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An amino acid that controls polypeptide helicity: β-amino alanine, the first strongly stabilizing C-terminal helix stop signal†

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Abstract

β-Amino alanine is shown to stop helix propagation in a polyalanine context while effectively stabilizing the resulting C-terminus. Structural evidence is provided by circular dichroism and t/c ratios from the reporting conformational template Ac-Hel.

Keywords: helicies; capping; 2,3-diaminopropionic acid.

N- and C-capping effects play a significant role in stabilizing the solution structures of polypeptide helices. In 1988 Presta and Rose reviewed evidence from the X-ray crystallographic protein data base for strong helix stabilization of polypeptides in proteins by amino acid residues sited at helix termini,[1,2] and subsequently helix capping has received much attention.[3,4] Asymmetric stabilizing effects of charged capping residues have been attributed to interactions of a side chain charge with an intrinsic helix dipole,[5] and stabilization by C-terminal arginine and lysine residues is abundantly documented by X-ray structural data,[6] NOE evidence,[7,8] and quantitation of stabilizing effects by positively charged synthetic C-capping functions.[9] The helical propensities of shorter lysine homologs has been studied by Padmanabhan et al.[10] who classified Dpr, 2,3-diaminopropionic acid or β-amino alanine, as a very strong helix breaker, based on the very weakly helical CD spectra of helically predisposed peptides containing Dpr residues in central sites. We now report complementary studies that reveal a more complex and interesting role for the short lysine homolog β-amino alanine (β).

To set the stage for our analysis we focus on two independent residue properties. A particular amino acid may be a strong helix breaker that by definition blocks the propagation of helical

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† Dedicated to Professor Harry Wasserman on the occasion of his 80th birthday.

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structure through its site in a peptide sequence (property ¬P). It may also be a strongly stabilizing helix cap that enhances helicity when sited at a peptide terminus (property ST). Logically speaking, there are four possible combinations of these properties (P∩ST), (P∩¬ST), (¬P∩ST), (¬P∩¬ST). All but one of these are demonstrated by the natural amino acids. Most amino acids neither break core structure nor act as strong stabilizers at termini (P∩¬ST). Proline or glycine can break core helical structure, but without demonstrating significant helix stabilization when sited at a terminus (¬P∩¬ST). Glutamate residues propagate core structure and can act as moderately stabilizing N-caps; lysine and arginine residues also propagate core structure and can act as strongly stabilizing C-caps (P∩ST).

The fourth category (¬P∩ST) is not represented among the natural amino acids. An amino acid with this feature would block core propagation and strongly stabilize helices when sited at a proper terminus. As shown in Fig. 1, in the parlance of Presta and Rose, it would act as a strongly stabilizing helix stop signal. We now show that β-amino alanine is the first amino acid that fulfills this role.

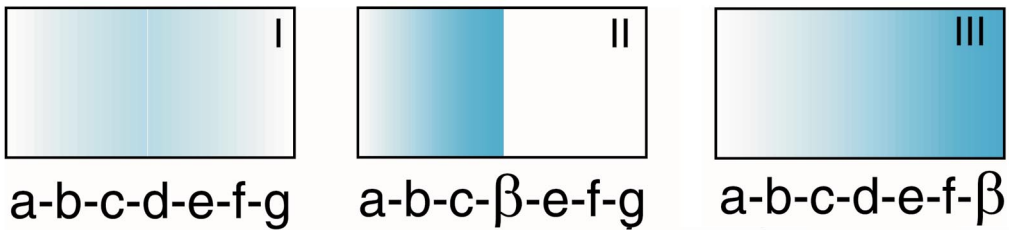

Figure 1. Increasing site helicity, noted by darkening color, depends upon the position of breakers and stabilizers. (I) A normal helical distribution. (II) and (III) Position dependent effects of β

Although circular dichroism CD is the conventional tool for quantitating peptide helicity, it is not well-suited for characterizing (¬P∩ST) candidates. CD ellipticity reports the mole fraction of amino acid α-carbons that appear within helical structures and is most sensitive to helicity changes within the helix core. It can readily establish the ¬P property of a particular amino acid, but provides intrinsically less definitive evidence concerning ST behavior. In 1988 we introduced a complementary quantitation technique based on conjugates of peptides with an N-terminal reporting helix-inducing template Ac-Hel. Distinct helical and nonhelical resonances appear in the ^{1}H NMR spectrum of such a conjugate, and the experimental parameter t/c, the ratio of their integrated intensities, is proportional to the peptide helicity at its N-terminal junction with the template.[11] As noted previously, t/c can be used qualitatively to establish the presence of conformational communication throughout an unbroken helix.[12] If an amino acid is added at the C-terminus of the peptide portion of the Ac-Hel conjugate, the t/c ratio will increase only if significant helical structure extends throughout the length of the peptide. Placement of an amino acid with the joint (¬P∩ST) property at the C-terminus of such a conjugate will result in a large increase in t/c, a test of the ST property, but extending the peptide length beyond the site of this amino acid will cause no t/c increase, a test of the ¬P property.

The results of such a test are shown in Fig. 2, which compares the dependence of t/c on peptide length for five series with Xxx = Arg, Lys, Orn, His and β-amino alanine for Ac-Hel-Ala_4-Xxx-Ala_n-NH_2, $1 \leq n \leq 5$. For the first four of these, t/c increases nearly linearly with the length of the C-terminal alanine chain. This result is consistent with the presence of helical conformations that extend throughout the peptide, confirming the P property for these amino

acids.[13] The different slopes reflect significantly different helical propensities for these four amino acids. The zero intercepts of these plots correspond to extrapolated t/c values expected for peptides that lack a C-terminal alanine sequence. The substantial scatter in each line prevents precise characterization of its intercept, which lies in the vicinity of t/c = 2.‡ A very different functional form is shown by t/c data for Ac-Hel-Ala_4-β-Ala_n-NH_2.

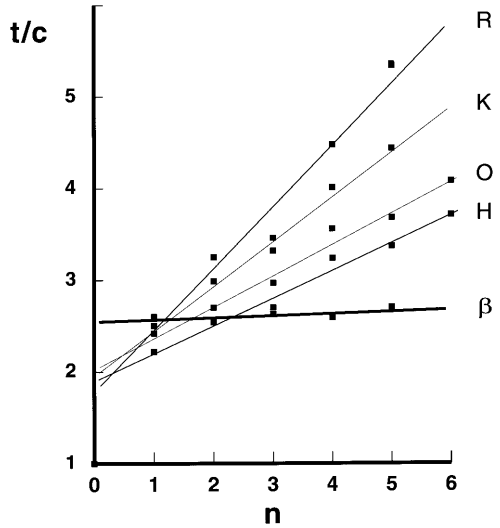

Figure 2. t/c ratios: Ac-Hel-A_4-Xxx-A_n-NH_2; Xxx = R, K, O, H, β; in water at 25°C

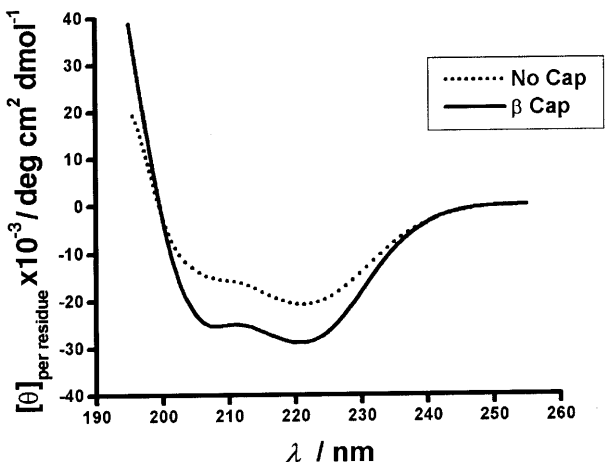

Figure 3. CD spectra comparing Ac-Hel-A_{12}-'L-Inp_2-K_4W with Ac-Hel-A_{12}-β-Inp-K_2W[14§] in water at 25°C. Ac-Hel and β are treated as caps and the length of the alanine core used was 12 residues

‡ Peptides were synthesized and t/c ratios obtained as previously described.[8,14] CD experiments were performed on an Aviv 62DS spectrometer equipped with a thermoelectric temperature controller. All data were collected as an average of five scans at 1.0 nm bandwidth and 0.5 nm step size. CD concentrations were determined by the UV absorption of W at 280 nm ($\varepsilon_{280\ nm} = 5560\ (M\ cm)^{-1}$) on a double-beam Varian Cary 100 Bio UV–vis spectrometer.

Consistent with earlier observations the significant increase of the zero intercept for the β-peptide implies a strong ST role. The invariance of t/c as the alanine chain is extended beyond β implies that the helix is terminated by this residue. Fig. 3 shows characteristic helical CD signatures for two Ac-Hel-A_{12} peptides, with and without a β cap. The 40% increase in the mean residue molar ellipticity ($-[\theta]_{222}$) reflects the efficiency of β as an ST function. Thus by independent NMR and CD evidence β stabilizes and terminates helicies initiated by Ac-Hel.

This result can be independently confirmed in nontemplated peptides by CD. Padmanabhan et al. assigned a very low helical propensity to β within their test peptide, but noted in passing an inconsistency when β replaced a C-terminal lysine at 0°C. From their data we calculate a 10–14% increase in $-[\theta]_{222}$ for this substitution. We scanned β through an alanine core of K_4-Inp_2-^{t}L-A_l-β-A_m-^{t}L-Inp_2-K_4W (**1**).[14§] A threefold increase of $-[\theta]_{222}$ was observed in water at 2°C when β was moved from the middle (l, $m=9$) to the C terminus ($l=18$, $m=0$), corroborating the ¬P property. The β-containing peptide **1** with ($l=18$, $m=0$) showed a 32% higher $-[\theta]_{222}$ at 2°C in water when compared with this sequence with β deleted; this result substantiates the ST property.

By what mechanism does β fulfill its (¬P∩ST) role when other amino acids do not? A modeling possibility is shown in the stereodiagram of Fig. 4, which depicts a plausible structure for a helix with a β residue sited at its C-terminus. Its short methylene side chain allows β to adopt a unique conformation in which its ammonium ion is able to interact highly efficiently with the three amide dipoles of the helix C-terminus, blocking propagation. NMR characterization of dihedral angles of isotopically labeled β residues may be able to test this model.

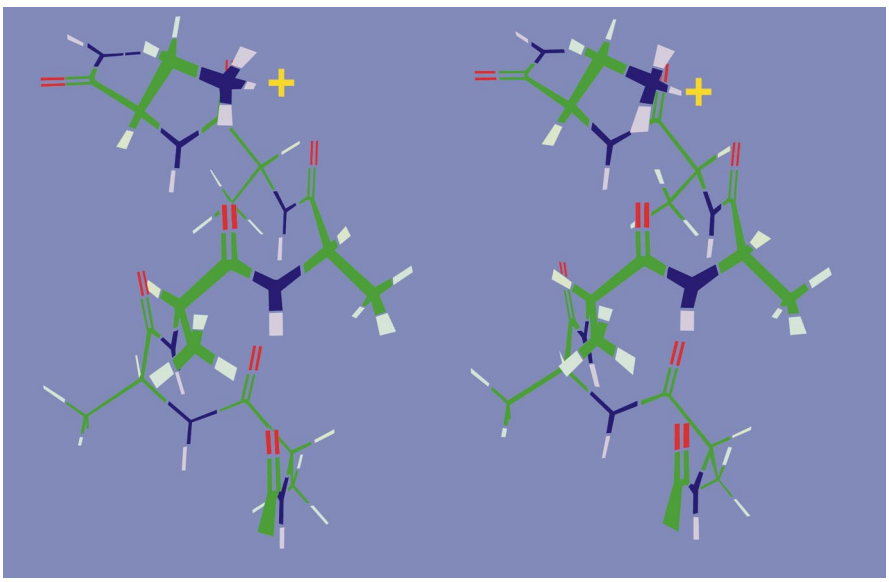

Figure 4. Stereodiagram of the interaction of β-amino alanine's interaction with the C-terminus

The structural C-capping role we have postulated for β-amino alanine suggests preparation and study of a variety of synthetic analogs, and we are currently engaged in such efforts. The exceptionally large value of $-[\theta]_{222}$ seen in Fig. 3 for the Ac-Hel-A_{12}-β-Inp-K_2W sequence is

§ The spacer, ^{t}L-Inp_2 prevents the solubilizing Lys residues from influencing the helicity of the alanine peptide core.

noteworthy and suggests the possibility of constructing highly helical peptides of defined length that could serve as calibration standards for relating CD ellipticity to fractional helicity. Uncertainties in this function significantly impede use of ellipticity as a tool for constructing rigorous helicity algorithms.[15,16] Moreover the availability of a library of amino acids that can serve as strong ($\neg P \cap ST$) helix stop signals at N- and C-termini would greatly facilitate the design of polypeptides that fold in predetermined ways and that mimic the conformational properties of natural proteins.

Acknowledgements

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