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Non-Hydrogen-Bonded Secondary Structure in β-Peptides: Evidence from Circular Dichroism of (*S*)-Pyrrolidine-3-carboxylic Acid Oligomers and (*S*)-Nipecotic Acid Oligomers

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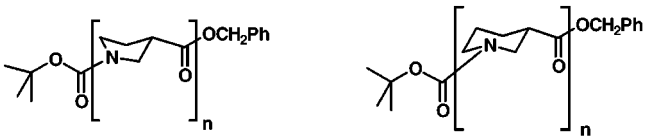
ABSTRACT



Homooligomers of β-amino acids (*S*)-3-pyrrolidine-3-carboxylic acid (PCA) and (*S*)-nipecotic acid (Nip) were studied by circular dichroism (CD) in methanol. In each series, a profound change in the far-UV CD spectrum was observed from monomer to tetramer, but little change was observed from tetramer to hexamer. A comparable pattern is observed in the CD spectra of short proline oligomers. We conclude that both PCA and Nip oligomers with ≥ four residues adopt a characteristic secondary structure.

Most regular secondary structures in proteins (α-helix, 3_{10}-helix, β-sheet) display a characteristic backbone hydrogen-bonding pattern that depends on the ability of secondary amide groups to serve as both hydrogen bond donors and acceptors. Proline is unique among the proteinogenic amino acids in having a disubstituted amino group, and Xxx-Pro linkages are therefore tertiary amides, which cannot serve as hydrogen bond donors. Despite the lack of internal hydrogen bonding, oligomers and polymers of proline adopt discrete secondary structures, e.g., polyproline I (PPI) and polyproline II (PPII) helices.[1] Non-hydrogen-bonded helices can also be formed by unnatural tertiary amide oligomers constructed from *N*-alkylglycine building blocks (peptoids).[2] The polyproline and peptoid helices appear to be specified largely by avoidance of steric repulsions.

Proline-rich segments that adopt the PPII helix conformation play important roles in protein−protein recognition,[3] e.g., interactions of SH3 domains.[4] Proline-rich sequences are crucial also for formation of the collagen triple helix, each strand of which has a PPII-like conformation.[5] It has recently been shown that peptoid and proline residues can be combined to create collagen mimics[6] and specific ligands for SH3 proteins.[7,8]

(1) For a review, see: Rabanal, R.; Ludevid, M. D.; Pons, M.; Giralt, E. *Biopolymers* **1993**, *33*, 1019.

(2) (a) Armand, P.; Kirshenbaum, K.; Goldsmith, R. A.; Farr-Jones, S.; Barron, A. E.; Truong, K. T.; Dill, K. A.; Mierke, D. F.; Cohen, F. E.; Zuckermann, R. N.; Bradley, E. K. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 4309. (b) Kirshenbaum, K.; Barron, A. E.; Goldsmith, R. A.; Armand, P.; Bradley, E. K.; Truong, K. T.; Dill, K. A.; Cohen, F. E.; Zuckermann, R. N. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 4303.

(3) Williamson, M. P. *Biochem. J.* **1994**, *297*, 249.

(4) (a) Yu, H.; Chen, J. K.; Feng, S.; Dalgarno, D. C.; Brauer, A. W.; Schreiber, S. L. *Cell* **1994**, *76*, 933. (b) Lim, W. A.; Richards, F. M.; Fox, R. O. *Nature* **1994**, *372*, 375.

(5) Dramer, R. Z.; Vitagliano, L.; Bella, J.; Berisio, R.; Mazzarella, L.; Brodsky, B.; Zagari, A.; Berman, H. M. *J. Mol. Biol.* **1998**, *280*, 623 and references therein.

(6) Jefferson, E. A.; Locardi, E.; Goodman, M. *J. Am. Chem. Soc.* **1998**, *120*, 7420. Kwak, J.; Jefferson, E. A.; Bhumralkar, M.; Goodman, M. *Bioorg. Med. Chem.* **1999**, *7*, 153 and references therein. For another class of collagen mimic, see: Holmgren, S. K.; Taylor, K. M.; Bretscher, L. E.; Raines, R. R. *Nature* **1998**, *392*, 666. Holmgren, S. K.; Bretscher, L. E.; Taylor, K. M.; Raines, R. R. *Chem. Biol.* **1999**, *6*, 63.

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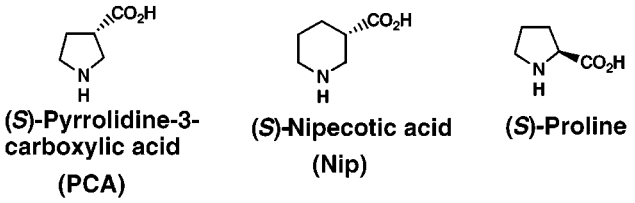
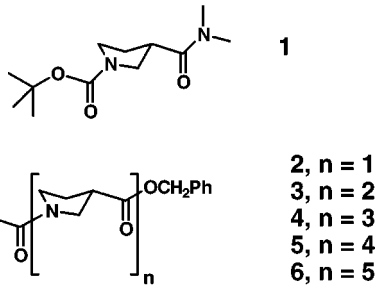
Here, we describe synthesis and preliminary conformational studies of a new type of oligo-tertiary amide, constructed from cyclic β-amino acids.[9] Circular dichroism (CD) data suggest that oligomers of (*S*)-pyrrolidine-3-carboxylic acid (PCA) or (*S*)-nipecotic acid (Nip) have distinct secondary structural preferences. PCA is an isomer of proline, and



Nip is a ring-expanded homologue of PCA. These results complement previous demonstrations that β-peptide oligomers can adopt conformations corresponding to each type of hydrogen-bonded secondary structure observed in proteins, helix,[10−12] sheet,[13,14] and reverse turn.[14]

Structural characterization of oligoprolines and proline-rich oligomers in solution has been limited to low-resolution methods such as CD because the PPII helix is too extended to allow NOEs between protons on nonadjacent residues.[15−17] (In contrast, the α-helix and the 3_{10}-helix are sufficiently compact that high-resolution structures can be deduced from NOE data.[18]) Peptide CD signals in the far-UV region (<250 nm) arise largely from amide groups, and each of the regular peptide secondary structures displays a characteristic far-UV CD signature.[19] The PPII helix formed by oligomers of (*S*)-proline gives rise to a weak maximum at 226 nm and a strong minimum at 206 nm in aqueous solution.[1,20] The polyproline I helix, favored in *n*-propanol and other aliphatic alcohols, is signaled by a weak minimum at 232 nm, a strong maximum at 215 nm, and a strong minimum at 200 nm.[1,20]

(*S*)-PCA was synthesized in its Cbz-protected form by a reported route from *trans*-4-hydroxy-L-proline.[21] This material was then converted to Boc-(*S*)-PCA or Boc-(*S*)-PCA-OBn by standard methods. In series **2**−**6**, each oligomer was prepared by using BOP-Cl to couple Boc-(*S*)-PCA to the appropriate N-deprotected oligomer. After purification by chromatography on silica, these oligomers all appeared to be pure by analytical HPLC.



Previous synthesis of optically active nipecotic acid derivatives has involved resolution of racemic ethyl nipecotate via cocrystallization with optically pure tartaric acid.[22] We developed an alternative resolution in which racemic nipecotic acid itself is cocrystallized with enantiomerically pure camphorsulfonic acid. This method provides either antipode of nipecotic acid in >99% ee.[23] The absolute configuration of the resolved material has been established crystallographically.[13,14a] Boc-(*S*)-Nip and Boc-(*S*)-Nip-OBn were prepared from the resolved material, and these building

(7) Nguyen, J. T.; Turck, C. W.; Cohen, F. E.; Zuckermann, R. N.; Lim, W. A. *Science* **1998**, *282*, 2088.

(8) Other approaches to PPII mimics: Zhang, R.; Madalengoitia, J. S. *J. Org. Chem.* **1999**, *64*, 330. Zhang, R.; Brownewell, F.; Madalengoitia, J. S. *J. Am. Chem. Soc.* **1998**, *120*, 3894.

(9) Oligomers of acyclic β-amino acids (β-peptoids): Hamper, B. C.; Kolodziej, S. A.; Scates, A. M.; Smith, R. G.; Cortez, E. *J. Org. Chem.* **1998**, *63*, 708.

(10) (a) Gellman, S. H. *Acc. Chem. Res.* **1998**, *31*, 173. (b) Appella, D. H.; Christianson, L. A.; Karle, I. L.; Powell, D. R.; Gellman, S. H. *J. Am. Chem. Soc.* **1996**, *118*, 13071. (c) Appella, D. H.; Christianson, L. A.; Klein, D. A.; Powell, D. R.; Huang, X.; Barchi, J. J.; Gellman, S. H. *Nature* **1997**, *387*, 381. (d) Applequist, J.; Bode, K. A.; Appella, D. H.; Christianson, L. A.; Gellman, S. H. *J. Am. Chem. Soc.* **1998**, *120*, 4891. (e) Appella, D. H.; Barchi, J. J.; Durell, S.; Gellman, S. H. *J. Am. Chem. Soc.* **1999**, *121*, 2309. (f) Appella, D. H.; Christianson, L. A.; Karle, I. L.; Powell, D. R.; Gellman, S. H. *J. Am. Chem. Soc.* **1999**, *121*, 6206. (g) Appella, D. H.; Christianson, L. A.; Klein, D. A.; Richards, M. R.; Powell, D. R.; Gellman, S. H. *J. Am. Chem. Soc.*, in press.

(11) (a) Seebach, D.; Overhand, M.; Kühnle, F. N. M.; Martinoni, B.; Oberer, L.; Hommel, U.; Widmer, H. *Helv. Chim. Acta* **1996**, *79*, 913. (b) Seebach, D.; Ciceri, P.; Overhand, M.; Juan, B.; Rigo, D.; Oberer, L.; Hommel, U.; Amstutz, R.; Widmer, H. *Helv. Chim. Acta* **1996**, *79*, 2043. (c) Seebach, D.; Matthews, J. L. *J. Chem. Soc., Chem. Commun.* **1997**, 2015−2022. (d) Seebach, D.; Abele, S.; Gademann, K.; Guichard, G.; Hintermann, T.; Jaun, B.; Matthews, J. L.; Schreiber, J.; Oberer, L.; Hommel, U.; Widmer, H. *Helv. Chim. Acta* **1998**, *81*, 932. (e) Seebach, D.; Abele, S.; Sifferlen, T.; Hänggi, M.; Gruner, S.; Seiler, P. *Helv. Chim. Acta* **1998**, *81*, 2218.

(12) Gung, B. W.; Zou, D.; Stalcup, A. M.; Cottrell, C. E. *J. Org. Chem.* **1999**, *64*, 2176.

(13) Krauthäuser, S.; Christianson, L. A.; Powell, D. R.; Gellman, S. H. *J. Am. Chem. Soc.* **1997**, *119*, 11719.

(14) (a) Chung, Y. J.; Christianson, L. A.; Stanger, H. E.; Powell, D. R.; Gellman, S. H. *J. Am. Chem. Soc.* **1998**, *120*, 10555. (b) Seebach, D.; Abele, S.; Gademann, K.; Jaun, B. *Angew. Chem., Int. Ed.* **1999**, *38*, 1595.

(15) (a) Okabayashi, H.; Isemura, T.; Sakakibara, S. *Biopolymers* **1968**, *6*, 323. (b) Rothe, M.; Rott, H.; Mazanek, J. *Peptides 1976*; Loffet, A., Ed.; p 309. (c) Helbecque, H.; Loucheux-Lefebvre, M. H. *Int. J. Pept. Protein Res.* **1982**, *19*, 94. (d) Dukor, R. M.; Kiederling, T. A. *Biopolymers* **1991**, *31*, 1747.

(16) The PPII helix has been characterized crystallographically in short proline oligomers (Benedetti, E.; Bavoso, A.; Benedetto, D. B.; Pavone, V.; Pedone, C.; Toniolo, C.; Bonora, G. M. *Biopolymers* **1983**, *22*, 305 and references therein) and in segments of folded proteins (Stapley, B. J.; Creamer, T. P. *Protein Sci.* **1999**, *8*, 587). High-resolution NMR evidence for the PPII helix has been obtained for proline-rich peptides bound to proteins, but in these cases the PPII conformation is defined by intermolecular NOEs between the proline-rich ligand and its structurally defined receptor (ref 4a and the following: Macias, M. J.; Hyvönen, M.; Baraldi, E.; Schultz, J.; Sudol, M.; Saraste, M.; Oschkinat, H. *Nature* **1996**, *382*, 646. Wittekind, M.; Mapelli, C.; Lee, V.; Goldfarb, V.; Friedrichs, M. S.; Meyers, C. A.; Mueller, L. *J. Mol. Biol.* **1997**, *267*, 933).

(17) CD data have recently been used to examine the conformational behavior of a number of unnatural oligomers, including peptoids (ref 2a), δ-peptides (Szabo, L.; Smith, B. L.; McReynolds, K. D.; Parrill, A. L.; Morris, E. R.; Gervay, J. **1998**, *J. Org. Chem. 63*, 1074), oligo-α-aminoxy acids (Yang, D.; Qu, J.; Li, B.; Ng, F.-F.; Wang, X.-C.; Cheung, K.-K.; Want, D.-P.; Wu, Y.-D. *J. Am. Chem. Soc.* **1999**, *121*, 589), and oligo-(phenylene ethynylene)s (Gin, M. S.; Yokozawa, T.; Prince, R. B.; Moore, J. S. *J. Am. Chem. Soc.* **1999**, *121*, 2643.

(18) Wüthrich, K. *NMR of Proteins and Nucleic Acids*; Wiley: New York, 1986.

(19) Johnson, W. C. *Annu. Rev. Biophys. Biophys. Chem.* **1988**, *17*, 145.

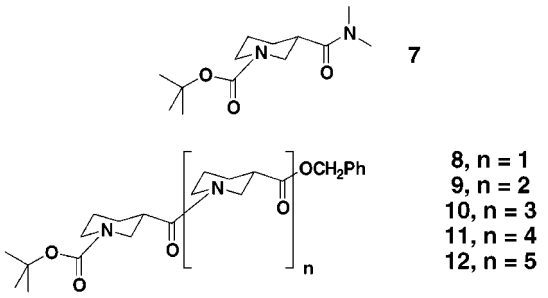
(20) Sreerama, N.; Woody, R. W. *Biochemistry* **1994**, *33*, 10022.

(21) Klein, S. I.; Czekaj, M.; Molino, B. F.; Chu V. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1773.

(22) Akkerman, A. M.; de Jongh, D. K.; Veldstra, H. *Recl. Trav. Chim. Pay-Bas* **1951**, *70*, 899.

(23) Chung, Y. J.; Huck, B. H.; Christianson, L. A.; Stanger, H. E.; Krauthäuser, S.; Powell, D. R.; Gellman, S. H., submitted for publication.

blocks were used for the synthesis of **7**−**12**. After purification by chromatography on silica, these oligomers all appeared to be pure by analytical HPLC.



Hydrogen-bonded β-peptide helices display characteristic CD signatures,[10−12] and we therefore used CD to examine the PCA and Nip oligomer series. Figure 1 shows CD data for the PCA series in methanol. We follow standard peptide practice in normalizing the data for both β-peptide concentration and number of amide groups, which facilitates comparisons among oligomers of different length. Tetramer **4**, pentamer **5**, and hexamer **6** display nearly identical CD spectra, with a minimum at ca. 214 nm and a zero crossing at ca. 203 nm. This signature is quite different from those of the smallest members of the PCA series, **1** and **2**. Trimer **3** displays a spectrum that is intermediate between those of **1** and **2** and those of **4**−**6**. Normalized spectra of 0.5 and 0.05 mM **5** were very similar, which suggests that there is no change in aggregation state (presumably monomeric) over this concentration range. The CD spectra of PCA pentamer **5** in methanol and 2-propanol are nearly identical; heating the 2-propanol solution from 25 to 75 °C causes little change in the CD spectrum.

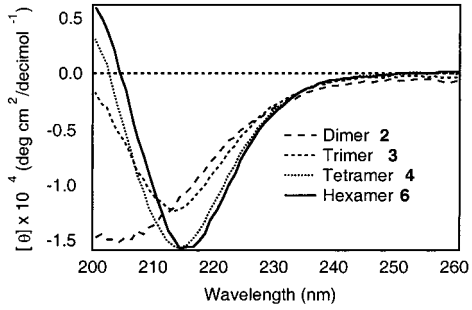


Figure 1. Circular dichroism data for PCA oligomers in methanol (25 °C): 1.0 mM **2**, 1.0 mM **3**, 0.5 mM **4**, and 0.5 mM **6**. The data for monomer **1** are similar to those shown for **2**, and the data for pentamer **5** are identical to those shown for **6**. The data were obtained on an Aviv instrument with 1 mm path length cells. The data have been normalized for β-peptide concentration and number of amide groups.

The length-dependent trend among PCA oligomers in Figure 1 is comparable to the length-dependent behavior of proline oligomers in aqueous and organic solvents as manifested in CD and other spectroscopic data.[15] Significant changes in the positions of minima and maxima are observed among the proline dimer, trimer, and tetramer. In contrast, relatively little change in shape is observed beyond the tetramer, although there is a gradual increase in the intensities of minima and maxima as proline residues are added. The proline hexamer displays a mean residue ellipticity of ca. −28 000 deg cm^2 decimol^{-1} at the 206 nm minimum,[15b] which may be compared with ca. −16 000 deg cm^2 decimol^{-1} for the 214 nm minimum of PCA hexamer **6** in methanol (Figure 1). We conclude from the data in Figure 1, by analogy to oligoproline precedents, that PCA oligomers can adopt a regular secondary structure in methanol and that the extent of secondary structure formation is maximal once four PCA residues are in place. Detailed elucidation of the PCA secondary structure will require a characterization method of higher resolution than CD.

Figure 2 shows CD data for Nip oligomers in methanol. The length-dependent pattern is comparable to the pattern shown for PCA oligomers (Figure 1) in that the CD signatures of monomer and dimer are very different from those of tetramer, pentamer, and hexamer. We therefore conclude that longer Nip oligomers adopt a specific secondary structure in methanol and that the extent of secondary structure formation is nearly maximal when four residues are in place. The CD spectra of Nip oligomers **10**−**12** shows a weak maximum at ca. 228 nm and a strong minimum at ca. 208 nm. This signature differs from that of the corresponding PCA oligomers, which suggests a difference between the secondary structures favored by these two β-peptide backbones. Nip pentamer **11** displays similar CD spectra in methanol and 2-propanol, and heating the 2-propanol solution from 25 to 75 °C causes very little change in the CD signature.

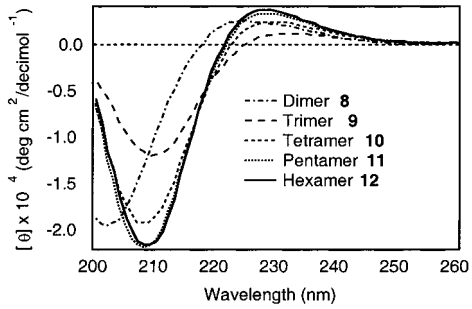


Figure 2. Circular dichroism data for Nip oligomers in methanol (25 °C): 1.0 mM **8**, 1.0 mM **9**, 0.5 mM **10**, 0.5 mM **11**, 0.5 mM **12**. The data for monomer **7** are similar to those shown for **8**. The data have been normalized for β-peptide concentration and number of amide groups.

The preliminary evidence we have presented for ordered folding of Nip and PCA homooligomers suggests that the conformational analogy between conventional peptides (constructed from α-amino acids) and β-peptides extends beyond hydrogen-bonded secondary structures[10−14] to non-hydrogen-

bonded structures such as the PPII helix. Adoption of well-defined folding patterns by oligo-Nip and oligo-PCA is of potential medicinal interest because these tertiary amide backbones should be less hydrophilic, and therefore more prone to diffuse across biological membranes,[24] than the secondary amide backbones common in conventional peptides and β-peptides. We are currently examining oligomers containing functionalized derivatives of Nip and PCA, which may be amenable to high-resolution structural analysis and/or display interesting biological activities.

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OL9909482

(24) Ito, Y.; Kwon, O. H.; Ueda, M.; Tanaka, A.; Imanishi, Y. *FEBS Lett.* **1997**, *415*, 285.