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Conformational Stability of Proline Oligomers

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Abstract: Reexamination of the solution conformation of a series of N-Boc O-benzyl protected proline oligomers (dimer-hexamer) by 500 MHz NMR reveals that these oligomers adopt the poly-L-proline type II conformation in solution and do not require a critical chain length for conformational stability as has been previously reported. Copyright © 1996 Elsevier Science Ltd

The poly-L-proline type II conformation (PPII) is emerging as an important secondary structure in the molecular recognition of peptides by proteins. A wealth of NMR and X-ray crystal structure data has shown that kinases , SH2 domains, SH3 domains, MHC class II proteins and other proteins bind peptide ligands in the PPII conformation or in extended conformations closely resembling this motif[1]. The poly-L-proline type II secondary structure is defined by ϕ = -75°, ψ = 145° and ω = 180°. PPII helices occur regularly in globular proteins and possess amino acids other than proline[2]. However, proline containing PPII helices are especially stable for two reasons: 1) the ϕ angle is defined at -75° by the constraints of the proline pyrrolidine ring and 2) the ψ angle is defined at 145° by minimization of 1,3-allylic-"like" strain in which the peptide bond represents the double bond surrogate (Figure 1). Although in proline containing oligomers, the *trans* peptide bond (ω = 180°)

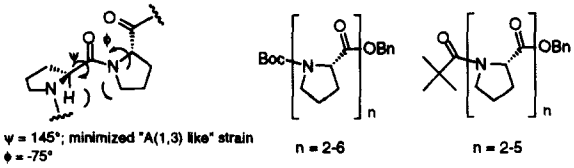

Figure 1

is favored (2.3-5.0 kcal/mol[3]), the *cis* conformation (ω = 0°) can be observed in some instances. For example, a study of the proline oligomers H-$(Pro)_n$-OH in D_2O has shown that the dimer (n = 2) exists as a mixture of *cis* and *trans* (35% and 65% respectively) conformations, however, for higher oligomers (n = 3-5) the percentage of peptide bonds in the *trans* conformation remains approximately constant (90%)[4]. In addition, a study of N-Boc O-benzyl protected oligomers (Boc-$(Pro)_n$-OBn) in $CDCl_3$ has reported that for n = 2-4 "these oligomers contained nearly random distributions of *cis* and *trans* peptide bonds"[5]. Furthermore, this study ascertained that a minimum of 5-6 prolines is required for the formation of a stable all-*trans* PPII helix. Due to the important role which peptides possessing short proline spans play in signal transduction pathways[1] and as part of our program aimed at the development of mimics of the PPII secondary structure, we wished to better understand the

structural parameters responsible for PPII helix stabilization. As such, we have reinvestigated the conformation of these Boc-$(Pro)_n$-OBn oligomers as well as the series Piv-$(Pro)_n$-OBn (Figure 1) which possess a clearly defined *trans* pivaloyl amide bond.

The N-Boc O-benzyl protected polyproline analogs were synthesized as previously described[6], while the N-pivaloyl protected analogs were synthesized by N-terminal deprotection (1M HCl/CH_3CO_2H) and acylation (PivCl, Et_3N) of the respective Boc-protected precursor. ^1H and ^{13}C NMR spectra were recorded on a Bruker 500 MHz Spectrometer. All spectra were collected in $CDCl_3$ unless otherwise noted.

Figure 2A shows the NMR region encompassing the α-proton resonances of the N-Boc protected proline oligomers. The dimer exhibits four clear double doublets at 4.65, 4.62, 4.49 and 4.37 ppm. The presence of these four signals is inconsistent with a mixture of *cis/trans* amide and *cis/trans* carbamoyl bonds since this

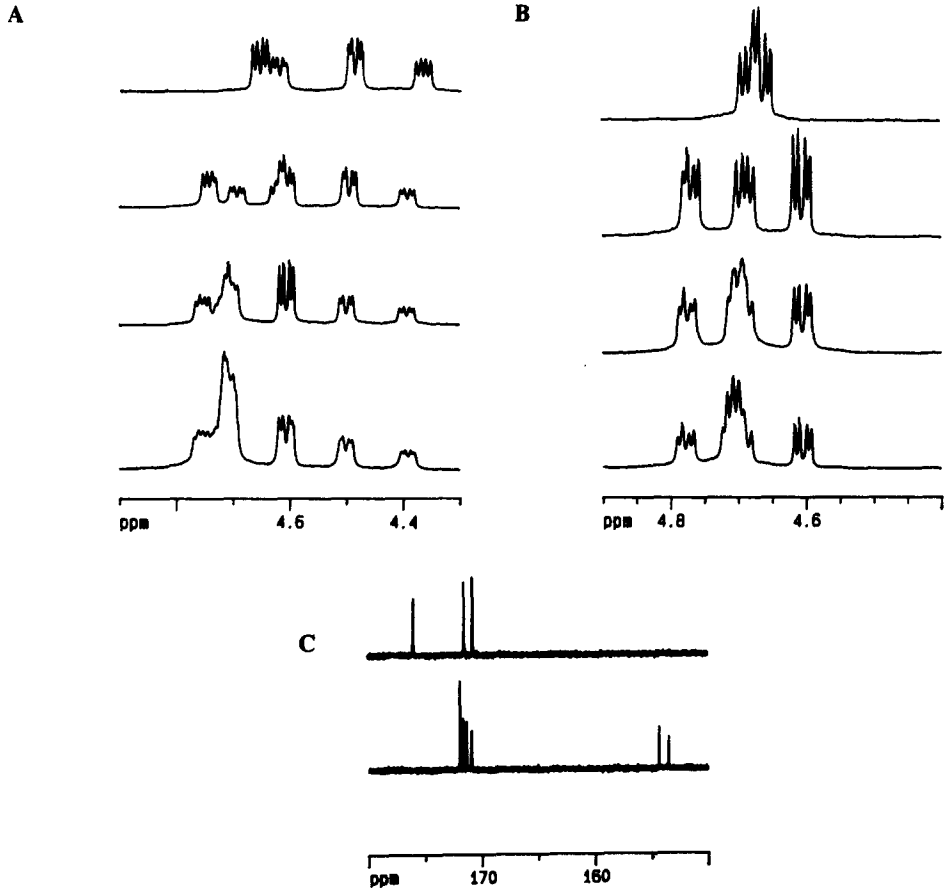

Figure 2. (A) ^1H NMR α-H region for the series Boc-$(Pro)_n$-OBn for n = 2-5 (top to bottom). (B) ^1H NMR α-H region for the series Piv-$(Pro)_n$-OBn for n = 2-5 (top to bottom). (C) ^{13}C NMR carbonyl region for Piv-$(Pro)_2$-OBn (top) and Boc-$(Pro)_2$-OBn (bottom).

number of conformational isomers would give rise to eight α-hydrogen signals. Since carbamoyl protected pyrrolidines exist as a mixture of *cis/trans* isomers, the four observed signals are due to the two conformations possible about the N-terminal Boc group and not to any lack of fidelity about the proline amide bond as has been

previously reported[5]. While we will not attempt to assign which signals correspond the N-terminal *cis/trans* conformers, we assign the 4.49 and 4.37 signals to the N-terminal proline based on the higher shift expected for the residue possessing the weaker electron withdrawing group (Boc Vs amide). We will henceforth use the nomenclature N-terminal, N+1, N+2 in describing the individual residues of proline oligomers. The presence of only two conformations caused by the lability of the N-terminal Boc group is also evident in the ^{13}C NMR carbonyl region (Figure 2C-bottom). The large shift difference between the two Boc-carbonyl resonances (154, 153 ppm) indicates that this is the site of *cis/trans* isomerization.

Further evidence of the conformational stability of short proline oligopeptides is evidenced in the higher oligomers. The ^1H NMR spectrum of trimer shows the presence of four clearly defined double doublets (4.74, 4.69, 4.49, 4.38 ppm) and two overlapping double doublets (4.61 ppm). This set of data is again consistent with the presence of two conformations and not with a random distribution of *cis/trans* conformations, which would give rise to 16 signals. Assignments for the trimer are as follows: 4.74, 4.69 (N+1); 4.61 (N+2); 4.49, 4.38 (N-terminal). The trend for this series of compounds, apparent from this set of data, is that as residues are placed away from the N-terminus, the two α-hydrogen resonances for each residue begin to coalesce. This is especially evident in the tetramer and pentamer in which the C-terminal α-proton resonances are clearly defined double doublets (~4.6 ppm). The presence of an electronic influence, which gives rise to two signals on the N-terminus and which decreases toward the C-terminus indicates that the complexity observed in the NMR spectra of these oligomers is due to the presence of two conformations about the N-terminal Boc group and not due to *cis/trans* isomerization about the backbone amide bonds. For all these cases the *trans* proline amide bond assignment is confirmed from the ^{13}C NMR shift of the proline γ and δ-carbons, as previously described[7].

To unambiguously establish the conformational stability of short proline oligomers, we synthesized the corresponding series of N-pivaloyl protected analogs, which possess a clearly defined N-terminal *trans* amide bond conformation. The ^1H NMR region encompassing the α-hydrogen resonances is shown in Figure 2B. While the dimer appears as two overlapping double doublets, the ^{13}C NMR spectrum clearly shows the presence of a single predominating conformation as exemplified by the signals observed in the carbonyl region (Figure 2C-top). In higher oligomers, the conformational stability of these polyproline analogs is best demonstrated by the ^1H NMR spectrum of the trimer, which shows the presence of three clearly resolved double doublets indicating the presence of one predominating conformation. Furthermore, the ^{13}C NMR spectra of the trimer, tetramer, and pentamer also exhibited the presence of a single predominating conformation (data not shown).

In addition to the Boc-$(Pro)_n$-OBn and Piv-$(Pro)_n$-OBn analogs we also examined the $Cl^-H_2^+$-$(Pro)_n$-OBn and Piv-$(Pro)_n$OH series. Both ^1H and ^{13}C NMR spectra of $Cl^-H_2^+$-$(Pro)_n$-OBn for n = 2-4 in D_2O again revealed this series existed as a single conformation within our detection limits. This result demonstrates that the conformational control elements responsible for PPII stabilization still function in aqueous medium. Furthermore, similar results were observed for the Piv-$(Pro)_n$OH series (n = 2-4) possessing the deblocked C-terminus.

We have also explored the role of intramolecular hydrogen bonding in influencing the conformational isomerism of the proline amide bond. Previously Naider[7a] had suggested that the Pro-Pro amide bond in the trimer Boc-Pro-Pro-Gly-OMe exists as a mixture of *cis/trans* conformations due to hydrogen bonding between the Gly NH and the Boc-carbonyl oxygen. To determine if hydrogen bonding of this sort could give rise to the *cis* conformation in our proline oligomers, we synthesized the analogs Piv-Pro-Pro-$N(CH_3)_2$ and Piv-Pro-Pro-

$NHCH_3$. The ^1H and ^{13}C NMR of Piv-$(Pro)_2$-$N(CH_3)_2$ again showed the presence of a single conformation possessing a *trans* Pro-Pro amide bond. However, Piv-$(Pro)_2$-$NHCH_3$ existed as an approximate 1:1 mixture of *cis/trans* conformations. The *cis* backbone amide conformation could be clearly assigned from the γ and δ carbon shifts of the N+1 residue[7]. In a strongly hydrogen bonding solvent like d_4-methanol, however, the *cis* conformation significantly diminished to account for only 15% of the material. It is thus expected that for oligopeptides in aqueous medium this interaction by itself does not contribute significantly to the *cis* conformation.

This study concludes that appropriately protected proline oligomers adopt the poly-L-proline type II conformation in solution and that a critical chain length is not required for helix stability as had been previously reported. This is highlighted by the fact that even a unit as small as an appropriately protected dimer is conformationally defined. Furthermore, although it is somewhat counterintuitive that a peptide dimer forms a well defined secondary structure especially since it does not possess enough residues to form a full turn of a helix, a protected proline dimer can be described by the set of ϕ and ψ angles which define a PPII helix.

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