

Infinite pleated β -sheet formed by the β -hairpin Boc- β -Phe- β -Phe-D-Pro-Gly- β -Phe- β -Phe-OMe

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A β -hairpin conformation and extended β -pleated sheet assembly have been characterized by single crystal x-ray diffraction for the synthetic peptide t-butoxycarbonyl- β -Phe- β -Phe-D-Pro-Gly- β -Phe- β -Phe-methyl ester [β -Phe: (S)- β^3 homophenylalanine]. The centrally located D-Pro-Gly segment nucleates a chain reversal in a type II' β -turn conformation. Two intramolecular cross-strand hydrogen bonds stabilize the peptide fold. Intermolecular NH \cdots O=C hydrogen bonds (two on each side of the hairpin) connect the hairpins into an infinitely extended β -sheet. The β -residues cause all C=O groups to point in the same direction, resulting in a "polar" sheet by the unidirectional alignment of NH \cdots O=C hydrogen bonds. In contrast, β -sheets formed by α -residues have alternating directions for the hydrogen bonds, thus resulting in an "apolar" sheet. The crystallographic parameters for C₅₃H₆₆N₆O₉·CH₃OH are: space group P2₁, *a* = 9.854(2) Å, *b* = 10.643(2) Å, *c* = 25.296(4) Å, β = 100.39(2)°, *Z* = 2, agreement factor *R*₁ = 0.065 for 3,706 data observed >4 σ (*F*) and a resolution of 0.90 Å.

A prominent feature of supramolecular chemistry is the self-assembly of like or unlike molecules into larger entities without the use of covalent bonds. The two common secondary structures adopted by polypeptide backbones, helices and sheets, can be generated in designed synthetic oligopeptides, which can then self-assemble into crystalline arrays (1). Peptide helices in hydrophobic sequences readily form single crystals in which cylindrical molecules pack into columnar arrangements, either parallel or antiparallel, while head-to-tail hydrogen bonds are formed between adjacent molecules in an individual column (2). Adjacent columns are held together in three dimensions by van der Waals forces. In contrast, in peptide β -sheets, strong networks of hydrogen bonds stabilize molecular arrangements in two dimensions and comprise one type of a supramolecular structure. The hydrogen bond between the C=O and NH moieties in amino acids, peptides, and proteins has been long recognized as an important attractive force between or within a molecule. The concept of an extended β -sheet (Fig. 1) was already applied over 70 years ago to the interpretation of the x-ray diffraction patterns obtained from silk and keratin (3–5). Pleated-sheet conformations for both the antiparallel chain sheet and the parallel chain sheet were proposed by Pauling and Corey in 1953 (6). Early experimental verification of the antiparallel pleated-sheet model was obtained for the fibrous polypeptide (Ala)_n by Arnott *et al.* (7). Early x-ray structures of single crystals of small peptides confirmed the antiparallel pleated-sheet for L-Ala-L-Ala-LAla (8) and the parallel pleated-sheet for Gly-L-Phe-Gly (9), although the occurrence of the parallel pleated-sheet motif in peptides is less common. The structure of [Leu⁵]enkephalin (Tyr-Gly-Gly-Phe-Leu) is an example of an extended antiparallel-pleated sheet formed by four quite different conformers of the pentapeptide crystallizing side by side in an orderly sequence (10). β -sheet structures are a common component of globular proteins, but these sheets are contained within a single molecule and have a limited number of β -strands, and most of the strands have a significant twist (11). Therefore the β -domains in protein molecules usually are dif-

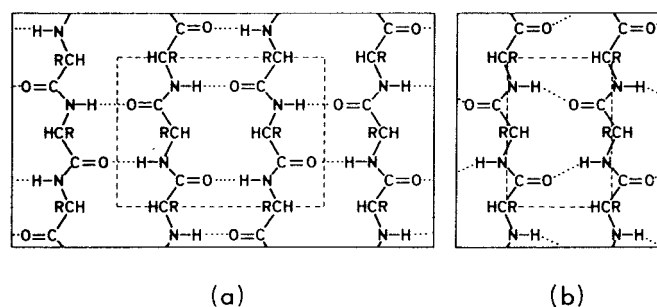


Fig. 1. Schematic diagram illustrating the hydrogen bonding arrangement in (a) the antiparallel-chain sheet and (b) the parallel-chain sheet model (5).

ferent from supramolecular structures, in which there is more regularity.

In this article we describe extended β -sheets formed in crystals of hexa- to decapeptides that have a β -hairpin conformation. A centrally positioned D-Pro-Gly or D-Pro-Ala segment had been used to facilitate type II' or type I' β -turn formations (12–15). Initially the two strands of the β -hairpins for which crystals were obtained were composed of all α -amino acid residues (16–18). Subsequently, β -residues were incorporated to ascertain the effect of the enhanced conformational versatility provided by the additional $-\text{CH}_2-$ groups in the backbone (19). A key difference between β -strands formed by α -amino acids and β -amino acids is that in the former, there is regular alternation in the direction of CO and NH groups, whereas in the latter, all of the CO groups point in one direction while NH groups face the opposite sides, resulting in distinctive strand polarity. In the present article, the crystallographic characterization of a designed β -hairpin peptide containing two facing pairs of β -Phe [(S)- β^3 -homophenylalanine] residues immediately adjacent to the β -turn, Boc- β -Phe- β -Phe-D-Pro-Gly- β -Phe- β -Phe-OMe (BHF4, where Boc is t-butoxycarbonyl and OMe is methyl ester), as well as its infinite sheet formation, will be emphasized.

Experimental Methods

The six-residue peptide BHF4 was synthesized by conventional solution-phase procedures by using a fragment condensation strategy. N- and C-terminal protection was provided by Boc and a methyl group, respectively. The Boc-(S)- β -Phe was synthesized by Arndt-Eistert homologation of Boc-Phe (20). Peptide coupling was mediated by *N,N'*-dicyclohexylcarbodiimide and 1-hydroxybenzotriazole. The crude peptide obtained as a white solid was poorly soluble in organic solvents. Repeated washing with cold methanol removed the contaminating dicyclohexylurea.

Abbreviations: Boc, t-butoxycarbonyl; β -Phe, (S)- β^3 -homophenylalanine; OMe, methyl ester.

Data deposition: The atomic coordinates have been deposited in the Cambridge Structural Database, Cambridge Crystallographic Data Centre, Cambridge CB2 1EZ, United Kingdom (CSD reference no. 172664).

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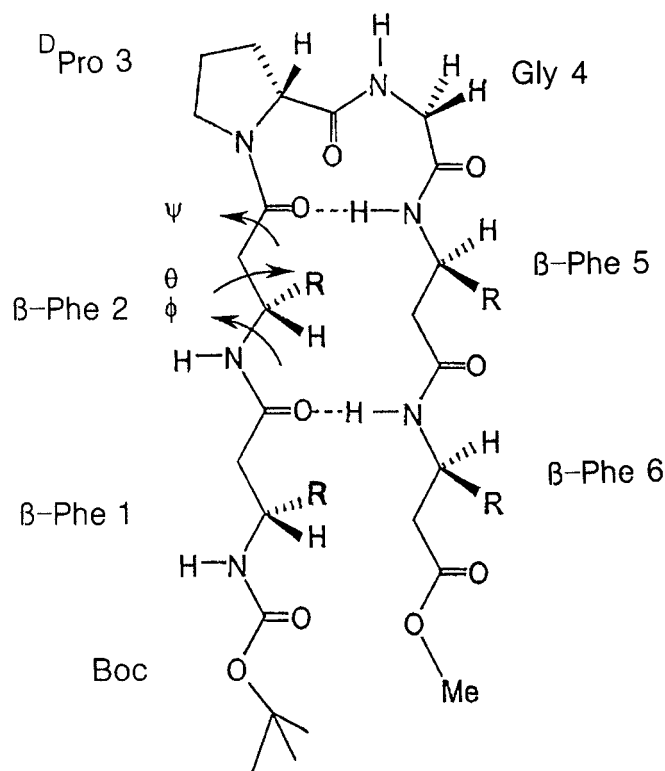


Fig. 2. Schematic representation of the β -hairpin in BHF4 with two pairs of facing β -Phe residues. The locations of the ϕ and ψ torsional angles, used for α -amino residues, and the additional θ torsional angle, needed to characterize β -amino residues, are shown.

The residual solid was characterized by electrospray MS (M_{calc} : 930 Da; M_{obs} : MH^+ , 931.5 Da and MNa^+ , 954.3 Da). The peptide was then dissolved in boiling methanol and crystals separated upon cooling.

A crystal in the form of a thin plate, $0.70 \times 0.60 \times 0.10$ mm, was coated with microscope oil and used for collecting x-ray diffraction data on a Bruker P4 diffractometer at -60°C . The

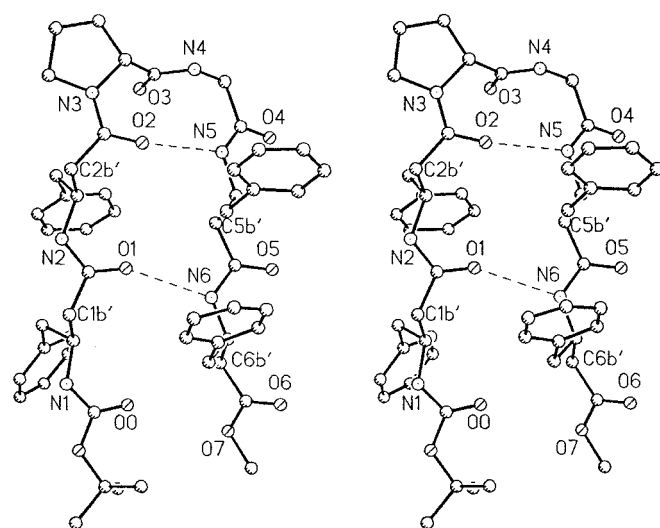


Fig. 3. Stereoradiogram drawn from coordinates obtained in the crystal structure of BHF4. Note the direction of all C=O moieties, except C=O(3), pointed to the right, and all NH moieties, except NH(4), pointed to the left.

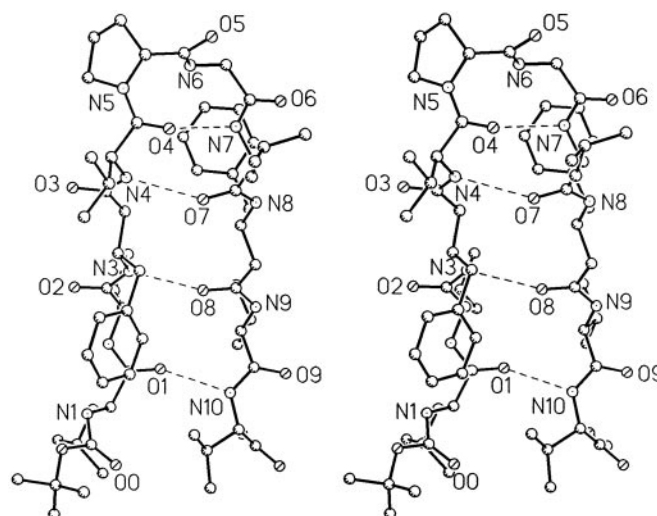


Fig. 4. Stereoradiogram of BBH10 with only one pair of facing β -Phe residues, 3 and 8 (19).

$\theta/2\theta$ scan mode was used with a $1.9^\circ + 2\theta(\alpha_1 - \alpha_2)$ scan width, $13^\circ/\text{min}$ scan speed, and $2\theta_{\text{max}} = 115^\circ$ ($0.90\text{-}\text{\AA}$ resolution). The crystal parameters were $\text{C}_{53}\text{H}_{66}\text{N}_6\text{O}_9 \cdot \text{CH}_3\text{OH}$, space group $P2_1$, $a = 9.854(2)\text{ \AA}$, $b = 10.643(2)\text{ \AA}$, $c = 25.296(4)\text{ \AA}$, $\beta = 100.39(2)^\circ$, $V = 2609.4(8)\text{ \AA}^3$, $Z = 2$, and $d_{\text{calc}} = 1.222\text{ gm/cm}^3$. The structure was solved by direct phase determination with the use of the tangent formula (21). Hydrogen atoms were placed in ideal positions and allowed to ride with the C or N atom, to which they were bonded, in a least-squares refinement on F^2 values. For 3,709 reflections with $|F| > 4.0\sigma$ the agreement factor R_1 was 6.53%. The OH group in the cocrystallized CH_3OH molecule is disordered between two sites with a 0.5/0.5 occupancy. The least-squares program used was Siemens SHELXTL, version 5.03 (Iselin, NJ).

Results and Discussion

The notation for the torsional angles in an α - and a β -amino acid residue is shown in Fig. 2. Two β -sheet conformations have been considered possible for all β -peptide chains that would conserve $\text{NH}\cdots\text{O}=\text{C}$ hydrogen bonding between two strands (22, 23). In these, the angle θ needs to have values near $\pm 60^\circ$ or 180° . In one case, the direction of the $\text{NH}\cdots\text{O}=\text{C}$ hydrogen bonds alternates along a pair of strands to produce an “apolar” sheet, whereas in the other case, all $\text{NH}\cdots\text{O}=\text{C}$ hydrogen bonds point in the same direction so that a “polar” sheet would be produced. For the present crystal structure of BHF4, that has two pairs of facing β -Phe residues (Figs. 2 and 3) the θ values for the $\text{C}^\alpha\text{-C}^\beta$ bonds range between $+153^\circ$ and $+171^\circ$ (close to the 180° predicted value). In the strands, all of the carbonyls are directed from left to right, whereas all of the NH groups are directed right to left, thus producing a “polar sheet.” In a previous structure, BBH10 (Boc-Leu-Val- β Phe-Val-D-Pro-Gly-Leu- β Phe-Val-Val-OMe) (19), in which there is only one pair of β -Phe residues facing each other (Fig. 4), among a number of α -residues, the θ values are 168° and 172° , also a bit less than 180° . In the latter structure, there is a mini-polar sheet encompassing the $\text{C}^\beta(3)$ and $\text{C}^\beta(8)$ region, whereas the remaining α -residues are arranged with the usual alternation of $\text{C}=\text{O}$ and NH groups that produce a local apolar sheet.

It should be noted that the β -turn made by the D-Pro-Gly segment in BHF4 is type II', the same as observed in other β -hairpins containing only α -residues (Fig. 5a). However, in the BBH10 structure that has only one pair of β -Phe residues, the β -turn is type I' (Table 1 and Fig. 5b). The least-squares fits of

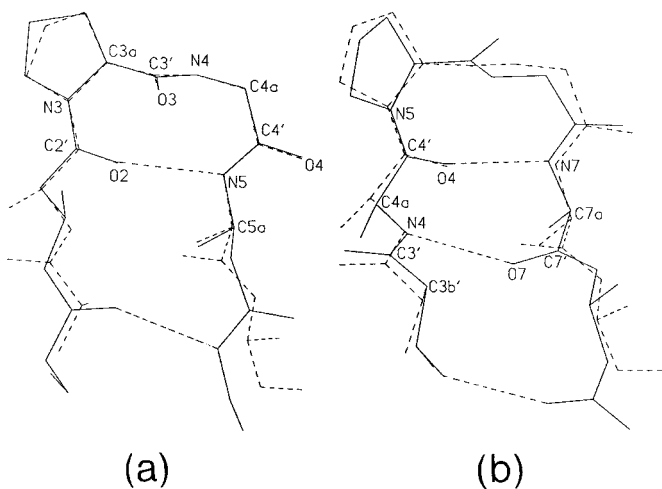


Fig. 5. Superpositions by least-squares fit of structures of β -hairpins at the β -bend. (a) Type II' β -bend in BHF4 (solid lines) and BH2L2 (dashed lines). (b) Type I' in BBH10 (solid lines) and type II' in BH2L2 (dashed lines).

the β -turns in molecules BHF4/BH2L2 (Boc-Leu-Val-Val-D-Pro-Gly-Leu-Val-Val-OMe) (16), both with type II' turns, show an excellent superposition, but the least-squares fit of type II' β -turn in BH2L2 and type I' in BBH10 (Fig. 5) shows the greatest deviation to be only 1.2 Å, at Gly C α . The comparable ϕ and ψ torsional angles listed in Table 1 show very similar values, without regard to the presence or absence of the -CH₂- group in the β -residues. It should be noted that β turns formed by D-Pro-Gly segments can adopt energetically favorable type II' turns ($\phi_{D-Pro} = +60^\circ$, $\psi_{D-Pro} = -120^\circ$, $\phi_{Gly} = -80^\circ$, and $\psi_{Gly} = 0^\circ$) or type I' turns ($\phi_{D-Pro} = +60^\circ$, $\psi_{D-Pro} = +30^\circ$, $\phi_{Gly} = +90^\circ$, and $\psi_{Gly} = 0^\circ$). In BHF4, the observed turn type is II', which also is observed in other β -hairpins containing only α -amino acids (Fig. 5 and see Fig. 7). It is likely that relatively small energy differences separate type II' and I' D-Pro-Gly conformations, with the choice being influenced by the constraints of the packing.

Side views of the β -hairpins illustrating the pleats in the backbones are shown for the classical α -amino acid residues in Fig. 6a, for the mixed α - and β -amino acid residues in Fig. 6b, and the all β -amino acid residues (except for D-Pro-Gly at the β -turn) in Fig. 6c. In Fig. 6a all of the pleats are equal in length and represent the planar segments C α -C(O)-N-C α' of each residue. In Fig. 6b, the long pleats contain the planar C α -C(O)-N-C α' or the planar C β' -C(O)-N-C α segments, whereas the short pleats con-

Table 1. Torsional angles for β -hairpins with β -residues

Residue	BHF4		Residue	BBH10*	
	Angle	Degree		Angle	Degree
β -Phe(1)	ϕ	-128	β -Phe(3)	ϕ	-116
	θ	163		θ	168
	ψ	115		ψ	106
	ω	178		ω	-177
β -Phe(2)	ϕ	-113	Val(4)	ϕ	-137
	θ	153		ψ	113
	ψ	110		ω	170
	ω	-173		ψ	37 [†]
D-Pro(3)	ϕ	55 [†]	D-Pro(5)	ϕ	56 [†]
	ψ	-133 [†]		ψ	-179
	ω	179		ω	88 [‡]
Gly(4)	ϕ	-92 [†]	Gly(6)	ϕ	-1 [†]
	ψ	8 [†]		ψ	175
	ω	-179		ω	-100
β -Phe(5)	ϕ	-84	Leu(7)	ϕ	-114
	θ	171		ω	-175
	ψ	115		ψ	-109
	ω	-173		θ	172
β -Phe(6)	ϕ	-99	β -Phe(8)	ϕ	128
	θ	166		ψ	177
	ψ	147		ω	
	ω	179			

*The comparable region for β -Phe and β -turn in Boc-Leu-Val- β -Phe-Val-D-Pro-Gly-Leu- β -Phe-Val-Val-OMe (19).

[†]Type II' β -turn.

[‡]Type I' β -turn.

tain the C α -C β' segments. In Fig. 6c, the long and short pleats alternate for the planar C β' -C(O)-N-C α' and C α -C β' segments of the β -amino acid residues. Sequences in which the β -residues do not face each other may not form the hairpin conformation because of the mismatch of C=O and NH positions between the separate strands.

It is noteworthy that in a strand containing all β -amino acids adjacent side chains project on the same face of the sheet, whereas in the α -amino acid counterparts, there is a regular alternation in side-chain orientation. In the antiparallel β -sheet structure formed by all β -amino acids, as in the case of BHF4, the side chains on facing strands of each β -hairpin point in opposite directions. In a single strand of BHF4, while the planes of the adjacent Phe-1 and Phe-2 as well as the adjacent Phe-5 and Phe-6 are not parallel, the dihedral angles between these planes are relatively small, 34° and 14°, respectively. The nearest ap-

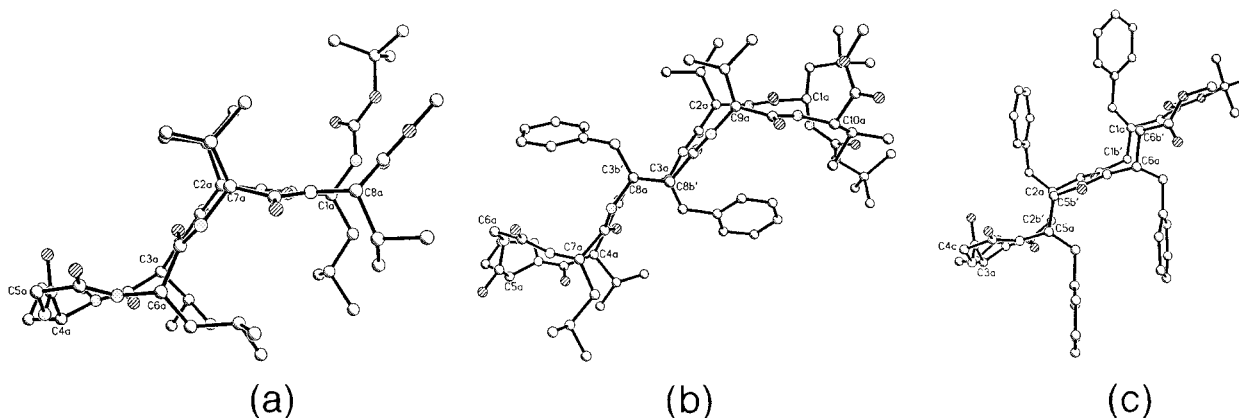


Fig. 6. Side views of β -hairpins with (a) classical α -amino acid residues, (b) mixed α - and β -residues, and (c) all β -residues in the strands.

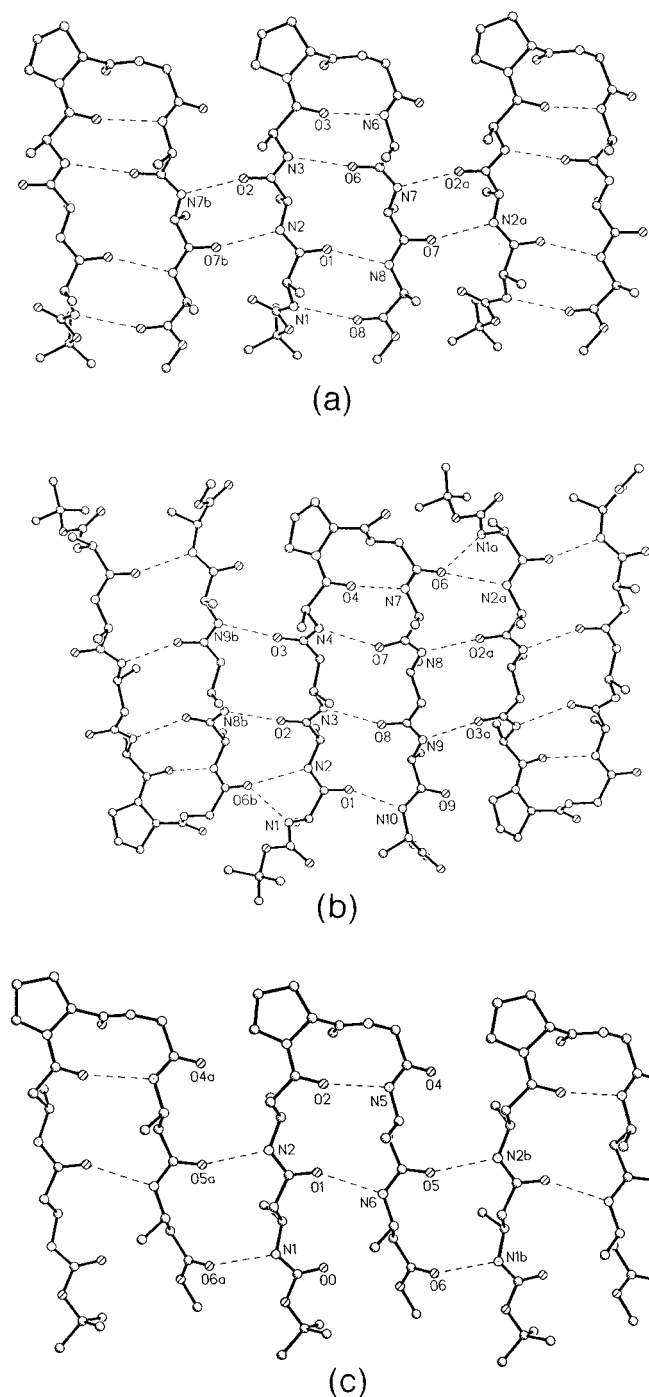


Fig. 7. Infinite β -sheets formed by (a) classical β -hairpins with all α -residues, (b) β -hairpins containing α - and β -residues, and (c) β -hairpins with all β -residues in the strands.

proaches between the pairs of phenyl groups are 4.11 Å and 4.59 Å, respectively. Distances between phenyl groups of neighboring molecules also are larger than van der Waals' approaches. There is no interleaving of any phenyl groups in this structure.

Supramolecular Assemblies of β -Hairpins

The perimeters of the three types of β -hairpins described above contain C=O and NH moieties that extend outward and are arranged in an orderly fashion in the average plane of the backbone atoms. They readily form strong C=O \cdots NH hydrogen

Table 2. Hydrogen bonds in BHF4

Type	Donor	Acceptor	D \cdots A, Å	H * -A, Å	D \cdots O=C angle degrees
Intermolecular	N1	O6 †	2.960	2.10	133
Intermolecular	N2	O5 †	2.980	2.09	160
	N3 (proline)				
Peptide-solvent	N4	O1MA ‡	2.829	2.14	
		O1MB ‡	2.752	1.92	
Intramolecular	N5	O2	2.942	2.08	147
Intramolecular	N6	O1	3.017	2.14	155
Solvent-peptide	O1MA ‡	O3	2.731		
Solvent-peptide	O1MB ‡	O3	2.491		

*Hydrogen atoms were placed in idealized positions with N-H = 0.90 Å.

† At symmetry equivalent $1 + x, y, z$.

‡ OH disordered on CH₃OH. At symmetry equivalent $2 - x, -0.5 + y, 1 - z$.

bonds with neighboring molecules on either side of the hairpin. The results are infinite ribbons with characteristics common with a β -sheet. The polar moieties forming the carbonyl-amide hydrogen bonds are in the plane of the sheet, whereas the side chains on the C $^\alpha$ atoms extend above and below the sheet. However, there are differences in the extended sheets formed by the three types of β -hairpins. The all α -residue β -hairpins, BH2L2 and an analog with the Gly in the β -turn replaced by L-Ala (18), form the sheet by simple translation of the hairpin in the direction perpendicular to the two strands of the hairpin. Appropriate hydrogen bonds are formed between the hairpins (Fig. 7a). The directions of the NH \cdots O=C bonds alternate throughout the sheet, thus canceling any polar effects. Fig. 7b shows the assembly by the hairpin with one pair of facing β -residues, BBH10. In this case, the adjacent hairpin molecules are antiparallel to each other. Because the molecules are related to each other by a horizontal 2-fold screw in the crystallographic space group, all of the individual strands are antiparallel to each other. It is not obvious why the hairpins pack in an antiparallel motif, rather than a parallel motif as in Fig. 7a, because the same number of intermolecular hydrogen bonds with the same direction can be made in either motif.

It should be noted in Fig. 7b that in a horizontal band through the middle of the hairpins, all of the NH \cdots O=C bonds point in the same direction because of the presence of the additional -CH₂- group in the β -residues and consequently the effective turning over of the -C(O)-N-groups. In view of the fact that uniformly directed hydrogen bonds are associated with polarity, the BHF4 molecule (Table 2) was synthesized with two pairs of facing β -residues. Its β -sheet packing in Fig. 7c shows that all of the C=O moieties are pointing to the right and all NHs are directed to the left (except for the top of the β -bend). Consequently all of the NH \cdots O=C hydrogen bonds point in the same direction and the β -sheet can be considered polar.

β -hairpins do not necessarily form extended sheets. The crystal structure of a synthetic 17-residue peptide consisting sequentially of a partially extended N terminus, approximately 1½ turns of an α -helix, a Schellman helix reversal segment, and an eight-residue β -hairpin (17) showed that the β -hairpin segment was very similar in conformation to the BH2L2 molecule shown in Fig. 7a. Yet it formed only a very limited β -sheet with the N-terminal extended portion from another molecule of the 17-residue peptide. The entire β -sheet contained only three peptide strands, plus some peripheral water molecules. Instead, the main assembly of the helix/ β -hairpin molecules was the head-to-tail hydrogen-bonded stacking of the helical portions (2) into an infinite pseudohelical column, even though the helical portion occurred in the middle of the molecule.

Conclusions

Crystal structure analyses have confirmed that β -hairpin molecules that have been constructed from β -amino acid residues or mixtures of α - and β -residues, as well as the classic β -hairpins containing only α -residues, have a strong propensity to assemble into regular, extended, infinite β -sheets by multiple and repeated hydrogen bonding. One major difference in the β -sheets is the directionality of the $\text{NH}\cdots\text{O}=\text{C}$ hydrogen bonds. α -Amino acid residues produce hydrogen bonds that are nearly parallel but alternate in direction, thus resulting in an apolar sheet, whereas β -residues produce nearly parallel hydrogen bonds that all point in the same direction, resulting in a polar sheet. Sheets containing both α - and β -residues have a mixture of polar and apolar

regions. The very poor solubility of peptide BHF4 is undoubtedly a consequence of the very strong intermolecular interactions present in the solid state. Attempts to construct β -hairpins with considerably higher strand lengths exclusively composed of β -Phe residues resulted in peptides with extremely poor solubility.

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1. Venkatraman, J., Shankaramma, S. C. & Balam, P. (2001) *Chem. Rev.* **101**, 3131–3152.
2. Karle, I. L. (1992) *Acta Crystallogr. B* **48**, 341–356.
3. Meyer, K. H. & Mark, H. (1928) *Ber. Deut. Chem. Ges.* **B61**, 1932–1936.
4. Astbury, W., T. & Street, A. (1932) *Philos. Trans. R. Soc. London A* **230**, 75–101.
5. Fraser, R. D. B. & Mac Rae, T. P. (1973) *Conformation in Fibrous Proteins* (Academic, New York), pp. 218–238.
6. Pauling, L. & Corey, R. B. (1953) *Proc. Natl. Acad. Sci. USA* **39**, 247–256.
7. Arnott, S., Dover, S. D. & Elliott, A. (1967) *J. Mol. Biol.* **30**, 201–208.
8. Fawcett, J. K., Camerman, N. & Camerman, A. (1975) *Acta Crystallogr. B* **31**, 658–665.
9. Marsh, R. E. & Glusker, J. P. (1961) *Acta Crystallogr.* **14**, 1110–1116.
10. Karle, I. L., Karle, J. Mastropaolo, D., Camerman, A. & Camerman, N. (1983) *Acta Crystallogr. B* **39**, 625–637.
11. Richardson, J. S. & Richardson, D. C. (1989) in *Principles and Patterns of Protein Conformation*, ed. Fasman, G. D. (Plenum, New York), pp. 1–98.
12. Awasthi, S. K., Ragothama, S. & Balam, P. (1995) *Biochem. Biophys. Res. Commun.* **216**, 375–381.
13. Haque, T. S., Little, J. C. & Gellman, S. H. (1996) *J. Am. Chem. Soc.* **118**, 6975–6985.
14. Haque, T. S. & Gellman, S. H. (1997) *J. Am. Chem. Soc.* **119**, 2303–2304.
15. Struthers, M. D., Cheng, R. P. & Imperiali, B. (1996) *Science* **271**, 342–345.
16. Karle, I. L., Awasthi, S. K. & Balam, P. (1996) *Proc. Natl. Acad. Sci. USA* **93**, 8189–8193.
17. Karle, I. L., Das, C. & Balam, P. (2000) *Proc. Natl. Acad. Sci. USA* **97**, 3034–3037. (First published March 21, 2000; 10.1073/pnas.070042697).
18. Das, C., Naganagowda, G. A., Karle, I. L. & Balam, P. (2001) *Biopolymers* **58**, 335–346.
19. Karle, I. L., Gopi, H. N. & Balam, P. (2001) *Proc. Natl. Acad. Sci. USA* **98**, 3716–3719. (First Published March 20, 2001; 10.1073/pnas.071050198)
20. Seebach, D., Overhand, M., Kuhnle, F. N. M., Martinoni, B., Oberer, L., Hommel, U. & Widmer, H. (1996) *Helv. Chim. Acta* **79**, 913–941.
21. Karle, J. & Karle, I. L. (1966) *Acta Crystallogr.* **21**, 849–859.
22. Krauthauer, S., Christianson, L. A., Powell, D. R. & Gellman, S. H. (1997) *J. Am. Chem. Soc.* **119**, 11719–11720.
23. Chung, Y. J., Christianson, L. A., Stanger, H. E., Powell, D. R. & Gellman, S. H. (1998) *J. Am. Chem. Soc.* **120**, 10555–10556.