}$$

and

$$U_{\text{pol}} = \frac{1}{2}\frac{|p_i|^2}{\alpha_i} \tag{5}$$

Inserting eqs 4 and 5 into eq 3 and using eq 1, we obtain the following expression for the interaction energy U_i between the atom *i* of the solvent and all atoms of the solute:

$$U_i = -\frac{1}{2}\alpha_i \mathbf{E}_i^2 \tag{6}$$

The total polarization energy is then

$$U = \sum_i U_i \tag{7}$$

Here *i* runs over the solvent atoms (*polarizable* particles) and *j* over the solute atoms (*polarizing* particles). A value of $\alpha' = f\alpha$ of 1.841×10^{-3} nm^3 was used for the isotropic polarizability of the CH_2 group of cyclohexane. This value was obtained by combining values from the literature[28] for the atomic polarizabilities of C (1.027×10^{-3} nm^3) and H (0.407×10^{-3} nm^3)

assuming that the atomic polarizabilities are additive. These values can be compared with values for the isotropic polarizability of CH_2 (1.947×10^{-3} nm^3) obtained from quantum mechanical calculations[29] and to a value of 1.835×10^{-3} nm^3 derived from the experimental dielectric constant of 2.023 using the Clausius−Mosotti equation.[27] The difference between these values results in a negligible difference in the overall interaction energy considering the assumptions within the model and only the results using the additive atom polarizabilities are reported.

The interaction energy U computed above corresponds to the dielectric solvation energy for the nonmutual polarization model. The dielectric solvation energy, often referred to as the Born energy in the case of single charges, is the work required to transfer a charged or polar solute molecule from vacuum into a polarizable medium. The dielectric solvation energy expressed as the energy density (energy per volume) u can be written generally in terms of the field energy as (see ref 27)

$$u = \frac{1}{2}\epsilon\epsilon_o \mathbf{E}\cdot\mathbf{E} - \frac{1}{2}\epsilon_o \mathbf{E}_o\cdot\mathbf{E}_o \tag{8}$$

where ϵ is the relative dielectric permittivity of the medium. The first term corresponds to the energy density of the field in the dielectric medium and the second term, to the energy density of the field in a vacuum. Comparing the value of the energy U deduced by integrating the general (continuum) expression for the energy density (eq 8) over all space (see eqs 9 and 10 below) with the value of U obtained using a microscopic model with discrete polarizable particles (eq 6), we can obtain a quantitative estimate of the error implicit in the nonmutual polarization model.

With this aim, the energy U is calculated below, for the case of a charge Q at the center a cavity of radius R, using both equations. Combining eq 8 with the expression for the electric field due to the charge Q at a distance $r > R$, the dielectric solvation energy can be expressed as

$$U = \frac{1}{2}\int_V \epsilon\epsilon_o\left(f\frac{Q}{\epsilon r^2}\right)^2 \mathrm{d}v - \frac{1}{2}\int_V \epsilon_o\left(f\frac{Q}{r^2}\right)^2 \mathrm{d}v \tag{9}$$

Integrating eq 9 over all space from $r = R$ to $r = \infty$ yields

$$U_B = \frac{1}{2}\left(\frac{1}{\epsilon} - 1\right) f\frac{Q^2}{R} \tag{10}$$

In a similar manner, using eq 6, the energy density (energy per volume) can be expressed in the discrete case as

$$u = -\frac{1}{2}\alpha n\left(f\frac{Q}{r^2}\right)^2 \tag{11}$$

where n is the number density of the polarizable sites within the environment. The dielectric solvation energy in this case can then be expressed as

$$U_p = -\frac{1}{2}\alpha n\int_V \left(f\frac{Q}{r^2}\right)^2 \mathrm{d}v \tag{12}$$

Integrating eq 12 over all space from $r = R$ to $r = \infty$ yields

$$U_p = -2\pi\alpha' n f\frac{Q^2}{R} \tag{13}$$

For convenience we introduce $\alpha' = f\alpha$, which has the dimensions of volume. This shows that the two ways of computing U differ by a fixed ratio dependent on the relative dielectric constant ϵ, the number density of the polarizable sites and their polarizabilities with the form

$$\frac{U_p}{U_B} = \frac{4\pi\alpha' n}{\left(1 - \frac{1}{\epsilon}\right)} \tag{14}$$

In the case of cyclohexane, the solvent used in this study, the dielectric constant $\epsilon = 2.023$ at 293 K and the number density of polarizable sites $n = 6 \times 5.514$ sites/nm^3. The value of n was obtained from the density of the liquid, 0.7731 g/mL, the molecular weight of cyclohexane, 84.16 (values taken from ref 30), and by noting that in the united atom representation used in the GROMOS96 force field there are 6 polarizable sites per molecule of cyclohexane. Substituting into eq 14 we obtain a value of 1.514. Thus, in the case of cyclohexane the value of U obtained with the nonmutual electronic polarization model overestimates the dielectric solvation energy by approximately a factor of 1.5.

It should be noted that in the current context this value corresponds to a correction due only to electronic polarizability to account for terms not explicitly included in the force field used in the simulations. It does not apply to the dielectric response associated with the reorientation of fixed dipoles. Thus, although a value of ~1.5 has been calculated for the specific case of cyclohexane, this value would be appropriate to correct for the effects of electronic polarizability in a wide range of condensed phases.

TABLE 1: Average Uncorrected and Corrected Electrostatic Interaction Energy U (± the Standard Deviation) between the Peptide and the Solvent (cyclohexane) Due to Electronic Polarization of the Solvent by the Peptide for the Different Peptide Conformations Shown in Figure 1

conformation	N	M [e nm]	$U_{\text{uncorrected}}$ ($\epsilon = 1$) [kJ/mol]	$U_{\text{corrected}}$ [kJ/mol]
α-helix	503	1	-51 ± 4	-34 ± 3
β-hairpin	500	0.1	-21 ± 3	-14 ± 2
extended	2170	0.2	-21 ± 6	-14 ± 4

The corrected values have been divided by a factor of 1.5 to correct for screening effects not included in the nonmutual polarization model (see section Electronic Polarization). The total number of cyclohexane molecules N in the simulation box and the average overall dipole moment M of the peptide is also given.

Results and Discussion

The values obtained for the electrostatic energy due to induced electronic polarization in the cyclohexane solvent for different conformations of the peptide are reported in Table 1. This is an estimate of the energetic term, not included in current force fields, which will contribute to the stability of the standard elements of secondary structure analyzed. Two values for each conformation are reported. The energies given in column 4 of Table 1 were calculated using the nonmutual polarization model. In this model the polarization induced interactions between the atoms of the solvent are neglected. Such interactions lead to a dielectric response of the medium which attenuates the electric field induced by the fixed charges. This means that the electric field experienced by atoms in the solvent is overestimated. To correct for this error the energies given in column 5 of Table 1 have been divided by a factor of 1.5 as is appropriate for liquid cyclohexane (see above).

As expected, the α-helical conformation, which has a large overall dipole moment shows the greatest stabilization ~50 or

~34kJ/mol, including the correction factor. For comparison Scholtz et al.[31] have estimated the total change in enthalpy associated with the formation of an α-helix in water to be between 4 and 5 kJ/mol per Ala residue (60−75 kJ/mol for this peptide). The extended and hairpin conformations have much smaller overall dipole moments but still a significant electrostatic interaction with the solvent due to the individual dipole moments of the backbone NH and CO groups. The net contribution to the stability of the helix relative to the hairpin or the extended conformations is ~30 or ~20 kJ/mol with the correction factor. This means that force fields parametrized to give the correct relative stability of an α-helical conformation in water might severely underestimate the relative stability of this conformation in a weakly polar or nonpolar environment. As the stabilization in this case is due to the collective action of many individual NH and CO dipoles, this missing term is difficult to correct for implicitly.

Conclusions

Using a very simple nonmutual polarization model we have estimated the potential contribution of electronic polarizability to the relative stability of an α-helical conformation, with a large overall dipole, compared to a β-hairpin and an extended conformation, in a nonpolar solvent. The calculations suggest the contribution is significant and that failure to include effects due to electronic polarizability could lead to a gross underestimation of the stability of the helical form. The work highlights the fact that the parametrization of current empirical force fields is dependent on the environment and that caution must be exercised when parameters are transferred between environments with significantly different dielectric responses.[32]

The nonmutual polarization model used only considers first-order effects. Assuming a value of $\epsilon = 1$ will lead to an overestimation of the effects due to electronic polarization. However, by comparing the results of this very simple approach to the results obtained using a continuum model for the dielectric solvation energy, it can been seen that a straightforward correction to the final energies can be made. Of course more sophisticated models could have been applied to this system. These would however have required: (i) the assignment of atomic polarizabilities to atoms within the peptide and/or (ii) the reassignment of the charge distribution in the peptide which already implicitly incorporates some degree of electronic polarization. In addition the nonmutual polarization model is noniterative and can, together with appropriate corrections, be easily incorporated in current simulation codes.

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