Stereochemistry of α-Aminoisobutyric Acid Peptides in Solution: Helical Conformations of Protected Decapeptides with Repeating Aib-L-Ala and Aib-L-Val Sequences

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Synopsis

The decapeptides Boc-(Aib-L-Ala)$_5$-OMe and Boc-(Aib-L-Val)$_5$-OMe have been studied by 270-MHz ^{1}H-nmr in $CDCl_3$ and $(CD_3)_2SO$ solutions. Intramolecular hydrogen-bonded NH groups have been delineated using the temperature and solvent dependence of the NH chemical shifts and differential broadening of the NH resonances, induced by addition of a nitroxide radical. Both peptides have eight solvent-shielded NH groups, suggesting that 3_{10}-helical conformations are maintained in the two solvents. In alternating Aib-X sequences, the Aib residues appear to play a dominant role in determining the preferred conformations, overriding the intrinsic stereochemical preferences of the X residues.

INTRODUCTION

The ability of α-aminoisobutyric acid (Aib) residues to promote helical folding in peptides is well documented.[1-5] There is, however, some doubt as to whether Aib residues favor 3_{10}- or α-helical conformations.[6-8] The two structures differ only slightly in the values of backbone dihedral angles (α_R-helix: $\phi \sim -55°$, $\psi \sim -45°$; 3_{10R}-helix: $\phi \sim -60°$, $\psi \sim -30°$) but are easily distinguishable on the basis of their intramolecular hydrogen-bonding patterns (α-helix $5 \rightarrow 1$, C_{13}, 3_{10}-helix $4 \rightarrow 1$, C_{10}).[9] While crystal structures of several Aib-containing peptides have yielded 3_{10}-helical conformations,[2,3,10-16] two recent structures of Boc-Ala-(Aib-Ala)$_2$-Glu(OBZ)-(Ala-Aib)$_2$-Ala-OMe[8] and Boc-Aib-Pro-Val-Aib-Val-OMe[11,17] have provided evidence for $5 \rightarrow 1$ (α-helical) hydrogen-bonding schemes. ^{1}H-nmr studies of Aib-containing fragments of alamethicin[18,19] and suzukacillin[20-22] favor 3_{10}-helical structures for several peptides. In order to determine whether specific sequence effects can modulate the nature of the helical conformations obtained, we have initiated a systematic investigation of the structural properties of sequences having Aib at alternate positions in a chain. The present report describes nmr studies on the decapeptides Boc-(Aib-X)$_5$-OMe, where X = L-Ala or L-Val. The choice of the X residue

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Biopolymers, Vol. 22, 2133–2140 (1983)
 CCC 0006-3525/83/092133-08$01.80

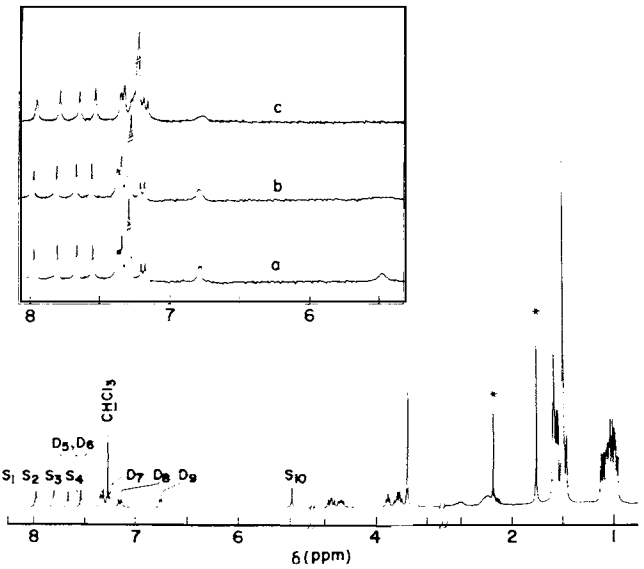

Fig. 1. The 270-MHz ^{1}H-nmr spectrum of Boc-(Aib-Val)$_5$-OMe in $CDCl_3$, $9.5 \times 10^{-3}M$. (Inset) NH resonances observed in the presence of varying concentrations (wt %) of the nitroxide, TEMPO: (a) 0.025% (b) 0.12%, and (c) 0.31%.

was dictated by the distinctly different preferences of Ala and Val residues for adopting helical conformations.[23] The results establish that both peptides adopt 3_{10}-helical conformations in solution, stabilized by eight intramolecular hydrogen bonds.

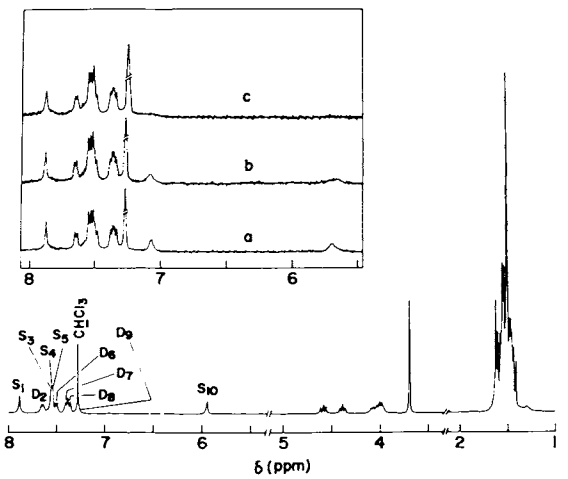

Fig. 2. The 270-MHz ^{1}H-nmr spectrum of Boc-(Aib-Ala)$_5$-OMe in $CDCl_3$, $1.75 \times 10^{-2}M$. (Inset) NH resonances observed in the presence of varying concentrations (wt %) of the nitroxide, TEMPO: (a) 0.025%, (b) 0.073%, and (c) 0.23%.

TABLE I
NH Chemical Shifts and Temperature Coefficients in Boc-(Aib-Val)$_5$-OMe

	δ NH			
	$CDCl_3$		$(CD_3)_2SO$–$CDCl_3$ (1:2)	$d\delta/dT^a \times 10^3$
Resonance	$9.5 \times 10^{-3}M$	$9.5 \times 10^{-4}M$	$9.5 \times 10^{-3}M$	(ppm/°C)
S_1	7.99	7.95	7.98	1.67
S_2	7.79	7.81	7.72	1.34
S_3	7.65	7.66	7.63	−0.03[b]
S_4	7.55	7.52	7.54	2.27
D_5	7.37	7.31	7.33	1.83
D_6	7.35	7.25	7.51	3.14
D_7	7.27	7.25	7.29	0.64
D_8	7.19	7.14	7.21	2.04
D_9	6.84	6.63	7.88	8.04
S_{10}	5.61	5.06	7.07	5.65

[a] These values were obtained in a 1:2 $(CD_3)_2SO$–$CDCl_3$ mixture.
[b] The negative sign indicates a slight downfield shift with increasing temperature.

MATERIALS AND METHODS

The peptides Boc-(Aib-Ala)$_5$-OMe (**A-10**) and Boc-(Aib-Val)$_5$-OMe (**V-10**) were synthesized by solution phase procedures. Dicyclohexylcarbodiimide (DCC) activation was used for urethane-protected amino acids or for peptides with C-terminal Aib. DCC-1-hydroxybenzotriazole-mediated couplings were used for peptides with L-Ala or L-Val at the carboxyl terminal to be activated. Peptides were isolated by standard work-up procedures.[24] The decapeptides were purified by column chromatography over silica gel using $CHCl_3$ and $CHCl_3$–CH_3OH (98:2) for elution. They were obtained as crystalline, chromatographically homogeneous solids, yielding 270-MHz ^{1}H-nmr spectra fully consistent with their structures (Figs. 1 and 2).

All nmr studies were carried out on a Bruker WH-270 FT nmr spectrometer, as previously described.[19,20]

RESULTS AND DISCUSSION

Delineation of Hydrogen-Bonded NH Groups

The decapeptides Boc-(Aib-Ala)$_5$-OMe (**A-10**) and Boc(Aib-Val)$_5$-OMe (**V-10**) show well-resolved amide NH resonances in 270-MHz ^{1}H-nmr spectra (Figs. 1 and 2). In both peptides five doublets (D_n) and five singlets (S_n) due to the X and Aib residues, respectively, are clearly observed over a wide range of solvent and temperature conditions. The chemical shifts of these resonances in $CDCl_3$ and $(CD_3)_2SO$ are summarized in Tables I and II. The subscripts n refer to the order of appearance of the resonance from low field in $CDCl_3$. The only resonance that can be unequivocally

TABLE II
NH Chemical Shifts and Temperature Coefficients in Boc(Aib-Ala)$_5$-OMe

	δ NH			
	$CDCl_3$		$(CD_3)_2SO$	$d\delta/dT^a \times 10^3$
Resonance	$1.75 \times 10^{-2}M$	$1.1 \times 10^{-3}M$	$1.75 \times 10^{-2}M$	(ppm/°C)
S_1	7.89	7.82	8.01	2.14
D_2	7.65	7.62	7.68	2.08
S_3	7.55	7.52	7.67	1.49
S_4	7.54	7.49	7.63	1.98
S_5	7.53	7.44	7.52	2.36
D_6	7.50	7.45	7.68	2.08
D_7	7.39	7.34	7.28	0.45
D_8	7.39	7.31	7.38	2.23
D_9	7.37	6.65	8.54	8.05
S_{10}	6.11	5.12	7.48	7.35

[a] These values were obtained in $(CD_3)_2SO$.

assigned in both the peptides is the urethane NH [Aib(1), S_{10}], by virtue of its high-field position in $CDCl_3$.[18-22] However, the conformational conclusions of this study are not critically dependent on specific assignments.

The presence of solvent-shielded (intramolecularly hydrogen-bonded) NH groups was established using three criteria: (1) temperature coefficients of NH chemical shifts ($d\delta/dT$) in a polar hydrogen-bonding solvent, $(CD_3)_2SO$ or $(CD_3)_2SO$–$CDCl_3$ mixtures[26]; (2) solvent dependence of NH chemical shifts in $CDCl_3$–$(CD_3)_2SO$ mixtures of varying composition[27]; (3) paramagnetic radical-induced broadening of NH resonances in $CDCl_3$.[28] The $d\delta/dT$ values for both peptides are listed in Tables I and II. For **V-10,** $d\delta/dT$ values were measured in a 1:2 $(CD_3)_2SO$–$CDCl_3$ mixture, since the peptide was largely insoluble in $(CD_3)_2SO$. For peptide **A-10,** the Aib(1) NH (S_{10}) and one Ala NH (D_9) show high $d\delta/dT$ values ($>5 \times 10^{-3}$ ppm/°C), characteristic of solvent-exposed NH groups. All the other eight NH groups have $d\delta/dT < 3 \times 10^{-3}$ ppm/°C, suggestive of their involvement in intramolecular hydrogen bonds. Similar results are obtained for **V-10,** with S_{10} and D_9 being the only solvent-exposed groups. Figure 3 shows the solvent dependence of NH chemical shifts in $CDCl_3$–$(CD_3)_2SO$ mixtures. It is clear that only resonances S_{10} and D_9 in peptide **A-10** and S_{10} and D_9 in peptide **V-10** show significant solvent shifts, diagnostic of their exposure to solvent. The addition of the nitroxide radical, 2,2,6,6-tetramethyl piperidine-1-oxyl (TEMPO) can result in the selective broadening of exposed NH groups. Figures 1 and 2 (insets) show the effect of increasing concentrations of TEMPO on the NH resonances of **A-10** and **V-10** in $CDCl_3$. The dependence of line width on radical concentration is summarized in Fig. 4. Quantitative measurements for all resonances were rendered difficult due to partial overlap. However, the spectra in Figs. 1 and 2 establish that only two NH resonances in each peptide—viz., S_{10}, D_9

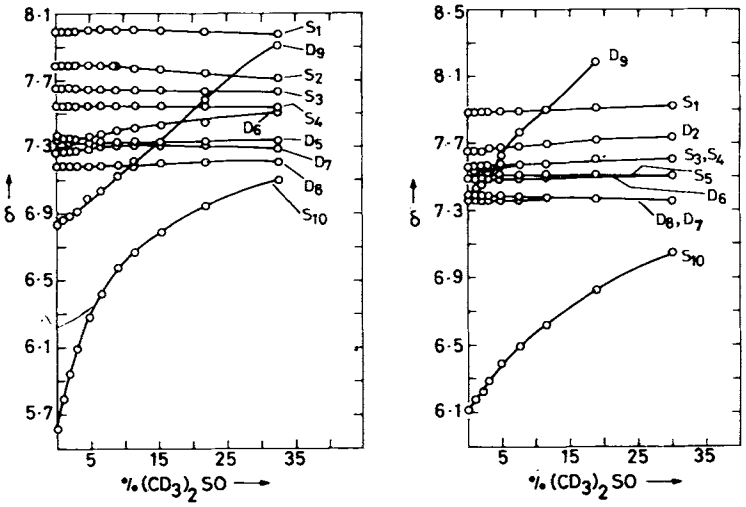

Fig. 3. Solvent dependence of NH chemical shifts: (left) Boc-(Aib-Val)$_5$-OMe, (right) Boc-(Aib-Ala)$_5$-OMe.

in **A-10** and S_{10}, D_9 in **V-10**—are appreciably broadened at the concentrations used. All three methods employed strongly suggest that in both peptides, *eight* NH groups (four Aib and four Ala or Val) are shielded from the solvent and presumably are hydrogen-bonded. The Aib(1) NH and one other Ala or Val NH group are exposed to solvent.

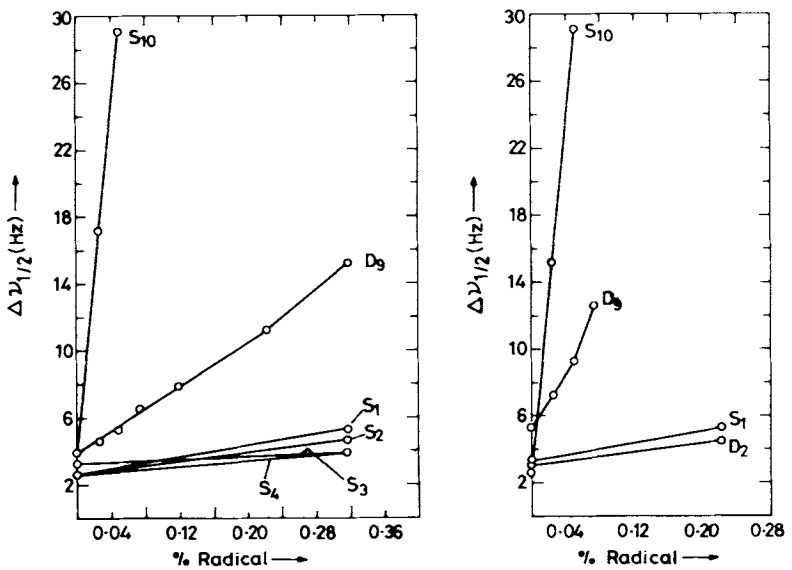

Fig. 4. Line widths ($\Delta\nu_{1/2}$) of NH resonances as a function of radical (TEMPO) concentration in $CDCl_3$: (left) Boc-(Aib-Val)$_5$-OMe, (right) Boc-(Aib-Ala)$_5$-OMe. Because of spectral overlap, not all NH resonances are included.

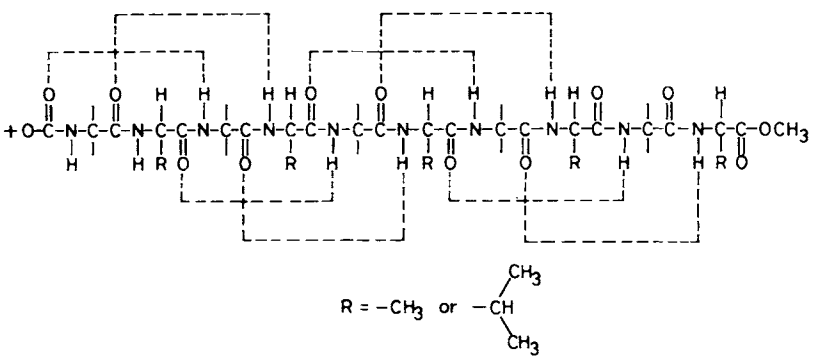

Fig. 5. Schematic hydrogen-bonding scheme proposed for the Boc-(Aib-X)$_5$-OMe, decapeptides. Note that the suggestion of *eight* intramolecular 4 → 1 hydrogen bonds formally corresponds to a 3_{10}-helical conformation.

In the preceding analysis of intramolecularly hydrogen-bonded NH groups, the effect of peptide association has not been explicitly considered. Detailed studies of the concentration dependence of peptide NH nmr parameters in $CDCl_3$ and $(CD_3)_2SO$ have been carried out for the peptides Boc-Aib-Pro-Val-Aib-Val-Ala-Aib-Ala-Aib-Aib-OMe,[29] Boc-Gln(Ala)-Aib-Leu-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-OMe,[30] and Z-(Aib-Pro)$_4$-OMe.[25] In these peptides there was little evidence for aggregation in $(CD_3)_2SO$ over the concentration range 10^{-2}–$10^{-3}M$. In $CDCl_3$, peptide association occurs at concentrations of $\sim 10^{-3}M$. However, *only* those NH resonances that *are solvent-exposed* show a marked concentration dependence of chemical shifts and $d\delta/dT$ values suggesting that peptide aggregation occurs via intermolecular hydrogen bonding involving free NH (not involved in *intramolecular* hydrogen bonds) groups. Helical peptides then associate by intermolecular hydrogen bonds formed by *amino terminal* NH groups. Crystal structures of several helical oligopeptides, in fact, provide evidence of head-to-tail association involving the amino terminal NH group in an intermolecular hydrogen bond.[12,14,17] For a 3_{10}-helical structure the NH group of residues 1 and 2 would be available for intermolecular association and would be expected to show a concentration dependence of nmr parameters. Interestingly, the data in Tables I and II establish that only the solvent-exposed NH groups, D_9, S_{10} in **A-10** and **V-10** show appreciable downfield shifts with increasing concentration.

The results presented above provide compelling evidence for the occurrence of helical conformations stabilized by eight intramolecular hydrogen bonds in peptides **A-10** and **V-10** in both $CDCl_3$ and $(CD_3)_2SO$. In contrast, smaller fragments (heptapeptides of the sequence Boc-X-(Aib-X)$_3$-OMe) show a loosening of hydrogen bonding in $(CD_3)_2SO$ as compared to $CDCl_3$ (unpublished). The schematic hydrogen-bonding scheme, consistent with these nmr results and the known stereochemical preferences of Aib residues, is shown in Fig. 5. The presence of *eight* hydrogen bonds

in the decapeptides strongly favors 3_{10}-helical folding, which would leave only Aib(1) NH and Ala(2) or Val(2) NH groups exposed to the solvent. An ideal α-helical structure requires only *seven* hydrogen bonds, leaving the Aib(1), Ala(2) or Val(2), and Aib(3) NH groups exposed to solvent. This is clearly not compatible with the observed nmr data. The alternative possibility of an α-helical conformation with one additional non-hydrogen-bonded NH group being strongly solvent shielded is unlikely, since in such a structure there does not appear to be strong steric shielding of the NH groups of residues 1–3. The possibility of maintaining 3_{10}-helical conformations in segments as large as 10 residues has been realized in the solid-state conformation of Boc-Aib-Pro-Val-Aib-Val-Ala-Aib-Ala-Aib-Aib-OMe.[32] The agreement between the results of nitroxide-broadening experiments in $CDCl_3$ and temperature coefficients determined in $(CD_3)_2SO$ supports the view that the 3_{10}-helical structures formed in these peptides are maintained even in strongly hydrogen-bonding media. The similarity of the results obtained for the Ala and Val peptides provides evidence for the dominant influence of Aib residues in dictating peptide folding in these sequences. The precise role of peptide chain length and sequence effects in modulating the nature of the helical conformations of Aib-containing membrane-channel-forming polypeptides[2,31] remains to be established. Further research in this area might usefully focus on the effect of the presence of stretches of two or three L-amino acids between Aib residues. Studies in this direction are under way.

This research was supported by a grant from the Department of Science and Technology.

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Received September 22, 1982
Accepted March 10, 1983