Molecular Dynamics Simulations of the Ala-Pro Dipeptide in Water: Conformational Dynamics of Trans and Cis Isomers Using Different Water Models

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Molecular dynamics simulations of a single dipeptide (Ala-Pro) molecule in water were carried out using different water models, the modified TIP3P (transferable intermolecular potential 3P), the refined SPC (simple point charge), and the original SPC/E (extended simple point charge). Both trans and cis isomers of the dipeptide were simulated, and conformations from simulations with the different water models were compared. Both isomers have several conformations, but the total conformational space is limited. Two experimentally obtained subconformations of the cis isomer were found in simulations with the different water models. The bioactive conformation, which is binding to Cyclophilin A, was the major cis conformation, and the conformation with an intramolecular hydrogen bond between the N-terminal and the C-terminal was found to exist as the minor cis conformation. The hydration of the dipeptide was found to depend both on its conformation and on the water model.

1. Introduction

To understand the mechanism of protein folding, it is very important to understand the conformational preferences of each of the 20 naturally occurring amino acid residues in water. In a recent review by Császár and Perczel[1] of a large number of studies of all naturally occurring amino acids, they point out the importance of detailed knowledge of the conformational behavior and the interactions of amino acids in solution, especially in water, and how this knowledge increases the understanding of hydration of peptides and proteins and also the role of water in biochemical and biological systems. The number of theoretical studies taking solvent effects into account is increasing, but still the majority of the ab initio studies were conducted in the gas phase. When water effects are included in ab initio studies, the results are closer to experimental vibration spectra. This shows the importance of hydration when the conformational space of amino acids is studied. As an example of an ab initio study where water effects were included, Ramirez et al.[2] present calculations of structural and vibrational dynamics of glutamine in solution, which give satisfactory agreement with the experimental data.

Different dipeptides have been used as simplified model structures to study the conformational dynamics of polypeptides. Most of the theoretical studies have applied Monte Carlo simulations,[3,4] molecular dynamics simulations,[5−10] stochastic dynamics simulations,[11,12] or ab initio calculations[1,13−15] to study the conformational space of the alanine dipeptide in water. Experimental studies of the alanine dipeptide in water solution are still today lacking the possibility to cover all details of the conformational space, and it is difficult to obtain estimates of populations of different conformations and kinetics of conformational changes between them.[15,16] The most recent ab initio study of the alanine dipeptide where water effects were also included[15] indicated the importance of the hydration and the specific surrounding water structure for the different conformations of this dipeptide, and the results were in satisfactory agreement with the experimental data. Several studies of dipeptides with residues different from alanine have also been performed.[1,7,16−18]

When using dipeptides as models for longer oligopeptides or proteins, the C-terminal and N-terminal ends have to be treated in such a way that electrostatic interactions between the charged end terminals, which would not be present in longer oligopeptides and proteins, do not interfere with the results.[3−18] In a study of the conformational space of five different dipeptide models (single residues with N-terminal acetyl and C-terminal *N*′-methyl amide blocking groups) simulated in water, using molecular dynamics simulations, significant differences were obtained compared with the database statistics of the "random-coil" state of proteins.[17] The database models were effected by the long-range interactions from the other amino acid residues present in the surrounding protein structures, interactions which are not present in the dipeptide models. Dipeptide model studies thus have to be interpreted with caution, with regard to the transferability of the results to polypeptides in water.

Simulations of the short peptide Leu-enkephalin (Tyr-Gly-Gly-Phe-Leu) as a zwitterion in water and DMSO solution, with charged end terminals, show that the salt bridge between the C-terminal and N-terminal ends stabilizes the conformation.[19] The surrounding environment and the charged end terminals thus affect the short peptide structures and the conformational space in water. Most of the short peptides with different residue combinations are believed to have a "random-coil" structure in water. The "random-coil" form of the short peptide includes all possible conformations, which exist in water, and the combination of different conformations depends on the amino acid residues in the short peptide. The conformational space of short peptides in the "random-coil" state has been studied by using molecular dynamics simulations[17,18,20] and by using NMR spectroscopy.[21] Only a few short peptides have been determined experimentally to have specific conformations in water. Some short proline-containing peptides that have been found in short turns show higher populations of specific structures in water

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using NMR and CD spectroscopy.[22−29] Several theoretical studies of the conformational space and dynamics in aqueous solution of tri-, tetra-, and pentapeptides from different short turns containing proline have been reported.[30−37]

The proline residue differs from the other naturally occurring amino acids by lacking the possibility to make hydrogen bonds with the N-terminal nitrogen. The heterocyclic pyrrolidine ring structure makes the proline residue side chain more restricted when compared with other naturally occurring amino acids. The planar peptide bond in polypeptides occurs predominantly in the trans conformation, but in recent years some cis conformations have been observed in high-resolution protein structures,[38] mainly for prolines. This unusual high probability to have cis conformation in different polypeptides makes the proline residue more interesting when compared with other naturally occurring amino acids, and several experimental studies of this unique amino acid have been reported in the literature.[39−44] Different short peptides were used when the equilibria between the cis and trans isomers of the Xaa-Pro bond in different solvents, including water, were measured,[39−41] and the isomerization kinetics and rotational barrier of the cis/trans isomerization of the peptide bond preceding proline have been examined experimentally[42−44] and theoretically[45] for proline and proline mutants, as well as for some other amino acids.[46]

As mentioned above, the proline residue has been found in short turns and some of these turns have a significant tendency to form specific structure in water. When the proline residue is connected with glycine (-Xaa-Pro-Gly-Xaa-), the proline has been found in the trans conformation and the predicted structure has a type II β turn conformation in water.[27−29] If the proline residue is connected on both sides with hydrophobic residues such as tyrosine (-Xaa-Tyr-Pro-Tyr-Xaa-), the proline residue has cis conformation and is packed together with hydrophobic residues and the peptide has a type VI β turn structure in water.[24−26] The YTGP (Tyr-Thr-Gly-Pro) peptide in aqueous solution shows an interaction between the aromatic ring of tyrosine and the backbone amide hydrogen atom of glycine.[22,23] Different simulation methods have been applied to model peptides,[30−37,47] and in most of the cases the results were in good agreement with experimental data, but some difficulties to predict the experimentally obtained conformations were also noted. Molecular dynamics simulations of type II β and type VI β turns are in agreement with experimental data,[30−35] but simulations of the YTGP (Tyr-Thr-Gly-Pro) peptide in aqueous solution are more complicated.[36,37] The conformational space of the YTGP peptide in aqueous solution is probably more complicated than the peptides supporting type II β and type VI β turn conformations, and for the sampling of the whole conformational space of the YTGP peptide, probably longer simulation times should be used. The different combinations of force fields for biomolecules and water models for liquid water were found to affect molecular dynamics simulation results of the YTGP (Tyr-Thr-Gly-Pro) peptide in water, and that was also discussed by van der Spoel et al.[36] To understand the effects of the force field on the simulation results several comparisons of different generally used force fields for biomolecules have been reported in the literature.[48−52] Many of the commonly used water models have been found to differ from experimental liquid water properties, and solvation effects depending on the water model used in molecular dynamics simulations have been reported.[53−58] The properties of water models, such as viscosity, have been proposed to effect the dynamics of the solute atoms in molecular dynamics simulations.[59−61]

TABLE 1: Nonbonded Parameters, Geometry, and Electrostatic Properties of the Three-Point Water Models

params and units	TIP3P modified	SPC refined	SPC/E original
dipole moment (D)	2.347	2.237	2.351
r_0^{OO} (Å)	3.5364	3.5257	3.5533
ϵ^{OO} (kcal mol^{-1})	0.1521	0.1553	0.1553
r_0^{HH} (Å)	0.449	0	0
ϵ^{HH} (kcal mol^{-1})	0.046	0	0
q^O (e units)	−0.834	−0.8068	−0.8476
q^H (e units)	0.417	0.4034	0.4238
b_0^{OH} (Å)	0.9572	1.0	1.0
θ_0^{HOH} (deg)	104.52	109.47	109.47

TABLE 2: Bulk Properties for Water Models at 25 °C

water model	TIP3P modified	SPC refined	SPC/E original	expt
self-diffusion coeff D[a] (10^{-9} m^2 s^{-1})	5.6	4.3	2.7	2.3[b]
tetrahedral struct[a]		increasing →		
viscosity (cP)	0.35[c]	0.54[d]	0.82[d]	0.83[d]
static dielectric constant ϵ_0[e]	96.7	64.5	68.2	78.3
Kirkwood G-factor G_K[e]	5.25	3.71	3.70	
Debye relaxation time τ_D[e] (ps)	6.9	7.6	12.1	9.3

[a] Present calculation. [b] Reference 67. [c] Reference 68. [d] Reference 69. [e] Reference 70.

In this study we report results of molecular dynamics simulations of the zwitterionic dipeptide Ala-Pro in water at 298 K. This dipeptide is in the cis conformation when bound to Cyclophilin A,[62] and our first interest with this study was the conformational sampling of both trans and cis isomers of Ala-Pro in water solution to investigate if the bioactive cis isomer conformation also exists for the free dipeptide. Furthermore we monitored the presence of an intramolecular hydrogen bond between the positively charged terminal amino group of the alanine residue and the terminal carboxylate group in the cis isomer of the zwitterionic species, as previously indicated by an NMR study[40] of the free Ala-Pro in water.

The properties of the water model used in a simulation may affect the solute, and we therefore studied the effect of different water models on conformational, hydration, and dynamical properties of the dipeptide by performing simulations using identical protocols with both trans and cis isomers with three commonly used water models. The water models used in this study, the modified TIP3P,[63,64] the refined SPC,[65] and the original SPC/E,[66] can be described as rigid, three-site models interacting with effective pair potentials composed of Lennard-Jones and Coulombic terms. The three models thus are similar in nature, but the Lennard-Jones and Coulombic terms differ (see Table 1) and give significant differences in their calculated bulk properties for liquid water[67−70] (see Table 2). The proline residue limits the conformational space of the dipeptide Ala-Pro, which makes it an attractive system for comparison of effects depending on the water models.

2. Methods

Molecular dynamics simulations of a single Ala-Pro molecule in water using different water models, the modified TIP3P (transferable intermolecular potential 3P),[63,64] the refined SPC (simple point charge),[65] and the original SPC/E (extended simple point charge)[66] were performed using the CHARMM[71] molecular mechanics program. The all-atom CHARMM22 force field for proteins[72] was used for the dipeptide. All simulations were performed at 298 K using 888 H_2O molecules and a single dipeptide molecule in a cubic box with periodic boundary conditions. The box side length was 30.0 Å, and for truncation

TABLE 3: Systems Simulated

simulatn	water model	simulatn period (ns)	temp[e] (K)	temp control	tot. energy[e] (kcal/mol)
1[a]	TIP3P modified	2.0[c]/1.0[d]	296.57(1.07)	no	−6917.1(0.2)
2[b]	TIP3P modified	5.0/4.0	298.00(0.03)	yes	−6896.2(14.9)
3[a]	SPC refined	2.0/1.0	297.82(1.14)	no	−6987.9(0.2)
4[b]	SPC refined	5.0/4.0	298.00(0.03)	yes	−6986.3(18.2)
5[a]	SPC/E original	2.0/1.0	298.83(1.31)	no	−8059.2(0.2)
6[b]	SPC/E original	5.0/4.0	298.00(0.03)	yes	−8076.0(22.6)

[a] The trans simulated isomer. [b] The cis simulated isomer. [c] Total time. [d] Time used for analysis. [e] Average calculated over the analyzed part of the simulation; standard deviation in parentheses.

TABLE 4: Starting Conformations Specified by Dihedral Angles

structural posn	dihedral[a]	trans (deg)	cis (deg)
(a) carboxylate rotation	^{a}OT1−^{a}C−^{a}CA−^{a}N	0.0	0.0
(b) proline puckering	^{a}HA−^{a}CA−^{a}CB−^{a}HB1	32.446	32.446
(c) phi (ϕ)	^{a}C−^{a}CA−^{a}N−^{b}C	−76.120	−76.120
(d) peptide bond (ω)	^{a}CD−^{a}N−^{b}C−^{b}O	−178.323	1.677
(e) psi (ψ)	^{b}N−^{b}CA−^{b}C−^{a}N	180.0[b]	180.0[b]
(f) methyl rotation	^{b}HA−^{b}CA−^{b}CB−^{b}HB1	−131.08	118.92
(g) amino rotation	^{b}HA−^{b}CA−^{b}N−^{b}HT1	60.0	60.0

[a] Residues: (a) proline; (b) alanine. [b] Psi (ψ) dihedral was changed to cover all possible conformations; 0, ±60, ±120 (deg) were used for different start structures.

of long-range interactions, an atom-based method, force shifting[73] with a spherical cutoff radius of 12.0 Å, was used. A spherical nonbonded list with a radius of 14.0 Å was used and updated when necessary using a heuristic test. The simulation temperature was controlled using the Hoover extended system constant temperature algorithm[74−76] when the cis isomer was simulated. No temperature control was needed for trans isomer simulations when the system temperature was allowed to vary ±5 K around 298 K. The SHAKE algorithm[77] was used to keep water molecules rigid and to constrain all hydrogen atom−heavy atom bond lengths for the dipeptide. The integration of Newton's equations of motion was carried out with the leapfrog Verlet algorithm[78] with a time step of 0.002 ps. The dielectric constant was 1.0, and all coordinates were saved every 100 steps. All simulations with the trans isomer were 2.0 ns long, and the simulations with the cis isomer were extended to 5.0 ns for better conformational space sampling. In all simulations the first 1.0 ns was used for equilibration and the rest of the simulation was used for analysis. A total of six simulations were performed (Table 3). Different start conformations (Table 4) were used to study the whole conformational space for both isomers.

Hydration of the dipeptide was estimated using the average number of hydrogen bonds to water and their average lifetime as the criteria for the hydration shell. The criteria used to define a hydrogen bond were hydrogen to acceptor distance ≤2.4 Å and acceptor−hydrogen−donor angle ≥135°. A water bridge was obtained when one water molecule had two hydrogen bonds with the dipeptide at the same time. All hydrogen bonds were calculated from the trajectory with 5.0 ps time resolution.

Rotational reorientation times for the dipeptide in water were calculated using the time-correlation function[79]

$$C(t_m) = \langle P_2[\mu_A(0)\cdot\mu_A(t_m)]\rangle \approx \frac{1}{N-m}\sum_{n=1}^{N-m} P_2[\mu_A(t_n)\cdot\mu_A(t_n+t_m)]$$

where N is the total number of time steps in the simulation and m is the number of time steps passed at time t_m. P_2[···] is the second-order Legendre polynomial, and μ_A is a unit vector,

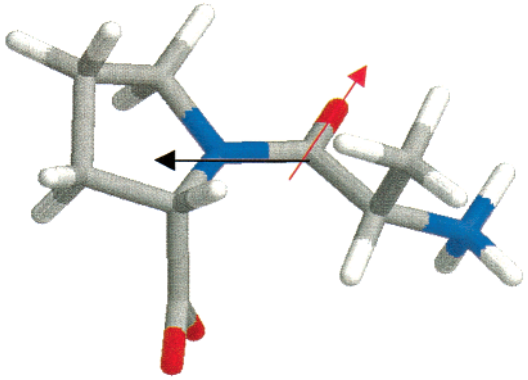

Figure 1. Cis isomer where vectors **V1** (black) and **V2** (red) are defined.

defined in the dipeptide molecule. The orientations of μ_A at times t_n and $t_n + t_m$, respectively, were calculated from the trajectory. Two different unit vectors, **V1** and **V2,** were defined in the dipeptide molecule (see Figure 1). We calculated the rotational reorientation time τ from the inverse of the slope of the linear part, 1 ps < t_m < 10 ps, of the decay of ln[$C(t_m)$] with time t_m. In some of the curves there is an initial fast (<0.2 ps) drop in $C(t_m)$, probably related to librational motion, which does not interfere with the analysis of the rotational reorientation time τ.

The translational diffusion coefficient for the dipeptide in water was calculated from the mean square displacement (MSD) of the nitrogen atom in proline, which is near the center of mass of the dipeptide, using the Einstein relation,[79]

$$\lim_{t\to\infty}\langle|\mathbf{r}(t'+t)-\mathbf{r}(t')|^2\rangle = 6Dt$$

where $\mathbf{r}(t)$ is the position of the nitrogen atom in the proline residue at time t, D is the translational diffusion coefficient, and the brackets denote averaging over the time origins t'.

The self-diffusion coefficient for water was calculated using the MSD of the oxygen atom of all water molecules.

3. Results and Discussion

3.1. Conformational Space. Both trans and cis isomers were simulated using three different water models. Trans and cis isomer simulations were performed separately, because the energy barrier for cis/trans or trans/cis isomerization of the peptide bond on the N-terminal side of proline (19−20 kcal/mol) is too high to be crossed over when simulated at room temperature.[35,44,45] No cis/trans or trans/cis isomerization was obtained in any of the simulations performed in this study.

3.1.1. Trans Isomer. All simulations were started with the same extended structure for the dipeptide where the peptide bond on the N-terminal side of proline was in trans conformation (Table 4). Different starting structures with the backbone dihedral ψ = (0°, ±60°, ±120°) were tested, but the trans

TABLE 5: Dihedral Angles for Specific Conformations Found in the Simulations

structural posn	dihedral[0]	trans[b] (deg)	cis[b] (deg)	cis[c] (deg)
(a) carboxylate rotation	[a]OT1−[a]C−[a]CA−[a]N	−33.0	−33.0	−33.0
(b) proline puckering	[a]HA−[a]CA−[a]CB−[a]HB1	−30.0, 30.0	−30.0, 30.0	−30.0, 30.0
(c) phi (ϕ)	[a]C−[a]CA−[a]N−[b]C	−69.0	−66.0	−66.0
(d) peptide bond (ω)	[a]CD−[a]N−[b]C−[b]O	162.0	−8.0	−8.0
(e) psi (ψ)	[b]N−[b]CA−[b]C−[a]N	162.0	155.0	90.0
(f) methyl rotation	[b]HA−[b]CA−[b]CB−[b]HB1	58.0	58.0	58.0
(g) amino rotation	[b]HA−[b]CA−[b]N−[b]HT1	60.0	60.0	60.0

[a] Residues: (a) proline; (b) alanine. [b] The major conformation. [c] The minor conformation.

TABLE 6: Hydrogen Bonds and Water Bridges

acceptor/donor	TIP3P modified	SPC refined	SPC/E original
Trans Isomer			
Hydrogen Bonds to Water			
ALA HT1	0.88[a]/8.4[b]	0.49/9.6	0.72/9.4
ALA HT2	0.90/8.8	0.56/8.6	0.79/11.1
ALA HT3	0.90/11.3	0.49/7.5	0.68/8.4
ALA O	0.91/5.7	0.55/5.6	0.65/6.9
PRO OT1	3.09/7.7	2.03/9.1	2.02/12.6
PRO OT2	2.96/8.2	1.85/8.0	1.77/9.7
Water Bridges			
PRO OT1−ALA O	0.16/6.2	0.03/5.0	0.0/0.0
PRO OT2−ALA O	0.10/5.3	0.08/5.7	0.06/6.0
PRO OT1−PRO OT2	0.28/5.6	0.19/6.1	0.18/5.8
Cis Isomer			
Hydrogen Bonds to Water			
ALA HT1	0.63/8.5	0.60/8.4	0.70/10.2
ALA HT2	0.59/8.1	0.63/8.5	0.67/9.9
ALA HT3	0.61/8.1	0.58/8.3	0.67/11.4
ALA O	0.71/5.7	0.77/6.1	0.83/7.2
PRO OT1	2.11/7.8	2.57/10.0	2.38/11.9
PRO OT2	2.10/7.6	2.48/9.9	2.42/11.8
Water Bridges			
PRO OT1−ALA HT	0.07/5.5	0.09/6.1	0.04/6.4
PRO OT2−ALA HT	0.03/5.4	0.09/5.8	0.09/6.2
PRO OT1−PRO OT2	0.19/5.6	0.20/5.5	0.18/5.4
Hydrogen Bonds within the Peptide			
PRO OT1−ALA HT	0.09/11.3	0.05/9.0	0.02/6.8
PRO OT2−ALA HT	0.04/8.1	0.06/9.4	0.06/11.1

[a] Average number. [b] Average lifetime (ps).

isomer always found the same conformation as seen in Table 5. The different dihedral angles as a function of time are shown in Figure 2 for the trans isomer simulated with all three different water models. In Figure 2 we can see that the dihedrals for the carboxylate, methyl, and amino groups in general rotate freely and sample their whole conformational space. The proline ring puckering shows similarities in fluctuations with all the dihedral angles. Both up and down conformations[80] of the proline ring were obtained (Figure 3), and the up conformation (the negative dihedral angle) for the proline ring was detected as the major conformation, populated 67% of the time. Similar results were obtained from simulations with all three water models. The trans conformation was similar from all simulations and is displayed in Figure 4.

3.1.2. Cis Isomer. All simulations were started with the same structure for the dipeptide with the peptide bond on the N-terminal side of proline in the cis conformation (Table 4). Different start structures were tested in a way similar to that for the trans isomer simulations. The cis isomer simulations show similarities with the trans isomer simulations and also always found the major cis conformation (Table 5) when started with different structures. This conformation, which is populated approximately 85% of the time for all three water models, does not exhibit any direct end-to-end interactions as can be seen in Figure 5. This major cis conformation was found to be the same conformation as that which binds to Cyclophilin A.[62] The experimentally obtained conformation[40] of the free dipeptide, where the N-terminal was interacting with the C-terminal making a salt bridge, was also found in all simulations and is called the minor cis conformation (Figure 6). The different dihedral angles as a function of time are shown in Figure 7 for the cis isomer simulated with all three different water models. The proline ring puckering was also observed to have both up and down conformations (Figure 3), with approximately equal populations.

3.2. Dipeptide Hydration. Hydration of both isomers was calculated from simulations with all three different water models. All calculated hydrogen bonds and water bridges from simulations are given in Table 6. The trans isomer was the most hydrated, but the cis isomer was the less hydrated with the TIP3P water model. The number of water molecules making hydrogen bonds with the trans isomer were significantly higher and with the cis isomer significantly lower when the calculations with the TIP3P water model were compared with the other water models. The longest lifetime of the hydrogen bonds was

TABLE 7: Rotational Reorientation and Translational Diffusion of the Dipeptide

	rotatnl reorientation time[a] (ps)					
	TIP3P modified		SPC refined		SPC/E original	
part of the trajectory (ns)	**V1**[b]	**V2**[c]	**V1**	**V2**	**V1**	**V2**
1.0−2.0	19.7	17.0	27.4	23.3	43.2	32.9
2.0−3.0	16.0	13.6	23.9	19.2	35.8	32.3
3.0−4.0	16.1	15.5	23.1	19.8	31.9	28.8
4.0−5.0	15.9	16.1	27.6	23.4	35.8	32.6
mean (std dev)	16.9(1.9)	15.6(1.4)	25.5(2.3)	21.4(2.2)	36.7(4.7)	31.7(1.9)
1.0−5.0[d]	16.9	15.4	25.4	21.4	36.3	31.5
	19.7[e]	15.6[e]	21.7[e]	19.6[e]	39.6[e]	34.2[e]
translatnl diffusion[f] (10^{-9} m^2 s^{-1})	1.1		0.9		0.6	

[a] The rotational reorientation of the **V1** and **V2** vectors for cis isomer were calculated using non-overlapping blocks of 1.0 ns. [b] **V1** vector: ALA C−PRO N. [c] **V2** vector: ALA C-ALA O. [d] The rotational reorientation times were calculated from the whole 4.0 ns trajectories. [e] The rotational reorientation of the **V1** and **V2** vectors for trans isomer were calculated from the whole 1.0 ns trajectories. [f] The translational diffusions for cis isomer were calculated from the whole 4.0 ns trajectories.

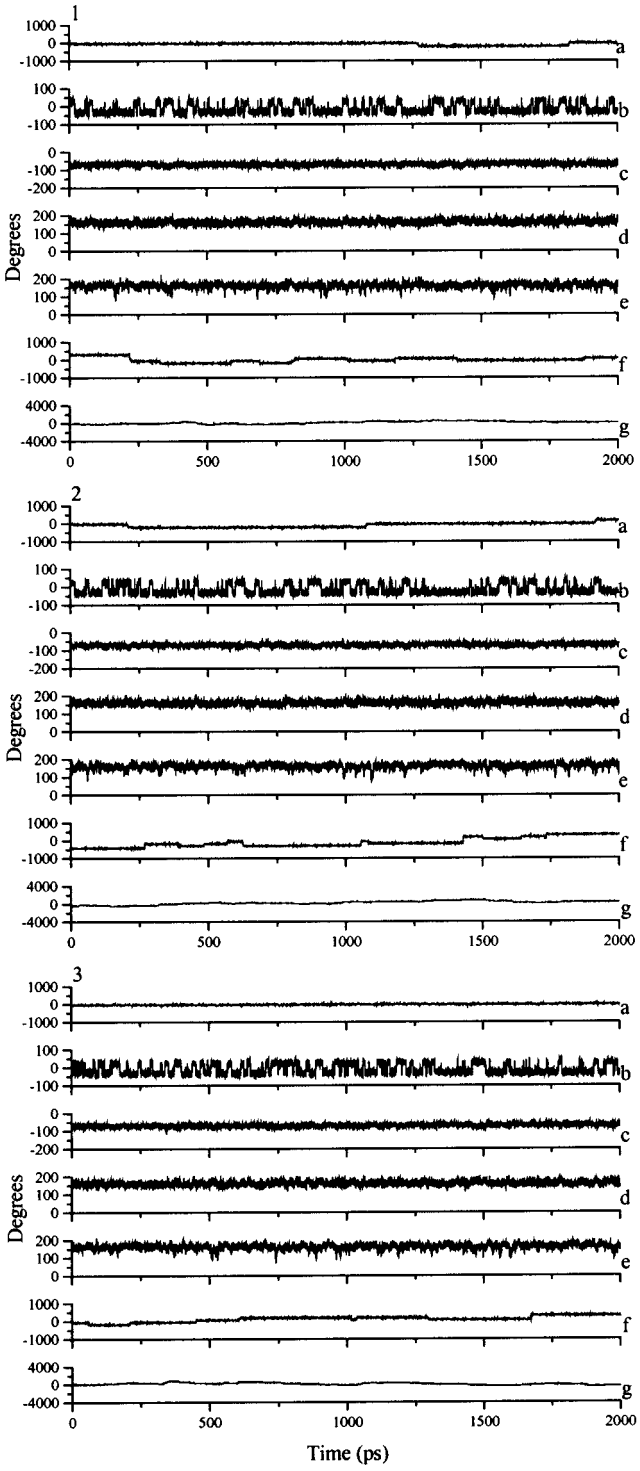

Figure 2. Different dihedral angles as a function of time for all simulations with the trans isomer. Water models: (1) TIP3P modified; (2) SPC refined; (3) SPC/E original. Key: (a) carboxylate group rotation; (b) proline puckering; (c) phi (ϕ); (d) peptide bond (ω); (e) psi (ψ); (f) methyl group rotation; (g) amino group rotation.

obtained with the SPC/E water model. With the cis isomer a hydrogen bond between the C-terminal and N-terminal ends was found but not with the trans isomer. The average number of hydrogen bonds within the dipeptide was significantly higher when the TIP3P water model was used. The TIP3P water model has a slightly shorter OH bond and a smaller HOH bond angle than the SPC and the SPC/E water models, which makes the TIP3P water model smaller in size than other two water models; the TIP3P water model also gives less structure for the bulk

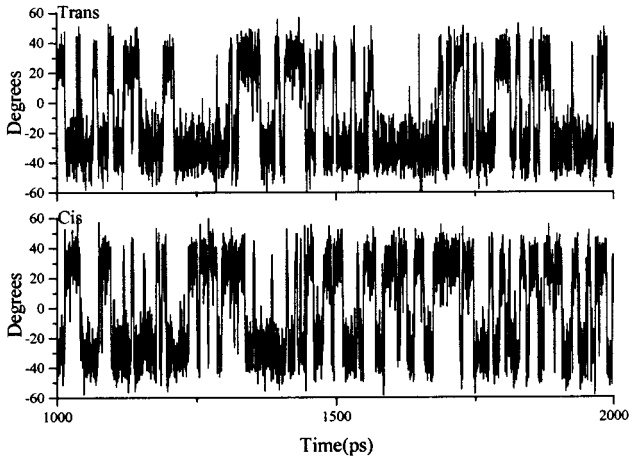

Figure 3. Ring puckering in the proline ring for both isomers as a function of time for simulations with TIP3P water model. In the down conformation, the torsion angle is positive, and in the up conformation, the torsion angle is negative.

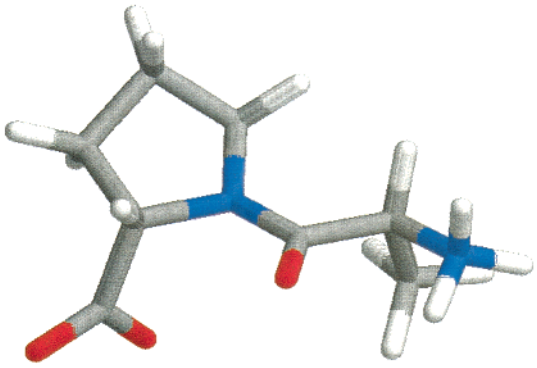

Figure 4. Major trans conformation in all simulations.

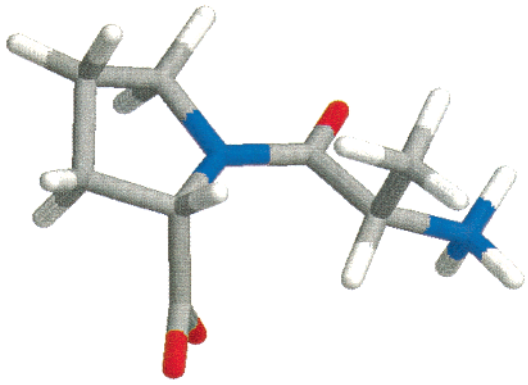

Figure 5. Major cis conformation in all simulations.

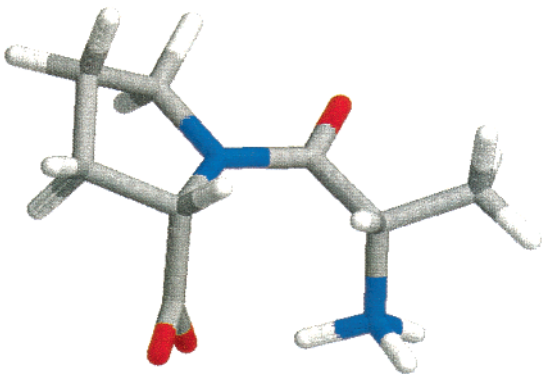

Figure 6. Minor cis conformation in all simulations.

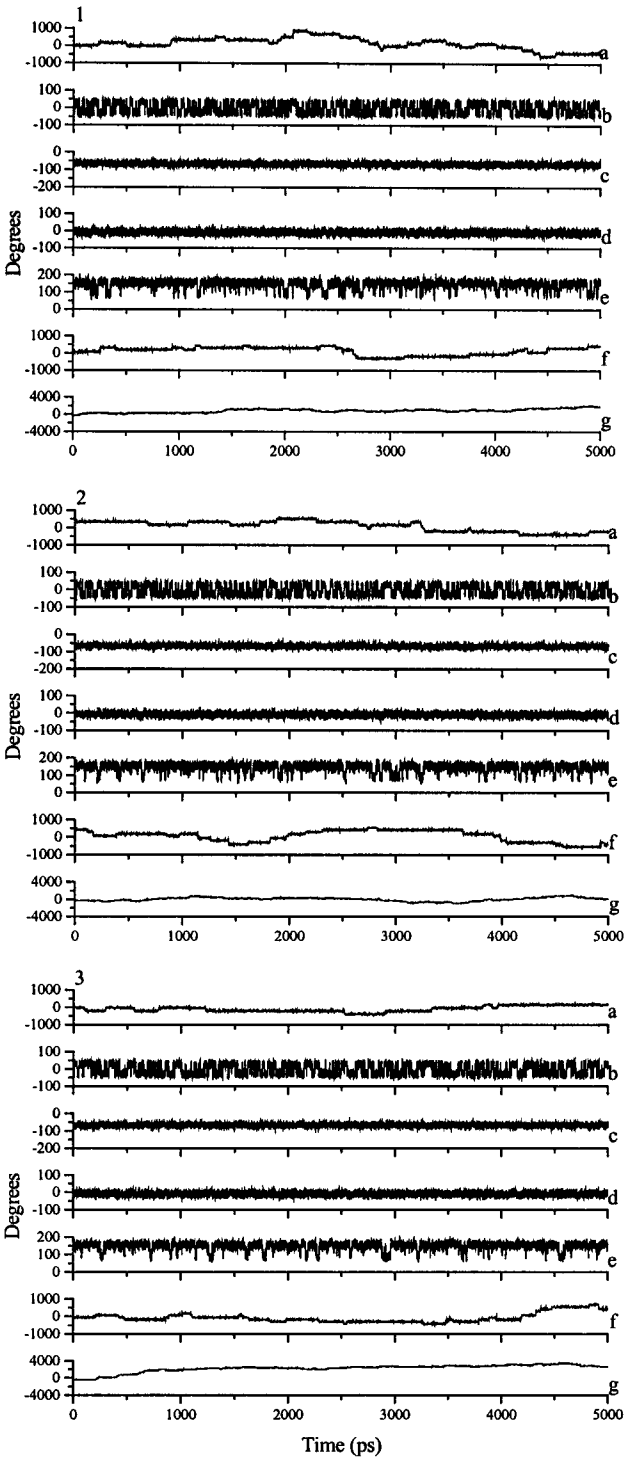

Figure 7. Different dihedral angles as a function of time for all simulations with the cis isomer. Water models: (1) TIP3P modified; (2) SPC refined; (3) SPC/E original. Key: (a) carboxylate group rotation; (b) proline puckering; (c) phi (ϕ); (d) peptide bond (ω); (e) psi (ψ); (f) methyl group rotation; (g) amino group rotation.

water than the other two models. Both the size and structure of the water model influence the hydration of the dipeptide, but the conformation of the dipeptide also has significant effects.

3.3. Rotational and Translational Diffusion. The rotational reorientation of the unit vectors **V1** and **V2** was calculated using the finite time intervals of 1.0 and 4.0 ns for the cis isomer and 1.0 ns for the trans isomer. Mean values and standard deviations for the rotational reorientation times for the cis isomer were calculated from four non-overlapping blocks of 1.0 ns. $C(t_m)$

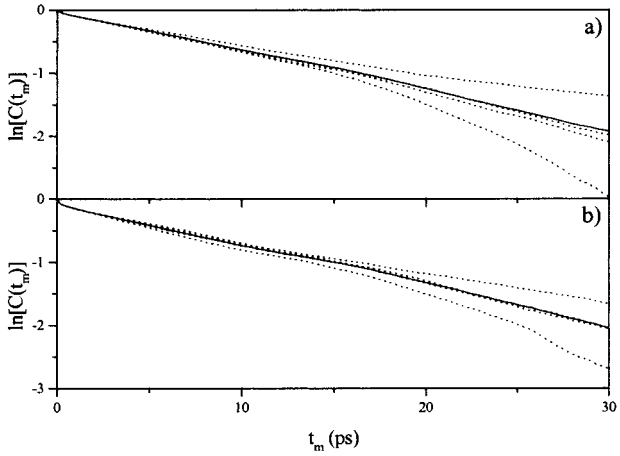

Figure 8. Time-correlation function $C(t_m)$ as a function of time (t_m) from simulation with cis isomer and TIP3P water: (a) ln[$C(t_m)$] calculated with vector **V1**, ALA C−PRO N; (b) ln[$C(t_m)$] calculated with vector **V2**, ALA C−ALA O. ln[$C(t_m)$] was calculated from non-overlapping blocks of 1.0 ns (dot) or from the full 4.0 ns simulation (line).

was found to decay exponentially with time, and the rotational reorientation time τ (Table 7) was calculated from the inverse of the slope of the linear decay of ln[$C(t_m)$] with time t_m (Figure 8). The calculated rotational reorientation times for 1.0 ns blocks have standard deviations of 11%, 9%, 13% and 9%, 10%, 6% for **V1** and **V2,** respectively when the TIP3P, SPC, and SPC/E water models were used; the error in the estimates from the total 4.0 ns will be roughly half as large.

We found that the rotational reorientation of the vector **V1** was always slower than the vector **V2**, consistent with the orientation of two vectors in the ellipsoid-shaped dipeptide; the vectors **V1** and **V2** point approximately along the long and short axes, respectively, of the ellipsoid (Figure 1). Both trans and cis isomers have similar rotational reorientation times (see Table 7), and due to the lack of the experimental data for the rotational reorientation time of Ala-Pro in water, we compare our simulation results with the experimental rotational reorientation time of tryptophan in water.[81] A single tryptophan molecule has approximately the same radius of gyration as the Ala-Pro dipeptide: 2.80, 2.75, and 3.14 Å for the Ala-Pro trans isomer, the Ala-Pro cis isomer, and tryptophan, respectively. The experimental value for the rotational reorientation time of tryptophan at 298 K is 21.8 ± 1.2 ps, and our simulation results for Ala-Pro in this study are of the same magnitude. The rotational reorientation time of the dipeptide increases with increasing viscosity of the model liquids.

The translational diffusion coefficients of the Ala-Pro dipeptide (cis isomer) were estimated from the long time slopes of the mean square displacements. The accuracy decreases for longer times, due to the decrease in sampling, and at very short times the Einstein relation is not valid. For this reason the intermediate interval, 4−100 ps, where the plot of MSD vs time is linear, was used to calculate the translational diffusion coefficients (Table 7). The translational diffusion coefficient of the dipeptide was also found to depend on the viscosity of the water models (Table 2). The radial distribution functions, g_{OO}, g_{OH}, and g_{HH} (not shown here), and the self-diffusion coefficients calculated from the dipeptide simulations (Table 2) are in good agreement with pure water simulations[82] (Mark and Nilsson, unpublished data). All of the properties collected from literature in Table 2 were computed at around 298 K but investigated by different groups using different programs and protocols, and comparisons are not always straightforward.

4. Conclusions

The Ala-Pro dipeptide was selected for this study because of its limited conformational space in water. All available conformations were sampled for both trans and cis isomers in the simulated trajectories. The two isomers were simulated separately because the isomerization barrier of the peptide bond on the N-terminal side of proline is too high to be crossed over in simulations at room temperature; this precludes a direct estimate of the conformational equilibrium between the two isomers from our simulations. Molecular dynamics simulations at high temperature or umbrella sampling methods could be used to investigate the trans/cis conformational equilibrium and the energy barrier between these isomers more carefully. For the zwitterionic form of the dipeptide the cis isomer is experimentally estimated[40] to be present 40% of the time at 298 K.

The conformational space sampling of the dipeptide in water was similar for all three water models, the main differences being in the hydration and in the viscosity-dependent dynamical properties of the dipeptide. Since the hydration of the cis and trans isomers depends on the water model (Table 6), it is conceivable that the diffusion properties of the dipeptide (Table 7) should reflect this difference, but this effect is negligible in comparison to the viscosity differences between the water models (Table 2) and cannot be evaluated separately.

The major conformation of the cis isomer in water was found to be the same as the bioactive conformation, which is the binding conformation when Ala-Pro is bound to Cyclophilin A.[62] The experimentally obtained cis isomer conformation[40] with the intramolecular hydrogen bond between the N-terminal and the C-terminal was found to exist as the minor cis conformation in all simulations with the different water models.

Most of the simulated system used in this study was water; only 13 water molecules out of 901 had to be removed to make room for the dipeptide, and the overall water dynamics and structure were not affected by the dipeptide. The water dynamics and structure close to the dipeptide is however influenced by interactions with the solute and may be different from bulk water properties.[3,10,56,57,83−86] The structure and dynamics of the model liquid may also be affected when a too small system is used, and this indirectly may affect the different dynamical motions of the solute. Recent studies of water models[82] (Mark and Nilsson, unpublished data) show that the dependence of the self-diffusion coefficient on the system size is almost negligible for systems as large as the one used in this study, and water properties calculated in the present system (Table 2) are indeed in agreement with results for these water models.

Molecular dynamics is a tool well suited to the study of the complicated conformational dynamics of flexible peptides that cannot be characterized by a single conformation, and even though the details depend on the water model, the overall properties of the dipeptide were consistently reproduced with all three water models. The main differences were found in the diffusion properties, and here the choice of water model is more critical than for structural properties. Experimental data on diffusion properties of short oligopeptides would be helpful in this respect.

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