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Molecular Dynamics Simulations of Polypeptide Conformations in Water: A Comparison of α, β, and poly(Pro)II Conformations

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ABSTRACT **A significant fraction of the so-called "random coil" residues in globular proteins exists in the left-handed poly(Pro)II conformation. In order to compare the behavior of this secondary structure with that of the other regular secondary structures, molecular dynamics simulations, with the GROMOS suite of programs, of an alanine octapeptide in water, in α-helix, β-strand, and left-handed poly(Pro)II conformations, have been performed. Our results indicate a limited flexibility for the α-helix conformation and a relatively larger flexibility for the β-strand and poly(Pro)II conformations. The behavior of oligopeptides with a starting configuration of β-strand and poly(Pro)II conformations, both lacking interchain hydrogen bonds, were similar. The (ϕ, ψ) angles reflect a continuum of structures including both β and P_{II} conformations, but with a preference for local P_{II} regions. Differences in the network of water molecules involved in hydrogen bonding with the backbone of the polypeptide were observed in local regions of β and P_{II} conformations. Such water bridges help stabilize the P_{II} conformation relative to the β conformation. Proteins 1999;36:400–406.** © 1999 Wiley-Liss, Inc.

Key words: MD simulations; poly(Pro)II conformation; α-helix; β-strand; water-bridge

INTRODUCTION

Among the well-characterized classes of protein secondary structures are α-helices, β-sheets, and β-turns. The α-helices and β-sheets have repeating torsion angles and are considered to be "ordered" or "regular" structures, whereas β-turns are considered to be "nonregular" structures because of non-repeating torsion angles defining the conformation.[1] Although there are different rules for assigning these secondary structure elements in protein crystal structures,[2–5] there is a general consensus on the basic geometric parameters, the Ramachandran ϕ and ψ angles, defining these structures. Residues that do not fall under any of these three well-defined secondary structures are generally grouped under the so-called "random coil" structure, though they are neither random nor coil.[6] This structure has been referred to by different names: other, unstructured, remainder, unordered, etc. We use the term "unordered" in this paper. This is generally believed to be a nonrepetitive structure and devoid of any ordered conformation. Each of these four structures gives rise to characteristic spectral features that are used in characterizing the structures of proteins and polypeptides.[7]

The electronic circular dichroism spectra of model polypeptides in the unordered structure are rather simple and are characterized by a strong negative band near 200 nm and, in many cases (for example, poly(Glu) and poly(Lys) in water), a weaker positive band near 217 nm.[7] Denatured proteins and some unordered polypeptides have circular dichroism spectra that are characterized by a strong negative band near 200 nm and a negative shoulder near 217 nm. Krimm and coworkers,[8] based on the similarities of the CD spectra of the unordered structure and those of collagen and poly(Pro)II, and a theoretical treatment of electrostatic interactions, proposed that a significant fraction of the unordered structure has short segments (4–7 residues) of poly(Pro)II helix (P_{II}) conformation, interspersed with sharp bends. Experimental results support their model, and these have been reviewed.[9]

The P_{II} conformation is a recognized secondary structural element of proline-rich synthetic polypeptides and fibrous proteins. In the last few years, algorithms have been developed to identify the P_{II} conformation in globular protein structures,[4,10,12] that has led to a new secondary structure assignment in globular proteins. These algorithms identify P_{II} segments in globular proteins, some of which lack any proline residues. Identification of the P_{II} structure in globular proteins has led to its inclusion in the analysis of protein circular dichroism spectra.[10,11] These studies have demonstrated that the P_{II} conformation forms a significant fraction of the residues in globular proteins previously considered to be without any identifiable structure.

In proline-rich polypeptides, the constraints of the pyrrolidine ring of proline stabilize the P_{II} conformation. What are the forces and interactions that stabilize the P_{II} conformation in the absence of constraints due to the pyrrolidine ring? The behavior of oligopeptides has been the subject of few investigations, which were directed towards understanding the stability of the α-helix and the

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characterization of the pathways of uncoiling of the α-helix in different solvents.[13–18] In order to examine the behavior of the P_{II} conformation and the interactions stabilizing this conformation, we have carried out molecular dynamics (MD) simulations on different conformations of polyalanine in water. Polyalanine in α-helix conformation, which is stabilized by intra-chain hydrogen bonds, showed limited flexibility with (ϕ, ψ) angles fluctuating around (−63°, −44°). Polyalanine with starting structures of β (ϕ = −120° and ψ = +120°) and P_{II} (ϕ = −8° and ψ = +145°) conformations, both lacking any stabilizing hydrogen bonds, showed a larger flexibility with (ϕ, ψ) angles, reflecting a continuum of structures including β and P_{II} conformations. The dynamics and the distribution of Ramachandran angles of these two conformations were similar. Differences in the network of water molecules involved in hydrogen bonding with the backbone of the polypeptide were observed in local regions of β and P_{II} conformations.

MATERIALS AND METHODS

The dynamics of $(Ala)_8$ in α, β, and P_{II} conformations were examined. The peptides were unblocked and in zwitterionic forms. All simulations were carried out using the GROMOS96 program package.[19] Standard united atom parameters were used for the peptide atoms in conjunction with the SPC/E model for water.[20] The dielectric constant was fixed at 1.0. The nonbonded pair list was generated by using a residue-based cutoff distance of 8.0 Å and was updated every 5 steps. The long-range electrostatic cutoff was set at 10–12 Å depending on the dimensions of the water box. All bond lengths were constrained using the SHAKE algorithm[21] with a tolerance of 0.0005 Å, allowing us to use a time step of 2 fs. Periodic boundary conditions were used to avoid edge effects in all calculations. The temperature was maintained at 300 K during the simulations by coupling to an external bath.[22]

The initial structures of the peptides were generated using standard bond lengths, bond angles, and torsion angles. These structures were subjected to steepest descent energy minimization in vacuum. The minimized structure was placed at the center of a rectangular box of SPC/E[20] water molecules, with walls at least 8 Å from any peptide atom. The resultant structure was subjected to steepest descent energy minimization, with the positions of the solute constrained, to remove unfavorable interactions between solute and solvent. The constraints were then removed, and the structure was subjected to energy minimization by the conjugate gradient method. The final structure was used as the starting point for MD simulations. During MD simulations, data were collected for 500 ps or more with an initial equilibration period of 50 ps. The results were analyzed using analysis programs written in our laboratory.

RESULTS AND DISCUSSION

The α-helix remained intact during the simulation. The ϕ and ψ angles fluctuated around −63° and −44°, respectively, in the middle of the helix, with RMS deviations less than 10°. Deviations from the canonical values of ϕ and ψ and larger RMS deviations (> 25°) were observed at the ends of the helix. The α-helix conformation is stabilized by intra-chain hydrogen bonds. Three intra-chain hydrogen bonds, 5→1, 6→2, and 7→3, are structurally possible and all were observed during the simulation. We also observed the making and breaking of the terminal hydrogen bonds, 5→1 and 7→3, and intermittent formation of 7→4, which corresponds to a 3_{10}-helix hydrogen bond. These results point to the limited flexibility of the α-helix and transitions between α-helix and 3_{10}-helix. These results are consistent with published results.[13–16]

The behavior of $(Ala)_8$ with the starting conformations of P_{II} and β, which we will henceforth call $(Ala)_8$-P_{II} and $(Ala)_8$-β, respectively, were similar. The (ϕ, ψ) angles deviated from the starting values of (−120°, +120°) for $(Ala)_8$-β and (−78°, +145°) for $(Ala)_8$-P_{II}, and the RMS deviations were large (16° to 32°). Large dihedral transitions were also observed in a couple of cases, which gave much larger deviations. The (ϕ, ψ) plots of these oligopeptides, which are shown in Figure 1, are similar, with both P_{II} and β regions populated. The (ϕ, ψ) angles reflect a continuum of structures including both β and P_{II} conformations, but with a preference to local P_{II} regions (see below).

The variations of the central six (ϕ, ψ) angles of $(Ala)_8$-P_{II} and $(Ala)_8$-β with simulation time, time series, are shown in Figures 2 and 3, respectively. The lines corresponding to the canonical (ϕ, ψ) angles of P_{II} conformation are also shown in these figures. The overall variations observed in (ϕ, ψ) angles of $(Ala)_8$-P_{II} and $(Ala)_8$-β are similar. The peptides span different conformations in the (ϕ, ψ) space and both P_{II} and β conformations are populated. For most of the simulation, the dihedral angle ψ fluctuates around approximately 135°, which is midway between P_{II} and β conformations, in both $(Ala)_8$-P_{II} and $(Ala)_8$-β. The dihedral angle ϕ shows larger variations than ψ, as the peptide spans the region surrounding both P_{II} and β conformations. Lack of stabilizing intramolecular hydrogen bonds in these conformations is the main reason for the observed flexibility.

The fraction of the time each set of (ϕ, ψ) angles corresponds to P_{II} and β conformations, which gives the population of P_{II} and β conformations, determined by allowing a rather conservative variation of ±15° in ϕ and ψ from their canonical values, are given in Table I. Each peptide unit spends less than 40% of the time in one of these two conformations and of these, the P_{II} conformation is populated 65% of the time and the β conformation is populated 35% of the time. It should be noted that we are here referring to a single strand in the β–conformation, while in proteins interstrand hydrogen bonds stabilize the structure of the β–conformation in a β–sheet. In the absence of stabilizing intermolecular hydrogen bonds of the β–sheet, the P_{II} conformation is populated approximately twice as frequently as the β–conformation.

The ϕ, ψ distributions from our simulations of $(Ala)_8$-P_{II} and $(Ala)_8$-β (Fig. 1) are very similar to that obtained by J. Hermans (personal communication) from a 2.5-ns simulation of Ace-Ala-NMe in water. The ϕ, ψ distribution

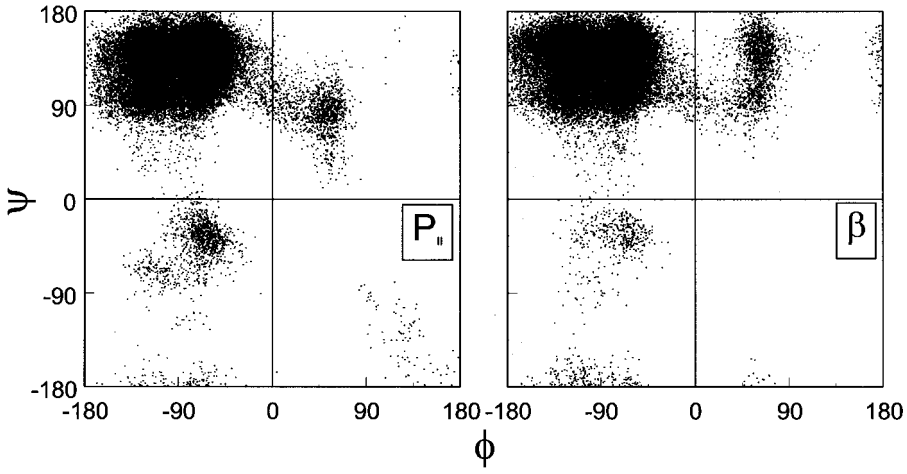

Fig. 1. The Ramachandran diagram of the $(Ala)_8$-P_{II} and $(Ala)_8$-β oligopeptides.

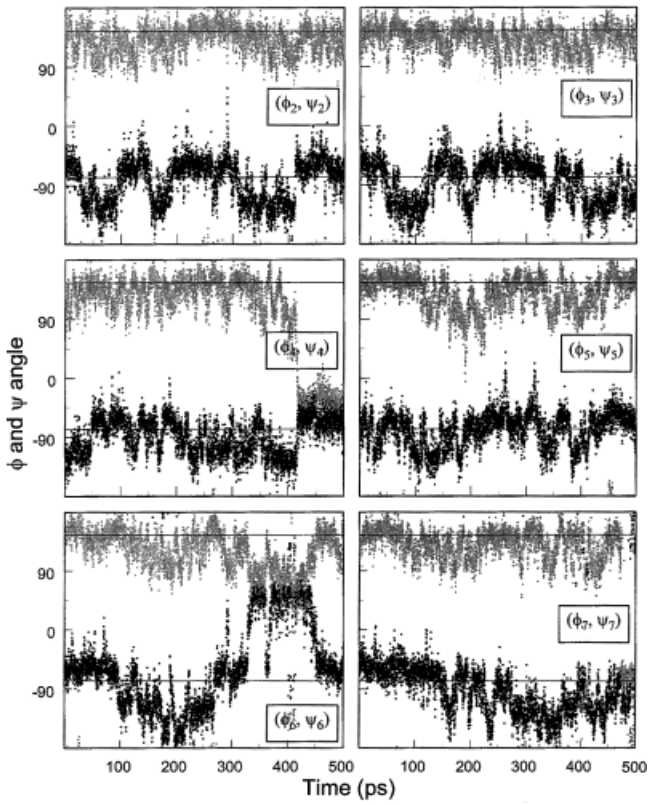

Fig. 2. Plot of (ϕ, ψ) dihedral angle variations with simulation time in $(Ala)_8$-P_{II} oligopeptide. The angles ϕ and ψ are shown in black and gray, respectively. Horizontal lines correspond to the canonical (ϕ, ψ) values of the P_{II}-conformation.

for the Ala dipeptide shows two lobes in the upper left quadrant of the Ramachandran map, a more heavily populated one corresponding to the P_{II} conformation, and a less populated one to the β conformation. Earlier simulations[23] over shorter times gave a single unresolved peak in the P_{II}–β region. The α_R region shows a rather broad distribution of conformations and, while much less populated than the P_{II}–β region, is more heavily populated than in our simulations. This probably reflects the shorter simulation time used in our calculations and that the starting point was that of a P_{II} or β conformation.

We analyzed the arrangement of water molecules around the oligopeptide in the different conformations to understand the behavior of these oligopeptides. Water molecules can hydrogen bond to the peptide backbone and among themselves, thus forming a "network" or "bridge" of water connecting two backbone atoms. The distance between the two polar atoms (nitrogen and oxygen atoms) involved is used to define a hydrogen bond. Distances of 3.3 Å between a water molecule and a backbone atom, and 2.8 Å between two water molecules, were used as upper limits. Figure 4 shows the polypeptide backbone and the numbering scheme for the water bridges considered. A water bridge involving one water molecule is formed if two backbone atoms are hydrogen bonded to a single water molecule, for example N3—*hb*—W38—*hb*—O3, where backbone atoms N3 and O3 are hydrogen bonded to water molecule W38 which is represented as N3-O3(1). The number of water molecules involved in the water bridge, *n*, can vary, and a smaller number indicates a greater influence on the conformational flexibility of the backbone dihedral angles connected by the water bridge. Water bridges with a maximum of three water molecules were considered in the analysis. Table II lists different types of water bridges connecting two polar atoms of the backbone at most two residues apart and involving *one to three* water molecules. Also listed in Table II are the backbone bonds and the dihedral angles influenced by such water bridges. Two examples of such water bridges are shown in Figure 4. The water bridge N1-O1(2) defines a hydrogen bonded network of two water molecules forming a bridge between the polar atoms N1 and O1, influencing the torsion angles ϕ_1 and ψ_1. The N4-O2(3) defines a water bridge formed by three water molecules connecting N4 and O2, influencing torsion angles ω_2, ϕ_3, ψ_3, and ω_2.

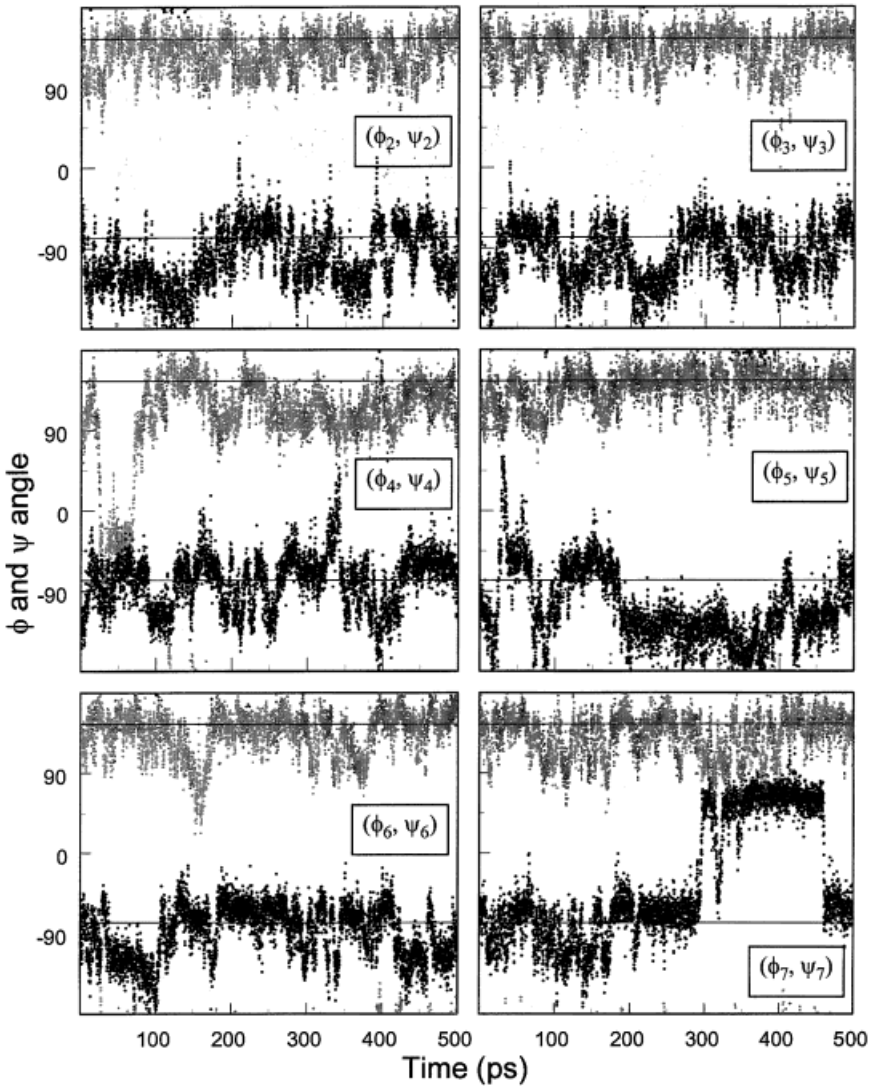

Fig. 3. Plot of (ϕ, ψ) dihedral angle variations with simulation time in $(Ala)_8$-β oligopeptide. The angles ϕ and ψ are shown in black and gray, respectively. Horizontal lines correspond to the canonical (ϕ, ψ) values of the P_{II}-conformation.

TABLE I. Populations of a Given Set of (ϕ, ψ) Angles of $(Ala)_8$-P_{II} and $(Ala)_8$-β in P_{II} and β Conformations†

	$(Ala)_8$-P_{II}		$(Ala)_8$-β	
(ϕ, ψ)	P_{II}	β	P_{II}	β
(ϕ_2, ψ_2)	20.2	13.5	12.7	15.6
(ϕ_3, ψ_3)	20.3	11.0	25.0	14.1
(ϕ_4, ψ_4)	18.9	11.6	12.4	7.7
(ϕ_5, ψ_5)	24.8	7.7	11.5	12.2
(ϕ_6, ψ_6)	14.4	7.2	20.8	7.8
(ϕ_7, ψ_7)	18.2	11.3	19.7	4.9

†The terminal (ϕ, ψ) angles are excluded. The population of a conformation is given as percent of the number of times each set of (ϕ, ψ) angles is observed in that conformation.

The MD trajectories were analyzed for such water bridges with the number of water molecules, $n \leq 3$, at every 0.1 ps interval. Examples of the network of water molecules observed during the simulation are shown in Figure 5, wherein snapshots of $(Ala)_8$-P_{II} at 40, 80, and 100 ps are presented. The peptide backbone and the water molecules are shown in Figure 5 as thick lines and the hydrogen-bonded atoms are connected by thin lines.

The types of bridges found in the α-helix were consistent with the geometry of the α-helix. The formation of these water bridges depends on the availability of the hydrogen-bonding partner from the peptide. In the α-helix conformation of $(Ala)_8$, O1, O2, O3, N5, N6, and N7 are not available for hydrogen bonding with water as they form intra-chain hydrogen bonds. The water bridges found in the α-helix were mainly at the helix termini.

Because of the similar behavior of $(Ala)_8$-P_{II} and $(Ala)_8$-β, the types and numbers of water bridges found from MD analysis are similar. These are given in Table III. The three dominant types of water bridges observed in $(Ala)_8$-

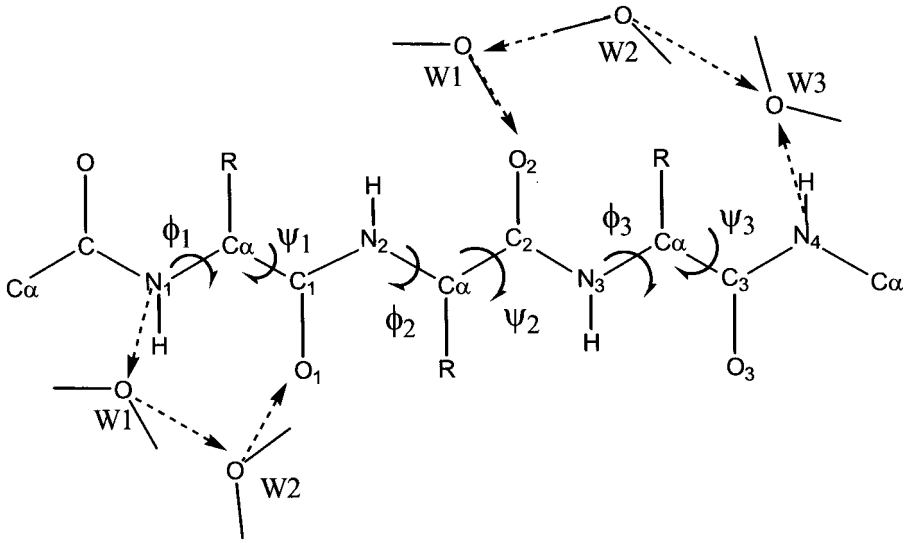

Fig. 4. The peptide backbone showing a part of the numbering scheme for the hydrogen-bonded network of water bridges. Examples of N1-O2(2) and N4-O2(3) water bridges are also shown.

TABLE II. Types of Hydrogen-Bonded Bridges of n Water Molecules and the Backbone Bonds and Torsion Angles Stabilized†

Type	Example(n)	Backbone bonds	Torsion angles
Ni-Oi	N4-O4(n)	N-Cα; Cα-C′	ϕ_4; ψ_4
Ni-N(i + 1)	N2-N3(n)	N-Cα; Cα-C′; C′-N	ϕ_2; ψ_2; ω_2
Oi-O(i + 1)	O2-O3(n)	C′-N; N-Cα; Cα-C′	ω_2; ϕ_3; ψ_3
Oi-N(i + 2)	N2-O4(n)	C′-N; N-Cα; Cα-C′; C′-N	ω_2; ϕ_3; ψ_3; ω_4
Ni-O(i + 1)	N3-O4(n)	N-Cα; Cα-C′; C′-N; N-Cα; Cα-C′	ϕ_3; ψ_3; ω_3; ϕ_4; ψ_4
Ni-N(i + 2)	N2-N4(n)	(N-Cα; Cα-C′; C′-N)$_2$	ϕ_2; ψ_2; ω_2; ϕ_3; ψ_3; ω_3
Oi-O(i + 2)	O2-O4(n)	(C′-N; N-Cα; Cα-C′)$_2$	ω_2; ϕ_3; ψ_3; ω_3; ϕ_4; ψ_4

†Water bridges connecting two backbone polar atoms at most two residues apart are given. Number of water molecules, *n*, in our analysis was varied from one to three. Water bridges spanning more than two residues are also possible, e.g., N2–N5, O2–O5, etc., but they generally involve a larger number of water molecules.

P_{II} and $(Ala)_8$-β are N*i*−O*i*, O*i*−O(*i+1*) and O*i*−N(*i+2*). Of these, the relative numbers of N*i*−O*i* and O*i*−O(*i+1*) bridges decrease as the number of water molecules involved increases from *one* to *three*. These three types of water bridges span a set of (ϕ, ψ) angles and one or two adjacent ω angles, thus influencing the conformation of one residue across a Cα atom. The water bridge formed with a smaller number of water molecules should have a greater influence on the bonds and dihedral angles that are spanned.

The types of water bridges that can be formed only in P_{II} and β conformations were obtained by an analysis of MD trajectories obtained by restraining the solute conformation. The number of water bridges observed in the β conformation is larger than that in P_{II}. The water bridge of the type O*i*−O(*i+1*) was observed only in the P_{II} conformation, while the other two dominant bridges were observed in both conformations.

In the absence of intermolecular hydrogen bonds, both P_{II} or β conformations show similar variations in their (ϕ, ψ) angles. The relative populations of these two structures are, however, different. The P_{II} structure is populated approximately twice as frequently as the β structure in water, according to molecular dynamics simulations. The hydrogen-bonding network of water molecules around these conformations is one factor that may be influencing the population difference between the two structures. Almost a quarter of the *one-water* bridges in $(Ala)_8$-P_{II} and $(Ala)_8$-β are of the type O*i*−O(*i+1*), that are formed only with the P_{II} structure.

MD simulations, Monte Carlo calculations and other analyses of the Ala dipeptide conformations, reviewed in Woody,[9] were in general agreement that water substantially stabilizes the P_{II} and α_R conformations relative to the C_7^{eq} conformer, which is the lowest energy conformation in vacuo.

There is experimental evidence that the P_{II} conformation in polypeptides is stabilized by hydration.[12,24] Analyses of protein crystal structures also indicate that short segments of P_{II} structure observed in globular proteins form hydrogen bonds with crystallographic water molecules.[4] Our MD results strongly support the idea that water molecules stabilize the P_{II} conformation in short stretches by hydrogen-bonding to the polar backbone atoms.

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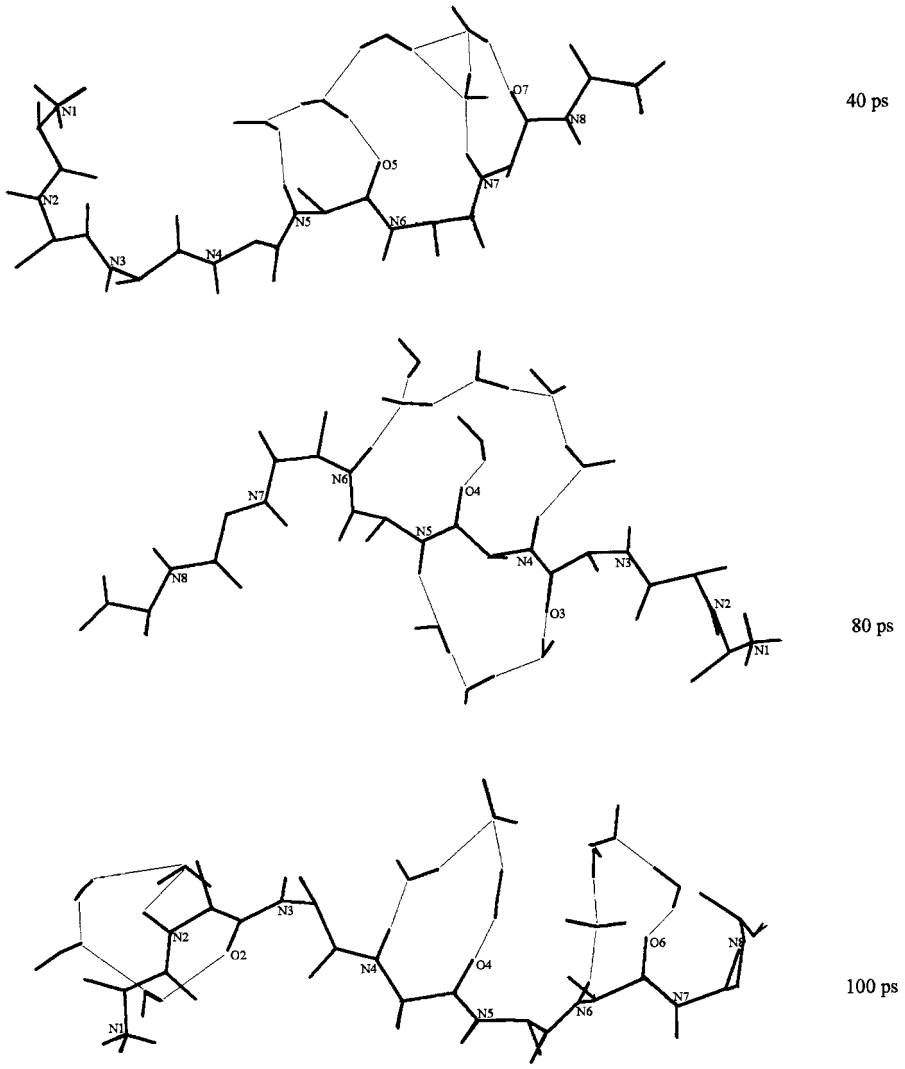

Fig. 5. The network of water molecules at 40-, 80-, and 100-ps snapshots of $(Ala)_8$-P_{II}. The peptide and the water molecules are shown as thick lines and the hydrogen bonding atoms are connected by thin lines.

TABLE III. Relative Populations of Different Types of Water Bridges Stabilizing $(Ala)_8$-P_{II} and $(Ala)_8$-β Oligopeptides†

Water bridge	$(Ala)_8$-P_{II}			$(Ala)_8$-β		
	1W[a]	2W	3W	1W	2W	3W
Ni-Oi	31.8	26.4	23.7	35.3	26.8	25.5
Ni-N(i + 1)	6.2	4.2	4.5	2.1	2.5	3.0
Oi-O(i + 1)	23.1	16.0	16.7	22.2	14.6	16.3
Oi-N(i + 2)	28.2	33.2	24.7	31.3	35.1	27.2
Ni-O(i + 1)	0.2	1.0	3.0	0.1	1.2	2.2
Ni-N(i + 2)	0.0	0.2	1.4	0.0	0.1	1.2
Oi-O(i + 2)	6.1	8.0	9.6	5.2	11.2	11.1
Average[b]	2.6	3.7	6.9	2.5	3.6	6.5

†The populations are given as percentages of the total number of water bridges observed with one to three water molecules.
[a]The number of water molecules involved in the water bridge.
[b]The average number of water bridges per configuration is reported. It was obtained by dividing the total number of water bridges observed by the number of configurations.

TABLE IV. Populations of Different Types of Water Bridges Stabilizing $(Ala)_8$ Oligopeptide in P_{II} and β Conformations†

Water bridge	P_{II}-conformation			β-conformation		
	1W[a]	2W	3W	1W	2W	3W
Ni-Oi	42.6	26.5	29.9	45.7	35.3	35.6
Ni-N(i + 1)	0.0	0.2	1.5	0.3	0.3	0.4
Oi-O(i + 1)	19.8	17.8	16.4	0.0	0.0	2.2
Oi-N(i + 2)	32.5	49.2	27.1	48.4	35.3	38.1
Ni-O(i + 1)	0.0	1.5	4.3	0.0	0.0	0.0
Ni-N(i + 2)	0.0	0.0	1.1	0.0	0.0	1.3
Oi-O(i + 2)	0.0	0.0	2.0	4.5	28.1	20.1

†The populations are given as percentages of the total number of water bridges observed with one to three water molecules. These were obtained by MD of $(Ala)_8$ oligopeptide constrained in P_{II} and β structures.
[a]The number of water molecules involved in the water bridge.

comments and Dr. Jan Hermans for communication of his unpublished results on Ace-Ala-NMe simulations.

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