Supporting Information for:

Consistent Helicities from CD and Template t/c Data for N-Templated Polyalanines: Progress Toward Resolution of the Alanine Helicity Problem

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I. Experimental

Peptides were synthesized, purified and identified as previously described.[1]

II. Characterization Table

Peptide	Electrospray Mass Spectrometry (M+zH)/z Found (Expected)
AcHel-A_4-^{t}LInp_2K_4W-NH_2	539.77 (539.32); 405.06 (404.74)
AcHel-A_5-^{t}LInp_2K_4W-NH_2	842.78 (844.00); 562.45 (563.00)
AcHel-A_6-^{t}LInp_2K_4W-NH_2	879.80 (879.52); 586.59 (586.68)
AcHel-A_7-^{t}LInp_2K_4W-NH_2	915.14 (915.03); 610.20 (610.36); 457.91(458.02)
AcHel-A_8-^{t}LInp_2K_4W-NH_2	950.83 (950.55); 634.58 (634.04); 476.01 (475.78)
AcHel-A_9-^{t}LInp_2K_4W-NH_2	986.12 (986.07); 657.94 (657.72); 395.08 (395.03)
AcHel-A_{10}-^{t}LInp_2K_4W-NH_2	1021.55 (1021.59); 681.30 (681.40); 511.15 (511.30)
AcHel-A_{11}-^{t}LInp_2K_4W-NH_2	1057.17 (1057.11); 705.76 (705.07); 528.76 (529.06)
AcHel-A_{12}-^{t}LInp_2K_4W-NH_2	1093.02 (1092.63); 728.97 (728.75); 546.97 (546.82)
AcHel-A_{13}-^{t}LInp_2K_4W-NH_2	1127.84 (1128.14); 752.31 (752.43); 564.41 (564.58)
AcHel-A_{14}-^{t}LInp_2K_4W-NH_2	1163.29 (1163.66); 775.87 (776.11); 582.11 (582.34)
(H)-^{t}LInp_2K_4W-NH_2	1051.71(1051.71); 526.30 (526.36); 351.18(351.18); 263.58 (263.58)
Ac-$^\beta$D-Hel-A_8 -β-NH_2	1067.29 (1067.49); 534.10 (534.25)
Ac-$^\beta$D-Hel-A_8 -β-InpK_2W-NH_2	840.70 (840.92); 540.81 (540.95)

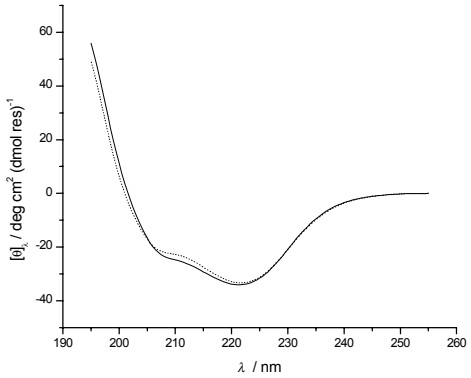
β = beta amino alanine, $^\beta$D= beta linked aspartate

[1] Maison, W.; Arce, E.; Renold, P.; Kennedy, R. J.; Kemp, D. S. *J. Am. Chem.Soc.* **2001**, *123, in press.*

III. Circular Dichorism and t/c Analysis

CD spectra were obtained on an Aviv 62DS spectrometer. Peptides were dissolved in water and concentration was determined by the UV absorbance of the tryptophan residue at 280 nm (ε_{280} = 5560 cm^{-1} M^{-1}).[1]

To isolate the $[\theta]_{222}$ of the alanine helix, the molar ellipticity of (H)-^{t}LInp$_2$K$_4$-NH_2 was subtracted from the molar ellipticity of the Ac-Hel-A$_n$- ^{t}LInp$_2$K$_4$-NH_2 series. The resulting molar ellipticity was divided by the number of alanine residues, yielding per residue molar ellipticity. The nonhelical CD contribution at 222 nm of the Ac-Hel was removed, by subtracting the limiting ellipticity.[2]



Plot of per residue molar ellipticity $[\theta]_{222}$ for Ac-Hel-A$_{14}$- ^{t}LInp$_2$K$_4$-NH_2. The original helical CD curve (dotted), - ^{t}LInp$_2$K$_4$-NH_2 corrected CD curve (solid).

The measurement of the t/c values was preformed as previously described.[3] The t/c values for the series Ac-Hel-A$_n$-^{t}LInp$_2$K$_4$W-NH_2 n= 5, 6, were consistent with AcHel-A$_n$-NH_2 as previously reported.[3]

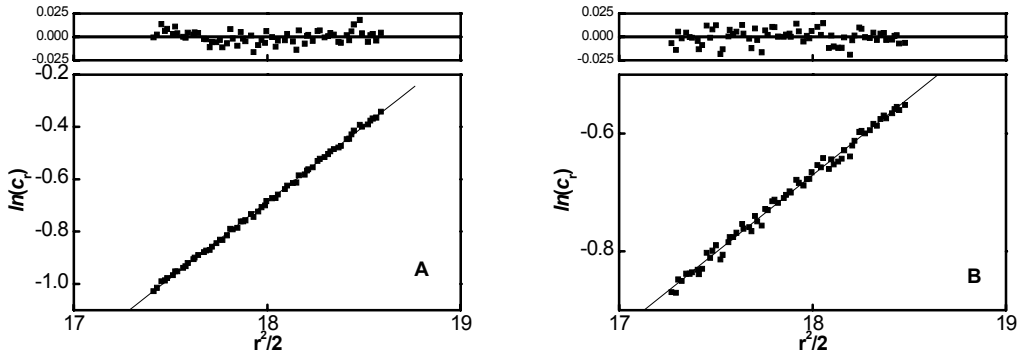
IV. Hydrogen Exchange

The decay of the amide NHs of Ac-$^{\beta}$D-Hel-A$_8$-β-NH_2 were monitored by 500 MHz NMR in 0.1M NaH_2PO_4 at pH=4 as a function of time. The integration values were fit to a first order exponential decay. Both single and overlapping chemical shifts gave reasonable fits. When compared with the rate constants for Ac-A$_3$-OH, the average protection factor for Ac-$^{\beta}$D-Hel-A$_8$-β-NH_2 was 17, which corresponds to an average fractional helicity of 0.94.

[2] Kemp, D. S.; Allen, T. J.; Oslick, S. L.; Boyd, J. G. *J. Am. Chem. Soc.* **1996**, *118,* 4240-4248.
[3] Renold, P.; Tsang, K-Y.; Shimizu, L. S.; Kemp, D. S. *J. Am. Chem. Soc.* **1996**, *118,* 12234-12235.

V. Analytical Ultra Centrifugation

Ac-Hel-A_9-^{t}LInp$_2$$K_4$W-$NH_2$, Ac-Hel-$A_{14}$-^{t}LInp$_2$$K_4$W-$NH_2$, Ac-$^\beta$D-Hel-$A_8$-β-$NH_2$, and Ac-$^\beta$D-Hel-$A_8$-βInp$K_2$W-$NH_2$ were all found to be monomeric species by sedimentation equilibrium analysis at 40,000 and 45,000 rpm. Data was collected on a Beckman Optima Xl-A centrifuge. Samples were tested at μM and mM concentration to take into account the solutions used in the CD and t/c – NMR experiments. Accurate molecular weights were obtained for μM samples in 0.1M NaCl. The consistent linear distribution of ln(c) vs. $r^2/2$ for μM and mM peptide samples in water are indicative of monomeric species.



Sedimentation Equilibrium Analysis at 45,000 rpm for AcHel-A_{14}-^{t}LInp$_2$$K_4$W-$NH_2$:
A 20 μM peptide in 0.1M NaCl; **B** 5 mM peptide in D_2O.