Structural Characterization of PPII Helical Oligoproline

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Introduction

Detailed knowledge of the geometry and conformational dynamics of molecules used as molecular scaffolds is crucial for the design of functional compounds based on this architecture. Oligoproline is such a molecular scaffold that adopts in aqueous environments a left-handed helix, the Polyproline II (PPII)-helix [1]. Powder diffraction studies provided in the 1950s valuable low-resolution information about the PPII-helix [2]. They revealed basic parameters *e.g.*, the all-*trans* configuration of the amide bonds and that every third residue is stacked on top of each other. However, in contrast to crystal structures of collagen and several other proteins with PPII-helical domains, crystallization of an oligoproline in a PPII-helical conformation had remained elusive. Our interest in the use of azidoproline-containing oligoprolines as functionalizable scaffolds [3-6] led us to revisit the crystallization of oligoprolines.

Results and Discussion

N-Terminal functionalization of a proline-hexamer with *p*-bromobenzoic acid provided *p*-Br-C₆H₄-Pro₆-OH that crystallized by vapor diffusion from acetonitrile to tetrahydropyran. This high-resolution structure provided detailed insight into the conformational properties of PPII-helical oligoprolines [7].

Fig. 2. Crystal structure of hexaproline *p*-Br-C₆H₄-Pro₆-OH (ORTEP).

Distance measurements between every N, C_α, and C_i revealed that the helical pitch between every third residue is 8.98 ± 0.14 Å in contrast to the 9.36 Å that was derived from the powder diffractogram [2]. Analysis of the distances between the carbonyl oxygens and the carbonyl carbons of neighboring residues (O_{*i-1*}...C_{*i*}) showed that they are all below the van der Waals' radii (3.2 Å) of these atoms (Figure 2). Such short distances are indicative of n → π* interactions between neighboring carbonyl groups, which involve delocalization of the non-bonding electrons of O_{*i-1*} into the π* orbital of the C_{*i*}=O_{*i*} bond [8]. Additional parameters that are indicative for n → π* interactions are the degree of pyramidalization of C_{*i*} (Δ) and the trajectory angle O_{*i-1*}...C_{*i*}=O_{*i*} (θ_{BD}) between adjacent carbonyl groups. Ideal trajectory angles of ~104° were observed in Pro1 and Pro2 that also show the highest degree of pyramidalization of C_{*i*} (Δ). These values of 0.040 Å and 0.023 Å are in fact the highest ever observed values for interactions between two amides. Additionally the crystal structure showed a correlation between the ring-pucker and the backbone dihedral angles and that water is not a necessity for PPII-helical oligoproline [7].

residue	θ _{BD}	<i>d</i> [Å]	Δ [Å]
Pro1	102.9 °	2.958	0.040
Pro2	106.5 °	2.923	0.023
Pro3	89.8 °	3.072	0.005
Pro4	98.9 °	3.063	0.022
Pro5	88.2 °	3.173	0.010
Pro6	98.2 °	3.047	nd ^a

^aThe high anisotropy of the O atoms within the carboxylic acid did not allow for determining a meaningful Δ value.

Fig. 1. Trajectory angles O_{*i-1*}...C_{*i*}=O_{*i*} (θ_{BD}), O_{*i-1*}...C_{*i*} distances (*d*) and pyramidalization of C_{*i*} (Δ).

Next, we explored the dynamic behavior of oligoprolines and performed double electron-electron resonance (DEER) measurements on a series of double spin-labelled oligoprolines to measure the dipole-dipole interactions between spin labels at distances on the nanometer scale with very high precision [8]. In contrast to commonly applied FRET chromophores, these spin-labels are significantly smaller and therefore reduce label-related line-broadening on the distance distribution. We used the super high-spin complex Gd^{3+} -DOTA and the nitroxide radical 2,2,5,5-tetramethyl-3-pyrrolin-1-oxyl as spin labels and varied the distances across a series of five oligoproline of the same length (Figure 3). Narrow distance distributions were obtained from the DEER-measurements both, in trifluoroethanol (TFE) and a water/glycerol mixture with maxima at distances that were expected for PPII-helices. The narrowest distance distributions were observed for probe **P5**, with distance distribution widths increasing with the number of repeating Pro_3 units between the labels in the other probes **P8-P17**. This implies that the width and shape of the distributions of **P11**, **P14**, and **P17** are governed by the flexibility of the oligoproline backbone. This observation is typical for shape-persistent oligomers, which is predicted by the worm-like-chain model (WLC) and related to the persistence length. [10]

Fig. 3 a.) Double spin-labeled oligoprolines **P5**, **P8**, **P11**, **P14**, and **P17** b.) C(4)-endo pucker of (4S)Amp.

Thus, using the WLC model the persistence length at ambient temperature (298 K) was determined to be between 3.0 and 3.5 nm.

Monte Carlo Simulations allowed to separate influences of backbone flexibility and the occurrence of *cis*-amide bonds that contribute to the observed line-broadening [11]. These computational studies allowed to quantify the probability of a *cis*-amide bond to be ~2% in both solvents [12].

In conclusion, we have gained insight into the crucial parameters of oligoprolines from the first crystal-structure of a PPII-helical oligoproline [7] and determined their shape-persistence [12]. The presented data are crucial for the use of oligoprolines as molecular scaffolds and will allow for the design of even better tailored molecular systems [7,12].

References

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