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Letter

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Factors Affecting the Stabilization of Polyproline II Helices in a Hydrophobic Environment

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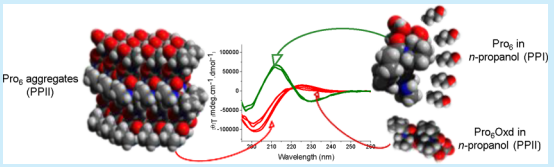
Supporting Information

ABSTRACT: Several parameters have a critical importance for the stabilization of either polyproline I (PPI) or polyproline II (PPII) helices in a hydrophobic environment. Among them, it was found out that the concentration is crucial as polyprolines at 3 mM concentration stably fold in PPII helices, that are organized in aggregates stable even after several days and are detectable by dynamic light scattering analysis. In more diluted concentration the same molecules stably fold in PPI helices, and no aggregates are found. In contrast, the introduction of a (4*S*,5*R*)-4-carboxy-5-methyloxazolidin-2-one (L-Oxd) moiety always inhibits the formation of the PPI helix, regardless of the L-Oxd position and the solution concentration.



Compared to generic peptide bonds, the peptidyl prolyl bond shows a strong propensity to adopt both the *cis* and the *trans* conformation, thus oligoprolines may stably fold into two different helical secondary structures, the right-handed polyproline I (PPI) with all amide bonds in the *cis* conformation and the left-handed polyproline II (PPII) with all the amide bonds in *trans*. The latter recently attracted significant attention because it has been frequently found in nature, mainly in the single strand of collagen.[1] Moreover, several proteins bind ligands in PPII conformation, such as SH3,[2] profilin protein,[3] WW domains,[4] MHC proteins, and EVH protein,[5] even if proline is not present in the sequence.[6] Finally, recent experimental studies indicate that in denatured proteins and peptides, the backbone occupies PPII conformations for a significant fraction of time.[7]

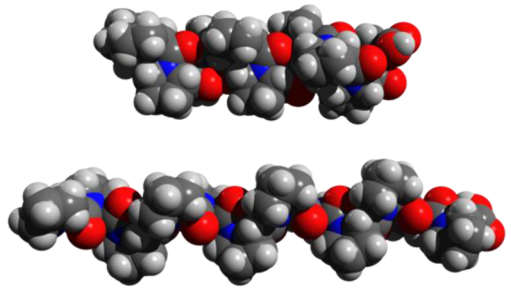


Figure 1. van der Waals surface of Pro_{12} in PPI conformation (top) and in PPII conformation (bottom).

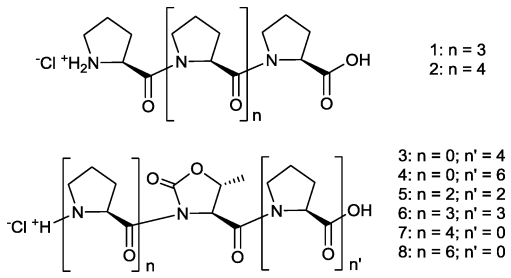


Figure 2. Chemical structure of the oligomers described in this work.

Table 1. Average Size of the Particles of a Solution of 2 or 8 in *n*-Propanol, as a Function of the Concentration and of the Time

compd	conc (mM)	size (d, nm) after 10 min	size (d, nm) after 24 h	size (d, nm) after 7 d
2	3	167	151	91.4
	0.3	110	89	
8	3	207	184	
	0.3	167	86	

Thus, oligoprolines are the perfect model compounds to study *cis*−*trans* conformational changes under different conditions,[8] as many factors may influence this equilibrium. Polar solvents such as either water or trifluoroethanol strongly favor the PPII helix, whereas the more hydrophobic *n*-propanol favors the PPI conformation. Indeed the PPI structure has dihedral angles of (ϕ, ψ, ω) = (−75°, 160°, 0°) with a helical pitch of 5.6 Å/turn and 3.3 residues/turn, while the PPII structure has backbone dihedral angles of (ϕ, ψ, ω) = (−75°, 145°, 180°) with helical pitch of 9.3 Å/turn and 3.0 residues/turn. Thus, the PPI structure is compact, the carbonyl groups are confined inside the helix, and the external surface shows only the aliphatic chains, hence favoring its formation in aliphatic environment (Figure 1). In contrast, PPII is more extended and the exposed carbonyl groups are able to interact with polar solvents.[9]

Further, temperature[10] and chain length[11] may induce conformational changes between PPI and PPII helices in *n*-propanol. Oka[12] found that Pro_{13} and Pro_6 adopt the PPI

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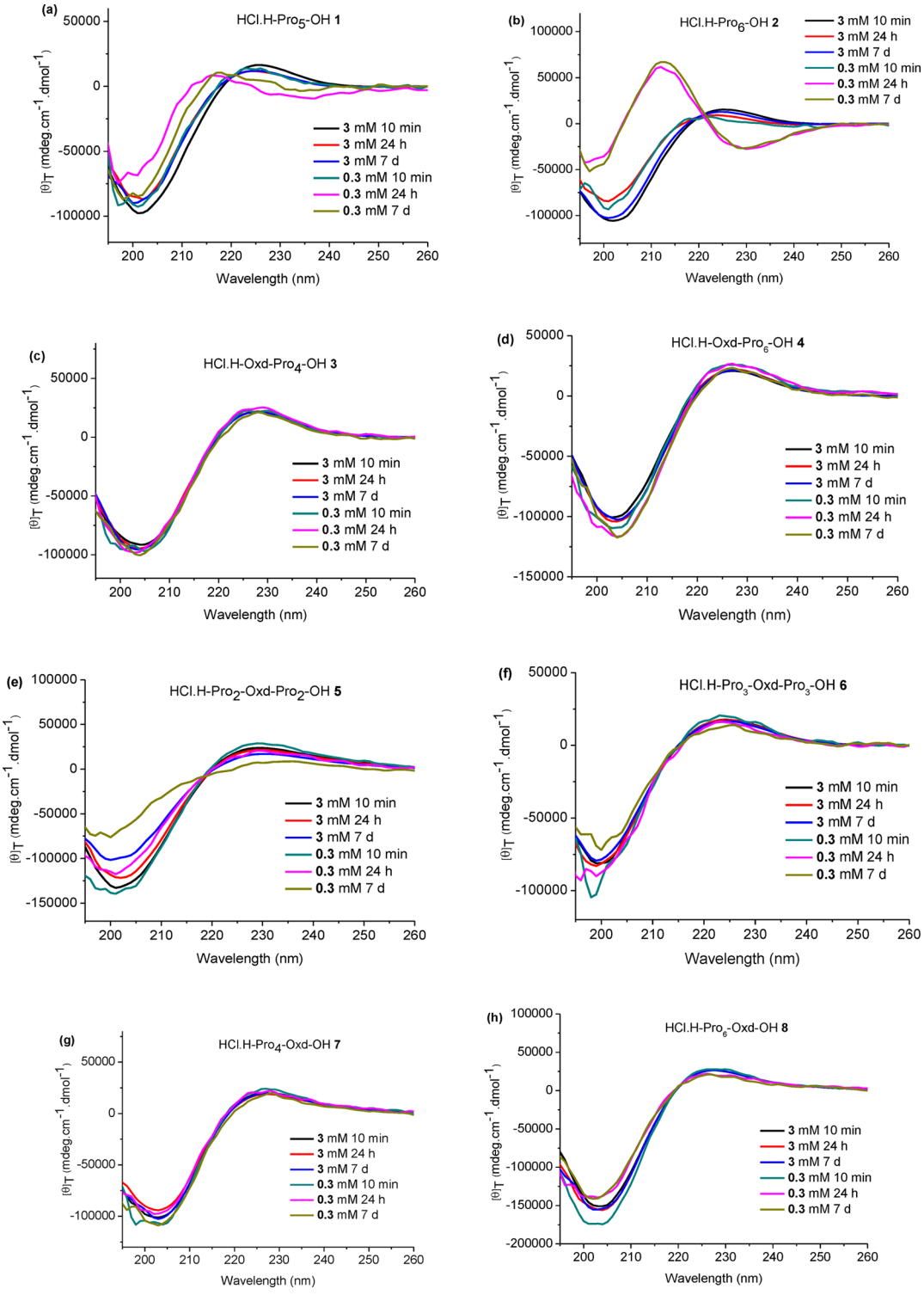

Figure 3. ECD spectra of **1**−**8** at 3 and 0.3 mM concentration after 10 min, 24 h, and 7 days.

conformation in *n*-propanol solution, while Pro_4 forms a stable PPII helix. Moreover, the PPII → PPI transition is slow, as days of incubation are necessary to obtain a stable PPI conformation.

Another parameter influencing the conformation preference is the presence and position of other amino acids in the peptide chain. Oka[13] showed that the oligomer Pro_6-Ala-Pro_6 in *n*-propanol maintains a PPII conformation even after 14 days, while Pro_6-Gly-Pro_6 slowly forms a PPI helix.

We may classify the preferred conformation assumed by oligoprolines, basing on the bands observed in the ECD spectra:[14]

- PPII: both strong negative band at 204 nm and weak positive band at 227 nm.
- PPI: strong negative band at 199 nm, strong positive band at about 214 nm, and weak negative band at about 232 nm.
- Unordered conformations (ensembles of conformers, rather than a well-defined conformation): strong negative

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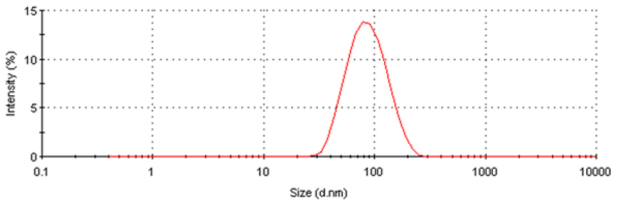

Figure 4. Size distribution by intensity of the particles from a 3 mM solution of molecule **2** in *n*-propanol after 7 days.

band at 199 nm. They may also display either a weak positive band approximately at 220 nm or a negative shoulder on the short-wavelength band, as the distribution of the ensemble in (φ, ψ) space depends upon the peptide sequence, the solvent, and the temperature.[15]

Considering the importance of and the great interest in these structures, in this letter we report our recent finding on the factors affecting the PPII → PPI isomerization on short oligomers with five to seven amino acid units. We chose short oligomers because the understanding of their behavior is still open, while clear-cut results on PPII → PPI isomerization in longer oligomers has been recently extensively described.[7,16]

We prepared eight fully deprotected oligomers, all containing L-Pro units, by solution phase synthesis. This synthetic strategy has been adopted as it is convenient for Oxd containing pseudopeptides and furnishes the compounds reported in Figure 2 with high purity and overall yields of about 20%.

Oligomers **1** and **2** are traditional polyprolines, while **3**−**8** contain the (4*S*,5*R*)-4-carboxy-5-methyloxazolidin-2-one moiety (L-Oxd) in various positions, to check if the imido-type function has a role in the stabilization of one of the two conformations, as in the past we demonstrated that oligomers of L-Oxd moiety always adopt the PPII conformation starting from the dimer in any condition.[17] This effect is due to the presence of a nitrogen atom connected both to an endocyclic and to an exocyclic carbonyl group that always adopt the *trans* conformation.[18]

The conformational analyses were based on the measurement of electronic circular dichroism spectra (ECD) in *n*-propanol. The measurements were made at 20 °C on freshly made solutions of compounds **1**−**8** at three different concentrations: 3, 0.3, and 0.03 mM. All the measurements were repeated both after 24 h and again after 7 days on the same samples. All the ECD spectra are reported in the Supporting Information, together with Table S1 that summarizes our observations concerning the presence of a positive Cotton effect between 200 and 240 nm, to check the presence of a preferred conformation (214 nm → PPI, 227 nm → PPII, no Cotton effect → unordered structure) in all the samples.

In Figure 3, we report the ECD spectra of compounds **1**−**8** at 3 and 0.3 mM concentrations as a function of the time. The spectra registered on solution at 0.03 M concentration were omitted here, as they do not display any significant modification compared with the spectra registered at 0.3 M concentration.

At first glance, we notice that the solution concentration, the equilibration time, and the position of the Oxd moiety (if included in the oligomeric sequence) have an effect on the preferred conformation. The sample concentration is a crucial parameter for the stabilization of the PPII conformation, as at 3 mM concentration oligomers **1**−**8** always display a positive Cotton effect at 227 nm, even after 7 days. In contrast, the more diluted solutions of pure polyprolines **1** and **2** at 0.3 mM concentration fold into a well-established PPI structure only after 24 h, which is still stable after 7 days.

The presence of a L-Oxd moiety in the oligomeric chain strongly affects the *trans*−*cis* isomerization, as none of the compounds **3**−**8** at any concentration folds in a PPI conformation. This outcome could be easily foreseen at 3 mM concentration, where the PPII is mainly stabilized by concentration effects. In diluted solutions the position of the L-Oxd moiety has an effect on the stabilization of the PPII conformation. Indeed oligomers **3**, **4**, **7**, and **8** that display the L-Oxd moiety either at the N-terminal or at the C-terminal position stably fold in the PPII helix at any concentration. The ECD spectra at 0.3 mM concentration of the oligomers **5** and **6**, which contain the L-Oxd moiety in the central position, present a decrease of the bands intensities as a function of time, thus suggesting a decrease of stability of the PPII conformation.

Thus, two are the crucial factors for the stabilization of PPII conformations under these conditions: the presence of a L-Oxd moiety, possibly in a terminal position, and the sample concentration, although all the samples readily dissolve into fully transparent solutions. To have a deeper insight on these phenomena, fresh solutions of **2** and of **8** at 3 and 0.3 mM concentrations were investigated by dynamic light scattering. The analysis was repeated after 24 h and after 7 days on the same samples (Figures S3−S6). In Table 1 the average size of the detected particles is reported.

After 10 min all the samples show the presence of aggregates with a variable diameter that is greater for more concentrated solutions. After 24 h all the assemblies have reduced sizes, while after 7 days aggregates may be detected only in **2** solution at the higher concentration.

This outcome may be due to the final synthetic workup with HCl dissolved in water and trifluoroethanol, as the polar environment could favor the formation of PPII helices. When the samples are dissolved in *n*-propanol, that is a hydrophobic environment, the molecules go in solution and tend to reorganize to the preferred PPI conformation. This process is slowed down in more concentrated solutions, as the molecules are aggregated and provide a local hydrophilic environment that stabilizes the PPII conformation.

At 0.3 mM concentration, the *trans*−*cis* isomerization takes place for compound **2**, to minimize the interactions between the lipophilic solvent and the polar carbonyl groups, in agreement with the ECD measurement. After 7 days the formation of the PPI helix is complete and no assemblies may be detected anymore. Finally, at 3 mM concentration, **2** is organized in larger aggregates that are still present after 7 days (Figure 4).

In contrast, **8** is always folded in PPII helices. While after 10 min and after 24 h it is aggregated at both concentrations, the aggregations disappear after 7 days, thus we can presume that the stabilization of the PPII helix is due to the presence of the L-Oxd moiety.

Two possible effects or a combination may play a role:

- the imide moiety is a "trans-nucleating" locus as it forces one peptide bond in the *trans* conformation and may induce the stabilization of all the peptide bonds in the same conformation;
- the Oxd methyl side chain may provide an enhanced lipophilicity to the whole structure, which favors the interaction with *n*-propanol.

In conclusion, we have described the synthesis in solution and the conformational analysis in a hydrophobic environment of

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eight short oligomers containing both L-Pro and the L-Oxd moieties. We have shown that short oligomers of L-Pro fold in a PPI helix only in diluted solution because, if the concentration rises, PPII helices are stabilized by the presence of aggregates that are stable even after several days and may be detected by dynamic light scattering analysis. Moreover, we have demonstrated that the L-Oxd moiety always inhibits the formation of PPI helices and strongly favors the formation of stable PPII helices.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b00532.

Synthetic procedure and characterization of compounds **1**−**8**. Copy of the ^{1}H and ^{13}C NMR spectra of compounds **1**−**8**. ECD spectra of compounds **1**−**8** in *n*-propanol at 3, 0.3, and 0.03 mM concentration. Size distribution by volume (DLS analysis) of the particles from a solution of molecules **2** and **8** in *n*-propanol at 3 and 0.3 mM concentration (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) (a) Shoulders, M. D.; Raines, R. T. *Annu. Rev. Biochem.* **2010**, *78*, 929. (b) Brodsky, B.; Thiagarajan, G.; Madhan, B.; Kar, K. *Biopolymers* **2008**, *89*, 345. (c) Engel, J. In *Collagen*; Brinckmann, J., Notbohm, H., Müller, P. K., Eds.; Springer Berlin Heidelberg: Berlin, Heidelberg, 2005; pp 7−33.

(2) Feng, S.; Chen, J. K.; Yu, H.; Simon, J. A.; Schreiber, S. L. *Science* **1994**, *266*, 1241.

(3) Mahoney, N. M.; Janmey, P. A.; Almo, S. C. *Nat. Struct. Biol.* **1997**, *4*, 953.

(4) Macias, M. J.; Hyvonen, M.; Baraldi, E.; Schultz, J.; Sudol, M.; Saraste, M.; Oschkinat, H. *Nature* **1996**, *382*, 646.

(5) Murthy, V. L.; Stern, L. J. *Structure* **1997**, *5*, 1385.

(6) Farrera-Sinfreu, J.; Zaccaro, L.; Vidal, D.; Salvatella, X.; Giralt, E.; Pons, M.; Albericio, F.; Royo, M. *J. Am. Chem. Soc.* **2004**, *127*, 474.

(7) (a) Rucker, A. L.; Creamer, T. P. *Protein Sci.* **2002**, *11*, 980. (b) Shi, Z.; Chen, K.; Liu, Z.; Kallenbach, N. R. *Chem. Rev.* **2006**, *106*, 1877.

(8) (a) Kuemin, M.; Schweizer, S.; Ochsenfeld, C.; Wennemers, H. *J. Am. Chem. Soc.* **2009**, *131*, 15474. (b) Garbuio, L.; Lewandowski, B.; Wilhelm, P.; Ziegler, L.; Yulikov, M.; Wennemers, H.; Jeschke, G. *Chem. - Eur. J.* **2015**, *21*, 10747. (c) Wilhelm, P.; Lewandowski, B.; Trapp, N.; Wennemers, H. *J. Am. Chem. Soc.* **2014**, *136*, 15829.

(9) Shi, L.; Holliday, A. E.; Khanal, N.; Russell, D. H.; Clemmer, D. E. *J. Am. Chem. Soc.* **2015**, *137*, 8680−8683.

(10) Kuemin, M.; Engel, J.; Wennemers, H. *J. Pept. Sci.* **2010**, *16*, 596.

(11) Sonar, M. V.; Ganesh, K. N. *Org. Lett.* **2010**, *12*, 5390.

(12) Kakinoki, S.; Hirano, Y.; Oka, M. *Pept. Sci.* **2004**, 453.

(13) Kakinoki, S.; Hirano, Y.; Oka, M. *Pept. Sci.* **2004**, 457.

(14) (a) Crisma, M.; De Zotti, M.; Formaggio, F.; Peggion, C.; Moretto, A.; Toniolo, C. *J. Pept. Sci.* **2015**, *21*, 148. (b) Zsila, F. *Pharm. Sci. Encycl.* **2010**, *2*, 1.

(15) Toniolo, C.; Formaggio, F.; Woody, R. W. In *Comprehensive Chiroptical Spectroscopy, Vol. 2: Applications in Stereochemical Analysis of Synthetic Compounds, Natural Products, and Biomolecules*; Berova, N., Polavarapu, P. L., Nakanishi, K., Woody, R. W., Eds.; John Wiley & Sons, Inc., 2012; pp 499−544.

(16) (a) Rabanal, F.; Ludevil, M. D.; Pons, M.; Giralt, E. *Biopolymers* **1993**, *33*, 1019. (b) Adzhubei, A. A.; Sternberg, M. J. E.; Makarov, A. A. *J. Mol. Biol.* **2013**, *425*, 2100. (c) Fillon, Y. A.; Anderson, J. P.; Chmielewski, J. *J. Am. Chem. Soc.* **2005**, *127*, 11798.

(17) (a) Bernardi, F.; Garavelli, M.; Scatizzi, M.; Tomasini, C.; Trigari, V.; Crisma, M.; Formaggio, F.; Peggion, C.; Toniolo, C. *Chem. - Eur. J.* **2002**, *8*, 2516. (b) Lucarini, S.; Tomasini, C. *J. Org. Chem.* **2001**, *66*, 727. (c) Tomasini, C.; Trigari, V.; Lucarini, S.; Bernardi, F.; Garavelli, M.; Peggion, C.; Formaggio, F.; Toniolo, C. *Eur. J. Org. Chem.* **2003**, *4*, 259.

(18) (a) Tomasini, C.; Luppi, G.; Monari, M. *J. Am. Chem. Soc.* **2006**, *128*, 2410. (b) Tomasini, C.; Angelici, G.; Castellucci, N. *Eur. J. Org. Chem.* **2011**, *2011*, 3648. (c) Angelici, G.; Castellucci, N.; Falini, G.; Huster, D.; Monari, M.; Tomasini, C. *Cryst. Growth Des.* **2010**, *10*, 923. (d) Milli, L.; Larocca, M.; Tedesco, M.; Castellucci, N.; Ghibaudi, E.; Cornia, A.; Calvaresi, M.; Zerbetto, F.; Tomasini, C. *J. Org. Chem.* **2014**, *79*, 5958. (e) Angelici, G.; Luppi, G.; Kaptein, B.; Broxterman, Q. B.; Hofmann, H. J.; Tomasini, C. *Eur. J. Org. Chem.* **2007**, *16*, 2713. (f) Zhang, R.; Madalengoitia, J. S. *Tetrahedron Lett.* **1996**, *37*, 6234.

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