Post-Print

This is the peer-reviewed version of the following article

Damien Barbaras and Karl Gademann

Stable Beta Turns of Tripeptides in Water through Cation-Pi Interactions

ChemBioChem **2008**, *9*, 2398-2401

which has been published in final form at:

http://dx.doi.org/10.1002/cbic.200800344

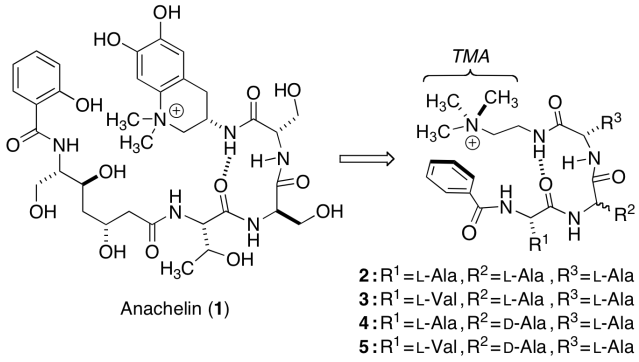
DOI: 10.1002/cbic.200800344

Stable β-Turns of Tripeptides in Water

Stable β-Turns of Tripeptides in Water through Cation-π Interactions

Damien Barbaras[b] and Karl Gademann*[a]

The protein folding problem remains as one of the significant challenges in the proteomics age.[1-3] Among the many factors that contribute to protein folding, the influence of charges remains unclear.[4] While their presence on the surface as well as close to the active site in proteins can be rationalized, the functional role of many charged groups in the interior of proteins remained unexplained.[5] Over the last years, several approaches have been undertaken to investigate the role of such groups and their relationship with neighboring amino acid functionalities. In particular, cation-π interactions[2,6] have been shown to play an important role: for peptides, the stabilization of folded structures was achieved through cation-π interactions of *e.g.* Lys-Trp side chains.[7] While in such observations large peptides or even proteins were examined, we asked whether the stabilization of a single structural unit, *i.e.* a β-turn, would be possible.[8] The structural lead for our studies was the natural product anachelin (**1**).[9] This cationic peptide alkaloid was shown to populate a folded structure by NMR,[10] and a cation-π interaction could be postulated as driving force. Based on this experimental evidence, the central hypothesis can be formulated that an aromatic substituent at the *N*-terminus with a cationic group at the *C*-terminus leads to folded tripeptide structures (**Scheme 1**). In this communication, we validate this hypothesis by demonstrating that tripeptides such as **2**-**5** can be folded into stable β-turn structures in water based on terminal cation-π interactions.



Scheme 1. The natural product anachelin (**1**) and derived tripeptides **2**-**5**.

The first tripeptide **2**, (L-Ala)$_3$ terminated by *N*-benzoyl and a trimethylammonium (TMA) group, displayed an interesting CD pattern in water (0.4 mM, 20°C, **Figure 1**, dark blue). The spectrum is characterized by a maximum at 234 nm, and two minima at 215 and 200 nm. Balaram and coworkers assigned these minima to a β-hairpin structure,[11] and Jung and coworkers postulated β-turns for such signals based on X-ray data.[8,12]

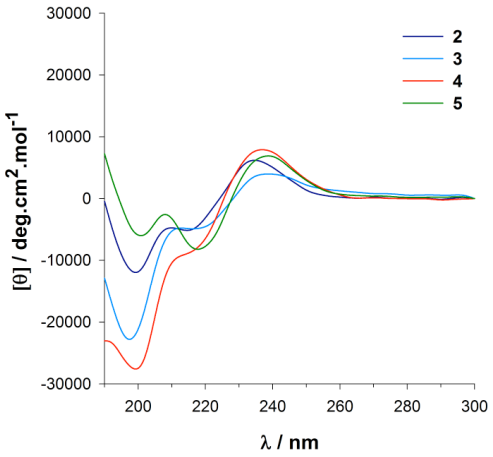


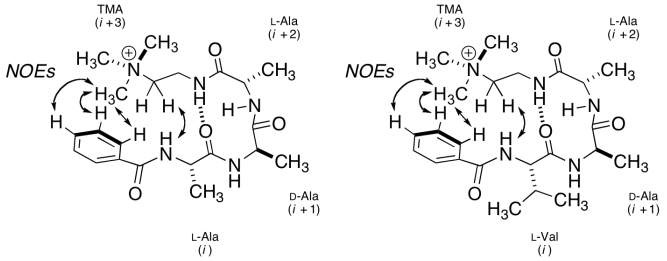
Figure 1: CD spectra of tripeptides **2**-**5** in H_2O (0.4 mM, 20°C). Abbreviation: [Θ] = molar ellipticity.

We prepared a series of different peptides **2**-**5** (**Scheme 1**) in order to probe the influence of sequence modifications on structure.[13] Replacement of Ala at position (*i*) by a branched Val residue gave the peptide **3**, whose CD spectrum is very similar to peptide **2** suggesting a very similar β-turn conformation in water (**Figure 1**). The configuration at position (*i*+1) was investigated next, and D-Ala introduced (peptides **4** and **5**). As expected, the Cotton effects at around 240 and 220 nm increased significantly, implying more stable β-turns.[14]

[a] *Prof. Dr. K. Gademann,*
Chemical Synthesis Laboratory
Swiss Federal Institute of Technology (EPFL)
CH-1015 Lausanne/Switzerland
Fax: (+)41 21 693 97 00
E-mail: karl.gademann@epfl.ch
Homepage: http://lsync.epfl.ch
[b] *Dipl. Ing. D. Barbaras, M. Sc*
Laboratorium für Organische Chemie
Swiss Federal Institute of Technology (ETH)
8093 Zürich/Switzerland

Supporting information for this article is available on the WWW under http://www.chembiochem.org or from the author.

We assign a type I β-turn conformation to peptides **2**-**5** to this pattern based on the available NMR data and correlations to CD spectra of larger peptides.[8,11,12,13] ROESY experiments for peptides **4** and **5** revealed strong interstrand NOE correlations between the methyl groups of the quaternary ammonium and the aromatic protons (**Scheme 2**). In addition, NOEs between the NCH_2 to the NH of residue (*i*) are supportive of the β-turn structure in solution (**Scheme 2**). Dilution NMR experiments established no change in the chemical shifts observed thus ruling out potential aggregation.[13] The observed NOEs are thus strongly considered arising from intramolecular interactions. In addition, the chemical shifts of the protons of the N-CH_2 as well as of the C(α)-H of residue (*i*) are also shifted when compared to random coil values, suggesting a folded, compact β-turn structure. These complementary NMR parameters (NOEs and chemical shifts) strongly support the presence of a type I β-turn structure of **2**-**5** in water.



Scheme 2: Selected NOE correlations in tripeptides **4** and **5**.

We then investigated the role of the cation-π interaction for folding. Replacement of the terminal aromatic Bz group in **4** by a cyclohexanecarboxylic acid (-› **6**), by Boc (-› **7**) or by acetate (-› **8**) (**Figure 2A**) led to disappearance of the typical CD pattern, consistent with loss of turn structure (**Figure 2B**). In particular, the characteristic minimum at around 220 nm disappeared and the minimum at around 200 nm flattened out. Substitution of the trimethylammonium group by control groups such as *N,N*-dimethylamine or as a methyl ester significantly reduced the characteristic β-turn CD signal of the peptides.[13] All these control peptides underline the importance of the cation-π interaction as driving force for peptide folding of tripeptides **2**-**5** in water.

The stability of the folded β-turn structure to chemical and thermal denaturation was addressed next. Chemical denaturation caused by the addition of hydrogen bond donors/acceptors such as guanidinium hydrochloride (Gdn·HCl) is frequently employed to assess the stability of peptides and proteins. All four peptides **2**-**5** were examined for chemical denaturation,[13] and it was generally found that the addition of over 0.5 M of the strong H-bond breaker Gdn·HCl starts to disrupt the central H-bond, as evident by the disappearance of the CD signal at 218 nm (**Figure 2C**). From these results, one can conclude that the addition of a roughly 2000 fold molar excess of Gdn·HCl to a 0.4 mM aqueous solution initiates disruption of the β-turn. With regard to thermal denaturation, the folded β-turn structure proved to be rather stable upon heating: Increasing the temperature from 0ºC to 50ºC of an aqueous solution of **2** led to a signal decrease of about 25%.[13] The corresponding values of **3** (50%), **4** (40%) and **5** (30%, **Figure 2D**) are the direct consequence of the configuration and sequence on folding. Interestingly, the L-Val-L-Ala-L-Ala peptide **3**, which has the least propensity for turn formation, was most sensitive to thermal denaturation. Supporting this notion, the $(L\text{-Ala})_3$ peptide **2** showed the best resistance and greater stability. From all these CD experiments, it can be concluded that the β-turn structures in water are rather stable towards both chemical and thermal denaturation, which can be rationalized as a consequence of the strength of the terminal cation-π interaction.

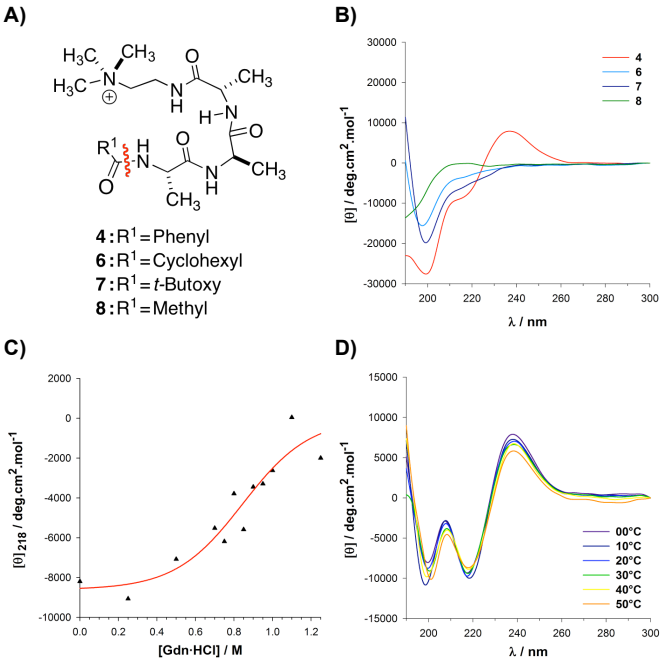


Figure 2. **A)** Control peptides **6**-**8** without a terminal aromatic group. **B)** CD spectra of folded peptide **4** vs. control peptides **6**-**8** (H_2O, 0.4 mM, 20°C). **C)** Chemical denaturation of **5** (0.4 mM, 20°C) by addition of Gdn·HCl. **D)** Thermal denaturation of **5** upon heating (H_2O, 0.4 mM). Abbreviations: [Θ] = molar ellipticity; $[\Theta]_{218}$ = molar ellipticity at the wavelength of 218 nm. [Gdn·HCl] = molar concentration of guanidinium hydrochloride.

In conclusion, we have shown that tripeptides such as **2**-**5** are present in a stable conformation in water, that was assigned a type I β-turn conformation using CD and NMR spectroscopy. This communication thus describes a method for the folding of very small peptides, which is difficult using other approaches. In addition, this study established the CD spectrum of a single β-turn, without the strand amino acids of a hairpin interfering. The dominant driving force for this remarkable peptide folding resides in the terminal cation-π interaction, and its absence leads to loss of the typical CD pattern. Moreover, terminal cation-π interactions lead to a stabilization of these type I β-turns that are formed independently of the primary sequence. The β-turns are rather stable in water, as judged by chemical and thermal denaturation experiments. The strong propensity of trimethylammonium groups to enforce cation-π interactions[15] has implications to biological processes, as histones carrying such modifications are involved in controlling gene expression.[16] In addition, these ultra-short β-turns thus offer applications in spectroscopy and biophysics or as peptidomimetics for pharmaceutical uses.

Experimental Section

All experimental details as well as additional spectra and full characterization data are given in the supporting information.

Acknowledgements

K. G. is a European Young Investigator (EURYI). We thank ETH Zürich for financial support (TH Gesuch 13/04-3, PhD fellowship to D. B.). NMR facilities (EPFL-ISIC) are gratefully acknowledged.

Keywords: peptide folding · peptidomimetics · bioorganic chemistry · structure determination · NMR spectroscopy

[1] V. Grantcharova, E. J. Alm, D. Baker, A. L. Horwich, *Curr. Opin. Struct. Biol.* **2001**, *11*, 70-82.

[2] V. Daggett, A. Fersht, *Nat. Rev. Mol. Cell Biol.* **2003**, *4*, 497-502.

[3] C. M. Dobson, *Nature* **2003**, *426*, 884-890.

[4] I. Gitlin, J. D. Carbeck, G. M. Whitesides, *Angew. Chem.* **2006**, *118*, 3090-3131; *Angew. Chem. Int. Ed.* **2006**, *45*, 3022-3060.

[5] I. Gitlin, K. L. Gudiksen, G. M. Whitesides, *ChemBioChem* **2006**, *7*, 1241-1250.

[6] a) M. T. Reetz, S. Hutte, R. Goddard, *J. Am. Chem. Soc.* **1993**, *115*, 9339-9340. b) J. P. Gallivan, D. A. Dougherty, *Proc. Natl. Acad. Sci. U. S. A.* **1999**, *96*, 9459-9464. c) E. A. Meyer, R. K. Castellano, F. Diederich, *Angew. Chem.* **2003**, *115*, 1244-1287; *Angew. Chem. Int. Ed.* **2003**, *42*, 1210-1250. d) R. S. Prajapati, M. Sirajuddin, V. Durani, S. Sreeramulu, R. Varadarajan, *Biochemistry* **2006**, *45*, 15000-15010.

[7] a) E. V. Pletneva, A. T. Laederach, D. B. Fulton, N. M. Kostic, *J. Am. Chem. Soc.* **2001**, *123*, 6232-6245. b) C. A. Olson, Z. S. Shi, N. R. Kallenbach, *J. Am. Chem. Soc.* **2001**, *123*, 6451-6452. c) Z. S. Shi, C. A. Olson, N. R. Kallenbach, *J. Am. Chem. Soc.* **2002**, *124*, 3284-3291. d) C. D. Tatko, M. L. Waters, *J. Am. Chem. Soc.* **2002**, *124*, 9372-9373. e) L. K. Tsou, C. D. Tatko, M. L. Waters, *J. Am. Chem. Soc.* **2002**, *124*, 14917-14921. f) C. D. Tatko, M. L. Waters, *Protein Sci.* **2003**, *12*, 2443-2452. g) S. E. Kiehna, M. L. Waters, *Protein Sci.* **2003**, *12*, 2657-2667. h) N. H. Andersen, K. A. Olsen, R. M. Fesinmeyer, X. Tan, F. M. Hudson, L. A. Eidenschink, S. R. Farazi, *J. Am. Chem. Soc.* **2006**, *128*, 6101-6110.

[8] For other studies on tripeptides in solution, see: a) F. Eker, X. Cao, L. Nafie, R. Schweitzer-Stenner, *J. Am. Chem. Soc.* **2002**, *124*, 14330-14341. b) F. Eker, K. Griebenow, R. Schweitzer-Stenner, *J. Am. Chem. Soc.* **2003**, *125*, 8178-8185. c) R. Schweitzer-Stenner, W. Gonzales, G. T. Bourne, J. A. Feng, G. R. Marshall, *J. Am. Chem. Soc.* **2007**, *129*, 13095-13109.

[9] a) H. Beiderbeck, K. Taraz, H. Budzikiewicz, A. E. Walsby, *Z. Naturforsch., C* **2000**, *55*, 681-687. b) Y. Itou, S. Okada, M. Murakami, *Tetrahedron* **2001**, *57*, 9093-9099. c) K. Gademann, Y. Bethuel, *Org. Lett.* **2004**, *6*, 4707-4710. d) K. Gademann, Y. Bethuel, *Angew. Chem.* **2004**, *116*, 3389-3391; *Angew. Chem. Int. Ed.* **2004**, *43*, 3327-3329. e) K. Gademann, Y. Bethuel, H. H. Locher, C. Hubschwerlen, *J. Org. Chem.* **2007**, *72*, 8361-8370.

[10] K. Gademann, H. Budzikiewicz, *Chimia* **2004**, *58*, 212-214.

[11] S. Aravinda, N. Shamala, R. Rajkishore, H. N. Gopi, P. Balaram, *Angew. Chem.* **2002**, *114*, 4019-4021; *Angew. Chem. Int. Ed.* **2002**, *41*, 3863-3865.

[12] a) G. Jung, R. Bosch, E. Katz, H. Schmitt, K.-P. Voges, W. Winter, *Biopolymers* **1983**, *22*, 241-246. (b) R. Bosch, G. Jung, K.-P. Voges, W. Winter, *Liebigs Ann. Chem.* **1984**, *6*, 1117-1128.

[13] See 'Supporting information' for details.

[14] D-Amino acids stabilize β-turns, see for examples: a) S. K. Awasthi, S. R. Raghothama, P. Balaram, *J. Chem. Soc., Perkin Trans. 2* **1996**, 2701-2706. b) I. L. Karle, S. K. Awasthi, P. Balaram, *Proc. Natl. Acad. Sci. U. S. A.* **1996**, *93*, 8189-8193. c) T. S. Haque, J. C. Little, S. H. Gellman, *J. Am. Chem. Soc.* **1996**, *118*, 6975–6985. d) S. H. Gellman, *Curr. Opin. Chem. Biol.* **1998**, *2*, 717–725. e) H. E. Stranger, S. H. Gellman, *J. Am. Chem. Soc.* **1998**, *120*, 4236–4237. f) G. Müller, G. Hessler, H. Y. Decornez, *Angew. Chem.* **2000**, *112*, 926-928; *Angew. Chem. Int. Ed.* **2000**, *39*, 894-897. g) J. F. Espinosa, S. H. Gellman, *Angew. Chem.* **2000**, *112*, 2420-2423; *Angew. Chem. Int. Ed.* **2000**, *39*, 2330-2333. h) I. L. Karle, H. N. Gopi, P. Balaram, *Proc. Natl. Acad. Sci. U. S. A.* **2001**, *98*, 3716-3719.

[15] a) R. M. Hughes, M. L. Waters, *J. Am. Chem. Soc.* **2005**, *127*, 6518-6519. b) R. M. Hughes, K. R. Wiggins, S. Khorasanizadeh, M. L. Waters, *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 11184-11188. c) R. M. Hughes, M. L. Benshoff, M. L. Waters, *Chem. Eur. J.* **2007**, *13*, 5753-5764.

[16] C. Martin, Y. Zhang, *Nat. Rev. Mol. Cell Biol.* **2005**, *6*, 838-849.

Received: ((will be filled in by the editorial staff))
Published online: ((will be filled in by the editorial staff))