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

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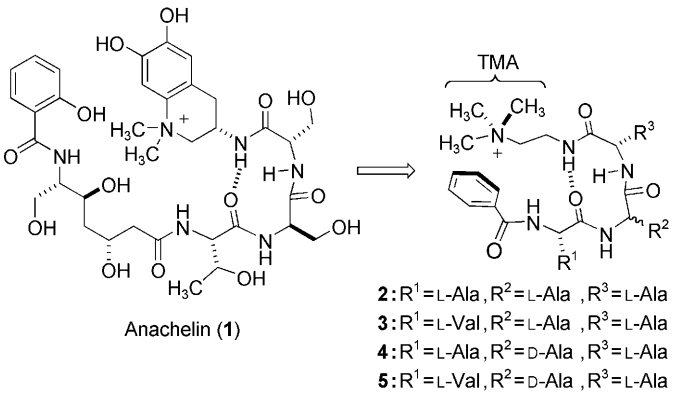
The protein-folding problem remains one of the significant challenges of the proteomics age.[1–3] Among the many factors that contribute to protein folding, the influence of charges remains unclear.[4] While their presence on the surface as well as close to the active site in proteins can be rationalized, the functional role of many charged groups in the interior of proteins remains unexplained.[5] Over the last years, several approaches have investigated the role of charged groups and their relationship with neighboring amino acid functionalities. In particular, cation–π interactions[2,6] play an important role in protein structure. For peptides, the stabilization of folded structures is achieved through cation–π interactions of, for example, Lys–Trp side chains.[7] While large peptides or even proteins have been 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 have a folded structure by NMR spectroscopy,[10] and a cation–π interaction could be postulated to be the driving force. Based on this experimental evidence, it can be hypothesized that an aromatic substituent at the N terminus together 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 trimethylammonium (TMA) groups—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 co-workers assigned these minima to a β-hairpin structure,[11] and Jung and co-workers postulated β turns for such signals based on X-ray data.[8,12]

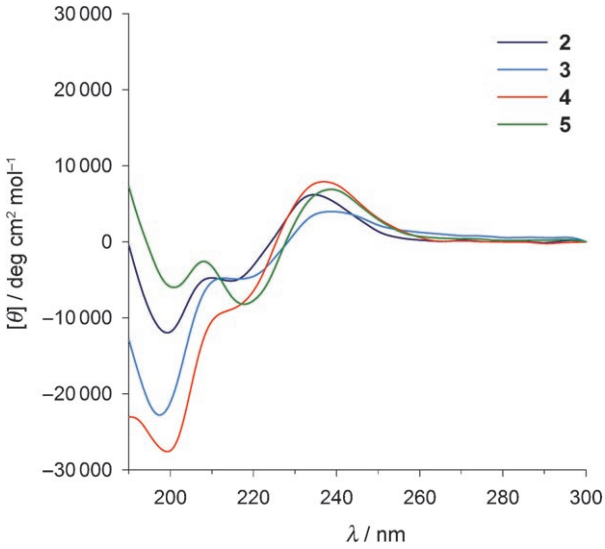


Figure 1. CD spectra of tripeptides **2**–**5** in H_2O (0.4 mM, 20 °C); [θ]: molar ellipticity.

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 peptide **3**, the CD spectrum of which was very similar to peptide **2**; this suggests a very similar β-turn conformation in water (Figure 1). The configuration at position (*i*+1) was investigated next, and D-Ala was introduced (peptides **4** and **5**). As expected, the Cotton effects at around 240 and 220 nm increased significantly, which implies more stable β turns.[14]

We assigned a type I β-turn conformation to this pattern in peptides **2**–**5** based on the available NMR spectroscopy 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 and NH of residue *i* sup-

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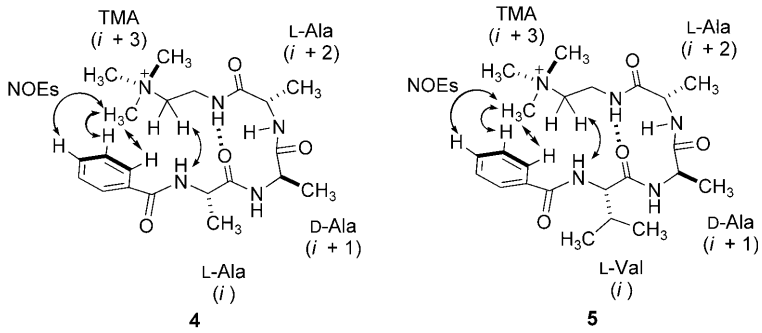

Scheme 2. Selected NOE correlations in tripeptides **4** and **5**.

port a β-turn structure in solution (Scheme 2). Dilution NMR spectroscopy experiments established that there is no change in the chemical shifts observed and ruled out potential aggregation.[13] The observed NOEs thus most likely arise from intramolecular interactions. In addition, the chemical shifts of the protons of the N–CH_2 as well as of the Cα–H of residue *i* were also shifted when compared to random-coil values; this suggests a folded, compact β-turn structure. These complementary NMR spectroscopy parameters (NOEs and chemical shifts) strongly support the presence of a type I β-turn structure of **2**–**5** in water.

We then investigated the role of the cation–π interaction in folding. Replacement of the terminal aromatic benzoyl (Bz) group in **4** by a cyclohexanecarboxylic acid (→**6**), butyloxycarbonyl (Boc; →**7**), or acetate (→**8**; Figure 2A) led to disappearance of the typical CD pattern, consistent with a loss of turn structure (Figure 2B).

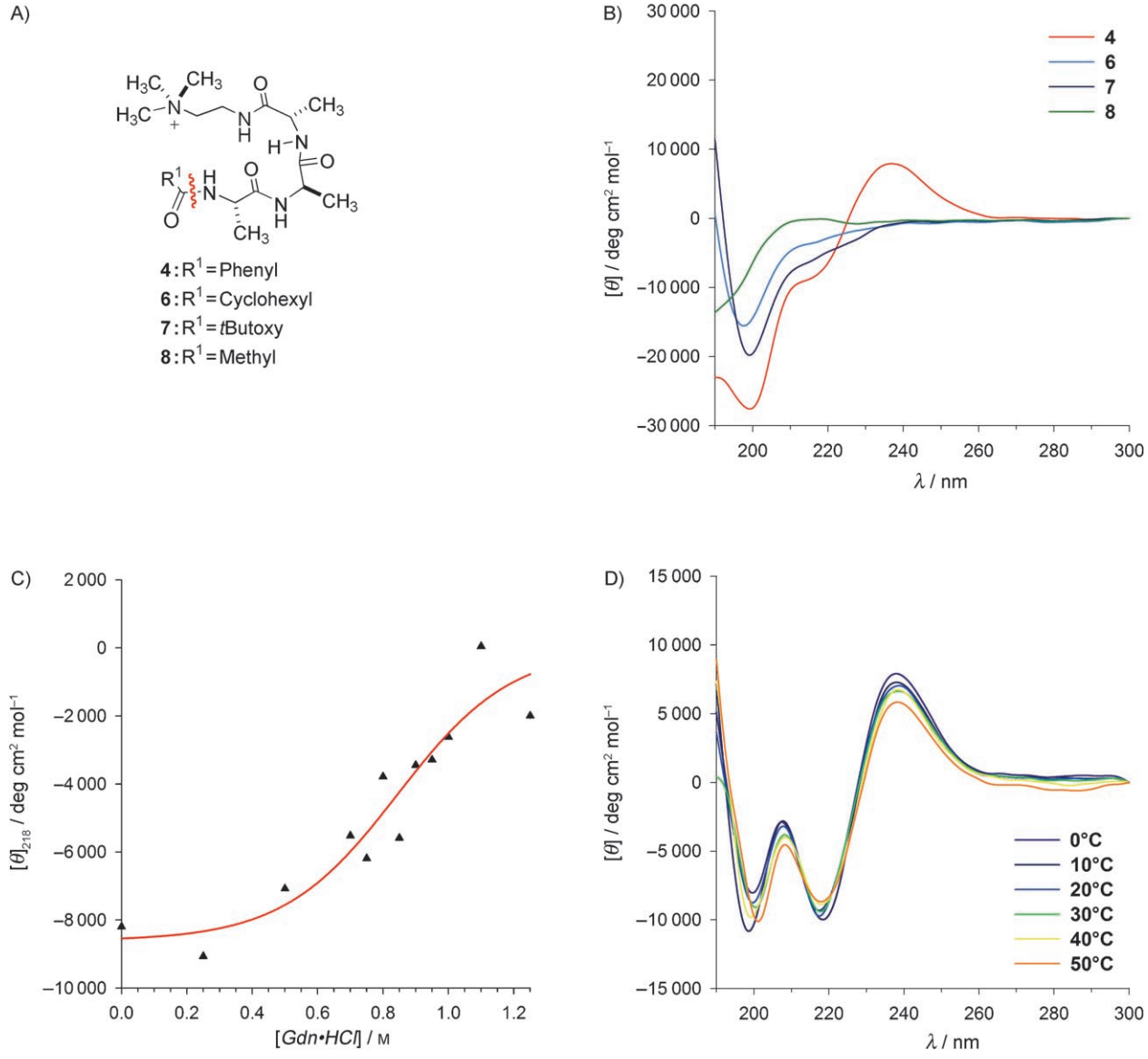

Figure 2. A) Control peptides **6**–**8** without a terminal aromatic group. B) CD spectra of folded peptide **4** versus control peptides **6**–**8** (H_2O, 0.4 mM, 20 °C). C) Chemical denaturation of **5** (0.4 mM, 20 °C) by addition of Gdn·HCl. D) Thermal denaturation of **5** upon heating (H_2O,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

In particular, the characteristic minimum at around 220 nm disappeared and the minimum at around 200 nm flattened out. Substitution of the trimethylammonium group with control groups, such as *N*,*N*-dimethylamine or 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 through 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; this was evident from 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 of an aqueous solution of **2** from 0 °C to 50 °C 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. In support of this notion, the $(L\text{-Ala})_3$ peptide (**2**) showed the best resistance to thermal denaturation and greatest stability. From these CD experiments, it can be concluded that β-turn structures in water are rather stable towards both chemical and thermal denaturation; this can be rationalized to be a consequence of the strength of the terminal cation–π interaction.

In conclusion, we have shown that tripeptides such as **2**–**5** adopt a stable conformation in water, which was assigned as a type I β-turn conformation by using CD and NMR spectroscopy. This communication thus describes a method for the folding of very small peptides, which is difficult with other approaches. In addition, this study established the CD spectrum of a single β turn, without the interference of strand amino acids of a hairpin. 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 stabilize 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 on biological processes, as histones carrying such modifications are involved in controlling gene expression.[16] In addition, these ultrashort β turns thus have 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.

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

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