

Circular Dichroism Studies of α -Aminoisobutyric Acid-Containing Peptides: Chain Length and Solvent Effects in Alternating Aib-L-Ala and Aib-L-Val Sequences

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Synopsis

The CD spectra of the peptides Boc-X-(Aib-X) $_n$ -OMe ($n = 1, 2, 3$) and Boc-(Aib-X) $_5$ -OMe, where X = L-Ala or L-Val have been examined in several solvents. The X = Ala and Val peptides behave similarly in all solvents, suggesting that the Aib residues dominate the folding preferences of these peptides. The decapeptides adopt helical conformations in methanol and trifluoroethanol, with characteristic negative CD bands at 222 and 205 nm. In the heptapeptides, similar spectra with reduced intensities are observed. Comparison with nmr studies suggest that estimates of helical content in oligopeptides by CD methods may lead to erroneous conclusions. The pentapeptides yield solvent-dependent spectra indicative of conformational perturbations. Peptide association in dioxane results in an unusual spectrum with a single negative band at 210 nm for the decapeptides. Disaggregation is induced by the addition of methanol or water to dioxane solutions. Aggregation of the heptapeptides is less pronounced in dioxane, suggesting that a critical helix length may be necessary to promote association stabilized by helix dipole-dipole interactions.

INTRODUCTION

The conformational flexibility of peptide backbones can be considerably restricted by the introduction of α -alkylated amino acids like α -aminoisobutyric acid (Aib).^{1,2} The occurrence of Aib-rich sequences in natural, membrane-channel forming polypeptides has led to an upsurge of interest in the stereochemistry of Aib peptides.³⁻⁶ The most definitive conclusions have been derived from single-crystal x-ray diffraction studies, which establish a marked tendency of Aib residues to promote 3_{10} -helical folding^{7,8} in short peptides up to five residues in length.²⁻¹⁵ In longer sequences, an α -helix has been observed in the 11-residue peptide, Boc-Ala-(Aib-Ala) $_2$ -Glu(OBz)-(Ala-Aib) $_2$ -Ala-OMe,¹⁶ while a 3_{10} -helix occurs in the solid state in Boc-Aib-Pro-Val-Aib-Val-Ala-Aib-Ala-Aib-Aib-OMe.¹⁷ In the natural 20-residue peptide alamethicin, a crystal structure analysis at 1.5-Å resolution supports an α -helical structure.¹⁸ Detailed ¹H-nmr studies of 10-

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11-, and 16-residue Aib-containing peptides¹⁹⁻²³ and shorter fragments^{24,25} have been interpreted in terms of 3_{10} -helical structures. CD studies of Aib oligopeptides have invariably been discussed in terms of α -helical conformations.^{15,26-29} In order to evaluate whether CD methods can be used to determine the nature of the folded conformation of these peptides, we have undertaken a systematic study of the effect of sequence, chain length, and solvent on the CD spectra of Aib oligopeptides. In this report we describe CD studies on tri-, penta-, hepta-, and decapeptides with alternating sequences of Aib and L-Ala or L-Val.

MATERIALS AND METHODS

The peptides Boc-Ala-Aib-Ala-OMe (**A-3**), Boc-Ala-(Aib-Ala)₂-OMe (**A-5**), Boc-Ala-(Aib-Ala)₃-OMe (**A-7**), Boc-(Aib-Ala)₅-OMe (**A-10**), and the corresponding Val analogs (**V-3**, **V-5**, **V-7**, and **V-10**) were synthesized by conventional solution phase methods. The procedures for the preparation of the tri-, penta-, and heptapeptides have been described.³⁰ The decapeptides were prepared by 3 + 7 coupling of Boc-Aib-X-Aib-OH to H₂N-X-(Aib-X)₃-OMe, using dicyclohexylcarbodiimide-1-hydroxybenzotriazole. Peptides were purified by silica-gel column chromatography and were homogeneous by tlc. Studies of the 270-MHz ¹H-nmr spectra have been reported for all peptides.^{30,31} The peptides **A-3**, **A-5**, and **A-10** have also been previously described.³²

CD spectra were recorded on a Jasco J-20 spectropolarimeter using 1-mm path-length cells. Band intensities are expressed as molar ellipticities, $[\theta]_M$ (deg cm² dmol⁻¹).

RESULTS AND DISCUSSION

Chain-Length Effects

Figure 1 shows the CD spectra in trifluoroethanol (TFE) of the tri-, penta-, hepta-, and decapeptides in both the Ala and Val series. TFE was chosen as a solvent in view of its known ability to promote folded peptide conformations. Studies of an 11-residue fragment of suzukacillin have established that aggregation of Aib peptides is insignificant at concentrations of ~ 1 mM, in this solvent, permitting analysis of CD data in terms of unassociated structures.³³ The CD spectra obtained for both series of peptides are largely similar, suggesting that both -Ala-Aib- and -Val-Aib-alternating sequences fold in the same fashion. The peptides Boc-Ala-Aib-Ala-OMe and Ac-Ala-Aib-Ala-OMe are reported to adopt Type III β -turn conformations in the solid state.³⁴ If conformational rigidity is maintained in solution, the CD spectra of **A-3** and **V-3** in TFE, comprising of a weak positive band at 202 nm and a very weak negative band at 232 nm, may be attributed to this structure. Ir studies in CHCl₃ solution provide

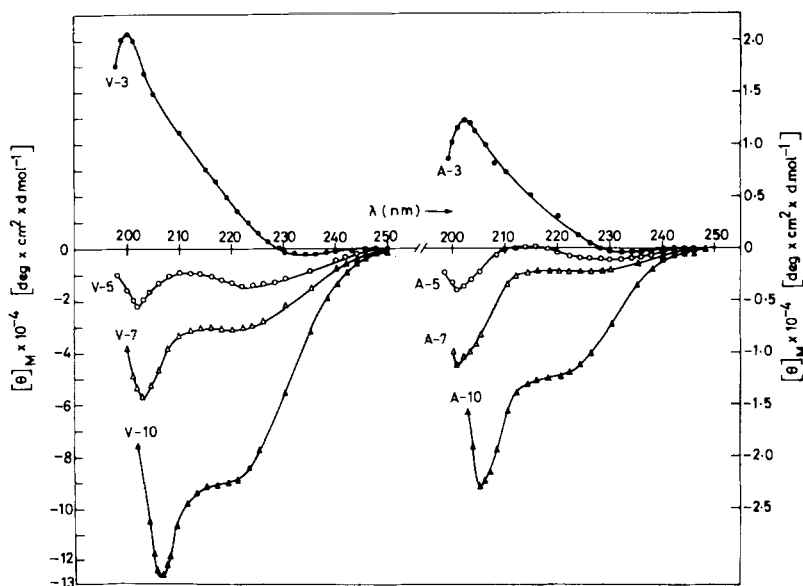


Fig. 1. CD spectra of the peptides Boc-Val-(Aib-Val) $_n$ -OMe, $n = 1, 2, 3$ (V-3, V-5, and V-7), Boc-(Aib-Val) $_5$ -OMe (V-10), Boc-Ala-(Aib-Ala) $_n$ -OMe, $n = 1, 2, 3$ (A-3, A-5, and A-7), and Boc-(Aib-Ala) $_5$ -OMe (A-10) in TFE. Concentration = 1 mg/mL. The molar ellipticity scale on the right is for V-3 and A-3.

evidence for the presence of intramolecularly hydrogen-bonded structures in Boc-Ala-Aib-Ala-OMe. This is evidenced by the ν_{NH} band at 3375 cm^{-1} . The observed CD pattern, however, does not correspond to those theoretically computed for classical β -turns.³⁵ It also does not resemble the spectra observed in β -turn models.^{36,37}

The 5-, 7-, and 10-residue peptides exhibit two negative bands at 220–230 and 201–205 nm in TFE (Fig. 1). There is a dramatic enhancement of ellipticity with an increase in chain length. The long-wavelength n - π^* band shifts to the blue with longer peptides, whereas there is a red shift of the π - π^* band. The CD parameters are summarized in Table I. The spectra of peptides A-10 and V-10 strongly resemble the patterns observed for helical polypeptides,³⁸ with the exception that the n - π^* band is significantly less intense than the π - π^* band. The ratio $[\theta]_{n-\pi^*}/[\theta]_{\pi-\pi^*}$ is typically 0.9–1.0 in helical polypeptides,³⁸ whereas it is ~ 0.5 for A-10 and ~ 0.7 for V-10, in TFE and MeOH. Earlier studies have attempted to estimate the “helical content” of Aib-containing oligopeptides using polypeptide standards.²⁸ In Boc-(Aib-L-Ala) $_5$ -OMe (A-10), these methods suggest that the CD spectra may be fitted to structures containing ~ 27 – 29% α -helix, 0– 8% β -sheet, and 64– 66% “random coil.” The same approach yields values of 0% α -helix, 29– 50% β -sheet, and 56– 71% “random coil” for A-5 in ethanol.²⁸ The structural significance of such estimates in small peptides (and to some extent in larger structures also) is obscure. Clearly, these con-

TABLE I
CD Parameters for Peptides in Different Solvents

Peptide	Methanol		Trifluoroethanol		Dioxane		Hydrogen Bonds (nmr) ^b CDCl ₃	
	λ (nm)	[θ] _M × 10 ⁻⁴ a	λ (nm)	[θ] _M × 10 ⁻⁴ a	λ (nm)	[θ] _M × 10 ⁻⁴ a	CDCl ₃	(CD ₃) ₂ SO
A-3 ^c	202	+2.21	202	+1.26	206	+1.12	—	—
	236	-0.03	235	-0.05	231 (230 ^d)	-0.59 (0.44 ^d)	—	—
	235	-0.19	229	-0.38	230	-0.78	3	3
A-5	212	+0.47	214	+0.08	207 (231 ^d)	+0.96 (-1.17 ^d)	3	3
	—	—	201	-1.52	—	—	—	—
A-7	225	-0.61	225	-0.87	227	-0.90	—	—
	203	-3.76	201	-4.43	207	-1.29	5	4
A-10	220	-4.61	220	-4.84	—	—	—	—
	205	-9.49	205	-9.09	208 (209 ^d)	-10.13 (-1.70 ^d)	8	8
V-3	202	+2.26	200	+2.08	207	+1.93	—	—
	—	—	234	-0.04	235	-0.33	—	—
V-5	231	-0.43	225	-1.32	235	-0.97	—	—
	212	+0.16	202	-2.22	209	+1.50	3	3
V-7	220	-1.80	220	-3.06	225	-0.87	—	—
	203	-3.76	203	-5.68	207	-1.82	5	4
V-10	220	-9.57	220	-8.82	—	—	—	—
	205	-13.63	206	-12.63	—	—	8	8

^a [θ]_M expressed as deg cm² dmol⁻¹.

^b Refs. 30 and 31.

^c Ref. 34 reports one 4→1 hydrogen bond in the solid state.

^d Spectra recorded at 86°C.

clusions are incompatible with the vast body of crystallographic and nmr studies, which favor consecutive β -turn, 3_{10} -, and α -helical conformations in these peptides in both solid state and solution.²⁻²⁵ The good correlation between x-ray and nmr studies implies that Aib peptides are conformationally homogeneous in solvents like chloroform or dimethylsulfoxide. The inescapable conclusion is that methods adopted for the estimation of secondary structures in globular proteins, based on high-molecular-weight synthetic polypeptides of defined conformation, may be inappropriate for the conformational analysis of small oligopeptides by CD techniques.

Detailed ¹H-nmr studies of the Ala and Val penta-, hepta-,³⁰ and decapeptides³¹ in CDCl₃ and (CD₃)₂SO reveal the presence of folded conformations in all the peptides, stabilized by intramolecular hydrogen bonds. For example, in both V-10 and A-10, the presence of *eight* solvent-shielded NH groups favors a 3_{10} -helical structure, incorporating eight 4→1 hydrogen bonds. The conclusions of the nmr studies are summarized in Table I. Since folded structures observed in CDCl₃ and (CD₃)₂SO would be expected to be maintained in TFE or MeOH, it is reasonable to suggest that the CD spectra in Fig. 1 characterize these structures. Thus, the pentapeptide spectrum is likely to correspond to $\sim 1\frac{1}{2}$ turns of a 3_{10} -helix, the hepta- and decapeptide spectra to 2 and 3 turns, respectively, of a 3_{10} -structure. In the cases of these folded helical oligopeptides, band ellipticities seem to depend strongly on helix length, precluding ready estimates of "helical content."

Solvent Effects

The CD parameters for the various peptides in MeOH, TFE, and dioxane are presented in Table I. There is little difference between the MeOH and TFE spectra in case of the tri- (A-3, V-3), hepta- (A-7, V-7), and decapeptides (A-10, V-10). Dioxane is a solvent of low polarity that is expected to sustain intramolecularly hydrogen-bonded conformations and also to enhance helical peptide association, stabilized by head-to-tail intermolecular hydrogen bonds and helix dipole-dipole interactions.³³

In the case of the tripeptides, a substantial enhancement of the long-wavelength negative band at ~ 230 nm is observed in dioxane, a feature retained at temperatures of 80°C (Fig. 2). This spectrum may be assigned to either folded -Ala-Aib- β -turn structures or may arise from C₇ (3→1) intramolecularly hydrogen-bonded conformations.³⁶ Presently, it is not possible to differentiate these structures unequivocally, using CD data alone. A similar negative band is also observed for A-5 and V-5 in dioxane. Since ¹H-nmr studies favor consecutive Type III β -turns in these cases,³⁰ it appears that the observation of a single negative band at ~ 230 nm may be a consequence of short segments of Type III β -turns.

An interesting feature of the pentapeptides is the pronounced solvent dependence of the CD spectra (Fig. 3). Large differences are observed between TFE and MeOH, in contrast to the tri-, hepta-, and decapeptides.

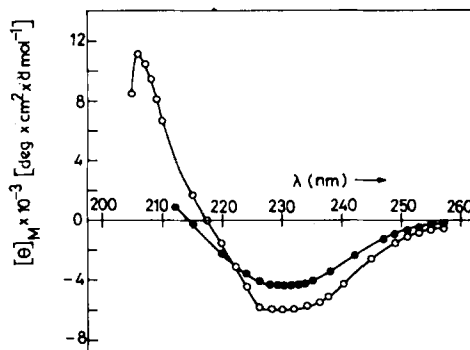


Fig. 2. Temperature dependence of CD spectra of Boc-Ala-Aib-Ala-OMe (A-3) in dioxane at 25 (O) and 86°C (●). Concentration = 1 mg/mL (~ 2.8 mM).

This may imply that helices are stabilized strongly only in the longer segments, while the small tripeptides have only a limited range of available backbone structures. In the pentapeptides opening of one of the repetitive β -turns or changes in β -turn type (Type II or II' structures are stereochemically possible for -L-Ala-Aib- and -Aib-L-Ala- sequences, respectively) are plausible reasons for the observed solvent dependence.

Peptide Association

^1H -nmr studies on several Aib-containing helical oligopeptides suggest that at concentrations of ~ 1 mM, peptide aggregation is insignificant in

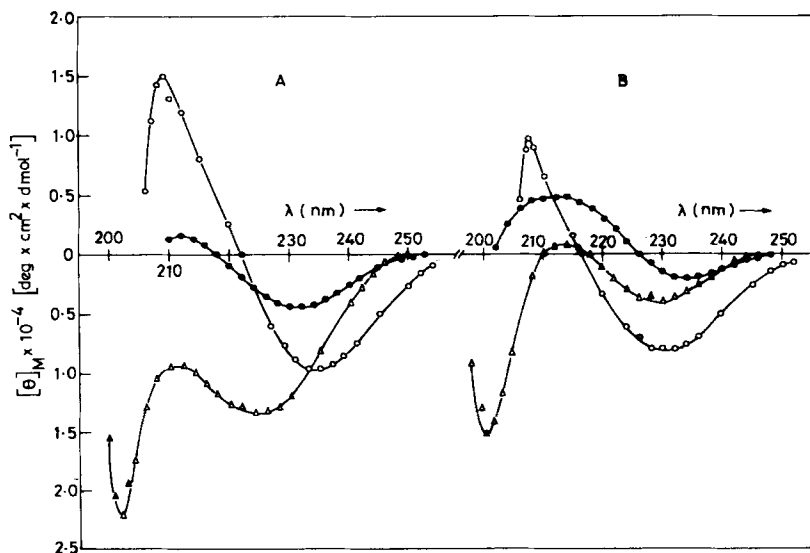


Fig. 3. CD spectra of the pentapeptides in methanol (●), TFE (Δ), and dioxane (O): (A) Boc-Val-(Aib-Val) $_2$ -OMe (V-5); concentration = 1 mg/mL (~ 1.7 mM). (B) Boc-Ala-(Aib-Ala) $_2$ -OMe (A-5); concentration = 1 mg/mL (~ 1.9 mM).

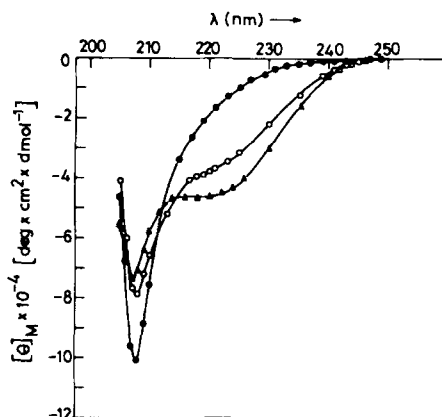


Fig. 4. CD spectra of Boc-(Aib-Ala)₅-OMe (A-10) in different mixed solvent systems: dioxane (●); dioxane-methanol, 1:1 (○); and dioxane-water, 1:1 (Δ). Concentration = 1 mg/mL (~1.1 mM).

polar, hydrogen-bonding solvents like (CD₃)₂SO.^{22,23} Association of helices without disruption of secondary structures occurs in relatively apolar solvents like CDCl₃.^{22,23} CD studies of a 11-residue, helical fragment of suzukacillin suggest that at concentrations up to 1 mM, largely monomeric peptides are observed in MeOH or TFE, but associated species are present in dioxane.³³

Figure 4 shows the spectrum of A-10 in dioxane at a concentration of ~0.83 mM. A single intense negative band at ~210 nm is observed, in marked contrast to MeOH or TFE, wherein two negative bands are observed at 220 and 205 nm (Fig. 1, Table I). Spectra in a mixture of dioxane-cyclohexane (1:1, v/v) were also characterized by an intense negative band at 206 nm. The spectrum of V-10 in dioxane was not recorded, due

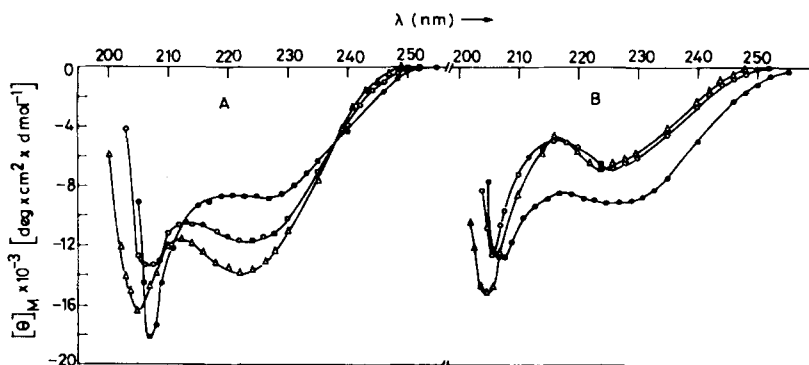


Fig. 5. CD spectra of the heptapeptides in different mixed solvent systems: (A) Boc-Val-(Aib-Val)₃-OMe (V-7); concentration = 1 mg/mL (~1.3 mM). (B) Boc-Ala-(Aib-Ala)₃-OMe (A-7); concentration = 1 mg/mL (~1.5 mM). Symbols: ●, dioxane; ○, dioxane-water (1:1); and Δ, methanol-water (1:1).

TABLE II
 CD Parameters for Peptides in Mixed Solvent Systems

Solvent System	V-7		A-7		A-10	
	λ (nm)	$[\theta]_M \times 10^{-4}$ ^a	λ (nm)	$[\theta]_M \times 10^{-4}$ ^a	λ (nm)	$[\theta]_M \times 10^{-4}$ ^a
Methanol-water						
3:1			228	-0.71		
			205	-2.31		
1:1	223	-1.37	226	-0.64		
	205	-1.65	205	-1.51		
1:3	221	-1.51	228	-0.51		
	205	-1.83	204	-1.55		
Dioxane-water						
3:1	224	-1.15	226	-0.69		
	208	-1.70	207	-1.67		
1:1	224	-1.17	226	-0.67	220	-3.76
	207	-1.34	206	-1.26	207	-7.39
1:3					220	-3.42
					205	-6.20
5:3	224	-1.34				
	208	-1.40				
Methanol-dioxane						
3:1	222	-1.07	226	-0.43		
	206	-2.16	207	-1.81		
1:1	224	-0.92	226	-0.46	220	-3.76
	208	-1.98	207	-1.52	207	-8.26
1:3	225	-0.77	230	-0.69		
	208	-1.88	208	-2.14		
Dioxane-cyclohexane						
1:1	228	-0.83	228	-0.49	—	—
	206	-1.80	207	-1.83	206	-5.98

^a $[\theta]_M$ expressed as deg cm² dmol⁻¹.

to insolubility of the peptide. The unusual spectrum of **A-10** in dioxane must arise from aggregation of peptides, since large changes in conformation in this solvent are extremely unlikely for this stereochemically constrained peptide. The spectrum in dioxane remained largely unchanged on heating up to 85°C, presumably a reflection on the stability of the associated species. Addition of water or MeOH to the dioxane solution resulted in a reversion of the spectrum to the "helical pattern" observed in pure MeOH or TFE (Fig. 4). This suggests that aggregates are effectively broken up on addition of a hydrogen-bonding solvent. It is also possible that helix dipole-dipole interactions³⁹⁻⁴¹ are attenuated with increasing dielectric constant of the medium.

The heptapeptides **A-7** and **V-7** yielded spectra in dioxane (Fig. 5) that are very similar to that observed in TFE or MeOH (Table I). Addition of water or MeOH to the dioxane solutions did not result in any significant spectral changes (Fig. 5). This behavior is in marked contrast to that of the decapeptides, suggesting that helical peptide association in apolar

solvents is promoted only after a critical length of helix is present. This is pertinent in view of the fact that helix dipole-dipole interactions are strongly dependent on helix length, for a fixed orientation, up to about 20 residues.³⁹

The CD spectra of A-7 and V-7 in MeOH-water mixtures are shown in Fig. 5. CD parameters in mixed-solvent systems are listed in Table II. Addition of water results in a significant decrease in the π - π^* band intensity at ~ 205 nm, leading to a dramatic increase in the ratio $[\Theta]_{n-\pi^*}/[\Theta]_{\pi-\pi^*}$. The largest decrease is between 0 and 50% (volume) of water, with the spectrum remaining unchanged on further increasing the water content (Table II). The spectral changes may be attributed to peptide association, driven by hydrophobic effects. Similar effects have also been noted for a 11-residue suzukacillin fragment.³³ The increase in $[\Theta]_{n-\pi^*}/[\Theta]_{\pi-\pi^*}$ on peptide association in aqueous media suggests that this parameter should also be cautiously employed in reaching conclusions about peptide helicity.

The results described above establish that the conformational characteristics of (Aib-X)_n sequences are similar for X = L-Ala and L-Val. This observation reinforces the view that the folding patterns of Aib-rich sequences are largely dominated by the stereochemical preferences of this residue.²⁻⁶ Chain-length effects are pronounced, with stable helical structures being present in the decapeptides. Estimates of helical content of oligopeptides from CD measurements are likely to be subject to considerable error. Considerable solvent effects are observed for the CD characteristics of the pentapeptides, suggestive of conformational heterogeneity. Peptide association profoundly influences the nature of the CD spectra obtained.

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