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PAPER

Concurrent display of both α- and β-turns in a model peptide†

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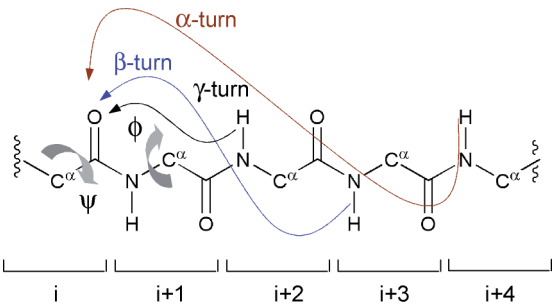
This article describes a model peptide that concurrently displays both α- and β-turns, as demonstrated by structural investigations using single crystal X-ray crystallography and solution-state NMR studies. The motif reported herein has the potential for the design of novel conformationally ordered synthetic oligomers with structural architectures distinct from those classically observed.

Introduction

Synthetic secondary structural motifs that promote unique/specific conformations are of considerable importance in the design and development of new peptidomimetics.[1] Furthermore, such motifs might also possess enzyme-like catalytic activities.[2,3] Intense interest in this area during the last two decades have resulted in the generation of a plethora of synthetic structural motifs that mimic/promote various protein secondary structures, in particular reverse turns.[4]

A turn in protein structure is defined as a site where a polypeptide chain reverses its overall direction. Reverse turns are generally categorized as α-turn, β-turn, and γ-turn, which are formed by five-, four-, and three-amino-acid residues, respectively.[5] According to the folding mode and backbone dihedral angles, each of such turns can be further classified into several different sub-types.[5]

Reverse turns play an important role in globular proteins from both a structural and functional point of view.[5,6] Among the reverse turns, β-turn, usually featuring a 10- membered ring intramolecular hydrogen-bonded network, has been extensively investigated in the past.[7] Recent studies suggest that α-turns,[8,9] although less frequently observed in proteins compared to β-turns, also play key roles in certain biological functions.[10] For instance, the role of α-turn motifs in molecular recognition processes involving HIV-neutralization has just begun to unfold. Investigations of crystal structure of the antibody F425-B4e8 in complex with a V3 peptide revealed a new binding mode for HIV-1 neutralization, involving a five-residue α-turn around the conserved GPGRA apex of the β-hairpin loop.[11] α-turn motif is likely to be one of the crucial structural factors effecting specific molecular recognition processes involving the recently isolated Asian scorpion toxin (BmK 17[4]).[12] The sustained efforts in the development of reverse turn mimetics as potential peptidomimetics have led to the discovery of structural motifs that display α-turns, although they are relatively limited in number.[13]



α-turn: 13-membered ring H-bonding; NH (i+4) → CO (i)
β-turn: 10-membered ring H-bonding; NH (i+3) → CO (i)
γ-turn: 7-membered ring H-bonding; NH (i+2) → CO (i)

Results and Discussion

The model peptides described in this article have been synthesized by straight forward routes, as described in the ESI.† Efforts to crystallize the model peptides **1a–d** resulted in the formation of crystals of **1a**, suitable for X-ray diffraction studies. In the model peptide **1a**, the amino acid residues proline (Pro) and glycine (Gly) occupies the i+1 and i+2 sites of a type-II β-turn (ϕ1 = −58°, ψ1 = +136°, ϕ2 = +69°, ψ2 = +15°; ideal type I1 β-turn,[14a] ϕ1 = −60°, ψ1 = +120°, ϕ2 = +80°, ψ2 = 0°), (fig. 1).

It should be noted that positioning of Pro and Gly at the i+1 and i+2 sites of peptide segments is known to promote type II β-turn formation.[14b] However, introduction of a N-acylated ethylene diamine (EDA) moiety at the i+3 site paved the way for

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† Electronic supplementary information (ESI) available: Experimental procedures, ^{1}H, ^{13}C, DEPT-135, 2D spectra of compounds and mass spectra. CCDC reference numbers 663336, 680778 and 680779. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c1ob05553d

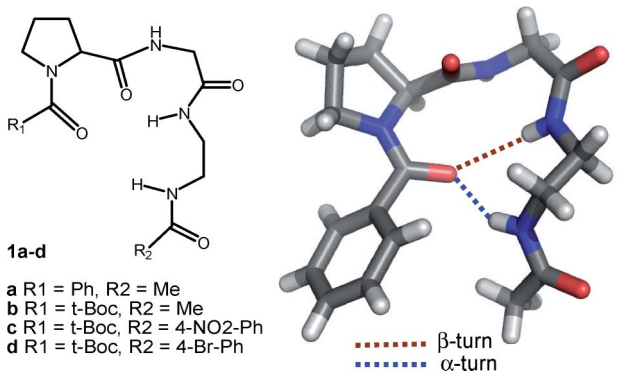

Fig. 1 Molecular structure of **1** (left) and crystal structure of **1a** in capped stick representation (right). Hydrogens other than at the hydrogen bonding sites have been deleted for clarity in the crystal structure image.

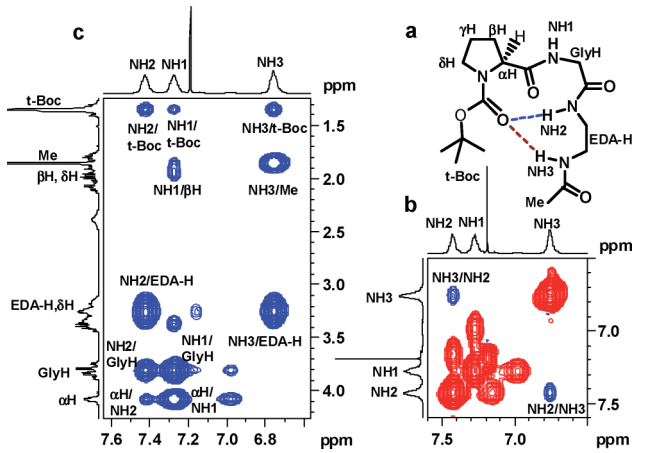

Fig. 2 Molecular structure (a), and expanded 2D NOESY spectra (b,c) of **1b** *Note*: The cross-peaks colored in red (Fig. 2b) are due to chemical exchange.

additional intramolecular hydrogen bonding interactions, forming an α-turn characterised by a three-centered hydrogen bond, as evident from its crystal structure. The molecule features two intramolecular hydrogen bonds; one coming from the expected type-II β-turn owing to the positioning of the Pro–Gly sequence at the i+1 and i+2 sites, respectively, and another intramolecular interaction featuring a 13-membered ring hydrogen bonding. A closer inspection of the crystal structure reveals that the α-turn interaction is relatively stronger than the β-turn interaction [H-bond geometric parameters for α-turn: bond distance d(N . . . O) = 2.8 Å, d(H . . . O) = 2.1 Å, bond angle (N–H . . . O) = 153°), and for β-turn: bond distance d(N . . . O) = 3.2 Å, d(H . . . O) = 2.5 Å, bond angle (N–H . . . O) = 150°]. Further inspection of the crystal structure reveals the formation of a self-assembled supramolecular structure involving the amide NH of glycine that does not participate in intramolecular interactions (ESI, S29†).

In order to investigate the solution-state conformation, we undertook a detailed NMR study of **1b**. The analog **1b** with all aliphatic substituents was preferred for solution-state NMR studies to minimize the "aromatic" chemical shift interferences. The three-centered hydrogen bonding interactions and the turn conformations, as observed in the solid-state, were confirmed by the observed dipolar couplings (nOes) from the 2D NOESY NMR spectra (400 MHz, $CDCl_3$) of **1b** (Fig. 2).

Analysis of the crystal structure had revealed that the most characteristic nOe that is quintessential to support the three-centered hydrogen-bonding interaction in the solution-state would be the requirement of the diagnostic dipolar coupling between the amide NHs involved in both α- and β-turns (Fig. 1) since they are closer in space (2.67 Å), held by the intramolecular turn conformations. Inspection of the 2D NOESY data indeed revealed the observation of the dipolar coupling between NH2 and NH3 (Fig. 2b), thereby confirming the prevalence of turn conformations in solution-state as well. Other selected nOes that supported the turn conformations were: NH2 *vs.* t-Boc, NH3 *vs.* t-Boc, NH2 *vs.* GlyH, NH2 *vs.* αH (Pro α-H), and NH1 *vs.* αH (Fig. 2c). It is noteworthy that the observed strong nOe between Pro α-H and NH1 is diagnostic of typical type II β-turns,[14] since these protons are positioned closely in such a conformation.

Solvent dilution experiments are particularly useful for differentiating the nature of hydrogen bonding interactions (inter *vs.* intra) in the solution-state, wherein intermolecular hydrogen bonds (solvent exposed NHs) are relatively more vulnerable to concentration effects. Further experimental support for the prevalence of three-centered intramolecular hydrogen bonding interactions, as observed in the solid-state, came from $CDCl_3$ dilution studies of **1b** (dilution graph and table available in ESI, S24). Notably, both the amide NHs participating in three-centered intramolecular H-bonds (NH2 and NH3, Fig. 2a) show a relatively small shift ($\Delta\delta$ NH2: 0.12 ppm, and $\Delta\delta$ NH3: 0.23 ppm) when solutions of **1b** in $CDCl_3$ was diluted. This observation suggested their involvement in intramolecular hydrogen bonding interactions; consistent with the X-ray structure of **1a**. However, the amide NH (NH1) that does not participate in three-centered intramolecular hydrogen bonding interactions showed a relatively larger shift ($\Delta\delta$ = 0.47 ppm), obviously because of its intermolecular interaction, again consistent with its crystal structure (*vide supra*).

Steric and electronic interactions often play a crucial role in the conformation of hydrogen bonded systems.[15] In an effort to investigate the effect of chain elongation from the N-terminal on the overall conformation, we synthesized **2**. Curiously enough, crystal structure studies showed the absence of an α-turn structure in **2**, although the β-turn conformation was still retained [H-bond geometric parameters for β-turn: bond distance d (N . . . O) = 3.0 Å, d(H . . . O) = 2.4 Å, bond angle (N–H . . . O) = 131°] (Fig. 3).

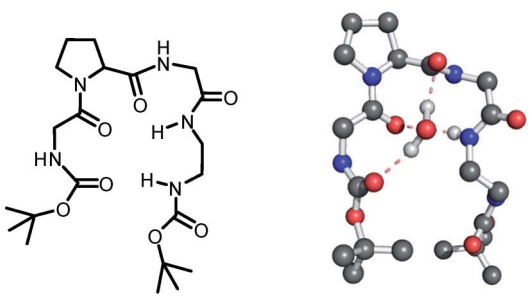

Fig. 3 Molecular structure (left) and single crystal X-ray crystal structure (right) of **2**. *Note*: Hydrogens, other than at the hydrogen bonding sites, have been deleted for clarity.

Further, a water molecule was found interacting with the peptide backbone, connecting the amide carbonyls. The strong interaction of the water molecule with the backbone amide groups was also

substantiated by 2D NOESY and DOSY NMR (diffusion ordered spectroscopy) experiments (*vide infra*).[16] It is noteworthy that water molecules strongly interacting with the peptide backbone have been shown to be play crucial roles in modulating the conformation and function of the host peptides/proteins.

Intrigued by the absence of an α-turn structure in **2**, as evident from its crystal structure, we undertook a detailed NMR study that would provide insights into its structural features in the solution-state. ^{1}H NMR studies at various temperatures, coupled with 2D NOESY NMR studies in various concentrations (ESI, S27, S28†), unambiguously suggested that the peptide **2** exists in multiple conformations in the solution state, presumably arising out of the *cis–trans* isomerisation of proline; a known property associated with N-acyl prolines lacking a compact conformation at the N-terminus.[17,18]

Variable temperature NMR experiments carried out in dichloromethane (CD_2Cl_2, 400 MHZ) at 100 mmol concentration (298 K down to 243 K) suggested that the two conformers freeze at 243 K (ESI, S25, 26†). Interaction of water molecules with the amide backbones of **2** was evident in the solution-state as well, as could be substantiated by 2D NOESY and DOSY studies.

2D NOESY studies carried out with varying parameters such as concentration, and temperature, were suggestive of water interactions with the amide backbone of **2** (Fig. 4). Strong interaction of water molecules with the backbone protons (including Gly CH_2), as evident from negative nOes (marked in blue), were consistently observed in the concentration of 265 mmol in CD_2Cl_2 at different temperatures (Fig. 4a, b). Interestingly, the interactions were observable even at a low concentration of 53 mmol in CD_2Cl_2, as evident from negative nOes with backbone CH_2s, (marked blue, Fig. 4c, d). However, positive cross peaks (marked red) are observed between water and all the amide protons since the exchange interactions dominate over the nOe interaction at low substrate concentration (Fig. 4c,d). As anticipated, the water–backbone interactions strengthen (re-appearance of nOes as indicated by the presence of negative cross peak with the amide NH, shown in blue color in Fig. 4d) as the systems is cooled from 298 K down to 278 K, obviously because of thermodynamic stabilization. The water–peptide interaction was further substantiated by the results of DOSY NMR (diffusion ordered spectroscopy) experiments (spectra and table available in ESI, S28, and S29†). Diffusion coefficient measurements at two concentrations (20 mg mL^{-1} and 100 mg mL^{-1}) at 298 K showed a significant decrease in self-diffusion coefficient of water with an increase in the substrate concentration, an observation which is also suggestive of water–substrate interactions in solution-state.

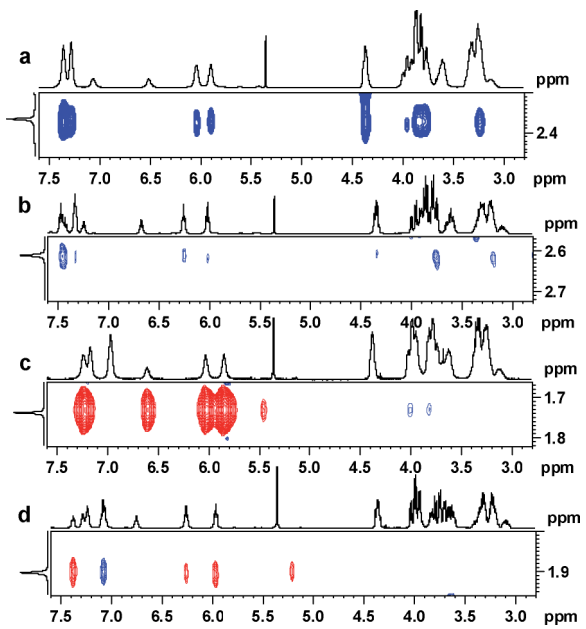

Fig. 4 Expanded 2D NOESY spectra of **2** showing the interaction of water with the peptide backbone. (a) 2D NOESY at 298 K in 265 mmol concentration; (b) at 278 K in 265 mmol concentration; (c) at 298 K in 53 mmol concentration; (d) at 278 K in 53 mmol concentration). Note: The cross-peaks colored in red (figure c, d) are due to chemical exchange at low concentration.

Conclusions

In summary, we describe herein a model peptide that concurrently displays both α- and β-turns within the molecule, as confirmed from structural investigations by single crystal X-ray crystallography[19] and NMR studies. The present findings also attest to the importance of utilizing three-centered hydrogen bonding interactions in stabilizing peptide/protein conformations which would also have a bearing on practical utility, for instance in the development of novel peptidomimetics that feature multiple reverse turns or novel structural features.[20,21] The model peptide described herein could become the starting point for the design of novel conformationally ordered synthetic oligomers with structural architectures distinct from those classically observed. We are currently in the course of developing large synthetic oligomers featuring this novel motif at regular intervals.

Experimental Section

N-Bz-Pro-Gly-NH-EDA-Ac 1a

To an ice-cold stirred solution of *N*-benzoyl-Pro-Gly methyl ester (0.5 g, 1.72 mmol, 1 equiv.) in methanol (10 mL), ethylenediamine (1.5 mL) was added. The resulting reaction mixture was stirred at 0 °C for 30 min, and continued at room temperature for 30 min. The solvent was stripped off under reduced pressure, the resultant residue was taken in toluene, and toluene was then stripped off under reduced pressure. The process was repeated twice to remove excess ethylenediamine. The residue was dried under vacuum to yield the desired ethylenediamine conjugated product as a thick liquid (0.54 g, 100%) which was used for the next reaction, without further purification. To a solution of *N*-Bz-Pro-Gly-NH-EDA, prepared as above, (0.5 g, 1.57 mmol, 1 equiv.) in dry pyridine (5 mL), acetic anhydride (0.44 mL, 4.71 mmol, 3 equiv.) was added. The resulting reaction mixture was stirred at room temperature for 5 h. The solvent was stripped off under reduced pressure to get the crude product, which on column chromatography (100% EtOAc) afforded the desired pure product **1a** (0.39 g, 69%); mp 157–160 °C; $[\alpha]^{26}_{D}$ −11.5 (c = 0.2, chloroform); IR ($CHCl_3$) ν (cm^{-1}): 3336, 3018, 1662, 1612, 1217, 771; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.24 (bs, 1H), 8.07 (bs, 1H), 8.05–7.95 (m, 2H), 7.88 (bs, 1H), 7.85–7.70 (m, 3H), 4.95–4.75 (m, 1H), 4.75–4.55 (m, 1H), 4.30–3.60 (m, 7H), 2.70–1.90 (m, 4H), 2.15 (s, 3H), 3; ^{13}C NMR (100 MHz,

$CDCl_3$): δ 172.5, 170.8, 170.0, 135.5, 130.6, 128.5, 126.9, 61.6, 50.7, 43.3, 39.6, 39.1, 29.4, 25.6, 22.7; ESI Mass: 383.17 (M+Na); Anal. Calcd. for $C_{18}H_{24}N_4O_4$: C, 59.99; H, 6.71; N, 15.55. Found: C, 59.82; H, 6.69; N, 15.40.

Boc-Gly-Pro-Gly-EDA-Boc 2

To an ice-cold stirred solution of Boc-Gly-Pro-Gly-OMe (1.0 g, 3.49 mmol, 1 equiv.) in methanol (10 mL), ethylenediamine (2 mL) was added. The resulting reaction mixture was stirred at 0 °C for 30 min, and continued at room temperature for 30 min. The solvent was stripped off under reduced pressure, and then the residue was taken in toluene, and again stripped off the solvent under reduced pressure. The residue was dried under vacuum to yield the desired ethylenediamine conjugated product as a thick liquid (1.09 g, 100%) which was used for the next reaction, without further purification. To an ice cold solution of the ethylenediamine conjugated product, prepared as above (0.54 g, 1.45 mmol, 1 equiv.) in tetrahydrofuran (10 mL), *tert*-butyldicarbonate (0.63 g, 2.91 mmol, 2 equiv.) was added and the resulting reaction mixture was stirred at room temperature for one hour. The reaction mixture was diluted with ethyl acetate (50 mL) and washed with water and saturated sodium chloride solution. Drying and concentration of the ethyl acetate extract under reduced pressure gave the crude product, which on column chromatography (100% EtOAc) afforded the desired pure product **2** (0.55 g, 75%); mp 195–198 °C; $[\alpha]^{26}_D$ −11.2 ($c = 0.2$, chloroform); IR ($CHCl_3$) ν ((cm^{-1})): 3325, 3018, 1666, 1612, 1215, 756; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.30–8.15 (d, 2H), 8.10–7.95 (d, 2H), 7.90 (bs, 1H), 7.79 (bs, 1H), 6.87 (bs, 1H), 4.20–3.85 (m, 3H), 3.65–3.30 (m, 6H), 2.30–1.75 (m, 4H), 1.40 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.8, 169.8, 169.6, 157.5, 156.6, 156.0, 79.8, 79.5, 61.3, 47.0, 43.0, 40.1, 29.0, 28.2, 24.9; ESI Mass: 494.20 (M+Na); Anal. Calcd. for $C_{21}H_{37}N_5O_7$: C, 53.49; H, 7.91; N, 14.85 Found: C, 53.15; H, 7.75; N, 14.67.

Acknowledgements

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