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@-Tide-Stabilized β-Hairpins

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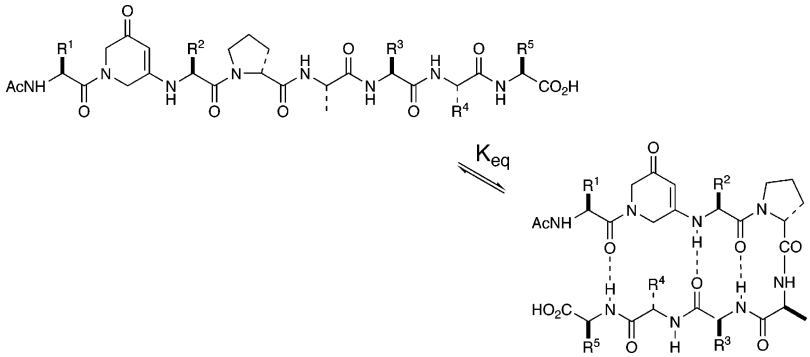

As minimalist versions of β-structure, two-stranded β-hairpins are commonly employed as platforms for assessing the interactions that stabilize β-sheets in proteins. We have found that the presence of a 1,6-dihydro-3(2*H*)-pyridinone moiety (the "@-unit") as an amino acid replacement at the $i - 1$ or $i + 4$ positions relative to a β-turn strongly stabilizes the hairpin conformation. Hybrids of this type bridge the gap between natural β-hairpins and unnatural β-sheets because the @-unit only replaces one residue in a peptide while stabilizing the hairpin conformation to a greater extent than a normal amino acid. In this report, we describe the synthesis of a variety of @-tide-templated hairpins and the NMR and CD characterization of their conformations in both polar and nonpolar solvents.

Introduction

Although the folded structure of a protein ultimately depends on its primary sequence, the discrete interactions that control local secondary and long-range tertiary structures remain poorly understood from a quantitative standpoint.[1,2] In the context of natural proteins, the interdependence of secondary and tertiary conformational effects complicates an analysis of the consequences of even simple changes in primary sequence.[3] Smaller, peptidomimetic models have therefore been developed to allow secondary structural motifs to be explored in the absence of competing tertiary interactions. Considerable success has been achieved in devising peptidomimetics of β-turns and α-helices, which depend on contacts between amino acids that are nearby in sequence. A greater challenge has been to devise mimics of the extended peptide conformation, in which intramolecular interactions are absent, or of β-sheets, in which the interactions are between strands.[1,2,4−7] Historically, β-sheet models have been notoriously difficult to study since the structural elements used to induce folding often promote aggregation as well.[5] As a consequence, the intrinsic propensities of individual amino acids to adopt the extended conformation, or the magnitudes of amino acid side chain−side chain interactions outside the context of folded proteins, are generally understood only qualitatively.[5,6,8,9]

Progress toward the development and quantitative analysis of well-folded monomeric β-sheets is being made on several fronts. One approach focuses on peptide

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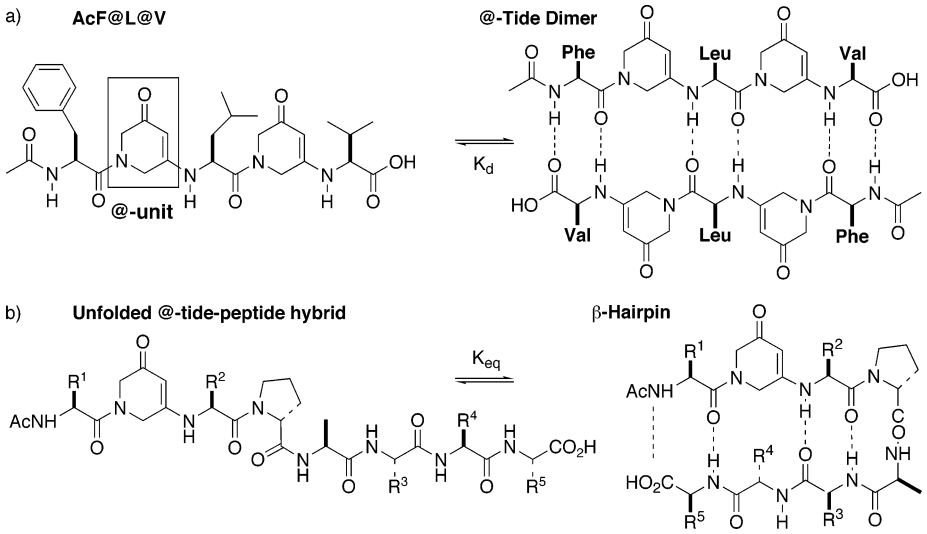

FIGURE 1. Dimerization of linear @-tides (a) and folding of @-tide-peptide β-hairpin structures (b).

sequences derived from β-sheet regions of known proteins[3,4,10,11] or that fold into β-hairpins.[5,10,12,13] Since these models are comprised entirely of natural amino acids, they are easily synthesized and readily varied; however, a significant folded population is usually observed only with fairly long peptides (12+ residues). An alternative approach has been to introduce structural components that stabilize a particular conformation, such as peptidomimetic templates[14−18] or covalent cross-links in macrocyclic analogues.[19−21] Ideally, the increased conformational control provided by these alternative approaches to β-strand stabilization should lead to greater tolerance for substitution in the peptide component.

The promise of the peptidomimetic strategy has led to the design of several β-sheet mimetics that template a peptide strand through hydrogen bonding. Among these designs are Kemp's diacylaminoepindolidiones,[18] Nowick's Hao-stabilized β-sheets,[22] and the hydrogen-bonded oligoamide duplexes described by Gong.[14] Although these aromatic systems are planar, they offer a pattern of hydrogen bond donors and acceptors with the same spacing as an extended peptide. When they are tethered to a peptide strand via a β-turn or other, nonpeptidic linkage, the hybrid structures fold into β-hairpin conformations with many of the characteristics of a β-sheet. While these systems fold or dimerize as β-sheets in organic solvents, they are much less ordered in water since the required conformational control is achieved through hydrogen bonding. Moreover, the templating groups lack the amino acid side chains; hence, to the extent that they replace amino acid units in the β-sheet model, they provide no information on the cross-strand interactions that are key for β-sheet sequence specificity.

As an alternative amino acid replacement, the 1,6-dihydro-3(2*H*)-pyridinone moiety (@-unit) adopts the extended conformation while retaining all the elements of the peptide backbone itself.[23] The conformational behavior of @-tide oligomers was explored previously, and their ability to mimic extended β-strands was demonstrated by their propensity to associate as head-to-tail dimers in chloroform−methanol solutions (Figure 1a). Since an @-tide strand should in principle associate in similar fashion with a peptide strand linked via a β-turn, we envisaged that it could serve as a small but effective template for the β-hairpin conformation (Figure 1b). By retaining the natural pattern of interstrand hydrogen bonds and most of the amino acid side chain contacts, an @-tide-β-turn-peptide hybrid may avoid some of the limitations encountered with previous models and bridge the gap between natural β-hairpins and unnatural β-sheet peptidomimetics. We now report spectroscopic studies of a variety of @-tide sequences that demonstrate an enhanced ability of the @-unit to stabilize a hairpin conformation, as well as a conformation-dependent CD signal of the @-tide vinylogous amide.

Results and Discussion

@-Tide β-hairpins of different lengths are readily accessible by solid-phase synthesis on Wang polystyrene resin, using conditions analogous to those developed for linear @-tides,[23] as detailed in Table 1. Amino acids were introduced as their *N*-Fmoc derivatives and the @-unit

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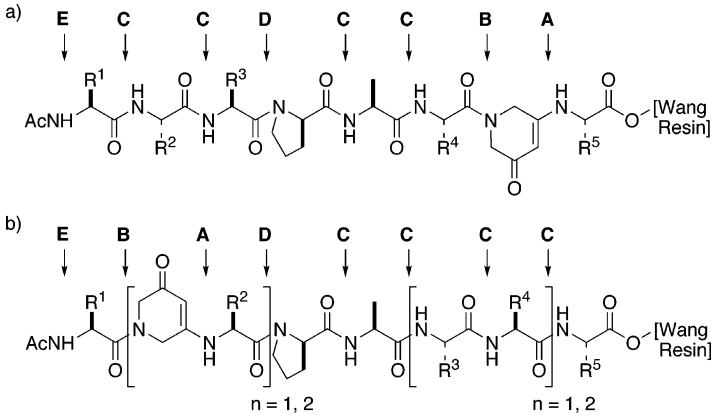
TABLE 1. Protocols for the Synthesis of @-Tide-Peptide hybrids with @-Units C-Terminal (a) and N-Terminal (b) to the β-Turn



protocol	*N*-deprotection conditions	coupling conditions[a]
A	Fmoc removal: 20% piperidine−DMF	Alloc-@-OSO_2Mes, $Sn(OTf)_2$, DIEA, 2:7 CH_2Cl_2:DMF
B	Alloc removal: $Pd(PPh_3)_4$, $TMSNMe_2$	Fmoc-amino acid,[b] HATU, DIEA
C	Fmoc removal: 20% piperidine−DMF	Fmoc-amino acid, HBTU, HOBt, DIEA
D	Fmoc removal: 20% piperidine−DMF	Fmoc-amino acid, HATU, DIEA
E	Fmoc removal: 20% piperidine−DMF	3:1:1 CH_2Cl_2:Ac_2O:pyridine

[a] Solvent = 1:1 CH_2Cl_2:DMF, unless otherwise indicated. [b] Alloc-@-Xaa is the amino acid component when coupling the second @-unit in extended *N*-terminal analogues.

was *N*-protected with an Alloc group and activated as the mesitylene sulfonate ("Alloc-@-OSO_2Mes"), as described previously.[23] While the shorter β-hairpins were assembled directly with use of this *N*-protected, *C*-activated @-unit, for the longer derivatives containing two @-units, the second @-unit was introduced by coupling the two-residue moiety Alloc-@-Xaa directly in 100% DMF, using HATU and DIEA. This strategy is not only more efficient, but appeared to be necessary to overcome the tendency of the growing chain to fold up in CH_2Cl_2−DMF mixtures, which inhibited the coupling reactions. After capping the terminal amino acid with acetic anhydride, the @-tide-peptide hybrid was cleaved from resin with 1:1 trifluoroacetic acid:CH_2Cl_2. All @-tide-peptide hybrids were purified by reverse phase HPLC with 0.1% TFA in acetonitrile−water mixtures, monitoring the eluant at 220 (peptide) and 284 nm (@-tide).

Evidence from Circular Dichroism of Solvent-Dependent Conformational Equilibria. The vinylogous amide moiety of the @-unit absorbs strongly at 284 nm (ϵ = 22 200 M^{-1} cm^{-1}). This chromophore also gives rise to a circular dichroic signal that falls conveniently outside the region of peptide CD spectra. However, like the peptide CD signature, the CD signal of the @-unit is sensitive to conformation. The sequence AcFGS^{D}PAL@I, in which the @-unit is in the *C*-terminal segment, was designed to probe the effectiveness of the @-tide template in the context of a short hairpin and with amino acids that disfavor the β-strand conformation (e.g., Ser and Gly). A control peptide, AcFGS^{D}PALSarI, containing sarcosine as a replacement for the @-unit, was synthesized as a comparison compound. As discussed below, the amino acid residues in these sequences were chosen to minimize overlapping resonances in the NMR spectrum. The objective of this experiment was not to optimize β-hairpin folding, but rather to gauge the influence of an @-unit versus an amino acid in a sequence with only modest conformational bias.

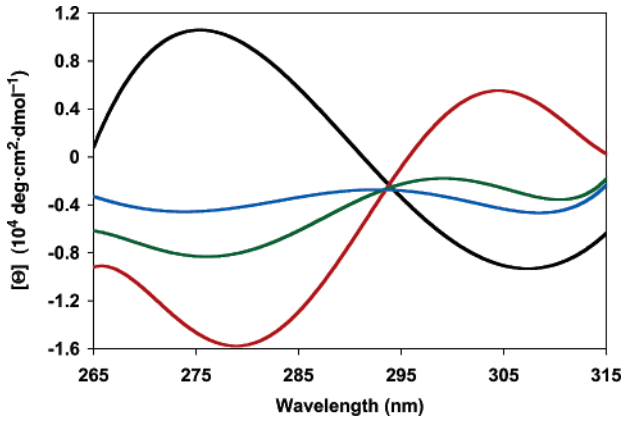
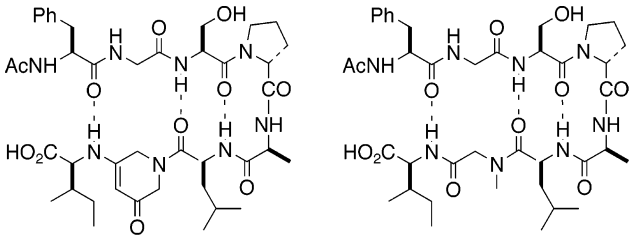


FIGURE 2. Circular dichroism spectrum of AcFGS^{D}PAL@I in $CHCl_3$ (black), with 5% (blue) and 15% (green) MeOH, and in pure MeOH (red) (367 μM, 25 °C).

Although the pattern of the CD spectrum for the β-hairpin AcFGS^{D}PAL@I is anomalous, as discussed below, the dramatic change observed on going from chloroform to methanol clearly signals a shift in conformational equilibrium (Figure 2). We reasoned that a folded β-hairpin conformation most likely predominates in chloroform, where intramolecular hydrogen bonding interactions are of paramount importance, and is disrupted with increasing amounts of methanol. We sought to verify this hypothesis through detailed NMR analysis, although the low solubility of the peptide derivative and related analogues in pure chloroform required these studies to be carried out in 5−10% methanol. The hairpin conformation is partially disrupted in this solvent mixture (see Figure 2); however the differences between the @-tide derivative and peptide control are more pronounced under these conditions.

NMR Analysis of @-Tide-Peptide Hybrids. The tendency of the @-tide-peptide hybrid AcFGS^{D}PAL@I to adopt a β-hairpin conformation in chloroform−methanol

TABLE 2. NMR Data for the @-Tide-Templated β-Hairpin AcFGS^{D}PAL@I, the Sarcosine Analogue AcFGS^{D}PALSarI, and Selected Values for the Random Coil Conformation[a]



AcFGS^{D}PAL@I (–@– analog) **AcFGS^{D}PALSarI** (–Sar– analog)

residue analogue	δ NH (ppm)		δ C_α–H (ppm)			$^3J_{HN\alpha}$ (Hz)[a]			$-\Delta\delta/\Delta T$ NH (ppb·K^{-1})	
	–@–	–Sar–	–@–	–Sar–	random[26]	–@–	–Sar–	random[30]	–@–	–Sar–
Phe	7.51	7.25	4.81	4.44	4.66	7.6	7.0	7.1	6.6	13
Gly	7.71	8.07	3.90	3.79	3.97	*a*	*a*	5.8	13	8.9
Ser	8.20	8.07	4.81	4.63	4.60	7.5	*a*	6.6	1.6	3.5
^{D}Pro			4.27	3.63	4.44					
Ala	7.54	7.62	4.40	4.22	4.35	*a*	7.5	5.8	9.0	7.8
Leu	7.89	7.56	4.98	4.88	4.17	8.5	7.5	6.6	5.2	4.7
Ile	7.56	7.14	4.04	4.40	3.95	*a*	8.5	7.2	8.2	6.1

[a] NMR parameters were determined in 5% CD_3OH–$CDCl_3$, 20 mM concentration, except for $^3J_{HN\alpha}$, for which 10% CD_3OH–$CDCl_3$ was necessary to resolve the amide protons. Even in this solvent, the resonances for Gly, Ala, and Ile in AcFGS^{D}PAL@I and Gly and Ser in AcFGS^{D}PALSarI were still not well enough resolved to determine the coupling constants.

solutions was evaluated by a variety of NMR techniques. Characteristic differences in the NMR spectral parameters for unstructured peptides and peptides in extended and intramolecularly hydrogen bonded conformations have been observed in both aqueous[24] and organic solvents.[15,25] Chemical shifts and coupling constants for the Cα hydrogens reflect the average conformations of individual amino acid residues,[26] while the chemical shifts of the NH hydrogens and their temperature dependence reveal whether they are solvent exposed or hydrogen bonded intramolecularly.[27,28]

(a) Evidence for a β-Hairpin from Chemical Shifts. The NH chemical shifts for AcFGS^{D}PAL@I in 5% CD_3OH–$CDCl_3$ provided the first evidence for the intramolecular hydrogen bonds expected in a β-hairpin conformation. Internally hydrogen bonded amides in peptides typically resonate around 8 ppm, approximately 2 ppm downfield of the values for solvent exposed amides.[15,29] In AcFGS^{D}PAL@I, the amide protons of the serine and leucine residues resonate at 8.2 and 7.9 ppm, consistent with the chemical shifts observed for artificial β-sheets in $CDCl_3$ solution (Table 2).[15] The NH resonances of the sarcosine analogue, AcFGS^{D}PAL-Sar-I, show smaller downfield chemical shifts, suggesting that it is less structured than the @-tide analogue.

The C_α–H chemical shifts also reflect a β-hairpin structure for AcFGS^{D}PAL@I. Relative to the chemical shifts observed for the α-hydrogens of a peptide in an unstructured, random coil conformation, those of a β-strand are more than 0.1 ppm downfield.[15,26] The relative downfield shifts observed for the α-hydrogens in the extended strands of AcFGS^{D}PAL@I range from 0.09 to 0.15 ppm for the terminal residues to 0.21–0.81 ppm for the amino acids flanking the β-turn, as shown in Table 2. These downfield shifts are generally larger than those observed for the sarcosine analogue.

(b) $^3J_{HN\alpha}$ Coupling Constants. The magnitude of the $^3J_{HN\alpha}$ coupling constant for a peptide residue is dependent on the φ-angle and, therefore, on the local conformation of the polypeptide backbone. Typical values for $^3J_{HN\alpha}$ for unstructured peptides range from ca. 6–7 Hz, with those for β-sheets at least 1 Hz larger.[15,30] The NH–C_α–H coupling constants for AcFGS^{D}PAL@I were determined in 10% CD_3OH–$CDCl_3$ for better resolution of the amide hydrogens. Even in this more dissociating solvent, coupling constants between 7.5 and 8.5 Hz were observed, which are also suggestive of a β-hairpin conformation (Table 2).

(c) Temperature Dependence of Amide Chemical Shifts. Whether an NH is hydrogen bonded or exposed to solvent is also revealed by the temperature dependence of its chemical shift: low values for $-\Delta\delta/\Delta T$ reflect persistent hydrogen bonds, intermediate values indicate an equilibrium between hydrogen bonded and nonbonded states, and high values are consistent with a solvent-exposed state.[27] In 5% CD_3OH–$CDCl_3$, the temperature dependence observed for the NH hydrogens is dependent on the location of the residue relative to the β-turn and whether internal hydrogen bonding is possible. For AcFGS^{D}PAL@I, high values are observed for Gly and Ala, whose NH's are exposed to the solvent when the oligomer adopts the hairpin conformation. In contrast, very low values are seen for Ser and Leu, which flank the β-turn,

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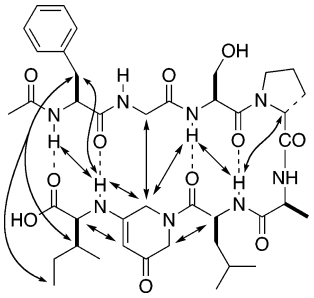

FIGURE 3. Key NOEs for AcFGS^{D}PAL@I indicative of a β-hairpin conformation (40 mM in 5% $CD_3OH/CDCl_3$, 20 °C).

and intermediate values are observed for Phe and Ile at the terminal positions, which are likely to be less structured.

(d) NOE Evidence for a β-Hairpin. In 5% $CD_3OH-CDCl_3$, AcFGS^{D}PAL@I shows nuclear Overhauser enhancements (NOEs) that are clearly indicative of a highly populated β-hairpin (Figure 3). Since the hairpin is not completely folded and minor conformations are present, care was taken to analyze only resonances for the major conformer, without potential overlap with other peaks in other residues or in minor conformers. As a consequence, some expected interactions were not assigned unambiguously due to resonance overlap. In addition to NOEs between backbone hydrogens on opposing strands, an extensive series of NOEs between side chain hydrogens on the Phe and Ile residues reflect their close proximity at the ends of the hairpin. In contrast, under the same conditions, the NOEs observed for the comparison peptide AcFGS^{D}PALSarI, with sarcosine in place of the @-unit, are consistent with less structured conformations. At a higher concentration of methanol (10% $CD_3OH-CDCl_3$), NOEs consistent with the hairpin structure are still observed for the @-tide templated hairpin, but they are diminished in number and in intensity.

(e) Evidence for Dimer Formation. At concentrations greater than 20 mM in 5% $CD_3OH-CDCl_3$, NOE cross-peaks and chemical shift changes consistent with a dimeric structure are observed for the templated hairpin, AcFGS^{D}PAL@I. At 40 mM, there is an NOE cross-peak between the β-hydrogens of the Phe and Ser residues; this cross-peak disappears at lower concentrations and it is eliminated on increasing the methanol concentration to 10%. The concentration dependence of the NH chemical shifts for AcFGS^{D}PAL@I in 5% $CD_3OH-CDCl_3$ reveals a dissociation constant of 25 mM in this solvent (Figure 4). Consistent with a 4-stranded β-sheetlike dimer, the Gly NH at the dimer interface shows the greatest sensitivity to concentration. Again as a comparison, the peptide AcFGS^{D}PALSarI shows no such chemical shift differences, even up to 230 mM.

Circular Dichroism Analysis of @-Tide-Peptide Hybrids. The CD spectra of other @-tide-peptide hybrids are solvent dependent as well, as illustrated by AcI@L^{D}PASGF, a templated hairpin in which the @-unit is incorporated in the *N*-terminal arm (Figure 5, red curves). The spectra are also dependent on the sequence and the stereochemistry. For example, the related hybrid AcI@L^{L}PASGF, in which the presence of L-proline strongly destabilizes the β-hairpin structure,[13] shows only a weak

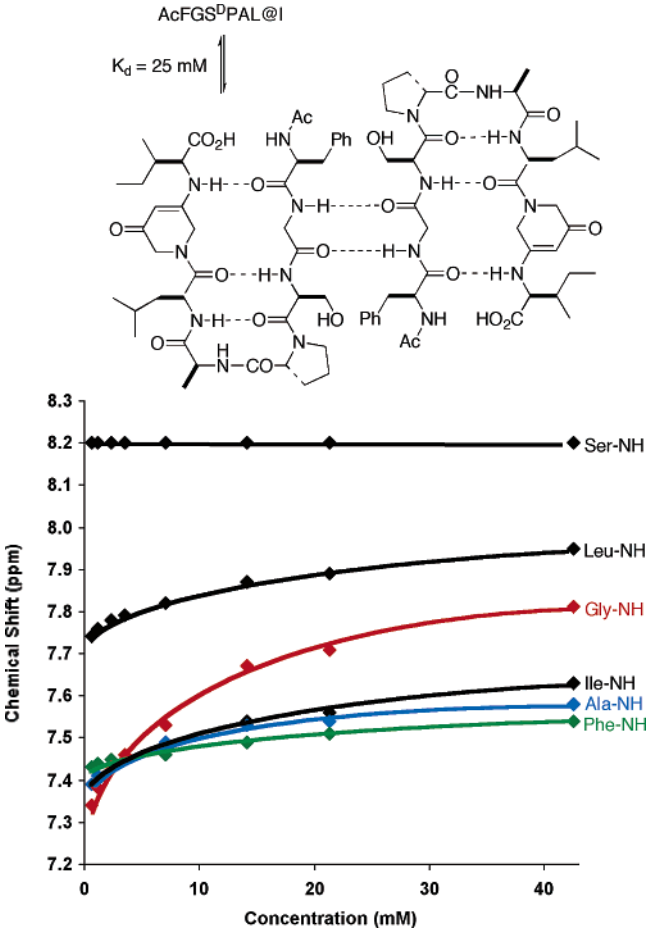

FIGURE 4. Concentration dependence of NH chemical shifts for AcFGS^{D}PAL@I in 5% $CD_3OH-CDCl_3$ at 22 °C.

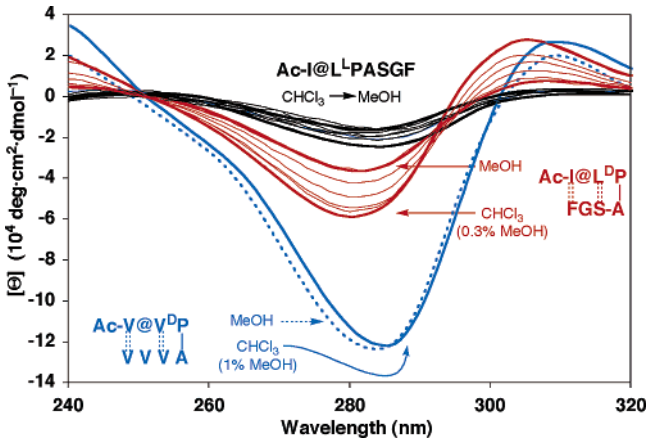

FIGURE 5. Solvent titration of AcI@L^{L}PASGF (400 μM, black), AcI@L^{D}PASGF (480 μM, red), and AcV@V^{D}PAVVV (180 μM, blue) from $CHCl_3$ to MeOH at 25 °C.

CD signal that is essentially invariant with solvent composition (Figure 5, black curves).

More stable β-hairpins can be obtained by incorporating amino acids that adopt the extended conformation to a greater extent than those in AcI@L^{D}PASGF and AcFGS^{D}PAL@I. For example, the combination of valine, a ^{D}Pro-Ala β-turn sequence, and the @-unit in AcV@V^{D}-PAVVV results in a hairpin whose stability far surpasses that observed for less favorable sequences. In fact, the

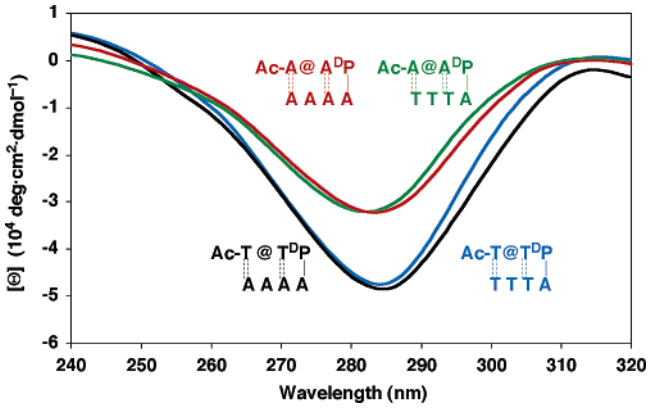

FIGURE 6. CD spectra for alanine mutations of AcT@T^{D}-PATTT in methanol at 25 °C (concentrations range from 3 to 34 μM).

hairpin conformation of AcV@V^{D}PAVVV is relatively impervious to the effect of methanol (Figure 5, blue curves). High values for the NH to C_αH coupling constants, which are generally greater than 9 Hz at 17 °C in 100% methanol, confirm that the valine residues in this hybrid are in the β-strand conformation.

The CD curves of Figure 5 clearly reveal the differences between unfolded and well-folded β-hairpins and suggest that the magnitude of the dispersion due to the @-unit may reflect the degree of folding for a β-hairpin. As a consequence, the @-unit may be useful as a spectroscopic probe of conformation, enabling the rapid analysis of peptide folding. For example, the impact of replacing the various threonine residues in AcT@T^{D}PATTT with alanines was readily evaluated by CD, much more expediently than would have been possible with other techniques. Threonine favors the β-sheet conformation and alanine does not,[31] so progressive replacement of Thr with Ala in the hairpin sidearms decreases the stability of the β-hairpin. Interestingly, even relatively small differences in β-hairpin population are reflected in the intensity of the @-unit signal at 280 nm. For example, Figure 6 shows the consequences of alanine substitution around the @-unit (AcA@A^{D}PATTT), in the opposing peptide strand (AcT@T^{D}PAAAA), and within the entire β-hairpin sequence (AcA@A^{D}PAAAA).

Figure 6 demonstrates the sensitivity of the @-unit to changes in folding caused by sequence variations and, moreover, illustrates the stabilizing effect that @-tides have on β-hairpin structure. Although alanine mutations in the peptide portion of the hairpin have little impact on folding, the corresponding substitutions around the @-unit have a definite weakening effect. While this result indicates that the @-unit is not acting alone in stabilizing a β-hairpin, it does reveal that the combination of an @-unit and favorable β-sheet forming amino acids have a more pronounced effect on β-hairpin folding than just a well-organized peptide strand.

Variations in the @-Unit CD Signal. The NMR evidence for a β-hairpin conformation of AcFGS^{D}PAL@I in chloroform and its disruption in methanol is supported by changes in the CD spectra obtained in these solvents (Figure 2). However, these CD spectra are quite different

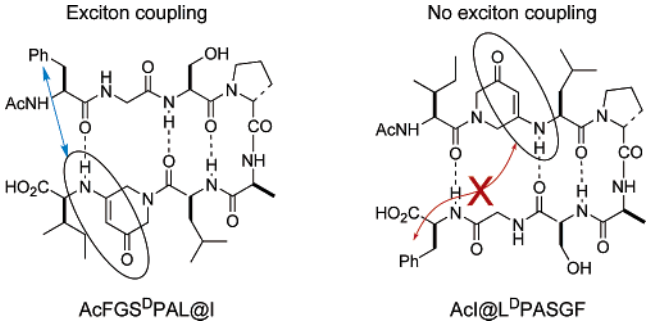

FIGURE 7. Exciton coupling and relationship between Phe side chain and @-unit vinylogous amide.

from those of all the other hairpins (compare Figure 2 with Figures 5 and 6). The dispersion is reduced in amplitude in all solvents, the pattern in chloroform is the opposite, and the curve is inverted, not merely attenuated, on transitioning from chloroform to methanol. Although some difference might be expected from the location of the @-unit in the C-terminal as opposed to the *N*-terminal arm of the hairpin, we attribute the anomalous behavior of AcFGS^{D}PAL@I to exciton coupling of the Phe chromophore and the vinylogous amide π-system of the @-unit, as illustrated in Figure 7.[32] For example, AcI@L^{D}PASGF, the structural isomer of AcFGS^{D}PAL@I in which the Phe residue is located on the opposite side of the vinylogous amide, has a "normal" CD spectrum (see Figure 5), presumably because the Phe side chain is at a distance where its electric transition dipole moment cannot interact with the excited state of the @-unit. Although these effects are not fully understood, we do find that placement of phenylalanine far from the vinylogous amide, as in AcI@L^{D}PASGF, avoids exciton-coupled perturbations in the CD spectra.

Conclusion

In combination with the β-turn-promoting dipeptide ^{D}Pro-Ala, the @-unit is effective as a template for stabilizing the β-hairpin conformation in a variety of short peptides in both nonpolar and hydroxylic solvents. These hairpins can be characterized by NMR and, more conveniently, by the circular dichroic signal of the vinylogous amide moiety of the @-unit itself. This peptidomimetic is more effective in promoting hairpin folding than the related acyclic amino acid sarcosine, and its chromophore provides, with some exceptions, a convenient method for assessing the conformational state of the peptide hybrid.

Materials and Methods[33]

General Procedures for Solid-Phase Synthesis of @-Tide-Peptide Hybrids. All @-tide-peptide hybrids were synthesized on solid phase with Wang resin in analogy to previously published procedures.[23] The peptide portions of @-tide-peptide hybrids were synthesized by using standard

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(33) Abbreviations used: @, the 1,2-dihydro-3(6*H*)-pyridinyl unit; DIEA, diisopropylethylamine; HOBt, 1-hydroxy-7-benzotriazole; HATU, *N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide; HBTU, 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate.

peptide coupling conditions with HBTU−HOBt activation.[34] The washing procedure involved agitating the resin for successive 2-min periods with 3 portions of DMF followed by 3 portions of CH_2Cl_2. Deprotection of the Fmoc group was accomplished by shaking the resin in 20% piperidine in DMF for 15 min. The following procedures are improved from that reported in ref 23 and are provided for clarity:

Fmoc−amino acid addition: The resin-bound peptide (0.1 g, 1.1 mmol/g) was suspended in 2 mL of DMF, the Fmoc-protected amino acid (2.5 equiv) was added, followed by HBTU (2.5 equiv), HOBt (2.5 equiv), and DIEA (5 equiv), and the reaction mixture was agitated at room temperature for 1 h. The resin was washed and immediately Fmoc-deprotected as described above.

Coupling to the secondary amine (i.e., the @-unit or proline): For the more difficult coupling reactions, a similar procedure was followed except that HATU (2.5 equiv) and DIEA (5 equiv) were employed as the coupling reagents and the reaction was allowed to proceed for 16 h.

Acetylation: The resin was agitated with 3:1:1 CH_2Cl_2:pyridine:Ac_2O for 3 h and then washed.

Cleavage from resin: The resin was dried in vacuo for at least 16 h prior to cleavage of the peptide. The resin (0.1 g, 1.1 mmol/g) was suspended in 1:1 CH_2Cl_2−TFA (3 mL) and rotated in a glass vial for 2 h. The solvent was removed and the resin was dried for 16 h under high vacuum. The resin was re-suspended in methanol, filtered, and washed (3 × 2 mL of MeOH and CH_2Cl_2). The combined organic solvents were concentrated and the crude product was purified immediately with preparative HPLC. Isolated yields ranged from 20% to 60% depending on the hydrophobicity of the sequence; high yields were obtained for nonpolar @-tide-peptide hybrids while lower yields were observed for polar derivatives.

Specific Procedures for the Syntheses of @-Tide-Peptide Hybrids. Three variations were required for @-tide-peptide hybrid syntheses, depending on the location of the @-unit. For a C-terminal @-tide-peptide hybrid, the @-unit was added directly to a resin-bound amino acid with use of conditions outlined in ref 23, including capping of excess resin-bound amino acid using Ac_2O. Extension of the β-hairpin was accomplished by using standard peptide coupling conditions as described above. Octamer @-tide β-hairpins with the @-unit on the *N*-terminus were also synthesized by using conditions analogous to those described above, except that Ac_2O capping after the @-unit addition was not necessary. Longer β-hairpins with *N*-terminal @-units required the addition of the second @-unit as a di-@-tide (2.5 equiv in relation to resin), using HATU (2.5 equiv) and DIEA (2.5 equiv) in DMF.

NMR Methods. Spectral data are reported as chemical shifts (multiplicity, number of hydrogens, coupling constants in Hz). ^{1}H NMR chemical shifts are referenced to TMS (0 ppm) in $CDCl_3$. Resonance assignments were obtained by the method of Wüthrich,[24] using TOCSY and NOESY spectra. Rigorous degassing was performed prior to the NOESY experiments, using 3 freeze−pump−thaw cycles.[35] NOESY experiments were performed by the method of Ananikov[36] at several mixing times. NOESY data were collected with 2048 data points in F2 and 512 data points in F1. ROESY data were collected with a Bruker 400 MHz spectrometer and the roesytp pulse program with 2048 data points in F1 and F2. NOESY and ROESY experiments gave similar NOE results for AcFGS^D-PAL@I and AcFGS^{D}PALSarI.

CD Methods. Compound concentrations were determined by UV absorbance (ϵ_{284} = 22 200 M^{-1} cm^{-1} per @-unit). Data were collected at a scan speed of 20 nm·min^{-1} with a bandwidth of 2 nm. The data were imported into Excel and corrected for solvent contributions, and the spectra are reported as molar ellipticities ([Θ] deg cm^2 $dmol^{-1}$) on a per @-unit basis (i.e., $[\Theta] = 100\psi/lcn$, where ψ = observable signal in millidegrees, l = path length in cm, c = concentration in millimolar, and n = number of @-units). Note: The success of the CD measurements is directly related to the purity of the sample and the care taken in sample preparation. It is critical that solvents are free from even trace amounts of other contaminating solvents and that samples are measured within a few hours of preparation.

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Supporting Information Available: Additional characterization of reported compounds and NMR methods and results. This material is available free of charge via the Internet at http://pubs.acs.org.

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