

β -Peptides Stabilized by Cation- π Interaction

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The cation- π interaction has been recognized as an important noncovalent interaction to provide significant stability in the structure of proteins, particularly at the solvent-exposed surface. Recent studies have shown that the cation- π interaction in various natural peptide model systems can stabilize the folded structure of a peptide just as in proteins. However, the strength of cation- π interactions and their role in protein/peptide folding is still in debate. The question of the relative strength of cation- π versus salt-bridge interactions led us to design an experimental study with an unnatural peptide model system, from which we could observe unseen aspect of the interactions. We have chosen a helical β^3 -peptide scaffold composed of acyclic residues for this purpose because the stabilization effect by salt-bridge interactions in aqueous solution has been well studied in the system.

β -Peptides (unnatural peptides composed of β -amino acids) have recruited enormous attention due to their ability to form diverse secondary structures found in proteins and their potential biological activities.^{1,2} β^3 -Peptides have proteinogenic side-chains and are known to adopt stable 14-helical conformation via intramolecular hydrogen bonding between backbone amides in organic solvent, but it requires additional stabilization strategy to enhance the helical propensity in aqueous solution. Several groups have reported that β^3 -peptides could be stabilized by charge-charge interaction (called “salt-bridge”) between residues at i and $i+3$ positions. One could expect that the cation- π interaction could be an alternative strategy to stabilize the 14-helical β^3 -peptides in aqueous solution because both theoretical and experimental studies suggested that the stabilization energy of cation- π interactions would be comparable to salt-bridge interactions. Despite of this prominence of evaluations, utilization of the interaction for β -peptide design has not been attempted yet. If the observed degree of stabilization effect in the α -peptide model systems can be simply extrapolated to β -peptide oligomers, the β^3 -peptides stabilized by cation- π interaction should have comparable helical stability with those stabilized by salt-bridge interaction. In this presentation, I will discuss about the degree of stabilization effect of cation- π interaction in β^3 -peptide system.³

In order to directly measure the relative stabilization effect in aqueous solution between cation- π versus salt-bridge in 14-helical β^3 -peptides by CD analysis, we prepared a series of β^3 -peptides

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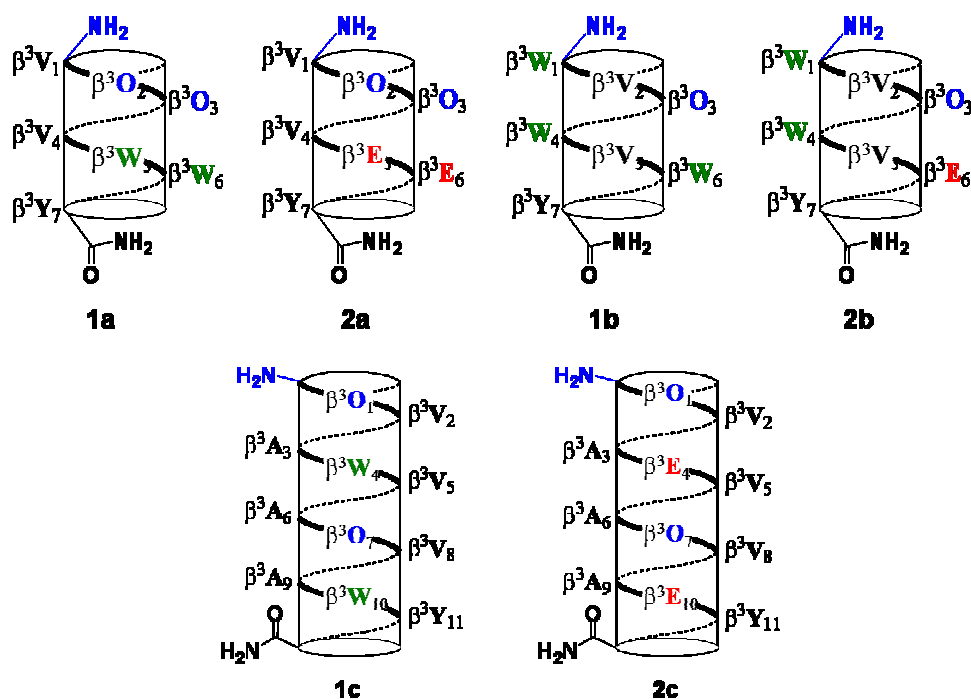


Figure 1. Schematic helical diagram of our designed β^3 -peptides

Table 1. Minimum MRE ($\text{deg}\cdot\text{cm}^2\cdot\text{dmol}^{-1}\cdot\text{residue}^{-1}$) values of β^3 peptides at 0.2 mM PBS buffer and 25 °C

	$-\theta_{\min}$ (pH 7)	$-\theta_{\min}$ (pH 3)	$-\theta_{\min}$ (pH 11)	$\% \Delta$ (pH 3/7)	$\% \Delta$ (pH 11/7)
1c	-2287	-1684	-1414	26	38
2c	-14365	-5740	-9147	60	36

as shown in Figure 1. The heptamer **2a** stabilized by salt-bridge showed the typical CD signal of 14-helical β -peptide with maximum at ~ 198 nm and minimum at ~ 214 nm, whereas the minimum value in the CD profile of **1a** was blue-shifted from that of **2a** (detected at ~ 227 nm) and the peak shape of **1a** was not smooth in the range from ~ 205 nm to ~ 230 nm. Complicated by the results of CD analyses, NMR study was performed to investigate the secondary structure of **1a**. Despite of the unusual CD signal, the NMR study strongly supported that the significant 14-helical population was observed in the β^3 -peptide **1a** stabilized by cation- π interaction. The unusual behavior in CD profiles observed in heptamers with β^3 -Trp residues was restored to the typical 14-helical CD signal in **1c**. However, the extent of helical stability of **1c** is much smaller than that of **2c**, the undecamer that is doubly stabilized by salt-bridge with identical sequences ($\theta_{214} = -2287$ and -14365 MRE at pH 7, respectively). To study the possible participation of Trp residues in the hydrophobic interaction with Val or Tyr residues in the peptide, we measured the fluorescence spectrum of Trp residues in **1a-c**. All the spectra showed the fluorescence maxima at 347-350 nm, indicating that the Trp residues are in solvent-exposed site, not under hydrophobic environment.

Finally we explored the relative helical stability of undecapeptides (**1c** and **2c**) by CD analysis in different pH environments (see Table 1). **1c** stabilized by cation- π interaction is the less sensitive to pH variation than **2c** stabilized by salt-bridge, especially in the range from pH 3 to pH 7. The minimum MRE value of **1c** decreased 26% whereas that of **2c** decreased significantly (60%) as the pH of the aqueous solution was changed from neutral to acidic. The observed better tolerance of 14-

helical propensity of the β^3 -peptide stabilized by cation- π interaction suggests that it can be applicable to biological targets in acidic environment.

In conclusion, we have explored whether the cation- π interaction can be used as an alternative strategy to promote the helical secondary structure of β^3 -peptides in aqueous solution. The CD study of heptameric β^3 -peptide was obscured by the effect of Trp residues, but that of the undecamer β^3 -peptide clearly showed that the typical 14-helical propensity exists when two cation- π interaction pairs were introduced on one helical face. Our newly designed β^3 -peptides with helical tolerance even in acidic condition will be a complementary structural motif for designing β -peptide tertiary structures.

References

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