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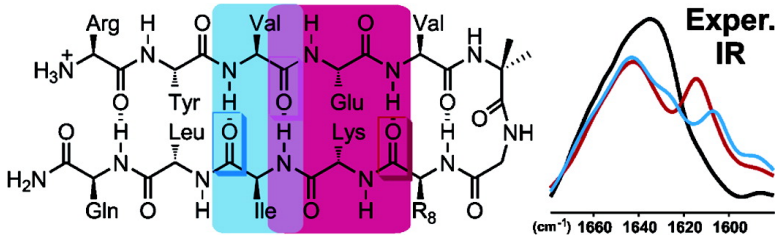

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IR Study of Cross-Strand Coupling in a β-Hairpin Peptide Using Isotopic Labels

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Studies of structure and stability of peptides as models of protein structural elements have long been a focus of biophysical studies. While good α-helix models exist, developing similar models for β-sheet structures has proven to be more difficult. Isolated β-sheets are generally less stable as monomers, being more subject to solvation and packing effects, which, moreover, are highly sequence-dependent. Several autonomously folding β-hairpin peptides have been reported with sequences stabilizing hairpin turns or enhancing cross-strand H-bonding and side-chain interactions.[1,2] Few of these β-hairpins mimic protein sequences, while most have been de novo designed to achieve stability.

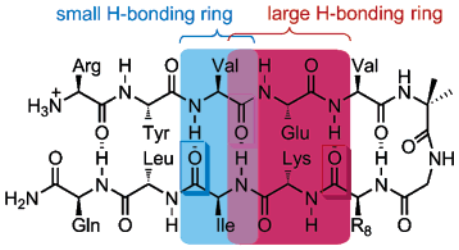
In one series of studies, ^{D}Pro-Gly sequences were used to form left-handed (type-II′ or type-I′) β-turns to stabilize water-soluble β-hairpins.[1,3,4] Aminoisobutyric acid (Aib) also promotes hairpin turn formation.[5] We hypothesized that Aib-Gly sequences might induce type-I/I′ β-turns due to their propensity to stabilize helical peptides[6] and their locally achiral nature, which allows formation of left or right turns due to their gauche dihedral angles (±30−60°).[7]

Isotopic labeling of peptides can provide site-specific information, even with the use of low-resolution spectroscopic techniques.[8] This is the first report of IR data for monomeric β-hairpin molecules with isotopic labeling correlated with ab initio theoretical modeling. Development of a stable β-hairpin model that allowed detection of the isotopically shifted transitions was critical.

We have prepared, by Fmoc solid-phase synthesis (see Supporting Information), an analogue of the 12-mer peptide amide originally studied by Gellman (**H^{D}PG**), in which an Aib residue (denoted B) is substituted for the ^{D}Pro in the $i + 1$ position of the β-turn.[9] The resulting sequence, RYVEV***BG***OKILQ (**HBG**; Scheme 1, C-terminally amidated),[10] has striking stability and spectral characteristics indicative of a β-hairpin (CD overlaps **H^{D}PG**,[4,9] min at 215 nm, max 200 nm, aromatic band ∼230 nm, and amide I′ IR peak at 1635 cm^{-1}). Two amide ^{13}C═O variants were synthesized by labeling (small boxes in Scheme 1) Val^{3} and Lys^{8} (**HBG-L**) or Val^{3} and Ile^{10} (**HBG-S**). **HBG-L** had Lys in place of Orn at position R^{8} (Scheme 1) due to the availability of l-^{13}C-labeled Fmoc L-Lysine(Boc) and its structural similarity to Orn. CD spectra for the three hairpins overlap, confirming the validity of this substitution. Assuming formation of an ideal hairpin, the two ^{13}C═O's will form a large (14-member, **HBG-L**, large filled red box) or small (10-member, **HBG-S**, smaller filled blue box) H-bonded ring (Scheme 1).

Infrared spectra were theoretically simulated using previously described methods[4,11−13] for a model, twisted β-hairpin. Spectral parameters were obtained from density functional theory (DFT) calculations for the turn and for two H-bonded antiparallel strand fragments (three amides each) and were transferred to the longer

Scheme 1



model peptide. Simulations for cross-strand isotope substitutions yielded different band shape patterns for the two isotope patterns,[13] large and small ring (Figure 1a), independent of position in the sequence. Each small ring had a larger ^{13}C shift from the ^{12}C band, but weaker relative intensity than did the ^{13}C in the large ring models. Since the ^{13}C═O groups are closer together in the small ring, they should give a larger exciton splitting then for the large ring, but ∼8 cm^{-1} was computed for both, due to end effects from the 3-amide strand model used.[13] The lower frequency component of the exciton-split pair of ^{13}C modes is the more intense transition for the small ring, while the higher frequency component is more intense for the large ring.[13] Thus, the apparent (most intense) **HBG-L** ^{13}C band is ∼8 cm^{-1} higher in frequency than the corresponding **HBG-S** band. These results suggested synthesis of small and large ring substituted peptides to test if labeling would indeed be diagnostic of hairpin formation and of the type of H-bond ring formed.

The experimental spectral patterns bear out this prediction for the ^{13}C═O bands (Figure 1b). **HBG-L** has a 1615 cm^{-1} ^{13}C band shifted down 28 cm^{-1} from its ^{12}C maximum. The **HBG-S** ^{13}C peak is ∼8 cm^{-1} higher. This difference is in remarkable (exact) agreement with the theoretical result. Such accuracy is a consequence of the local coupling (off-diagonal interactions) being computed more reliably than the diagonal force constants with our method. The ^{13}C substitution has an effect on the ^{12}C band, since the 5 °C amide I′ band maxima occur at 1635 cm^{-1} for **HBG** but at 1643 cm^{-1} for both **HBG-S** and **HBG-L**. This is not predicted in the theoretical simulations and is further obscured by the broadening of the ^{12}C band. The amide I′ IR band shape of the unlabeled **HBG** peptide is typical for peptides with high β-sheet content[4,11−14] and results from in-strand coupling plus cross-strand, hydrogen bond interaction. The experimental ^{12}C bandwidths, in contrast to the prediction, are evidence of structural variation (the simulation is for a uniform hairpin). Fraying of the terminal segments, seen in other hairpins,[3−5] reduces the exciton coupling magnitude and broadens the spectra, giving rise to bands between the intense low- and weak high-frequency β-strand components. Solvation of the turn residues (H-bond to water) and the outward directed strand amides will lead to frequency variations not adequately represented in vacuum simulations.[13]

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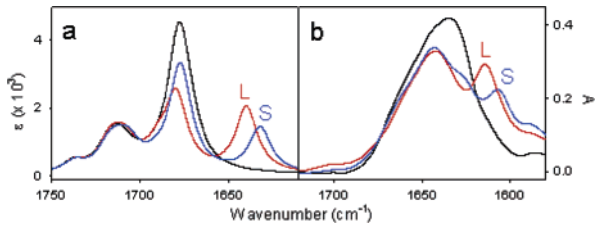

Figure 1. (a) Theoretical amide I′ IR absorption for a 12-residue β-hairpin determined from coordinates of intestinal fatty acid binding protein (PDB **1IFC)**. Spectra were simulated using parameters from BPW91/6-31G* level DFT computations for two three-amide strands (all Ala) and the corresponding turn, which were transferred to the larger peptide.[4] (b) Experimental IR absorption for **HBG** (black), **HBG-L** (red), and **HBG-S** (blue), normalized to a constant area, obtained for 10−50 mg/mL solutions in 75 mM phosphate/D_2O buffer (pH = 7.0 uncorrected) in a 50-μm path CaF_2 cell, by averaging 940 scans, 4 cm^{-1} resolution. The peptides were lyophilized twice from 10 mM DCl solution for H/D exchange and reduction of TFA interference. ECD of these samples matched dilute results and previous hairpins (see Supporting Information).[9,10]

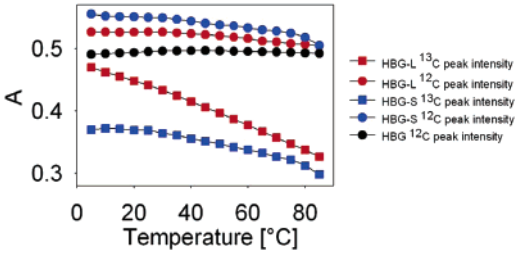

Figure 2. Thermal variation of the ^{12}C (●) and ^{13}C (■) amide I′ intensity for **HBG** (black), **HBG-L** (red), and **HBG-S** (blue).

Upon heating the samples to 85 °C (in 5 °C increments), we observed a gradual shift of the IR amide I′ absorption maxima from 1635 to 1641 cm^{-1} and from 1643 to 1648 cm^{-1} for **HBG** and both **HBG-L** and **HBG-S**, respectively. These equivalent shifts indicate a partial transition from β-hairpin to "random coil" (or PII, proline II-type) conformation caused by the heating. Such a frequency shift is expected for sheet-to-coil transitions and is supported by calculations for 3_1-(PII) helices.[8a,15] By contrast, the hairpin ^{13}C bands are higher in frequency than those observed for coils.[8a] The transitions were fully reversible on cooling for dilute samples but not complete (fully sigmoidal) by 85 °C (our highest practical temperature). Incomplete unfolding is also evident in the ECD (see Supporting Information) where residual ellipticity at 215 nm results from the stability of the Aib-Gly turn, as seen for ^{D}Pro-Gly.[4] Multistate unfolding is suggested by the lack of an isosbestic point in the amide I′ IR variation, but temperature dependence of the component spectra could also cause this. Factor analysis of the total band shape shows a contribution from a third component that grows in and disappears with increasing temperature.

Labeling provides a particularly sensitive way to judge the thermal transition via a site-specific probe. The ^{13}C feature shows the largest intensity variation in these hairpin-to-coil thermal transitions, dropping sharply at high temperatures without much shift. The variation for **HBG-L** is larger than that for **HBG-S** and has a different character, indicating site-specific behavior. The $^{12}C{=}O$ band varies little in intensity and only shifts ~6 cm^{-1}. The relative variances are illustrated in Figure 2. The ^{13}C amide I′ is a local oscillator shifted from the centroid of the band (representing uncoupled $^{12}C{=}O$ modes). The $^{13}C{=}Os$ are coupled to each other but are not strongly affected by exciton splitting of the ^{12}C modes, which is the source of the characteristic β-amide I. By contrast, the ^{12}C mode loses such coupling in the coil form, thus shifting up in frequency, but maintains most of its intensity. The ^{13}C intensity variation in the **HBG-L** transition apparently arises from loss of coupling to the normally intense β-strand mode with out-of-phase C=O motion in the strand, which disappears on unfolding.[12,13] Figure 2 summarizes the temperature dependence of the ^{12}C and ^{13}C peak intensities of the **HBG**, **HBG-S**, and **HBG-L** hairpins. The thermal transition is incomplete, which prevents ideal thermodynamic analysis but emphasizes the stability of the Aib-Gly turn. A comparative study of similar sequences will be reported in detail separately. Such increased sensitivity to folding change should have a positive impact on kinetic studies now being applied to hairpins.[16]

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Supporting Information Available: Detailed peptide synthesis and characterization, comparison ECD, and thermal spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Gellman, S. H. *Curr. Opin. Chem. Biol.* **1998**, *2*, 717−725. (b) Searle, M. S.; Ciani, B. *Curr. Opin. Struct. Biol.* **2004**, *14*, 458−464. (c) Galzitskaya, O. V. *Mol. Biol.* **2002**, *36*, 207−216.
(2) (a) Kelly, J. W. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 930−932. (b) Cochran, A. G.; Skelton, N. J.; Starovasnik, M. A. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 5578−5583. (c) Tatko, C. D.; Waters, M. L. *J. Am. Chem. Soc.* **2002**, *124*, 9372−9373. (d) Santiveri, C. M.; Rico, M.; Jimenez, M. A. *Protein Sci.* **2000**, *9*, 2151−2160.
(3) (a) Stanger, H. E.; Gellman, S. H. *J. Am. Chem. Soc.* **1998**, *120*, 4236−4237. (b) Zhao, C.; Polavarapu, P. L.; Das, C.; Balaram, P. *J. Am. Chem. Soc.* **2000**, *122*, 8228−8231.
(4) Hilario, J.; Kubelka, J.; Keiderling, T. A. *J. Am. Chem. Soc.* **2003**, *125*, 7562−7574.
(5) Aravinda, S.; Shamala, N.; Rajkishore, R.; Gopi, H. N.; Balaram, P. *Angew. Chem., Int. Ed.* **2002**, *41*, 3863−3865.
(6) Toniolo, C.; Crisma, M.; Formaggio, M.; Peggion, C. *Biopolymers* **2001**, *60*, 396−419.
(7) Wysong, C. L.; Yokum, T. S.; McLaughlin, M. L.; Hammer, R. P. *CHEMTECH* **1997**, *27*, 26−33.
(8) (a) Silva, R. A. G. D.; Kubelka, J.; Decatur, S. M.; Bour, P.; Keiderling, T. A. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 8318−8323. (b) Decatur, S. M.; Antonic, J. *J. Am. Chem. Soc.* **1999**, *121*, 11914−11915. (c) Paul, C.; Wang, J.; Wimley, W. C.; Hochstrasser, R. M.; Axelsen, P. H. *J. Am. Chem. Soc.* **2004**, *126*, 5843−5850. (d) Brauner, J. W.; Dugan, C.; Mendelson, R. *J. Am. Chem. Soc.* **2000**, *122*, 677−683. (e) Kubelka, J.; Keiderling, T. A. *J. Am. Chem. Soc.* **2001**, *123*, 6142−6150. (f) Halverson, K.; Sucholeiki, I.; Ashburn, T. T.; Lansbury, P. T., Jr. *J. Am. Chem. Soc.* **1991**, *113*, 6701−6703. (g) Fang, C.; Wang, J.; Charnley, A. K.; Barber-Armstrong, W.; Smith, A. B., III; Decatur, S. M.; Hochstrasser, R. M. *Chem. Phys. Lett.* **2003**, *382*, 586−592.
(9) Haque, T. S.; Gellman, S. H. *J. Am. Chem. Soc.* **1997**, *119*, 2303−2304.
(10) Thomas, C. L.; Etienne, M. A.; Wang, J.; Setnicka, V.; Keiderling, T. A.; Hammer, R. P. *Peptide Revolution: Genomics, Proteomics, and Therapeutics*; Kluwer Academic Publishers: Dordrecht, The Netherlands, 2004; pp 381−382.
(11) Bour, P.; Sopkova, J.; Bednarova, L.; Malon, P.; Keiderling, T. A. *J. Comput. Chem.* **1997**, *18*, 646−659.
(12) Kubelka, J.; Keiderling, T. A. *J. Am. Chem. Soc.* **2001**, *123*, 12048−12058.
(13) Bour, P.; Keiderling, T. A. *J. Phys. Chem. B* **2005**, *109*, 5348−5357.
(14) (a) Kuznetsov, S. V.; Hilario, J.; Keiderling, T. A.; Ansari, A. *Biochemistry* **2003**, *42*, 4321−4332. (b) Keiderling, T. A.; Silva, R. A. G. D. In *Synthesis of Peptides and Peptidomimetics*; Goodman, M., Herrman, G., Eds.; Georg Thieme Verlag: Stuttgart, Germany, 2002; *E32*, pp 715−738. (c) Haris, P. I.; Chapman, D. *Biopolymers* **1995**, *37*, 251−263. (d) Jackson, M.; Mantsch, H. H. *Crit. Rev. Biochem. Mol. Biol.* **1995**, *30*, 95−120.
(15) Kubelka, J.; Keiderling, T. A. *J. Phys. Chem. B* **2005**, *109*, 5348−5357.
(16) (a) Du, D.; Zhu, Y.; Huang, C.-Y.; Gai, F. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 15915−15920. (b) Xu, Y.; Oyola, R.; Gai, F. *J. Am. Chem. Soc.* **2003**, *125*, 15388−15394. (c) Munoz, V.; Thompson, P. A.; Hofrichter, J.; Eaton, W. A. *Nature* **1997**, *390*, 196−199. (d) Dyer, R. B.; Maness, S. J.; Peterson, E. S.; Franzen, S.; Fesinmeyer, R. M.; Andersen, N. H. *Biochemistry* **2004**, *43*, 11560−11566. (e) Yang, W. Y.; Pitera, J. W.; Swope, W. C.; Gruebele, M. *J. Mol. Biol.* **2004**, *336*, 241−251. (f) Jas, G. S.; Eaton, W. A.; Hofrichter, J. *J. Phys. Chem. B* **2001**, *105*, 261−272.

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