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Conformational Analysis of Linear Peptides. 4. Association Properties of Protected Oligomers of α-Aminoisobutyric Acid in Chloroform

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Abstract: Activity coefficients, γ, measured by vapor-pressure osmometry, are reported for Z-(Aib)$_n$-O*t*Bu, $n = 1$–5, in chloroform solutions at 27 °C. No osmotic nonideality was detectable in the solutions of Z-Aib-O*t*Bu and Z-(Aib)$_2$-O*t*Bu. Association constants for the dimerization of the other peptides are as follows: Z-(Aib)$_3$-O*t*Bu, $K_2 = 1.2\ \text{m}^{-1}$; Z-(Aib)$_4$-O*t*Bu, $K_2 = 5.0\ \text{m}^{-1}$; Z-(Aib)$_5$-O*t*Bu, $K_2 = 11\ \text{m}^{-1}$. Corollary infrared absorption data are presented as independent evidence of molecular association, which would have to be taken into account in interpreting other spectroscopic (e.g., nuclear magnetic resonance) data.

The unusual, achiral, α,α-dialkylated amino acid, α-aminoisobutyric acid (Aib),[3] is found in large amounts (35–50%) in the *peptaibophol* class of antibiotics[4-16] which also have an amino alcohol (Pheol) at the C terminus. They include alamethicins, antiamoebins, emerimicins, suzukacillins, and zervamicins. Antiamoebins, emerimicins, suzukacillins, and zervamicins also contain a chiral α,α-dialkylated amino acid, Iva.[13] Most of these antibiotics alter the ionic permeability of membranes by forming channels. Other antibiotics, hypelcin A, trichotoxins A-40 and A-50, and trichopolyns I and II, contain Aib, but Pheol is replaced by different amino alcohols.[13,14,17-19] (We therefore propose the name *peptaibol* to include all the aforementioned peptide antibiotics containing several Aib residues and an amino alcohol.)

Table I. Expansion Coefficients Obtained from Fits of Eq 2 and 4

peptide	K_2, m^{-1}
Z-Aib-O*t*Bu	0.6 ± 0.6
Z-(Aib)$_2$-O*t*Bu	0.4 ± 0.8
Z-(Aib)$_3$-O*t*Bu	1.2 ± 0.9
Z-(Aib)$_4$-O*t*Bu	5.0 ± 1.4
Z-(Aib)$_5$-O*t*Bu	11 ± 2.0

We are interested in the connection between the biological properties of peptides containing α,α-dialkylated amino acids and their stereochemistry. According to conformational energy calculations the ϕ,ψ torsional angles of an Aib residue are restricted to values near those associated with right- or left-handed 3_{10} or $\alpha(3.6_{13})$ helices.[20-22] We have recently determined by X-ray diffraction the solid state structures of *t*-Boc-Aib-OH, Z-(Aib)$_{2,4}$OH, Z-(Aib)$_{3,5}$O*t*Bu, Z-Aib-L-Pro-Aib-OMe, Z-(Aib)$_3$L-Val-OMe, and Z-(Aib)$_3$L-Val-Gly-OMe. Intramolecularly hydrogen-bonded folded structures (incipient 3_{10} helices) occur whenever possible, i.e., in the N-blocked tri-, tetra-, and pentapeptides.[23-27]

In this work we examined the tendency of the Z-(Aib)$_n$O*t*Bu ($n = 1$–5) peptides to self-associate in relatively nonpolar environments. Self-association is relevant to the potential role of aggregates in channel formation.[28,29] It is also important for proper interpretation of spectroscopic data (e.g., NMR), as we have previously shown for other peptides.[30]

Experimental Section

The synthesis and characterization of Z-(Aib)$_n$-O*t*Bu, $n = 1$–5, have been described.[24,26,27,31-35]

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(3) The abbreviations used are as follows: Ac, acetyl; *t*-Boc, *tert*-butyloxycarbonyl; O*t*Bu, *tert*-butoxy; OMe, methoxy; Aib, α-aminoisobutyric acid; Iva, isovaline, or α-ethylalanine; Pheol, phenylalaninol; Z, benzyloxycarbonyl.

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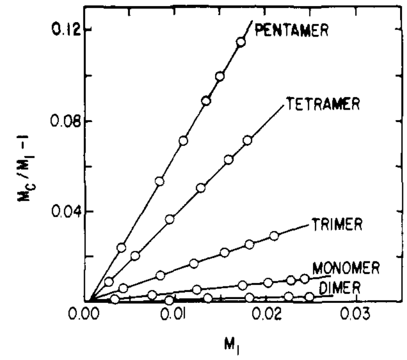

Figure 1. Values of $(m_c/m_1) - 1$ plotted against m_1 according to eq 4.

Vapor-pressure osmometry data were obtained on a Model 232A apparatus manufactured by Wescan Instruments, Santa Clara, CA. Fisher Certified chloroform was used as solvent after passing through an alumina column. Benzil, from Aldrich Chemical Co., was used in the calibration of the osmometer to obtain the difference between thermistor resistances, ΔR, as a function of the stoichiometric molal concentration, m_s. Solutions of all other compounds were prepared to known stoichiometric concentrations, and the measured values of ΔR were used to read colligative concentrations, m_c, from the calibration curve. This procedure gives values of the practical osmotic coefficient, $\phi = m_c/m_s$.[36]

Numerical integration of osmotic data as a function of concentration, according to

$$\ln \gamma = (\phi - 1) + \int_0^{m_s} \frac{(\phi - 1)}{m_s} \mathrm{d}m_s \qquad (1)$$

gives the activity coefficient, $\gamma = m_1/m_s$, where m_1 is the molal concentration of free monomer.[36] Fitting m_s/m_1 vs. m_1, according to a series expansion

$$(m_s/m_1) - 1 = 2K_2m_1 + 3K_3(m_1)^2 + \ldots \qquad (2)$$

gives the association constants $K_2 = m_2/(m_1)^2$, $K_3 = m_3/(m_1)^3$, etc., where m_2 is the molal concentrtion of dimer, m_3 of the trimer, etc.[36]

As a check on the internal consistency of the data, we also used eq 3[36]

$$\ln X_1 = \int_0^{m_c} \frac{(\phi - 1)}{m_c} \mathrm{d}m_c \qquad (3)$$

to evaluate the colligative mole fraction of free monomer, $X_1 = m_1/m_c$, and fit m_c/m_1 vs. m_1 to extract association constants according to

$$(m_c/m_1) - 1 = K_2m_1 + K_3(m_1)^2 + \ldots \qquad (4)$$

All measurements were carried out at 27 °C.

The infrared absorption spectra were recorded using a Perkin-Elmer Model 580 spectrophotometer. Spectrograde deuteriochloroform (99.8%, *d*) was purchased from Merck, Darmstadt, West Germany. The band positions are accurate to ±1 cm^{-1}.

Results

In obtaining fits of the experimental data to eq 2 and 4, we found that including the quadratic term in m_1 was not warranted; i.e., the standard deviation of the fit to the term linear in m_1 was smaller than the uncertainty in the individual experimental data points. Thus, any trimerization that occurred was not detectable by the present experimental method. Expansion coefficients, K_2, obtained using the two different treatments of data agreed to within ±10%. Averaged values of the expansion coefficients are given in Table I. Figure 1 shows plots of $(m_c/m_1) - 1$ vs. m_1 according to eq 4.

Table II. Osmotic Activity Coefficients, γ, for Z-(Aib)$_n$-O*t*Bu, n = 3-5, as a Function of Molal Concentration, m_s

	γ		
m_s	Z-(Aib)$_3$-O*t*Bu	Z-(Aib)$_4$-O*t*Bu	Z-(Aib)$_5$-O*t*Bu
0.001	0.998	0.990	0.979
0.005	0.988	0.954	0.909
0.010	0.977	0.916	0.843
0.015	0.967	0.883	0.793
0.020	0.956	0.854	0.752
0.025	0.946	0.828	0.717

Table III. Infrared Absorption Data of the Z-(Aib)$_n$-O*t*Bu Peptides in Deuteriochloroform Solution in the 3500-3300 cm^{-1} Frequency Range

	2×10^{-2} M concn		2×10^{-4} M concn	
n	band positions	A_H/A_F[a]	band positions	A_H/A_F[a]
1	3447, 3414	1.2	3447, 3413	1.2
2	3435, 3399	1.2	3434, 3400	1.1
3	3429, 3377	1.6	3430, 3378	1.2
4	3428, 3360	3.4	3428, 3362	2.3
5	3428, 3350	5.1	3428, 3352	3.6

[a] Ratios of the areas of the hydrogen-bonded (A_H) to free (A_F) N-H stretching bands.

The expansion coefficients for Z-Aib-O*t*Bu and Z-(Aib)$_2$-O*t*Bu (Table I) are zero to within the uncertainty of the experimental data. For the other three compounds increasing osmotic nonideality was observed with increasing chain length (Table I). Table II shows the activity coefficient, γ, as a function of molal concentration, m_s, for n = 3–5. Table III illustrates the concentration dependence of the infrared absorption of the Z-(Aib)$_{1-5}$O*t*Bu peptides in deuteriochloroform at concentrations 0.02 M and 2 × 10^{-4} *M*.

Discussion

Infrared absorption data provide independent evidence for the existence of self-associated species when there is a concentration dependence in the ratios of the areas of the hydrogen-bonded (A_H) to free (A_F) N-H bands. In deuteriochloroform (Table III) the former bands are seen at 3414–3350 cm^{-1}, the latter at 3447–3428 cm^{-1}.[24,26,27,36] The A_H/A_F ratios (Table III) at the lower concentration examined (2 × 10^{-4} M) can safely be considered as arising only from intramolecularly hydrogen-bonded N-H groups. At 0.02 M concentration self-association is substantial only for the pentamer and the tetramer, whereas it is of minor significance for the trimer and negligible for the dimer and monomer. We therefore take the expansion coefficients for n = 3–5 (Table I) to have the significance of association constants.

Values of K_2 (Table I) reported in the present study for the di- and tripeptides, compared to those observed previously (e.g., *t*-Boc-Gly-L-Val-Gly-OMe, Ac-L-Val-Gly-OMe),[36] are significantly smaller. This is likely to be the result of steric factors in the α,α-dialkylated peptides.

The dimerization constants for the longer homologues of the series are, nevertheless, significant. For example, given the K_2 values for n = 4 and n = 5, 12% and 20%, respectively, of the molecules are present as dimerized species in a 0.02 M chloroform solution. Hence, self-association of tetra- and higher peptides rich in Aib residues in solvents of low polarity at high concentration would have to be taken into account in spectroscopic studies, particularly in NMR investigations where chemical shifts (or the temperature dependence of chemical shifts) are used to determine conformational features.

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Registry No. Z-Aib-O*t*Bu, 4512-31-6; Z-(Aib)$_2$-O*t*Bu, 83537-94-4; Z-(Aib)$_3$-O*t*Bu, 4512-37-2; Z-(Aib)$_4$-O*t*Bu, 4512-38-3; Z-(Aib)$_5$-O*t*Bu, 4512-39-4.

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