JCTC Journal of Chemical Theory and Computation

Letter

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Effects of Polarizable Solvent Models upon the Relative Stability of an α-Helical and a β-Hairpin Structure of an Alanine Decapeptide

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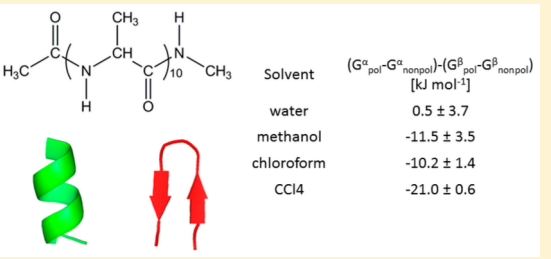
Supporting Information

ABSTRACT: The free enthalpy of changing a nonpolarizable solvent into a polarizable solvent is calculated for an alanine decapeptide solvated in water, methanol, chloroform, or carbon tetrachloride. Introducing polarizability into a water solvent does not change the relative stability between the α-helix and the β-hairpin, while for methanol and chloroform the α-helix is stabilized by about 1 kJ mol^{-1} per residue and for carbon tetrachloride by about 2 kJ mol^{-1} per residue. These results suggest that the less polar the solvent is, the more the α-helical structure is stabilized with respect to the β-hairpin structure by the use of a polarizable solvent model instead of a nonpolarizable one. This highlights that inclusion of polarizability in models for less polar and nonpolar solvents or protein environments is as important as, if not more important than, including polarizability in models for liquid water.



1. INTRODUCTION

In the past few decades, the simulation of biomolecules at the atomic level of resolution has become an important tool to understand their properties and behavior. The quality of such simulations, however, critically depends on the accuracy of the molecular model or force field employed. The most widely used biomolecular force fields treat nonbonded interactions as a pairwise additive,[1−4] where many-body contributions are included in an average manner. This approach usually gives a satisfactory description for homogeneous systems, such as pure liquids or mixtures of compounds of similar polarity, but may break down for spatially electronically inhomogeneous ones, such as a protein or membrane in water, interfaces, or mixtures of different solvents. For this kind of systems the introduction of polarizability should and does lead to an improved description.[5−7]

As water plays an important role in biomolecular processes, large efforts have been undertaken to find good polarizable models for liquid water,[8−10] while less attention has been paid to less polar or nonpolar liquids such as methanol,[11] chloroform,[12] and carbon tetrachloride.[13] Soto and Mark[14] investigated the effect of neglecting electronic polarization on the relative stability of different conformations of a polyalanine in a nonpolar environment. Their results suggested that the stability of conformations that have an enhanced molecular electric dipole moment, e.g. an α-helix, can be significantly underestimated by neglecting the effects of electronic polarization in the solvent force field.[14]

The effect of introducing polarizability into the solvent model upon the stability of peptide/protein conformations has been studied by means of molecular dynamics (MD) simulations. Using a polarizable methanol solvent, helical structures, which have nonzero dipole moments, are stabilized, while no obvious effect was detected in the simulations of peptides with hairpin structure as the dominant fold.[15] It was concluded that the introduction of electronic polarizability into the solvent model appears of importance to a proper description of folding equilibria if these are determined by competing solute conformations that have different dipole moments. Other studies in the literature focused on the effect of including polarizability into the peptide model on the secondary structure of the solute[16−18] or the stability of the native protein fold.[6,7]

In this work, we quantitatively investigate the effect of introducing polarizability into the solvent model for four solvents of different polarity on the relative stability between an α-helical and a β-hairpin secondary structure of a deca-alanine solute by calculating the free enthalpy of polarization, i.e. by assessing the free enthalpy[19] or Gibbs energy[20] change of these conformations induced by changing a nonpolarizable solvent into a polarizable one at constant temperature (300 K) and pressure (1 atm).

2. METHODS

The model system considered is an alanine decapeptide capped at both termini with methyl groups, acetyl-(Ala)$_{10}$-*N*-methyl (Figure S1), solvated in a rectangular box with 3204 water molecules, 1428 methanol molecules, 725 chloroform molecules, or 597 carbon tetrachloride molecules, respectively. The GROMOS force field 54A7[21] was used for the peptide. The SPC model[22] and the COS/B2 model[9] were used as the nonpolarizable model and the polarizable model for water molecules, respectively. Standard GROMOS96 solvent force-

Received: March 4, 2015

DOI: 10.1021/acs.jctc.5b00210
J. Chem. Theory Comput. XXXX, XXX, XXX−XXX

field models[23] (referred to as G96 below) were used as the nonpolarizable models for methanol,[24] chloroform,[25] and carbon tetrachloride,[26] and the COS/M,[11] COS/C,[12] and a one-site polarizable carbon tetrachloride model[13] were used as the polarizable models for methanol, chloroform, and carbon tetrachloride, respectively. The simulations were carried out using the GROMOS11 simulation package.[27]

The free enthalpies of polarization were calculated by thermodynamic integration (TI).[28,29] At each of the 21 equidistant λ-values, the system was equilibrated for 0.2 ns followed by 2 ns of production. The statistical uncertainties were estimated using block averaging.[30] In order to keep the peptide into the α-helical and β-hairpin conformation during the TI simulations, hydrogen-bond restraints were applied. These restraints are defined as an attractive half-harmonic function V_{rest} applied to the hydrogen-bonding pairs of H and O atoms that characterize the α-helical or β-hairpin conformations (Table S1). The reference H−O distance is set to 0.25 nm, and the restraining force constant is set to 5000 kJ mol^{-1} nm^{-2} for all simulations. With this choice, we expect the restraining forces, which are zero as long as the H−O distances of the hydrogen-bonding pairs are smaller than the indicated distance, i.e. when the solute conformation stays close to the respective conformation of interest, to have a negligible effect on the equilibrium distributions within each of the conformational states.[31] Details of simulation setup can be found in the Supporting Information.

Since distance restraints were applied in the TI simulations (vertical arrows in the middle part of Figure 1), reweighting needs to be carried out to obtain the free enthalpy differences between the nonpolarizable and polarizable Hamiltonians, i.e. to remove the effects of conformational restraining. Reweighting can be conducted in two different ways. A most simple way of reweighting (referred to as One-Step Reweighting below) only uses the TI simulations at the end states $\lambda = 0$ and $\lambda = 1$, i.e. only the two end state ensembles are reweighted

$$\Delta G^{\alpha}_{\text{pol,pol+rest}} = G^{\alpha}_{\text{pol}} - G^{\alpha}_{\text{pol+rest}} = -k_{\text{B}}T \ln\langle e^{\beta V^{\alpha}_{\text{rest}}}\rangle_{\text{pol+rest}} \quad (1)$$

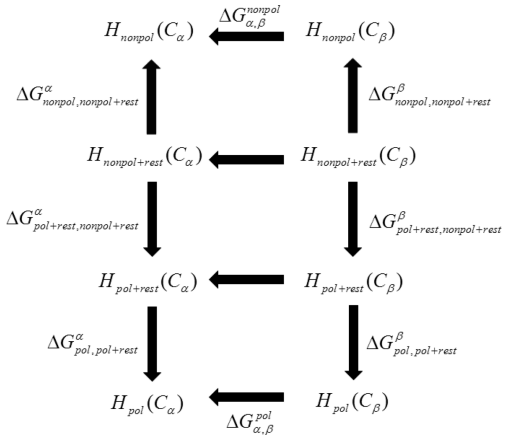


Figure 1. Schematic representation of a thermodynamic cycle in which H_{nonpol} and H_{pol} denote a deca-alanine solute in a nonpolarizable and a polarizable solvent, respectively; $H_{\text{nonpol+rest}}$ and $H_{\text{pol+rest}}$ denote a deca-alanine solute with α-helical or β-hairpin hydrogen-bond restraining in a nonpolarizable and a polarizable solvent, respectively; C_α and C_β denote the α-helical and the β-hairpin conformation, respectively.

where V^{α}_{rest} is the restraining potential energy of an α-helix conformation, the subscript "pol" denotes the use of a polarizable solvent model in the Hamiltonian of the simulations, and the subscript "rest" denotes the use of distance restraining in the Hamiltonian of the simulations. $\Delta G^{\alpha}_{\text{nonpol,nonpol+rest}}$, $\Delta G^{\beta}_{\text{pol,pol+rest}}$, and $\Delta G^{\beta}_{\text{nonpol,nonpol+rest}}$ are calculated correspondingly. This yields

$$\Delta G^{\alpha}_{\text{pol,nonpol}} = G^{\alpha}_{\text{pol}} - G^{\alpha}_{\text{nonpol}} = \int_{\lambda=\text{nonpol}}^{\text{pol}} \left\langle \frac{\partial H}{\partial \lambda} \right\rangle_{\lambda,\text{rest}} \text{d}\lambda + \Delta G^{\alpha}_{\text{pol,pol+rest}} - \Delta G^{\alpha}_{\text{nonpol,nonpol+rest}} \quad (2)$$

A schematic representation of the thermodynamic cycle of this One-Step Reweighting is shown in Figure 1. An alternative way of reweighting (referred to as TI reweighting below) uses the TI simulations of all λ values, i.e. the $\partial H/\partial\lambda$ values are reweighted at all intermediate λ values of the restraint simulations[32]

$$\Delta G^{\alpha}_{\text{pol,nonpol}} = \int_{\lambda=\text{nonpol}}^{\text{pol}} \frac{\left\langle \frac{\partial H}{\partial \lambda} e^{\beta V^{\alpha}_{\text{rest}}} \right\rangle_{\lambda,\text{rest}}}{\langle e^{\beta V^{\alpha}_{\text{rest}}}\rangle_{\lambda,\text{rest}}} \text{d}\lambda \quad (3)$$

Note that both ways of reweighting do not require additional simulations.

3. RESULTS AND DISCUSSION

The results of the TI calculations in the presence of the conformational restraints (TI(rest)) are shown in Table 1 as well as in Figures S2−5. Since the systems contain many solvent molecules, the value of $\Delta G_{\text{pol,nonpol}}$ for the solute conformations α or β divided by the number of solvent molecules yields a good approximation of the free enthalpy difference per molecule between the polarizable and nonpolarizable solvent models. The free enthalpy per molecule of changing SPC water to COS/B2 water is 1.0 kJ mol^{-1}. Since the potential energy per molecule of liquid COS/B2 water is about 0.5 kJ mol^{-1} more negative than for SPC water,[9] this indicates that COS/B2 water has a lower entropy per molecule, by 5.0 J mol^{-1} K^{-1} at 300 K, than SPC water. The free enthalpy per molecule of changing G96 methanol to COS/M methanol is 3.5 kJ mol^{-1}. The potential energy per molecule of liquid COS/M methanol is 1.5 kJ mol^{-1} more positive than that of G96 methanol,[11] which indicates that COS/M methanol has a lower entropy per molecule, by 6.7 J mol^{-1} K^{-1} at 300 K, than G96 methanol. The free enthalpy per molecule of changing G96 chloroform to COS/C chloroform is −1.5 kJ mol^{-1}. The inversed sign of the free enthalpy change compared with the previous two solvents is probably due to a 2.1 kJ mol^{-1} more negative potential energy per molecule of liquid COS/C chloroform than G96 chloroform.[12] So liquid COS/C chloroform has a lower entropy per molecule, by 2.0 J mol^{-1} K^{-1} at 300 K, than G96 chloroform. For these three solvents the polarizable solvent model yields a lower entropy in the liquid phase compared with the nonpolarizable model, which is consistent with a previous conclusion[9] that polarizable solvent models yield a more structured liquid than nonpolarizable ones.

The much smaller values $\Delta G_{\text{pol,nonpol}}$ (TI(rest)) obtained in the TI calculations for carbon tetrachloride compared with the other three solvents are due to the absence of permanent partial charges in both CCl_4 models. The only difference between the G96 and the polarizable CCl_4 model is the introduction of the

DOI: 10.1021/acs.jctc.5b00210
J. Chem. Theory Comput. XXXX, XXX, XXX−XXX

Table 1. Free Enthalpy Differences between Polarizable and Nonpolarizable Solvent Models for α-Helical and β-Hairpin Conformations of Deca-Alanine in Four Different Solvents Calculated in Two Different Ways[a]

	deca-alanine in α-helical or β-hairpin			pure liquid (per molecule)			
	$\Delta G^{\alpha}_{\mathrm{pol,nonpol}}$ [kJ mol^{-1}]	$\Delta G^{\beta}_{\mathrm{pol,nonpol}}$ [kJ mol^{-1}]	$\Delta\Delta G^{\alpha\beta}_{\mathrm{pol,nonpol}}$ [kJ mol^{-1}]	$\Delta H_{\mathrm{pol,nonpol}}$ [kJ mol^{-1}]	$\Delta G^{\alpha}_{\mathrm{pol,nonpol}}$ [kJ mol^{-1}]	$T\Delta S_{\mathrm{pol,nonpol}}$[b] [kJ mol^{-1}]	$\Delta S_{\mathrm{pol,nonpol}}$[c] [J mol^{-1} K^{-1}]
H_2O: SPC (nonpol) to COS/B2 (pol)							
TI(rest)	3184.8 ± 2.4	3186.5 ± 2.4	−1.7 ± 3.4	−0.5[d]	1.0	−1.5	−5.0
OSR	3184.8 ± 2.4	3184.3 ± 2.8	0.5 ± 3.7				
TIR	3179.4 ± 407.8	3183.8 ± 1039.8	−4.4 ± 1116.9				
MeOH: G96 (nonpol) to COS/M (pol)							
TI(rest)	4956.3 ± 2.6	4967.6 ± 2.3	−11.3 ± 3.5	1.5[e]	3.5	−2.0	−6.7
OSR	4956.1 ± 2.6	4967.6 ± 2.3	−11.5 ± 3.5				
TIR	4954.8 ± 208.8	4972.5 ± 1131.9	−19.7 ± 1151.0				
$CHCl_3$: G96 (nonpol) to COS/C (pol)							
TI(rest)	−1092.3 ± 0.5	−1079.5 ± 0.6	−12.8 ± 0.8	−2.1[f]	−1.5	−0.6	−2.0
OSR	−1090.1 ± 1.3	−1079.9 ± 0.6	−10.2 ± 1.4				
TIR	−1093.0 ± 150.0	−1078.5 ± 133.9	−14.5 ± 201.1				
CCl_4: G96 (nonpol) to G96 (pol)							
TI(rest)	−32.7 ± 0.2	−10.8 ± 0.1	−21.9 ± 0.2				
OSR	−32.7 ± 0.3	−11.7 ± 0.5	−21.0 ± 0.6				
TIR	−32.2 ± 6.4	−10.4 ± 1.5	−21.8 ± 6.6				

[a]TI(rest): results of TI with hydrogen-bond restraining; OSR: results of One-Step Reweighting (eq 2); TIR: results of TI Reweighting (eq 3). [b]$T\Delta S_{\mathrm{pol,nonpol}} = \Delta H_{\mathrm{pol,nonpol}} - \Delta G^{\alpha}_{\mathrm{pol,nonpol}}$. [c]$\Delta S_{\mathrm{pol,nonpol}} = T\Delta S_{\mathrm{pol,nonpol}}/300$. [d]Reference 9. [e]Reference 11. [f]Reference 12.

polarizability in the latter,[13] whereas for the other solvents, other nonbonded force-field parameters are also different.

The results of applying reweighting are shown in Table 1, and the detailed results for One-Step Reweighting (eq 2) are shown in Figures S2−5. Because the hydrogen-bond restraining potential energies are quite small in all simulations, the free enthalpy changes $\Delta G_{\mathrm{pol,pol+rest}}$ and $\Delta G_{\mathrm{nonpol,nonpol+rest}}$ calculated through eq 1 are not very big, i.e. in the range of −0.2 to −4.2 kJ mol^{-1} (Figures S2−5). As a result, the free enthalpy differences $\Delta G^{\alpha,\beta}_{\mathrm{pol,nonpol}}$ obtained from One-Step Reweighting are similar to the TI(rest) results. They differ by 2.2, −0.2, 2.6, and 0.9 kJ mol^{-1} for water, methanol, chloroform, and carbon tetrachloride, respectively. These values are smaller than k_BT and are of the order of the statistical uncertainty of such calculations.

Using reweighting at all λ values (TI Reweighting, eq 3), very large statistical uncertainties of 10^2 to 10^3 kJ mol^{-1} were obtained for the three polar solvents. This is due to noise induced by the restraining potential energy, which amplifies through the exponential factor the fluctuations in $\partial H/\partial\lambda$ in the calculations of eq 3. Yet, despite the very large statistical uncertainties, the values of $\Delta G^{\alpha,\beta}_{\mathrm{pol,nonpol}}$ from TI Reweighting are not very different from the ones obtained from One-Step Reweighting,[33] being 1 to 8 kJ mol^{-1} lower. So TI Reweighting is less reliable than One-Step Reweighting, and thus only the latter values will be used in the following discussion.

According to the $\Delta G^{\alpha,\beta}_{\mathrm{pol,nonpol}}$ values calculated using One-Step Reweighting upon a change from the nonpolarizable to the polarizable model for the solvent, the α-helical conformation is stabilized over the β-hairpin one by −0.5 ± 3.7, 11.5 ± 3.5, 10.2 ± 1.4, and 21.0 ± 0.6 kJ mol^{-1} for water, methanol, chloroform, and carbon tetrachloride, respectively (Table 1). These numbers correlate weakly with the polarizabilities of 0.93, 1.32, 9.5, and 10.5 $4\pi\varepsilon_0$ 10^{-3} nm^3 of the polarizable models of the four solvents, respectively. The less polar the solvent is, the more the α-helical structure is stabilized by the use of a polarizable solvent model. The relative stability between the α-helix and the β-hairpin does not change upon introduction of solvent polarizability with water as solvent, while the α-helix is stabilized by about 1 kJ mol^{-1} per residue with methanol or chloroform as solvent and by about 2 kJ mol^{-1} per residue with carbon tetrachloride as solvent. These results suggest that nonpolarizable solute force fields parametrized to give the correct relative stability between the α-helix and the β-hairpin in water may underestimate the relative stability of the α-helix in a less polar or nonpolar environment represented by a nonpolarizable model by about 1−2 kJ mol^{-1} per residue. Therefore, including polarizability in models for less polar and nonpolar solvents is as important as, if not more important than, including polarizability in models for liquid water.

■ ASSOCIATED CONTENT

Supporting Information

The details of the simulation setup are given with the chemical formula of the solute in Figure S1, and the hydrogen-bond atom pairs used in the restraining in Table S1. Figures S2−S5 contain the TI (nonpolarizable to polarizable solvent) and one-step perturbation (restrained to nonrestrained for both cases) results in the α-helical and β-hairpin configurations for the four solvents. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors would like to thank Daan Geerke and Haiyan Liu for useful discussions. This work was financially supported by grant number 200020-137827 of the Swiss National Science Foundation and by grant number 228076 of the European Research Council (ERC), which is gratefully acknowledged.

DOI: 10.1021/acs.jctc.5b00210
J. Chem. Theory Comput. XXXX, XXX, XXX−XXX

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DOI: 10.1021/acs.jctc.5b00210
J. Chem. Theory Comput. XXXX, XXX, XXX−XXX