Synthetic Peptide Helices in Crystals: Structure and Antiparallel and Skewed Packing Motifs for α-Helices in Two Isomeric Decapeptides

ISABELLA L. KARLE,[1] JUDITH L. FLIPPEN-ANDERSON,[1] K. UMA,[2] and P. BALARAM[2]

[1]Laboratory for the Structure of Matter, Naval Research Laboratory, Washington, DC 20375-5000, USA, and
[2]Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560-012, India

SYNOPSIS

The isomeric decapeptides Boc-Aib-Ala-Leu-Ala-Aib-Aib-Leu-Ala-Leu-Aib-OMe (II) and Boc-Aib-Ala-Aib-Ala-Leu-Ala-Leu-Aib-Leu-Aib-OMe (III), are predominantly α-helical with little effect on the conformation with interchange of Aib/Ala residues or Aib/Leu residues. The packing motif of helices in crystal II is antiparallel, whereas the helices pack in a skewed fashion in crystal III, with a 40° angle between neighboring helix axes. Crystal III contains a water molecule in a hydrophobic hole that forms hydrogen bonds with two carbonyl oxygens that also participate in 5 → 1 type hydrogen bonds. Values for helical torsional angles ϕ and ψ assume a much wider range than anticipated. Crystal II: $C_{49}H_{88}N_{10}O_{13}$, space group $P2_1$, with a = 16.625(2) Å, b = 9.811(5) Å, c = 18.412(3) Å, β = 99.79(1)°, Z = 2, R = 5.7% for 4338 data with $|F_0| > 3\sigma(F)$. Crystal III: $C_{49}H_{88}N_{10}O_{13} \cdot 1/2H_2O$, space group $P2_1$ with a = 11.072(2) Å, b = 34.663(5) Å, c = 16.446(3) Å, β = 107.85(1)°, Z = 4, R = 8.3% for 6087 data with $|F_0| > 3\sigma(F)$.

INTRODUCTION

The stabilization of helical peptide conformations by incorporation of α-aminoisobutyric acid (Aib) into peptide sequences is a well-established phenomenon.[1-5] Aib-containing sequences have therefore been chosen for the construction of stereochemically rigid helical segments in a modular approach to synthetic protein design.[5] The role of Aib content, positioning, and precise sequence effects are being systematically investigated by examining the solid state and solution conformations of synthetic oligopeptides with lengths of at least 9 residues or greater.[5-7] High-resolution x-ray diffraction analyses of helical peptides in crystals provide valuable information on helix packing,[4,8-10] solvation,[6,7,10] and conformational heterogeneity.[11,12] Studies of hydrophobic helix aggregation in crystals are also of relevance in developing structural models for transmembrane channels formed by peptide clusters in lipid bilayers.[1,7] In this report we present the crystal structures of the decapeptides II and III, and compare them to the related decapeptide I described earlier.[13]

I Boc-Aib-Ala-Leu-Ala-Leu-Aib-Leu-Ala-Leu-Aib-OMe
II Boc-Aib-Ala-Leu-Ala-Aib-Aib-Leu-Ala-Leu-Aib-OMe
III Boc-Aib-Ala-Aib-Ala-Leu-Ala-Leu-Aib-Leu-Aib-OMe

The five molecules involved—I occurring in two different crystal forms, II and III occurring with two independent molecules in the asymmetric unit of a single crystal—all form α-helices, either entirely or with a 3_{10}-helix type hydrogen bond at the N-terminus. The distribution of ϕ and ψ values is considerably wider than anticipated, and is definitely influenced by packing forces. Three different packing motifs for the α-helices of these closely related peptides are found in the four crystals: completely parallel, antiparallel, and skewed.

EXPERIMENTAL

The decapeptides were synthesized by conventional solution-phase procedures using a fragment con-

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Table I Diffraction Data for Boc-Aib-Ala-Leu-Ala-Aib-Aib-Leu-Ala-Leu-Aib-OMe (II) and Boc-Aib-Ala-Aib-Ala-Leu-Ala-Leu-Aib-Leu-Aib-OMe (III)

	II	III
Empirical formula	$C_{49}H_{88}N_{10}O_{13}$	$C_{49}H_{88}N_{10}O_{13} \cdot 1/2H_2O$
Color habit	Clear plate	Clear plate
Crystal size (mm)	0.6 × .40 × .16	.24 × .15 × .30
Space group	$P2_1$	$P2_1$
Cell parameters (Å)	a = 16.625 (2)	11.072 (2)
	b = 9.811 (5)	34.663 (5)
	c = 18.412 (3)	16.446 (3)
	β = 99.79 (1)°	107.85 (1)°
Volume (Å^3)	2959.4	6008.1
Z	2	4
Molecular weight	1025.2	1025.2 + 9.0
Density (g/cm^3)	1.150	1.143
$F(000)$	1112	2240
Radiation (Å)	$CuK_\alpha(\lambda = 1.54184)$	CuK_α
Temperature	−70°C	−70°C
Mounted in capillary	No	No
2θ range (deg)	1–110	1–110
Scan type	$2\theta - \theta$	$2\theta - \theta$
Scan speed	Variable	Variable
Index ranges h	−18–18	0–12
k	0–11	0–38
l	0–20	−18–18
Independent reflections	5026	9270
Observed reflections		
$[\|F_0\| > 3\sigma(F)]$	4338	6087
Final R indices	R = 5.7%	R = 8.3%
(observed data)	R_w = 6.7%	R_w = 9.2%
Goodness of fit	2.48	1.60
Data-to-parameter ratio	6.4 : 1	9.3 : 1
Largest difference peak (eÅ^{-3})	0.30	0.51
Largest difference hole (eÅ^{-3})	−0.26	−0.39

densation approach.[14] Crystals were grown by slow evaporation from $CH_3OH \cdot H_2O$ solutions.

Dry crystals of both II and III were cooled to −70°C during the x-ray data collection by a stream of cold nitrogen. It is interesting to note that for some of the apolar peptides, scattering at higher angles improves considerably at lowered temperatures, whereas in many other related peptides, the crystals shatter upon cooling. For each crystal, x-ray diffraction data were measured on an automated four-circle diffractometer with a graphite monochromator. Three reflections used as standards, monitored after every 97 measurements, remained constant within 3% in the two data sets. Pertinent diffraction data are listed in Table I.

The structures were solved by direct-phase determination. Full-matrix anisotropic least-squares refinement was performed on the C, N, and O atoms before hydrogen atoms were added in idealized positions and allowed to ride with the C or N atom to which each was bonded. Fractional coordinates for C, N, and O atoms in crystals II and III are listed in Tables II and III, respectively.* Conformational angles are listed in Table IV.

RESULTS AND DISCUSSION

The Peptide Molecules

Molecules II, IIIA, and IIIB form α-helices with seven 5 → 1 type hydrogen bonds for II and IIIA and six 5 → 1 type for IIIB. Additionally, the car-

* Supplementary material consisting of bond lengths, bond angles, anisotropic thermal parameters, and coordinates for hydrogen atoms will be deposited with the Cambridge Crystallographic Data File. Observed and calculated structure factors are available from ILK or JFA.

Table II Atomic Coordinates ($\times 10^4$) and Equivalent Isotropic Displacement Coefficients ($Å^2 \times 10^3$)

	x	y	z	U (eq)[a]		x	y	z	U (eq)[a]
C(1)	−4508 (3)	9223	9051 (3)	55 (2)	N(6)	−105 (2)	12548 (8)	6701 (2)	37 (1)
C(2)	−5253 (4)	8917 (14)	9333 (4)	111 (4)	C^{α}(6)	427 (3)	13718 (9)	6947 (3)	39 (2)
C(3)	−4079 (8)	10372 (15)	9440 (5)	172 (7)	C′(6)	1173 (3)	13204 (9)	7485 (3)	38 (2)
C(4)	−3928 (6)	8102 (14)	9202 (6)	154 (6)	O(6)	1871 (2)	13545 (8)	7433 (2)	56 (1)
O	−4729 (2)	9481 (9)	8289 (2)	79 (2)	C_1^{β}(6)	−46 (3)	14691 (9)	7360 (3)	50 (2)
C′(0)	−4222 (3)	9596 (9)	7806 (3)	49 (2)	C_2^{β}(6)	693 (3)	14424 (9)	6298 (3)	53 (2)
O(0)	−3488 (2)	9850 (8)	7971 (2)	55 (1)	N(7)	1011 (2)	12424 (8)	8038 (2)	38 (1)
N(1)	−4623 (2)	9461 (8)	7112 (3)	46 (2)	C^{α}(7)	1662 (3)	12076 (9)	8654 (3)	45 (2)
C^{α}(1)	−4198 (3)	9183 (9)	6495 (3)	47 (2)	C′(7)	2247 (3)	11009 (9)	8451 (3)	43 (2)
C′(1)	−3562 (3)	10328 (9)	6443 (3)	40 (2)	O(7)	2973 (2)	11058 (8)	8715 (2)	61 (2)
O(1)	−2863 (2)	10072 (8)	6363 (2)	49 (1)	C^{β}(7)	1296 (3)	11638 (10)	9314 (3)	57 (2)
C_1^{β}(1)	−4814 (3)	9223 (10)	5771 (3)	68 (2)	C^{γ}(7)	841 (3)	12770 (11)	9655 (3)	75 (3)
C_2^{β}(1)	−3772 (4)	7823 (9)	6576 (4)	72 (3)	C_1^{δ}(7)	1406 (5)	13826 (13)	10029 (5)	117 (4)
N(2)	−3840 (2)	11594 (8)	6471 (3)	41 (1)	C_2^{δ}(7)	352 (4)	12150 (14)	10208 (4)	112 (4)
C^{α}(2)	−3305 (3)	12713 (8)	6343 (3)	39 (2)	N(8)	1936 (2)	10026 (8)	7981 (2)	41 (1)
C′(2)	−2542 (3)	12795 (8)	6944 (3)	37 (2)	C^{α}(8)	2458 (3)	8969 (9)	7755 (3)	46 (2)
O(2)	−1895 (2)	13219 (8)	6795 (2)	49 (1)	C′(8)	3015 (3)	9507 (9)	7248 (3)	38 (2)
C^{β}(2)	−3766 (3)	14070 (9)	6297 (4)	59 (2)	O(8)	3639 (2)	8882 (8)	7179 (2)	55 (1)
N(3)	−2643 (2)	12477 (8)	7620 (2)	38 (1)	C^{β}(8)	1949 (3)	7803 (10)	7375 (4)	72 (3)
C^{α}(3)	−1944 (3)	12543 (9)	8225 (3)	44 (2)	N(9)	2779 (2)	10644 (8)	6869 (2)	37 (1)
C′(3)	−1334 (3)	11418 (9)	8183 (3)	40 (2)	C^{α}(9)	3272 (3)	11267 (8)	6371 (3)	38 (2)
O(3)	−599 (2)	11627 (8)	8353 (2)	51 (1)	C′(9)	3875 (3)	12326 (9)	6763 (3)	42 (2)
C^{β}(3)	−2186 (3)	12519 (10)	8978 (3)	63 (2)	O(9)	4486 (2)	12608 (8)	6495 (2)	57 (1)
C^{γ}(3)	−2767 (5)	13665 (12)	9129 (5)	104 (4)	C^{β}(9)	2739 (3)	11891 (9)	5705 (3)	45 (2)
C_1^{δ}(3)	−2545 (6)	15011 (12)	8857 (6)	145 (6)	C^{γ}(9)	2517 (4)	10933 (10)	5048 (3)	63 (2)
C_2^{δ}(3)	−2842 (6)	13629 (14)	9936 (5)	163 (6)	C_1^{δ}(9)	1938 (4)	11578 (11)	4442 (4)	82 (3)
N(4)	−1633 (2)	10184 (8)	7963 (2)	39 (1)	C_2^{δ}(9)	2193 (4)	9571 (10)	5239 (4)	76 (3)
C^{α}(4)	−1064 (3)	9075 (9)	7894 (3)	43 (2)	N(10)	3670 (3)	12961 (9)	7338 (3)	48 (2)
C′(4)	−538 (3)	9439 (9)	7327 (3)	40 (2)	C^{α}(10)	4114 (3)	14173 (10)	7655 (3)	60 (2)
O(4)	205 (2)	9252 (8)	7463 (2)	52 (1)	C′(10)	4209 (3)	15173 (10)	7063 (4)	63 (2)
C^{β}(4)	−1530 (3)	7765 (9)	7695 (3)	57 (2)	O(10)	4786 (3)	15891 (8)	7054 (3)	94 (2)
N(5)	−918 (2)	9955 (8)	6685 (2)	38 (1)	C_1^{β}(10)	3604 (4)	14832 (11)	8180 (4)	82 (3)
C^{α}(5)	−484 (3)	10364 (8)	6093 (2)	36 (2)	C_2^{β}(10)	4943 (4)	13734 (11)	8094 (4)	89 (3)
C′(5)	169 (3)	11450 (9)	6381 (3)	38 (2)	O(11)	3534 (3)	15244 (8)	6553 (3)	72 (2)
O(5)	882 (2)	11316 (8)	6321 (2)	51 (1)	C(11)	3562 (5)	16170 (11)	5960 (5)	100 (4)
C_1^{β}(5)	−79 (3)	9138 (9)	5790 (3)	52 (2)					
C_2^{β}(5)	−1118 (3)	11005 (9)	5476 (3)	49 (2)					

[a] Equivalent isotropic U defined as one third of the trace of the orthogonalized U_{ij} tensor.

bonyl oxygen of the Boc end group, O(O), forms 4 → 1 type hydrogen bonds in II and IIIB, but not in IIIA (Figures 1 and 2 and Table V). Peptide II differs from peptide I[13] only in the replacement of Leu(5) by Aib(5). The effect of such a replacement on the conformation is miniscule as can be seen from the superposition of the two molecules in Figure 3a. The rms deviation of the backbone atoms is only 0.12 Å. This deviation is less than differences found between two molecules of the same sequence that crystallize in different polymorphs[12,13] or two conformers crystallized in the same cell.[11]

Peptide III differs from peptide II by the interchange of Leu and Aib at residues 3 and 5, and the interchange of Ala and Aib at residues 6 and 8. Even with changes at four sites, the conformations of the two peptides are very similar (Figure 3b), with a rms deviation of only 0.26 Å for backbone atoms $C^{\alpha}(3)$ to $C^{\alpha}(9)$. The rms deviation for the same backbone atoms in II and IIIB is 0.25 Å. The conclusion that can be drawn from this experiment is that an Aib residue can readily replace an apolar residue or vice versa, without disturbing the α-helix.

The Boc-blocking group on the N-terminus in

Table III Atomic Coordinates ($\times 10^4$) and Equivalent Isotropic Displacement Coefficients ($Å^2 \times 10^3$)

	x	y	z	U (eq)[a]
Molecule A				
C(1)	6014 (13)	4755 (5)	6363 (10)	70 (5)
C(2)	7355 (12)	4842 (3)	6429 (6)	91 (6)
C(3)	6125 (12)	4571 (3)	7231 (6)	75 (5)
C(4)	5302 (14)	4509 (4)	5645 (7)	92 (6)
O	5237 (7)	5109 (3)	6375 (6)	58 (3)
C′(0)	4819 (10)	5325 (5)	5686 (8)	50 (4)
O(0)	5170 (7)	5316 (3)	5049 (6)	56 (3)
N(1)	3914 (7)	5571 (3)	5759 (6)	45 (3)
$C^{\alpha}(1)$	2997 (9)	5749 (4)	5062 (8)	43 (3)
C′(1)	3706 (9)	5990 (4)	4556 (8)	40 (4)
O(1)	3458 (6)	5952 (3)	3753 (5)	49 (3)
$C_1^{\beta}(1)$	2194 (10)	6018 (6)	5391 (9)	66 (5)
$C_2^{\beta}(1)$	2166 (11)	5445 (5)	4450 (9)	67 (5)
N(2)	4470 (7)	6263 (3)	4959 (6)	43 (3)
$C^{\alpha}(2)$	5000 (10)	6540 (4)	4521 (8)	47 (4)
C′(2)	5876 (8)	6353 (4)	4065 (7)	34 (4)
O(2)	5848 (6)	6439 (3)	3340 (5)	50 (3)
$C^{\beta}(2)$	5669 (11)	6870 (4)	5050 (8)	62 (5)
N(3)	6677 (7)	6085 (3)	4524 (6)	41 (3)
$C^{\alpha}(3)$	7562 (9)	5865 (4)	4175 (8)	40 (4)
C′(3)	6781 (9)	5663 (4)	3368 (8)	36 (4)
O(3)	7048 (6)	5676 (3)	2684 (5)	52 (3)
$C_1^{\beta}(3)$	8212 (11)	5563 (5)	4836 (8)	61 (5)
$C_2^{\beta}(3)$	8535 (10)	6127 (5)	3990 (10)	63 (5)
N(4)	5785 (8)	5443 (3)	3437 (6)	40 (3)
$C^{\alpha}(4)$	5069 (10)	5212 (5)	2681 (8)	51 (4)
C′(4)	4355 (9)	5465 (4)	1928 (8)	42 (4)
O(4)	4306 (6)	5371 (3)	1195 (5)	54 (3)
$C^{\beta}(4)$	4146 (13)	4961 (5)	2936 (10)	78 (6)
N(5)	3828 (7)	5794 (3)	2094 (6)	43 (3)
$C^{\alpha}(5)$	3182 (9)	6056 (4)	1402 (7)	44 (4)
C′(5)	4096 (9)	6217 (4)	952 (8)	41 (4)
O(5)	3779 (6)	6242 (3)	167 (5)	52 (3)
$C^{\beta}(5)$	2567 (9)	6384 (5)	1737 (8)	48 (4)
$C^{\gamma}(5)$	1380 (9)	6263 (5)	1963 (9)	60 (5)
$C_1^{\delta}(5)$	1085 (12)	6548 (6)	2572 (11)	89 (7)
$C_2^{\delta}(5)$	233 (10)	6254 (7)	1118 (10)	85 (6)
N(6)	5234 (7)	6327 (3)	1421 (6)	39 (3)
$C^{\alpha}(6)$	6168 (9)	6480 (4)	1050 (8)	42 (4)
C′(6)	6600 (8)	6177 (4)	566 (7)	35 (4)
O(6)	6747 (6)	6257 (3)	−158 (5)	46 (3)
$C^{\beta}(6)$	7287 (9)	6653 (5)	1741 (8)	51 (4)
N(7)	6828 (7)	5822 (3)	904 (6)	39 (3)
$C^{\alpha}(7)$	7241 (9)	5512 (4)	453 (8)	41 (4)
C′(7)	6217 (11)	5421 (4)	−419 (9)	46 (4)
O(7)	6499 (6)	5354 (3)	−1058 (5)	58 (3)
$C^{\beta}(7)$	7602 (10)	5166 (4)	1043 (9)	55 (5)
$C^{\gamma}(7)$	8820 (11)	5225 (5)	1781 (9)	61 (5)
$C_1^{\delta}(7)$	9968 (12)	5197 (6)	1482 (11)	94 (7)
$C_2^{\delta}(7)$	8848 (14)	4891 (6)	2436 (11)	97 (7)
N(8)	5005 (7)	5417 (3)	−374 (6)	41 (3)
$C^{\alpha}(8)$	3905 (9)	5325 (5)	−1130 (8)	46 (4)
C′(8)	3871 (9)	5613 (4)	−1852 (8)	41 (4)
O(8)	3420 (6)	5533 (3)	−2612 (5)	53 (3)
$C_1^{\beta}(8)$	3959 (11)	4920 (5)	−1441 (9)	65 (5)
$C_2^{\beta}(8)$	2714 (10)	5384 (5)	−890 (8)	58 (5)
N(9)	4236 (7)	5983 (3)	−1607 (6)	40 (3)
$C^{\alpha}(9)$	4157 (9)	6273 (5)	−2218 (8)	48 (4)
C′(9)	5315 (10)	6320 (4)	−2522 (8)	47 (4)
O(9)	5223 (7)	6451 (3)	−3230 (5)	58 (3)
$C^{\beta}(9)$	3811 (11)	6684 (5)	−1903 (11)	74 (6)
$C^{\gamma}(9)$	2471 (12)	6689 (7)	−1853 (13)	100 (8)
$C_1^{\delta}(9)$	1555 (14)	6607 (13)	−2540 (17)	303 (25)
$C_2^{\delta}(9)$	2260 (17)	7061 (8)	−1467 (16)	153 (12)
N(10)	6431 (8)	6209 (4)	−1967 (7)	53 (4)
$C^{\alpha}(10)$	7587 (10)	6181 (5)	−2209 (9)	53 (5)
C′(10)	7867 (11)	6558 (7)	−2617 (11)	78 (7)
O(10)	8225 (9)	6551 (6)	−3250 (8)	124 (6)
$C_1^{\beta}(10)$	8681 (11)	6094 (6)	−1426 (10)	78 (6)
$C_2^{\beta}(10)$	7531 (15)	5859 (7)	−2771 (12)	107 (8)
O(11)	7777 (9)	6853 (4)	−2135 (9)	95 (5)
C(11)	8248 (15)	7207 (6)	−2553 (17)	170 (13)
Molecule B				
C(1)	9495 (13)	7419 (6)	4469 (9)	83 (6)
C(2)	8893 (13)	7635 (3)	5082 (6)	123 (8)
C(3)	8498 (13)	7280 (3)	3717 (6)	98 (6)
C(4)	10316 (18)	7096 (4)	4941 (9)	136 (8)
O	10187 (8)	7701 (3)	4096 (6)	67 (3)
C′(0)	11225 (11)	7871 (4)	4565 (9)	53 (4)
O(0)	11657 (8)	7865 (3)	5353 (5)	63 (3)
N(1)	11822 (8)	8053 (3)	4083 (5)	53 (3)
$C^{\alpha}(1)$	13149 (11)	8172 (4)	4390 (9)	54 (4)
C′(1)	13406 (10)	8419 (4)	5187 (8)	44 (4)
O(1)	14244 (6)	8362 (3)	5846 (5)	58 (3)
$C_1^{\beta}(1)$	13481 (11)	8403 (4)	3722 (8)	63 (5)
$C_2^{\beta}(1)$	14019 (13)	7809 (5)	4608 (9)	79 (6)
N(2)	12585 (7)	8727 (3)	5144 (6)	43 (3)
$C^{\alpha}(2)$	12909 (11)	9012 (4)	5841 (8)	57 (5)
C′(2)	12935 (10)	8840 (5)	6703 (9)	52 (4)
O(2)	13685 (6)	8940 (3)	7325 (6)	54 (3)
$C^{\beta}(2)$	11983 (12)	9349 (5)	5634 (9)	77 (6)
N(3)	12004 (8)	8577 (4)	6670 (7)	53 (4)
$C^{\alpha}(3)$	11918 (10)	8380 (5)	7440 (8)	51 (4)
C′(3)	13163 (10)	8172 (4)	7855 (8)	46 (4)
O(3)	13688 (6)	8190 (3)	8652 (6)	57 (3)
$C_1^{\beta}(3)$	10888 (11)	8082 (5)	7193 (10)	65 (5)
$C_2^{\beta}(3)$	11618 (11)	8677 (5)	8062 (9)	69 (5)
N(4)	13624 (8)	7966 (3)	7346 (7)	47 (3)
$C^{\alpha}(4)$	14741 (9)	7736 (4)	7672 (8)	46 (4)
C′(4)	15925 (9)	7981 (4)	8078 (8)	44 (4)
O(4)	16731 (7)	7881 (3)	8737 (6)	60 (3)
$C^{\beta}(4)$	14916 (11)	7503 (28)	6946 (7)	60 (5)
N(5)	16056 (8)	8300 (3)	7671 (7)	45 (3)
$C^{\alpha}(5)$	17125 (9)	8563 (4)	8016 (8)	42 (4)
C′(5)	17047 (11)	8737 (4)	8836 (9)	49 (4)

Table III *(Continued from the previous page.)*

	x	y	z	U (eq)[a]		x	y	z	U (eq)[a]
O(5)	18038 (6)	8769 (3)	9454 (6)	56 (3)	O(8)	20951 (8)	8060 (4)	12199 (6)	84 (4)
$C^{\beta}(5)$	17131 (10)	8882 (5)	7387 (9)	55 (4)	$C_1^{\beta}(8)$	19706 (11)	7907 (5)	10152 (9)	66 (5)
$C^{\gamma}(5)$	17832 (12)	8843 (7)	6757 (11)	85 (7)	$C_2^{\beta}(8)$	19436 (15)	7452 (23)	11309 (11)	88 (9)
$C_1^{\delta}(5)$	17909 (15)	9192 (8)	6262 (12)	129 (10)	N(9)	19703 (8)	8544 (4)	11446 (7)	49 (4)
$C_2^{\delta}(5)$	17317 (13)	8489 (7)	6150 (11)	98 (8)	$C^{\alpha}(9)$	20427 (10)	8845 (5)	11978 (8)	60 (5)
N(6)	15947 (8)	8885 (4)	8866 (7)	50 (4)	C'(9)	20106 (11)	8904 (4)	12799 (8)	44 (4)
$C^{\alpha}(6)$	15875 (11)	9073 (5)	9631 (9)	60 (5)	O(9)	20921 (7)	9014 (3)	13457 (6)	51(3)
C'(6)	16205 (10)	8790 (6)	10383 (10)	63 (5)	$C^{\beta}(9)$	20255 (15)	9222 (6)	11478 (9)	112 (8)
O(6)	16865 (7)	8892 (4)	11106 (6)	73 (3)	$C^{\gamma}(9)$	20851 (24)	9156 (15)	10585 (22)	247 (21)
$C^{\beta}(6)$	14559 (12)	9256 (6)	9475 (11)	100 (7)	$C_1^{\delta}(9)$	20442 (30)	9642 (15)	10310 (26)	602 (52)
N(7)	15663 (8)	8443 (5)	10248 (7)	63 (4)	$C_2^{\delta}(9)$	22144 (32)	9004 (13)	10871 (14)	440 (42)
$C^{\alpha}(7)$	15883 (11)	8179 (6)	10919 (10)	84 (7)	N(10)	18895 (9)	8852 (4)	12725 (7)	54 (4)
C'(7)	17292 (13)	8036 (5)	11293 (9)	73 (6)	$C^{\alpha}(10)$	18363 (11)	8926 (5)	13422 (9)	63 (5)
O(7)	17793 (8)	8000 (4)	12062 (6)	98 (4)	C'(10)	18748 (11)	9331 (6)	13796 (10)	66 (6)
$C^{\beta}(7)$	14972 (14)	7821 (6)	10695 (11)	103 (8)	O(10)	18886 (9)	9416 (4)	14552 (7)	97 (5)
$C^{\gamma}(7)$	14592 (28)	7623 (10)	11236 (20)	267 (21)	$C_1^{\beta}(10)$	18900 (13)	8644 (5)	14154 (10)	78 (6)
$C_1^{\delta}(7)$	14479 (19)	7763 (8)	12038 (15)	182 (15)	$C_2^{\beta}(10)$	16943 (10)	8906 (7)	13088 (10)	79 (6)
$C_2^{\delta}(7)$	13737 (21)	7276 (9)	10932 (24)	182 (16)	O(11)	18819 (8)	9596 (4)	13213 (7)	78 (4)
N(8)	17907 (9)	7984 (4)	10702 (7)	59 (4)	C(11)	19139 (15)	9960 (5)	13526 (12)	102 (7)
$C^{\alpha}(8)$	19248 (12)	7880 (5)	10931 (9)	64 (5)	W(1)	6551 (18)	7133 (7)	9565 (13)	211 (11)
C'(8)	20042 (11)	8164 (6)	11603 (9)	65 (6)					

[a] Equivalent isotropic U defined as one third of the trace of the orthogonalized U_{ij} tensor.

molecules II, IIIA, and IIIB is almost fully extended with torsional angles about the C-O bond of −169°, −173°, and −175°, and torsional angles about the O-C'(O) bond of 163°, 166°, and 168°, respectively. In all three peptides, the helix reverses from right-handed to left-handed at the esterified Aib residue at the C-terminus where the ϕ torsional angles are +49, +53, and +51, and the ψ torsional angles are +40, +49, and +35, respectively, for II, IIIA, and IIIB. The geometry of the Aib residues in the right-handed portions of the helices shows consistent differences in bond angles between those involving the methyl group in the L-position (that is, the same configuration as in L-amino acids) and those with the methyl in the D-position. Average values for the six angles about the C^{α} atom in the nine Aib residues are $NC^{\alpha}C'$, 109.2°; $NC^{\alpha}C^{\beta}$(L), 108.8°; $NC^{\alpha}C^{\beta}$(D), 110.8°; $C'C^{\alpha}C^{\beta}$(L), 107.9°; $C'C^{\alpha}C^{\beta}$(D), 109.8°; C^{β}(L)$C^{\alpha}C^{\beta}$(D), 110.1°. Six of the nine leucyl side chains have conformations (Table IV) near the preferred conformation g^+, (tg^+).[16,17] Two other Leu residues have χ^1 values at −90° and one at −150°.

Conformational Angles in 5 → 1 Helices

Although the apolar decapeptides, reported in this paper and elsewhere, are totally or predominantly α-helical, there is a considerable spread among the values of the ϕ and ψ angles (torsions about $N\text{-}C^{\alpha}$ and $C^{\alpha}\text{-}C'$ bonds[15]). Data for the plots of the distributions of ϕ, ψ, and ω, shown in Figure 4, are taken from nine decapeptides (Refs. 11–13 and present paper), an 11-residue peptide[2] and a 13-residue peptide.[5] All of these structures are well refined (5–8% R values) at high resolution (0.89–1.00 Å), so that the ESDs are in the range of 0.5°–1.0°. The first residue has been omitted in the plots since often it is involved in a 4 → 1 hydrogen bond rather than a 5 → 1 type. Also, the last residue has been omitted and sometimes the penultimate residue because of an occasional helix reversal. The torsional values for those Aib residues occurring in the body of the α-helix are plotted separately from the values for Ala, Val, Leu, Ile, and Glu. All the helices are right handed.

The ω values (torsion about $C'_i\text{-}N_{i+1}$) have a median value of −179° with more than 90% of the observations within ± 6° of −179°. The 26 Aib residues have well-defined ϕ values falling between −50° and −59°; however, the spread for the ψ values, −36° to −54° and one outlier at −28°, is more diffuse.

For the Ala, Val, Leu, Ile, and Glu residues, ϕ values occur from −53° to −90°, with more clustering (75%) between −58° and −71°. The distribution

Table IV Torsion Angles[a,b]

Residue	ϕ	ψ	ω	χ^1	χ^2
		Peptide II			
Aib(1)	−58[c]	−48	−173		
Ala(2)	−65	−35	−179		
Leu(3)	−71	−38	178	−58	−43, −171
Ala(4)	−61	−47	179		
Aib(5)	−57	−54	−175		
Aib(6)	−56	−51	−169		
Leu(7)	−75	−36	180	−66	−79, 168
Ala(8)	−71	−23	179		
Leu(9)	−91	−28	−167	−90	175, 50
Aib(10)	+49	+40[d]	180[e]		
		Peptide IIIA			
Aib(1)	−59[c]	−59	−169		
Ala(2)	−63	−44	178		
Aib(3)	−57	−52	−174		
Ala(4)	−66	−37	177		
Leu(5)	−63	−43	179	−73	159, −81
Ala(6)	−67	−42	179		
Leu(7)	−62	−42	−178	−70	−75, 166
Aib(8)	−58	−36	−176		
Leu(9)	−89	−27	171	−68	−57, 174
Aib(10)	+53	+49[d]	173[e]		
		Peptide IIIB			
Aib(1)	−54[c]	−50	−170		
Ala(2)	−64	−39	179		
Aib(3)	−57	−47	−176		
Ala(4)	−65	−42	−178		
Leu(5)	−65	−48	−176	−91	−172, 60
Ala(6)	−62	−46	−177		
Leu(7)	−64	−37	175	−150	26, −178
Aib(8)	−53	−43	−175		
Leu(9)	−81	−36	−175	−62	−179, −52
Aib(10)	+51	+35[d]	179[e]		

[a] The torsion angles for rotation about bonds of the peptide backbone (ϕ, ψ, and ω) and about bonds of the amino acid side chains (χ^1, χ^2) are described in Ref. 15.
[b] ESDs ~ 1.0°.
[c] C′(0), N(1), C$^\alpha$(1), C′(1).
[d] N(10), C$^\alpha$(10), C′(10), O(OMe).
[e] C$^\alpha$(10), C′(10), O(OMe), C(OMe).

of ψ values, −17° to −49° and one outlier at −59°, is equally diffuse, with some concentration (75%) between −35° and −49°. The ϕ,ψ plot[18] of the same data points shows overlap of the Aib residues (+) with the other apolar residues (·) on the right side (Figure 5). Although the points representing the ϕ and ψ values for Ala, Val, Leu, Ile, and Glu residues cluster near ϕ = −65°, ψ = −41° (idealized values for α-helices[19]), there is a wide range in the values actually observed. The spread of ϕ and ψ values for Aib residues is much smaller, with a median near ϕ = −56° and ψ = −44° (compared to theoretically computed values of ϕ = −57° and ψ = −47°[20,21]). The comparative rigidity of the Aib residue is consistent with its propensity for helix formation.

The 65 5 → 1 hydrogen bonds have a continuous distribution of N···O lengths from 2.9 to 3.3 Å. If the N-H distance is fixed at 0.96 Å, with the H atoms placed on the N atoms in idealized positions, the H···O distances range from 2.0 to 2.5 Å, with the larger concentrations between 2.0 and 2.2 Å. The wide range of N···O distances is consistent with the irregularities in the α-helices, as indicated by the spread of the ϕ and ψ values. The distribution of values for the C=O···N angle is approximately Gaussian, from 146° to 169°, with a median at 156°.

Some interesting features emerge from a limited comparison of peptides I–III, all of which contain only three types of residues, viz., Aib, Ala, and Leu, and have Aib residues at both N- and C-terminii. Peptides II and III are isomeric, and have an Aib content of 40%. Peptide I[13] differs from II solely by replacement of the Aib residue at position 5 by Leu and has a total Aib content of 30%. In all three cases the largest deviation in ϕ,ψ torsion angles from ideal helical values are observed for the Leu(9) residue: IA, −91°, −31°; IB, −100°, 5°; II, −91°, −28°; IIIA, −89°, −27°; IIIB, −81°, −36°. The 5 → 1 hydrogen bond between Leu(9)NH and the CO of residue 5 is also appreciably weaker in most cases with the following N9···O5 distances: IA, 3.285 Å; IB, 3.056 Å; II, 3.212 Å; IIIA, 3.237 Å; IIIB, 3.322 Å. The Leu residues in these peptides also appear to yield ϕ,ψ values significantly scattered from idealized helical values. Using a total of 17 Leu, 15 Ala, and 13 Aib residues in peptides I–III, the following averaged ϕ,ψ values were obtained: Aib, ϕ = −56.6 ± 3.2°, ψ = −48° + 6.6°; Ala, ϕ = −66° ± 6.7°, ψ = −35.9° ± 10.9°; Leu, ϕ = −74.9° ± 13.4°, ψ = −36.4° ± 11.3°. In all cases the C-terminal Aib residue [Aib(10)] was excluded from the analysis. The crystal structure of a decapeptide Boc-Aib-(Ala-Leu-Aib)$_3$-OMe, which is isomeric with II and III, is also available.[7] However, this was not used for comparison because of backbone hydration and attendant distortion of the helical conformation. It is noteworthy that helix hydration in that sequence is accompanied by significant conformational distortion at the Leu(3) residue.[7] It is likely that the observed deviations in backbone torsion angles at Leu residues, particularly ϕ values, are a consequence of packing constraints involved in accommodating the bulky isobutyl side chains in helical aggregates.

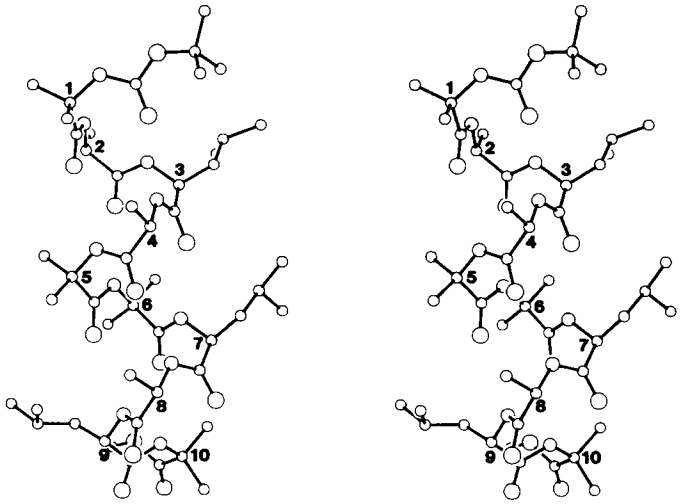


Figure 1. Stereodiagram of peptide II drawn with the experimentally determined coordinates. C^{α} atoms are labeled 1–10.

Aggregation of Peptides in Crystals

Peptides I–III, as, generally, all apolar helical peptides with 7 or 8 or more residues, form continuous columns by head-to-tail NH · · · OC hydrogen bonding. The hydrogen-bond lengths for II and III are listed in Table V. The N(3)H moiety in molecule IIIA does not participate in any hydrogen bonding. The peptide columns are straight in space group P1, and may be somewhat zigzag in space groups $P2_1$ and $P2_12_12_1$ where the molecules in a column are related by a twofold screw.

The columns of peptides assemble into sheets with *all* the helix axes pointed in the same, or nearly the same, direction (Figure 6). If there are two conformers in the same crystal, each conformer assembles into separate sheets, each with parallel packing of helices (Refs. 10 and 11; present paper). The column and sheet motif appear to be common to all the helical peptides studied so far.

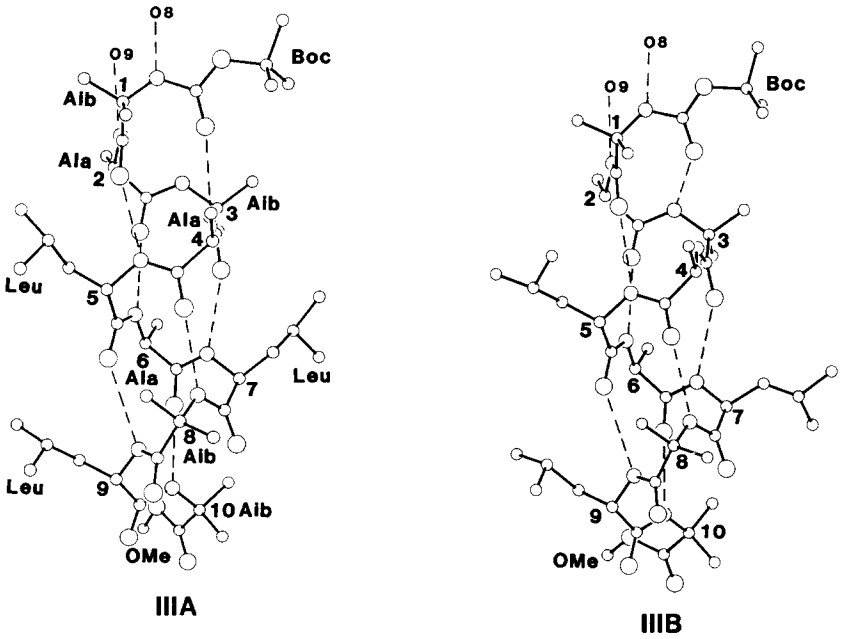


Figure 2. Diagrams of independent peptide molecules IIIA and IIIB coexisting in the same cell. Hydrogen bonds are indicated by dashed lines.

Table V Hydrogen Bonds[a]

Type	Donor	Acceptor	N···O, Å	H···O,[b] Å	Angle (deg) C=O···N
		Peptide II			
Head to tail	N(1)	O(8)[c]	2.968	2.19	139
	N(2)	O(9)[c]	2.964	2.00	140
3_{10}-Helix (4 → 1)	N(3)	O(0)	3.056	2.40	126
α-Helix (5 → 1)	N(4)	O(0)	3.104	2.33	165
	N(5)	O(1)	3.188	2.53	160
	N(6)	O(2)	3.081	2.31	146
	N(7)	O(3)	2.939	1.99	153
	N(8)	O(4)	2.972	1.95	156
	N(9)	O(5)	3.212	2.26	156
	N(10)	O(6)	3.079	2.37	153
		Peptide IIIA			
Head to tail	N(1)	O(8)[d]	2.898	2.00	143
	N(2)	O(9)[d]	2.910	1.97	144
	N(3)	None			
α-Helix (5 → 1)	N(4)	O(0)	2.965	2.02	169
	N(5)	O(1)	2.932	2.00	160
	N(6)	O(2)	3.042	2.00	156
	N(7)	O(3)	2.907	1.98	161
	N(8)	O(4)	2.918	2.02	155
	N(9)	O(5)	3.237	2.44	148
	N(10)	O(6)	2.893	1.99	159
Water to peptide	W(1)	O(6)	3.066		
		Peptide IIIB			
Head to tail	N(1)	O(8)[e]	2.950	2.06	140
	N(2)	O(9)[e]	2.991	2.14	142
3_{10}-Helix (4 → 1)	N(3)	O(0)	*3.228*	*2.67*	128
	N(4)	O(1)	3.078	2.53	120
α-Helix (5 → 1)	N(5)	O(1)	3.064	2.13	169
	N(6)	O(2)	2.973	2.03	158
	N(7)	O(3)	2.989	2.05	155
	N(8)	O(4)	3.112	2.20	148
	N(9)	O(5)	*3.322*	2.46	150
	N(10)	O(6)	2.914	2.07	157
Water to peptide	W(1)	O(4)[f]	2.966		

[a] Carbonyl oxygens O(7) and O(10) do not participate in hydrogen bonding in peptides II, IIIA, and IIIB.
[b] The H atoms were placed in idealized positions with the N-H distance equal to 0.96 Å.
[c] Symmetry equivalent $-1 + x, y, z$ to coordinates listed in Table II.
[d] Symmetry equivalent $x, y, 1 + z$ to coordinates listed in Table III.
[e] Symmetry equivalent $-1 + x, y, -1 + z$ to coordinates listed in Table III.
[f] Symmetry equivalent $-1 + x, y, z$ to coordinates listed in Table III.

The differences in aggregation of peptides are the manner in which sheets assemble: parallel, antiparallel, and skewed. Peptides I–III exhibit all three modes of assembling sheets. The sheets in peptide I assemble in the parallel mode, with all helix axes pointed in the same way, in one polymorph and in the antiparallel mode in another polymorph (Figures 3–6 in Ref. 13). Peptide II assembles with sheets in the antiparallel mode (Figure 7), and peptide III containing the two independent conformers in separate sheets assembles with the direction of the helices skewed ~ 40° with respect to each other [Fig-

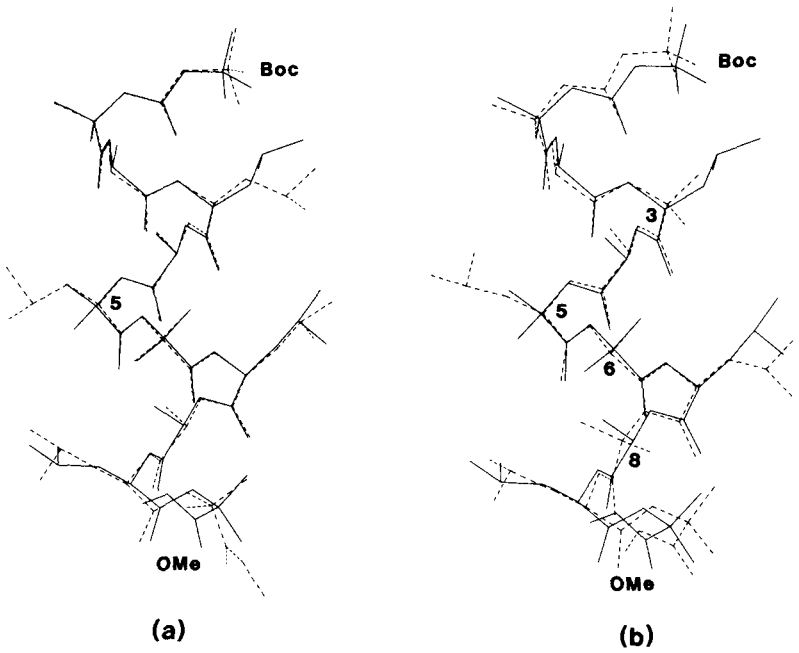


Figure 3. (a) Superposition of peptide I (dashed line) and peptide II (solid line). The two peptides differ at residue 5 where Leu has been replaced by Aib in II. (b) Superposition of peptide II (solid line) with peptide IIIB (dashed line). The two peptides differ at residues 3 and 5 where Leu and Aib have been interchanged and at residues 6 and 8 where Ala and Aib have been interchanged.

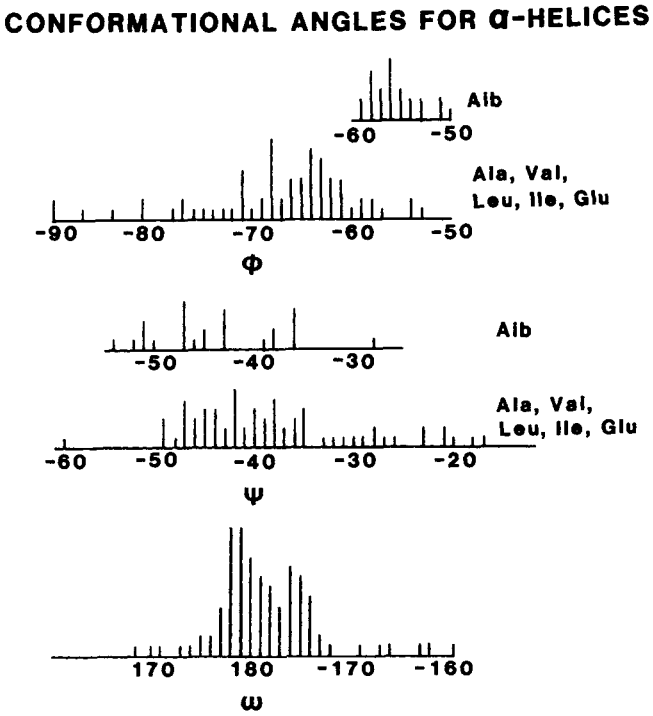


Figure 4. Conformational angles for α-helices as determined for eleven 10–13-residue peptides by crystal structure analyses at high resolution, 0.9 Å. Values for the first and last residues were omitted because of possible end effects. Values of ϕ and ψ for the Aib residues have been plotted separately from the other hydrophobic residues.

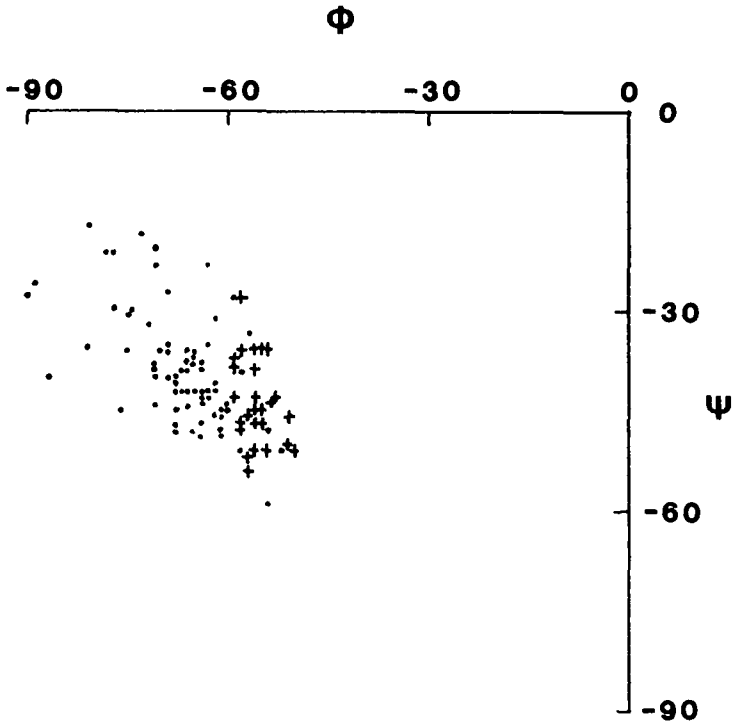


Figure 5. The same data as in Figure 4 is shown plotted on a ϕ,ψ map. Values from Aib residues are shown as a plus, while values from other hydrophobic residues are shown as a dot.

ure 8(a)]. Although there are many examples of skewed packing of helices in proteins[22] there are very few among peptides. Melittin is one such example.[23]

Figure 8(b), projection down the x axis, shows the skewed sheet structure in crystal III with an edge-on view of each of four sheets composed of molecules IIIB, IIIA, IIIB′, and IIIA′, respectively, where B′ and A′ are related to its neighbors by a twofold screw operation. Each sheet is turned by 40° with respect to its neighbors. However, in adjacent sheets B and A, as well as B′ and A′, the helical axes are skewed 40° from an antiparallel arrangement, whereas in adjacent sheets A and B′, as well as A′ and B, the helical axes are skewed 40° from a parallel arrangement. Chothia et al., in their paper on the skewed packing of helices,[22] discussed the optimum fitting of the ridges and grooves of adjacent helices. In the present crystal, molecules in adjacent sheets A and B′ (closer to the parallel arrangement) have ridges fitting into grooves, whereas in adjacent sheets A and B (closer to the antiparallel arrangement), ridges come into contact with ridges. This difference in the efficiency of fitting of helices in adjacent sheets is reflected by the shorter distance between helix axes in sheets A and B′, 8.4 Å, as compared to 8.9 Å between helix axes in sheets A and B (measured along the y direction).

Furthermore, the inefficient packing of ridge/ridge and groove/groove between sheets A and B leaves enough space in the groove/groove area for the insertion of a water molecule W. The water molecule forms hydrogen bonds with carbonyl oxygens O(6) in molecule A, 3.066 Å, and O(4) in molecule B, 2.966 Å, that is, with carbonyl oxygens that also participate in α-helix hydrogen bonding. Similar occurrences of water molecules in close proximity to hydrophobic helices have been observed elsewhere.[5]

In the other view of the skewed packing in crystal III, shown in Figure 8(a), the projection is along the z axis and coincides with an end-on view of the helix for A and A′.

CONCLUSIONS

The crystal structures of peptides II and III, together with the earlier reported structure of I,[13] establish that stable helical conformations can be maintained in decapeptides containing intervening triads and

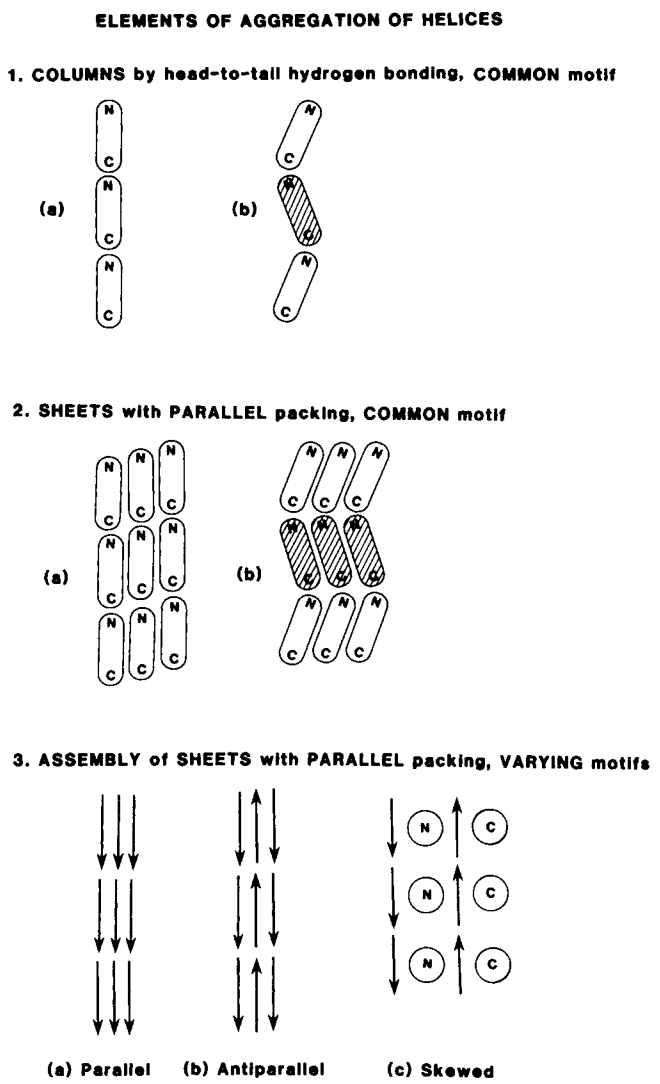
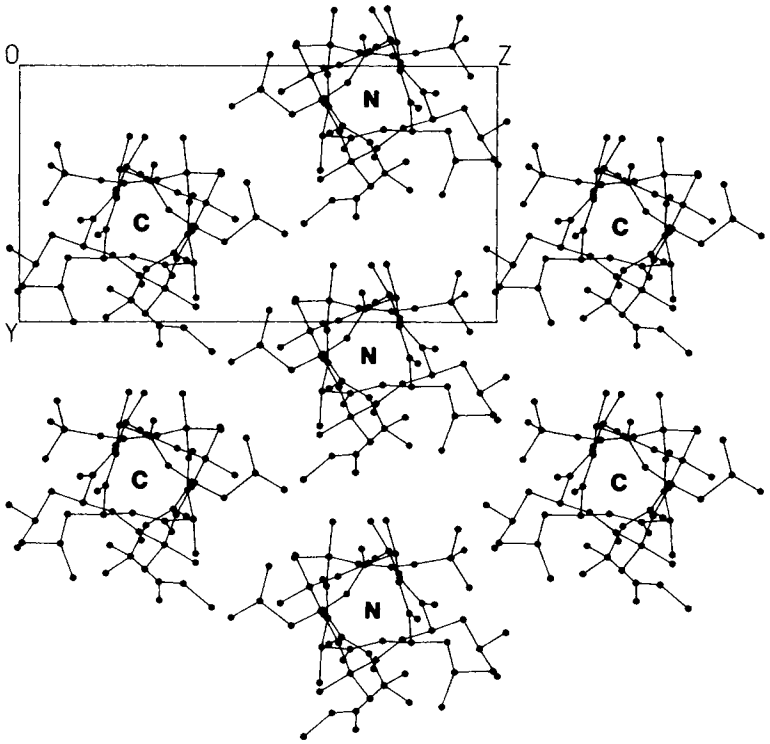
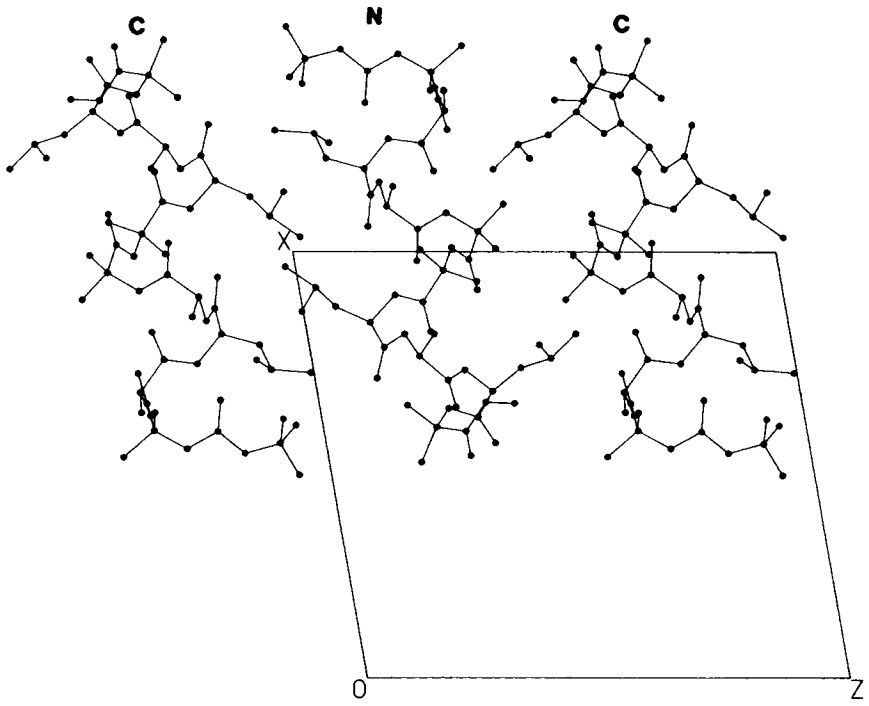


Figure 6. A schematic description of the assembly of helical hydrophobic peptides. All peptides form long columns by head-to-tail hydrogen bonding: (1a) straight column by repetition as in space group P1; (1b) slightly zig zag column by a twofold screw operation as in $P2_1$ or $P2_12_12_1$. In all peptides the columns appear to assemble into sheets with all helix axes pointing in the same direction: (2a) parallel columns may be displaced in vertical direction as in P1; (2b) slightly zig zag columns (from 1b) assemble by repetition, as in $P2_1$ with twofold screw axis vertical. Large differences occur in the assembly of the sheets: (3a) sheets repeat in a *parallel* fashion for an all parallel packing as in P1 or $P2_1$ with a vertical twofold screw axis; (3b) sheets assemble in an antiparallel mode as in $P2_1$ with a horizontal twofold screw axis or P1 with two independent peptide molecules;[11] (3c) neighboring sheets are rotated with respect to each other as in $P2_1$ with two independent molecules per asymmetric unit and a horizontal twofold screw axis.

tetrads of non-Aib residues. The precise positioning of Aib residues in these cases is without effect on the overall helical nature of the backbone. The structures of II and III add to the growing body of information on helix packing in crystals, and in the case of III, provide a rare example, in peptides, of skewed arrangement of helices. The helical conformations of diverse sequences containing 15–50% Aib residues, suggest that stable helical segments can be reproducibly constructed, providing an important element in a modular approach to synthetic protein design.



(a)



(b)

Figure 7. Antiparallel packing of helices in peptide II. (a) View down the x axis shows characteristic hexagonal array with sheets of parallel helices assembled in an antiparallel mode. N and C indicate the amino or carboxyl terminus at the top of each helix. (b) View down the y axis.

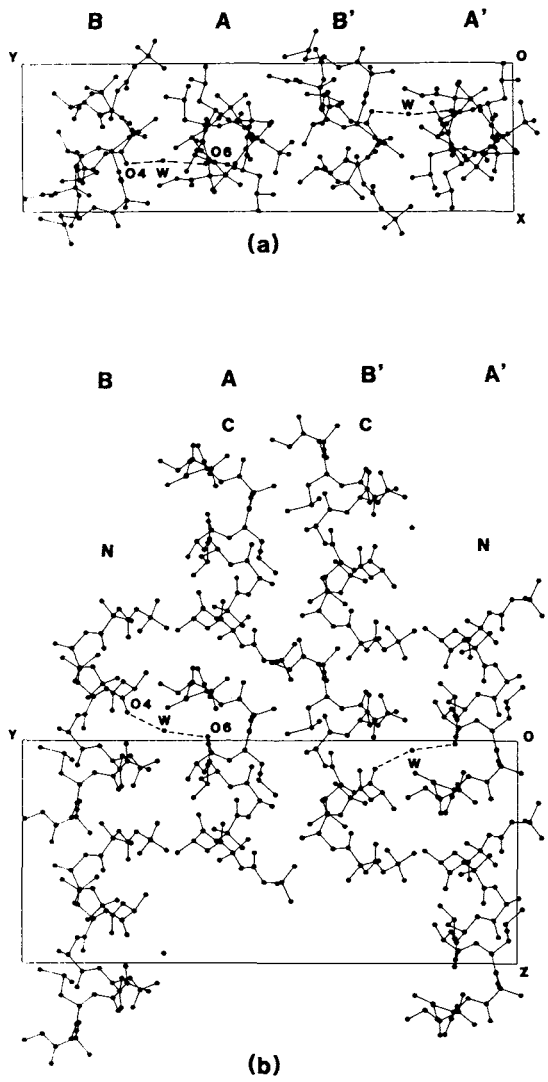


Figure 8. Skewed packing of helices in peptide III with two independent molecules, A and B, per asymmetric unit. (a) View down the *z* axis directly into the helix of A and symmetry equivalent A′ (by a twofold screw along the *y* axis). Helix B is rotated ~40° with respect to A. (b) View down the *x* axis, edge-on to sheets of parallel helices where each sheet contains only one type of helix, A, A′, B, or B′. Water molecules W are inserted between helices where the skewed helices meet groove to groove, rather than ridge to groove.

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