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Dominant Solvation Effects from the Primary Shell of Hydration: Approximation for Molecular Dynamics Simulations

A simple approximation is developed to account for the dominant effects of solvation in molecular dynamics simulations of biopolymers. A small number of water molecules are included explicitly in the primary hydration shell around the biopolymer. A nonspherical confining potential responding dynamically to the conformational changes of the biopolymer is applied to prevent evaporation and to approximate the conditions of constant pressure of a bulk solution. Simulations of a spherical system of 25 water molecules are used to adjust the empirical restraining potential to yield a uniform density distribution close to that in the bulk liquid. The primary hydration shell approach is tested with molecular dynamics simulations of simple hydrated peptides. The conformational equilibrium of alanine dipeptide and alanine tripeptide is examined using umbrella sampling calculations. The relative free energies of the C_{7ax} ($\phi = 60$, $\psi = -80$) and α_L ($\phi = 60$, $\psi = 60$) conformations of the alanine dipeptide and the opened and closed conformations of a reversed β-turn modeled with the alanine tripeptide were calculated. The results indicate that the primary hydration shell can reproduce the influence of solvent on small peptides that was observed in simulations involving a much larger number of water molecules. © *1995 John Wiley & Sons, Inc.*

INTRODUCTION

Because the influence of solvent on the conformation of flexible biopolymer is of such central importance in biophysics, considerable efforts are devoted in developing accurate and effective computational methods for its treatment. Approximate schemes taking into account the dominant effects of solvation implicitly are of particular interest since they can often provide accurate results and remain computationally inexpensive. Among those are the phenomenological approaches representing solvation effects in terms of atom- or group-based solvent-exposed area,[1,2] hydration shell models,[3–5] continuum electrostatic approximations,[6–8] and statistical mechanical liquid state theories based on integral equations.[9–11] In particular, approximations based on continuum electrostatics, in which the solvent is represented as a featureless dielectric material, can reproduce the dominant electrostatic contribution to the solvation free energy of many different small solutes.[12] Nevertheless, descriptions in which all atomic and structural details of the solvent molecules are ignored may not always be appropriate, e.g., water molecules are involved directly in the unfolding process of an α-helix.[13]

Detailed computer simulations of atomic models, in which a large number of solvent molecules are included explicitly, provide a more realis-

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tic treatment of solvation.[13–16] Such a detailed treatment, involving a very large number of solvent molecules, may not always be necessary. For example, it has been observed in computer simulations, as well as in experiments, that partial hydration is sufficient to restore dominant solvation effects.[17,18] A description of dominant solvation effects with a small number of solvent molecules located near the surface of a biopolymer is an attractive idea. For a useful computational method, it is necessary to introduce some effective boundary potential to localize the water molecules near the solute and prevent their evaporation. Such intermediate approaches have been developed to describe dominant solvation effects on a flexible biopolymer with smaller number of explicit solvent molecules, e.g., molecules included in a small region are simulated explicitly and the influence of the surrounding bulk is taken into account with an effective solvent boundary potential.[19–23] However, the solvent boundary potentials that are presently available are inappropriate because they have been developed in terms of a fixed spherical shape.[19–23] Fixed-shape boundary potentials cannot adjust dynamically according to the conformational fluctuations of a flexible biopolymer and thus still require a very large number of explicit water molecules.

The goal of the present article is to introduce a new computationally inexpensive approximation for the treatment of solvent effects on a flexible peptide molecule with an arbitrary shape. The approximation may be considered as an intermediate between implicit and explicit solvent treatments. In the "Primary Hydration Shell" approach, a small number of water molecules are included and maintained close to the surface of a polypeptide using a flexible nonspherical restraining potential. The potential is introduced to prevent the evaporation of the primary waters and to mimic the environment of a bulk liquid at constant pressure. Its shape is adapting dynamically to the conformational changes of the solute. The ability of the approach to reproduce dominant solvent effects correctly is tested by calculating the influence of solvent on the conformational equilibrium of two flexible peptides, the alanine dipeptide and the alanine tripeptide. Using umbrella sampling calculations, the relative free energy of the C_{7ax} ($\phi = 60$, $\psi = -80$) and α_L ($\phi = 60$, $\psi = 60$) conformations of the alanine dipeptide and the opened and closed conformations of a reversed β-turn modeled with the alanine tripeptide are compared with previous results obtained in bulk solution with a large number of water molecules.[23–25]

METHOD AND COMPUTATIONAL DETAILS

A small number of explicit solvent molecules are placed in the vicinity of a biopolymer solute. A flexible restraining shell potential is introduced to prevent the evaporation of the primary waters and to mimic conditions of constant pressure in the system. It is constructed from half-harmonic restraining potentials acting between the ith water oxygen and the jth solute atom corresponding to the nearest van der Waals surface,

$$U_{\text{shell}}(r_{ij}, R_{\text{shell}}) = \begin{cases} \frac{1}{2}K_{\text{shell}}x^2 & \text{if } x \geq 0 \\ 0 & \text{if } x < 0 \end{cases} \quad (1)$$

where x is $(r_{ij} - R_{\text{vdw}}^{(j)} - R_{\text{shell}})$, is the distance between particle i and j, $R_{\text{vdw}}^{(j)}$ is the van der Waals radius of the jth solute atom, R_{shell} is the shell radius, and K_{shell} is the shell force constant (nb: the jth solute atom corresponds to the atom for which the quantity $r_{ij} - R_{\text{vdw}}^{(j)}$ is the smallest).

The shell potential, as defined by Eq. (1), is a continuous function that provides a spatial restraint surrounding the solute and adapted to its instantaneous conformation. Its purpose is to prevent the evaporation of the water molecules. Displacements of the waters along the van der Waal surface around the solute are allowed, but displacements away from the surface are prevented with a shell force constant K_{shell}. Preliminary tests indicated that a time-independent constant shell radius R_{shell} was inappropriate. Large conformational fluctuations, in which peptide atoms are shielded from the solvent, can result in a significant decrease in the primary shell volume that can be occupied by solvent molecules. In those situations, a shell potential Eq. (1) with constant shell radius gives rise to large spurious forces. To account for arbitrary variations in the conformation of the polypeptide, the shell radius is adjusted dynamically in reaction to the instantaneous "pressure" inside the primary shells. The shell radius was varied according to the simple empirical relation,

$$\frac{dR_{\text{shell}}}{dt} = C_{\text{relax}}(\overline{F_{\text{shell}}(t)} - F_{\text{ref}}) \quad (2)$$

where C_{relax} is a relaxation constant, F_{ref} is a reference value and $\overline{F_{\text{shell}}(t)}$ is the instantaneous boundary force averaged over the primary waters. Such first-order dynamical equation is similar to other computational techniques used to enforce a constant pressure by adjusting the volume size in molecular dynamics simulations with periodic boundary conditions.[26] In Eq. (2), the average boundary force $\overline{F_{\text{shell}}(t)}$ corresponds to a measure of the instantaneous pressure inside the primary shell, e.g., when the shell is too small, $\overline{F_{\text{shell}}(t)}$ is larger than the reference value F_{ref}, and the radius is increasing with time. To obtain a rapid estimate of the pressure independent of the number of water molecules inside a primary shell, the average shell pressure is calculated by including only

the waters feeling a nonzero force $U'_{shell}(r_{ij}, R_{shell})$ from the boundary. The primary hydration shell model was realized for molecular dynamics calculations as a part of the CHARMM program.[27] The parameters F_{ref}, K_{shell}, and C_{relax} were adjusted empirically from preliminary tests with spherical systems of pure water: K_{shell} = 3 kcal/(mol Å^2), F_{ref} = 0.9 kcal/(mol Å), and C_{relax} = 0.5 Å^2/(ps kcal/mol).

The performance of the primary hydration model was tested with two small model peptides. The conformational flexibility of the alanine dipeptide, CH_3-CO-Ala-NH-CH_3, and of the alanine tripeptide, CH_3-CO-Ala-Ala-NH-CH_3, was examined. These systems were chosen because they represent prototypical models of the protein backbone and have already been the object of previous molecular dynamics calculations with a large number of explicit water molecules.[23-25] The initial configuration of the peptide molecules with its primary hydration shell was constructed in the following way. The peptide molecule was overlaid at the center of a large sphere of 500 waters equilibrated at 300 K. The water molecules with their oxygen lying closer than 3 Å or farther than 5 Å from any peptide atoms were removed. The resulting number of waters were 43 for the alanine dipeptide and 50 for the alanine tripeptide. The initial systems were equilibrated during 40 ps with Langevin dynamics in the presence of the primary shell potential, keeping the peptide atoms fixed in space. A time step of 0.002 ps was used for all simulations and a friction constant of 25/ps was applied to all nonhydrogen atoms. All calculations were done using the extended atom CHARMM[27] potential function for the peptides and the

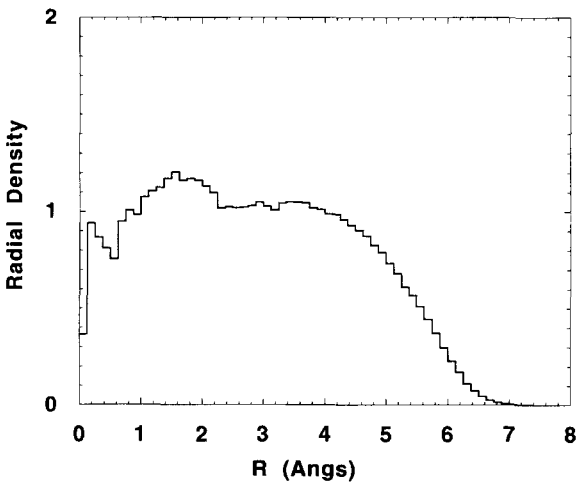

FIGURE 1 Normalized radial distribution of water oxygens from the center of a sphere containing 25 explicit TIP3P[28] waters. The density distribution function was divided by 0.0334/Å^3, the density of bulk water. The average was calculated from the trajectory of Langevin dynamics of 500 ps using a friction of 5/ps on the oxygens. The average value of the shell radius was 5.7 ± 0.3 Å.

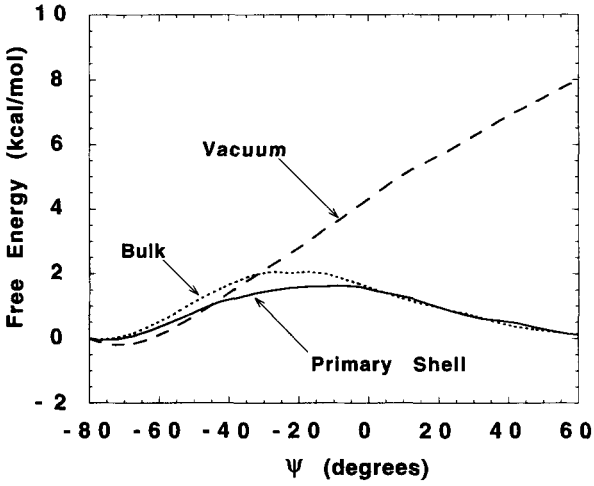

FIGURE 2 Relative free energy difference of the α_L to C_{7ax} conformations for the alanine dipeptide, CH_3-CO-Ala-NH-CH_3. The PMF along the ϕ dihedral angle, calculated in gas phase (vacuum) and with 43 explicit water molecules using the primary hydration shell model, are shown. The results from Beglov and Roux (Bulk1)[23] and from Tobias and Brooks (Bulk2)[24] are also shown. The PMF calculations were done using the umbrella sampling method. The potential energy function of the system was biased with a harmonic dihedral umbrella potential $0.5k(\psi - \psi_i)^2$ for nine windows successively centered at $\psi_i = -100°, -80°, \ldots, 60°$; the force constant k was equal to 4.0 kcal/mol/rad^2. The value of the dihedral ϕ was restrained around 60° with a strong harmonic potential. The configurational sampling was performed from Langevin dynamics trajectories of 200 ps. Each window was equilibrated during 40 ps starting from the last configuration of the previous window. The histograms were unbiased and combined using a statistical weighting method.[17]

TIP3P[28] water model. The relative conformational free energy was obtained from the potential of mean force (PMF) along the ψ dihedral angle of the alanine dipeptide and the O-H distance between terminating carbonyl oxygen and amide hydrogen for the alanine tripeptide. The PMFs were calculated using the umbrella sampling technique; further computational details are given in the figure captions.

RESULTS AND DISCUSSION

The normalized radial density distribution in a spherical system containing 25 water molecules is shown in Figure 1. It is observed that the average density of bulk water (0.0334/Å^3) is well reproduced in the interval from 0.5 to 4.0 Å. The average density decays to zero over a distance of about 2.5

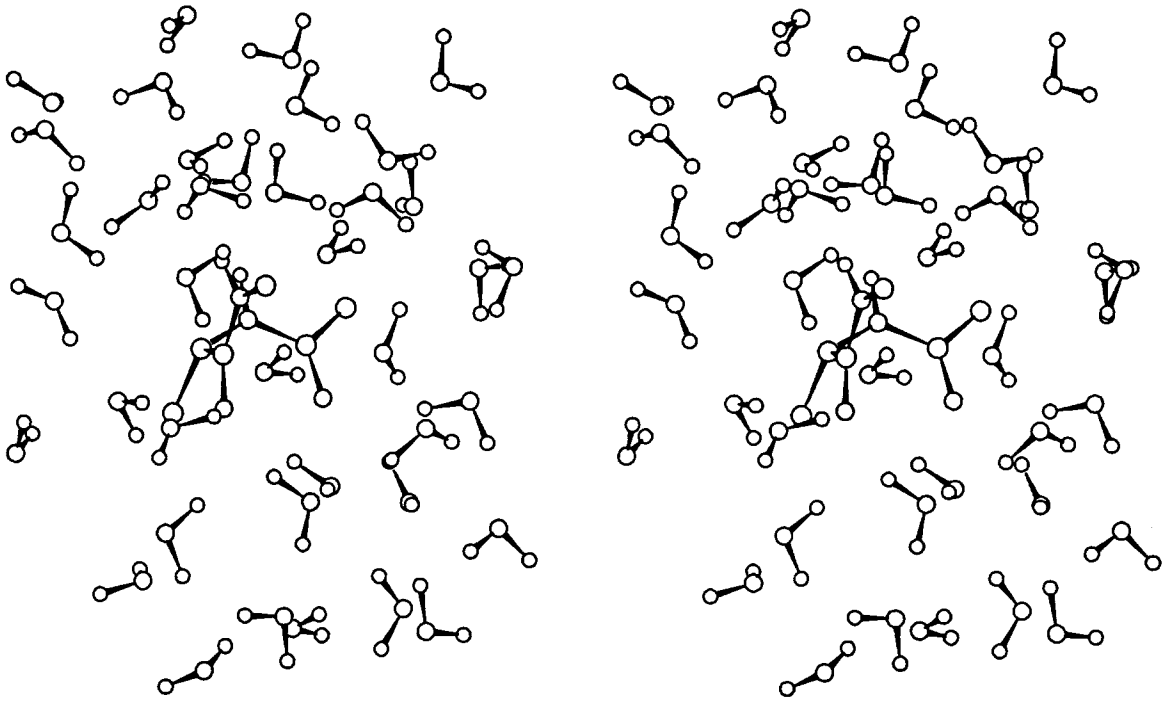

FIGURE 3 An illustration of the alanine dipeptide hydration in the α_L conformation. The configuration is taken from the simulation of the last window in the umbrella sampling calculation corresponding to $\psi_n = 60°$ (see Figure 2). The average value of the shell radius was 4.3 Å with rms fluctuations on the order of 0.2 Å.

Å. The value of the density near the center of sphere is not statistically meaningful since it corresponds to a very small volume element. Overall, the equilibrium density of the water sphere was not very sensitive to the choice of parameters of the flexible restraining potential. The mean value of the shell radius R_{shell} was 5.7 Å, with rms fluctuations on the order of 0.3 Å. The value of the force constant K_{shell} of the half-harmonic shell potential was fixed at 3 kcal/mol/Å^2. The relaxation constant C_{relax}, was chosen such that the equilibration of the water sphere took place in a reasonable time. For values of C_{relax} larger than 5.0 Å^2/(ps kcal/mol), the response of the shell radius to the fluctuations in the shell forces is too rapid and abrupt; the shell radius oscillates and becomes unstable. For values of C_{relax} less than 0.05 Å^2/(ps kcal/mol), the shell radius does not adjust to the solute conformation in a reasonable time. After several tests, a value of 0.5 Å^2/(ps kcal/mol) was chosen. The most sensitive parameter is the reference boundary force F_{ref}. The value of 0.9 kcal/mol/Å was calculated from a molecular dynamics simulations of a sphere of 25 waters with a radius fixed at a value of 5.633 Å, corresponding to the density of the bulk liquid (0.0334/Å^3). As it can be observed in Figure 1, allowing the radius to vary dynamically, according to Eq. (2), did not modify the average radial density in the water sphere. However, experimenting with the value of the reference boundary force indicated that the bulk density was too high in simulations of a sphere of 25 waters when the parameter F_{ref} is increased from 0.9 to 1.0 kcal/mol/Å. For smaller values, the simulated sphere expanded and the density was too small.

The ability of the primary shell model in reproducing the influence of bulk solvation on the conformational equilibrium of model peptides was examined. The solvent-induced conformational free energy of two small peptides, the alanine dipeptide and the alanine tripeptide, was calculated. These two peptides were chosen because they represent prototypical models of the protein backbone and have already been the object of molecular dynamics calculations involving a large number of explicit water molecules.[23–25] To obtain a quantitative estimate of the performance of the primary hydration shell model, relative free energies between specific conformations of the peptides were calculated using the umbrella sampling procedure.[29]

As a first test of the primary hydration model, the conformational equilibrium of the alanine di-

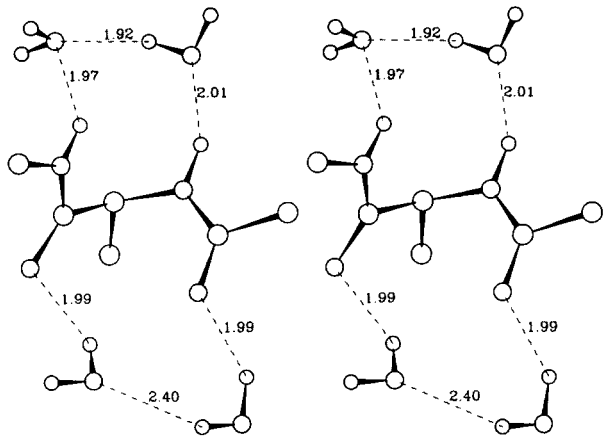

FIGURE 4 Detail of the alanine dipeptide hydration in the α_L conformation showing the presence of two water molecules forming bridges between the carbonyl oxygens or amide hydrogens.

peptide in water was considered. The relative free energy of the C_{7ax} ($\phi = 60, \psi = -80$) and α_L ($\phi = 60$, $\psi = 60$) conformations of the alanine dipeptide was calculated from the PMF along the dihedral ψ with a constant value of 60° for the dihedral ϕ. The PMF calculated with the primary hydration model is shown in Figure 2. For comparison, the PMF calculated in vacuum as well as the results taken from two previous calculations[23,24] are also shown. Further details are given in the caption. In the study of Tobias and Brooks,[24] the PMF was calculated with the extended atom CHARMM potential function in the presence of 200 water molecules using molecular dynamics simulations with a free energy perturbation technique based on holonomic internal coordinate constraints. In the study of Beglov and Roux,[23] the PMF was calculated using the umbrella sampling technique from simulations of the dipeptide inside a sphere of 100 water molecules with a solvent boundary potential generated with the same potential function. Only 43 explicit water molecules were included in the present simulations, which is almost five times less than the number of water molecules used in the calculations of Tobias and Brooks. Despite the differences in simulation systems and computational methodologies, the PMF calculated with the primary hydration shell approximation is in good agreement with the previous results.[23,24] The largest difference is at the barrier between the C_{7ax} and α_L conformations. In the present calculations, the barrier is lower by about 0.5 and 1.5 kcal/mol with respect to the two previous calculations.[23,24] It should emphasized that the presence of the primary waters provides a sufficient hydration to stabilize the α_L conformation. In vacuum, this conformation is highly unstable. The free energy difference between the α_L and C_{7ax} conformations is 0.1 kcal/mol with waters while it is 8 kcal/mol in vacuum. In vacuum, the C_{7ax} conformation is stabilized by a hydrogen bond formed between the terminating carbonyl oxygen and amide hydrogen of alanine dipeptide. One particular configuration of the alanine dipeptide in the α_L conformation surrounded by its primary hydration shell is shown in Figure 3. A structural detail of the hydration is presented in Figure 4. The hydration of the α_L conformation of alanine dipeptide is characterized by the formation of two water bridges connecting the amide hydrogens or the carbonyl oxygens. Each bridge consists of two water molecules coupled by a hydrogen bond (see Figure 4). Generally, implicit treatments of solvation[1-8] cannot take the presence of such correlated hy-

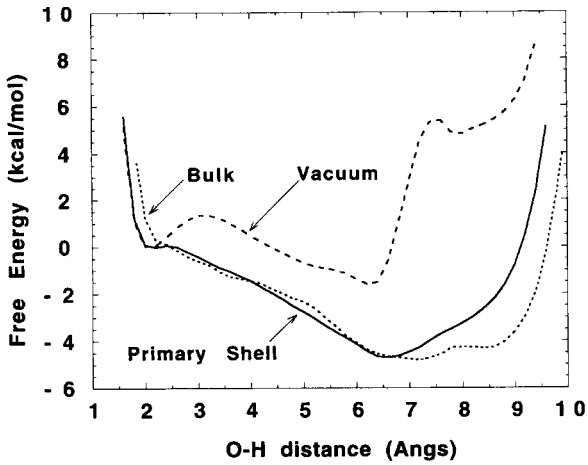

FIGURE 5 Relative free energy difference of the close and open reverse β-turn conformations for the alanine tripeptide, CH_3-CO-Ala-Ala-NH-CH_3. The PMFs, calculated in vacuum and with 50 explicit water molecules using the primary hydration as a function of the carbonyl oxygen–amide hydrogen distance of the blocking groups shell model, are shown. The results from Tobias and Brooks (Bulk) are also shown. The PMF was obtained using the umbrella sampling method following a protocol similar to that used with the alanine dipeptide calculations. The potential energy function of the system was biased by a harmonic umbrella potential $0.5k(r_{OH} - r_i)^2$ depending of the distance r_{OH} between the blocking carbonyl oxygen and the blocking amide hydrogen; a force constant k equal to 3.0 kcal/mol/Å^2 was used for twelve windows, successively centered at $r_i = 1.8, 2.6, \ldots, 10.6$ Å (one more window was generate for r_i equal to 1.7 Å with a force constant of 6.0 kcal/mol/Å^2 for a better accuracy at short distances).

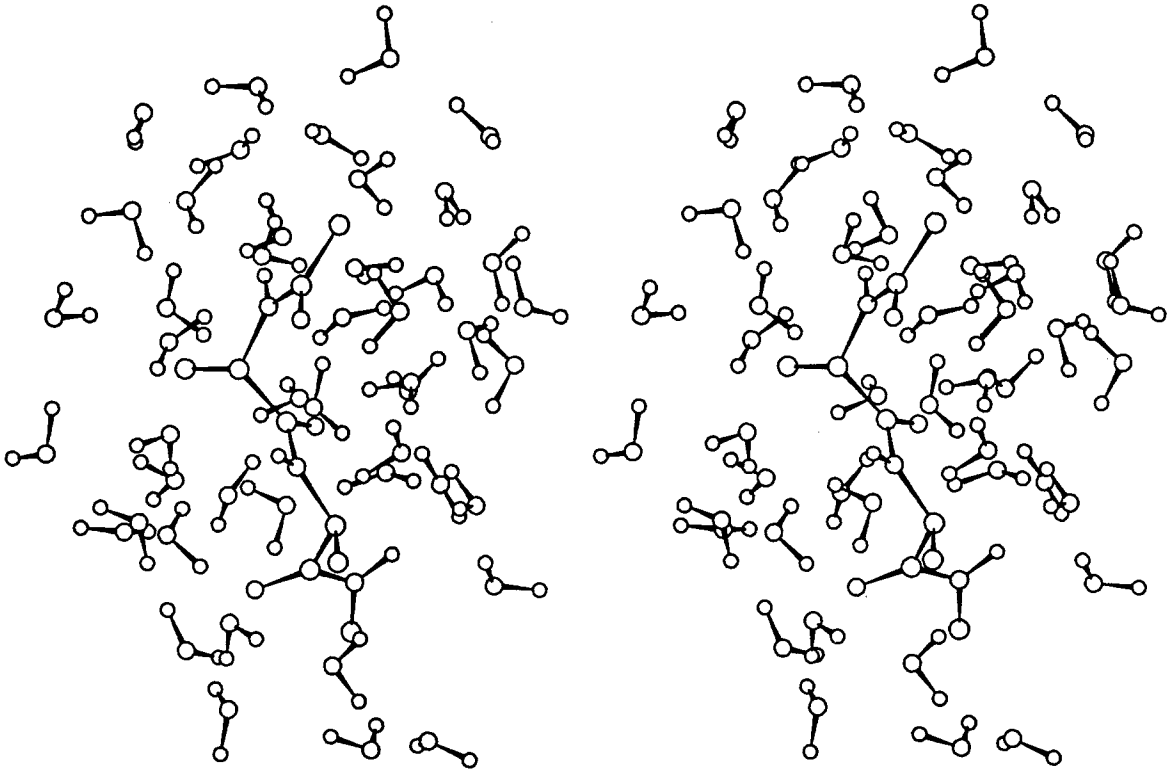

FIGURE 6 Stereo view of the hydrated alanine tripeptide in the open conformation. The configuration is taken from the trajectory in the umbrella sampling calculation corresponding to r_i = 6.6 Å (see Figure 5). The average value of the shell radius was 4.5 Å with rms fluctuations on the order of 0.4 Å.

drogen-bonded structures into account, though some solvent–solvent correlation are included in the RISM-HNC integral equation approach.[9–11]

As a second test, the conformational equilibrium of a reversed β-turn, modeled with an alanine tripeptide, was considered. This system was chosen because it has been the object of an extensive theoretical study by Tobias and Brooks.[25] To characterize the conformation of the β-turn, Tobias and Brooks calculated the PMF as a function of the O-H distance, corresponding to the intramolecular hydrogen bond between the blocking carbonyl oxygen and the blocking amide hydrogen. Approximately 240 explicit waters treated with periodic boundary conditions were included in their calculations. The PMF calculated with the primary hydration shell model, using the umbrella sampling technique, is shown in Figure 5. For comparison, the PMF calculated in vacuum and the result from the calculation of Tobias and Brooks[25] are also shown. Further computational details are given in the caption. The agreement of the primary shell model with the result of Tobias and Brooks[25] is remarkable considering the fact that the number of water molecules included explicitly in the present simulation is almost five times smaller. The main conclusion of their study is supported by the present calculations: a closed β-turn in water is less stable than an open one by about 5 kcal/mol. It can be noted that, relative to vacuum, the 50 primary waters are sufficient to stabilize the extended conformation (O-H distance of 8 Å) by 5 kcal/mol. In vacuum, the open hydrogen bond (O-H distance of 6.6 Å) is more stable due to the formation of hydrogen bond between the terminating carbonyl oxygen and the middle amide hydrogen. This hydrogen bond is also a stabilizing factor of the 6.6 Å conformation in water. A typical configuration of the alanine tripeptide with the primary hydration shell is shown in Figure 6. To obtain more quantitative information about the hydration structure around the solute, a 500 ps unrestrained Langevin trajectory of the tripeptide and the 50 primary waters was generated and the solute–solvent radial distribution functions were calculated. The results for a carbonyl oxygen, amide hydrogen, and a methyl group are shown in Figure 7. In all cases, a well-structured first peak is observed in the radial

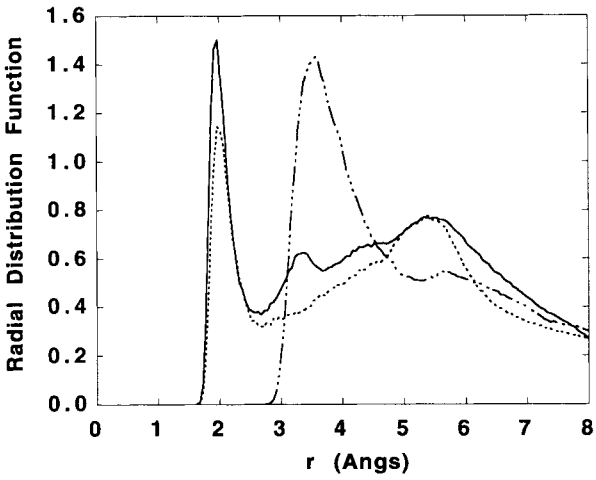

FIGURE 7 Radial distribution functions of water around particular atoms of the alanine tripeptide. The distribution of water hydrogens around the second carbonyl oxygen (solid line), the distribution of water oxygen around the first amide hydrogen (dashed line), and the distribution of water oxygen around the third methyl group (long-dashed line) are shown (counting from the CH_3CO to the CH_3NH terminating group). The distribution functions were calculated from a 500 ps Langevin trajectory of the unconstrained tripeptide in the presence of 50 waters. The radial distribution functions were normalized using the density of bulk water, 0.0334/Å^3.

distribution function. The primary water molecules are forming stable hydrogen bonds to the carbonyl oxygen and the amide hydrogen. Furthermore, even the nonpolar methyl group is well hydrated on average although some vacancies with no waters appear transiently around the hydrophobic group due to statistical fluctuations (e.g., see Figure 6). The radial distribution functions around other atoms of the tripeptide were very similar. Finally, detailed analysis of the hydration structure showed that a bridge of hydrogen-bonded water molecules, similar to that shown on Figure 4, is present between the terminating and the middle amide hydrogen. Again, it is likely that implicit solvent treatments cannot take such correlated hydrogen-bonding structures into account.

CONCLUSION

A simple and inexpensive computational scheme was developed to account for the dominant effects of solvation in molecular dynamics simulations of biopolymers. In the primary hydration model, a small number of water molecules are included explicitly, and a nonspherical confining potential, responding dynamically, is applied to prevent evaporation. The approach was tested with typical systems. The results of the calculations show that the dominant effects of hydration of simple peptides can be reproduced by the primary hydration model. This indicate that the present approach may be most useful to include the influence of solvation when simulations with a large number of explicit water molecules are too expensive computationally, e.g., for conformational searches of small- and medium-sized peptides,[30] or for the refinements of structures using nuclear Overhauser effect restraints derived from two-dimensional nmr.[31] In particular, it is well known that nmr refinement with restrained dynamics calculations performed in vacuum may lead to unrealistic polar–polar interactions between solvent-exposed groups.[32] Including explicit solvent molecules in the restrainted dynamics calculations can improve the structure refinement,[18,32–34] but is computationally expensive. The present approach has been designed to provide the dominant hydration effects and prevent such unrealistic protein–protein interactions.[18,32]

The approach may be extended in several directions. For example, the shell potential Eq. (1) could be applied with respect to a selected subset of atoms in a larger complex molecule. This may be useful in providing a limited solvation in studying flexible fragments of a large protein.[35] In addition, the approach could be extended by incorporating the long-range influence of the surrounding bulk solvent based on a dielectric continuum approximation by solving the Poisson–Boltzmann equation using finite difference algorithms.[6] Such a extended primary hydration model may be considered as intermediate to the fully detailed simulations involving a large number of explicit water molecules[13–16] and the implicit solvent treatment in the continuum dielectric approximations.[6] Present efforts include the developments of the primary shell in these directions and studies of the unfolding of an α-helix (D. Beglov, R. Bruschweiler, and B. Roux, in preparation).

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