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Rapid Communication

Disruption of the β-Sheet Structure of a Protected Pentapeptide, Related to the β-Amyloid Sequence 17–21, Induced by a Single, Helicogenic C^{α}-Tetrasubstituted α-Amino Acid

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Abstract: Fifteen years ago it was shown that an α-aminoisobutyric acid (Aib) residue is significantly more effective than an L-Pro or a D-amino acid residue in inducing β-sheet disruption in short model peptides. As this secondary structure element is known to play a crucial role in the neuropathology of Alzheimer's disease, it was decided to check the effect of Aib (and other selected, helix inducer, C^{α}-tetrasubstituted α-amino acids) on the β-sheet conformation adopted by a protected pentapeptide related to the sequence 17–21 of the β-amyloid peptide. By use of FT-IR absorption and ^{1}H NMR techniques it was found that the strong self-association characterizing the pentapeptide molecules in weakly polar organic solvents is completely abolished by replacing a single residue with Aib or one of its congeners.

Keywords: amyloid-related sequence; helix inducer; infrared absorption; nuclear magnetic resonance; β-sheet breaker; β-sheet structure; C^{α}-tetrasubstituted α-amino acid

INTRODUCTION

About 20 years ago we were the first to describe an IR absorption method to titrate quantitatively the extent of β-sheet structure in peptides [1]. In particular, β-sheet disruption was easily monitored following the disappearance of the intense amide I C=O stretching band at about 1630 cm^{-1} of strongly intermolecularly H-bonded molecules. It was also shown that the increasing tendency of peptides to self-aggregate is paralleled by a decrease in their chemical reactivity and solubility. Subsequently this method, applied to numerous biologically relevant peptides including *C*-terminal sequences of substance P [2], highlighted the β-sheet breaker properties of the helicogenic Aib (α-aminoisobutyric acid) residue [3,4]. Studies on model peptides unravelled similar properties for the L-Pro residue [5,6]. In 1989, using a series of $(L\text{-Val})_n$ homo-oligomers as model host sequences, it was reported that the rank order observed for the destabilization of the β-sheets formed by protected peptides containing a selected, single guest residue replacement at an internal position was Aib ≫

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L-Pro > D-Val ≫ L-Val [7]. In a solvent of low polarity the Aib/L-Val hexapeptide was found to be folded into a helix structure.

Elimination of neuritic plaques composed of the fibrillar β-amyloid (Aβ) peptide is a potential target in the prevention or therapy of the Alzheimer's disease. The strongest peptide ··· peptide self-interactions are mediated by short hydrophobic sequences (e.g. 17–21, -L-Leu-L-Val-(L-Phe)$_2$-L-Ala-) in the middle of Aβ [8–11]. A number of recent investigations demonstrated that the β-sheet structure of Aβ, of its central segments, and of related fibril forming peptides may be disrupted by incorporating *N*-alkylated L-α-amino acids (including L-Pro), D-α-amino acids or α-hydroxy acids [8–16].

This study examined by FT-IR absorption and ^{1}H NMR techniques in CH_2Cl_2 or $CHCl_3$ solution the formation and stability of the self-associated species generated by the protected pentapeptide Z-L-Leu-L-Val-L-Phe-L-Phe-L-Asp(O*t*Bu)-O*t*Bu, where Z is benzyloxycarbonyl and O*t*Bu is *tert*-butoxy, related to the Aβ sequence 17–21 [8], and the disruptive effect on the β-sheet structure induced by a single point replacement. More specifically, the L-Val residue was substituted for Aib or the related, helix inducer, C$^\alpha$-tetrasubstituted α-amino acids L-(αMe)Val (C$^\alpha$-methyl valine) and Ac$_6$c (1-aminocyclohexane-1-carboxylic acid) [4].

MATERIALS AND METHODS

Synthesis and Characterization of Peptides

Melting points were determined using a Leitz (Wetzlar, Germany) model Laborlux 12 apparatus and are not corrected. Optical rotations were measured using a Perkin-Elmer (Norwalk, CT, USA) model 241 polarimeter equipped with a Haake (Karlsruhe, Germany) model D thermostat. Thin-layer chromatography was performed on Merck (Darmstadt, Germany) Kieselgel 60F$_{254}$ precoated plates using the following solvent systems: 1, ($CHCl_3$–EtOH, 9 : 1); 2, (Bu^n-OH–AcOH–H_2O, 3 : 1 : 1); 3, (toluene–EtOH 7 : 1). The chromatograms were examined by UV fluorescence or developed by chlorine–starch–potassium iodide or ninhydrin chromatic reaction as appropriate. All compounds were obtained in a chromatographically homogeneous state. The physical properties and analytical data for the newly synthesized peptides are listed in Table 1. All final products and their synthetic intermediates were also characterized by ^{1}H NMR (data not shown). Peptide bond formation was achieved in CH_2Cl_2 solution using N-ethyl, N′-(3-dimethylaminopropyl)-carbodiimide in the presence of 7-aza-1-hydroxy-1,2,3-benzotriazole as the hydroxylamine-based additive [17].

Infrared Absorption

The solid-state infrared absorption spectra (KBr disk technique) were recorded with a Perkin-Elmer model 580 B spectrophotometer equipped with a Perkin-Elmer model 3600 IR data station and a model 660 printer. The solution spectra were obtained using a Perkin-Elmer model 1720 X FT-IR spectrophotometer, nitrogen flushed, equipped with a sample-shuttle device, at 2 cm^{-1} nominal resolution, averaging 100 scans. Cells with path lengths of 0.1, 1.0 and 10 mm (with CaF_2 windows) were used. Spectrograde methylene chloride, deuterochloroform (99.8% d), dimethylsulfoxide (DMSO) (99.9% d$_6$), 2,2,2-trifluoroethanol (TFE) and 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) were purchased from Fluka (Buchs, Switzerland). Solvent (baseline) spectra were recorded under the same conditions.

^{1}H NMR

The ^{1}H NMR spectra were recorded with a Bruker (Karlsruhe, Germany) model AM 400 spectrometer. Measurements were carried out in deuterochloroform (99.96% d; Aldrich, Milwaukee, WI, USA) and deuterated dimethylsulfoxide (99.96% d$_6$; Acros Organics, Geel, Belgium) with tetramethylsilane as the internal standard. The free radical 2,2,6,6-tetramethylpiperidinyl-1-oxy (TEMPO) was purchased from Sigma (St Louis, MO, USA).

RESULTS AND DISCUSSION

Figure 1 shows that the molecules of the fully protected pentapeptide Z-L-Leu-L-Val-(L-Phe)$_2$-L-Asp(O*t*Bu)-O*t*Bu are strongly intermolecularly H-bonded in CH_2Cl_2 solution at 40 mM concentration. By diluting the solution to 1 mM the intensity of the characteristic IR absorption band at about 1635 cm^{-1} progressively decreases, eventually disappearing between 5 and 1 mM concentration. A parallel trend may be seen, although less clearly, in the NH stretching (amide A) region by following the absorption near 3290 cm^{-1} (not shown). Alternatively, the β-sheet structure present at 40 mM concentration is sensitive to the addition of solvents capable of interacting with the peptide C=O or the N-H groups (not shown). In

Table 1 Physical and Analytical Properties for the Newly Synthesized Peptides

Peptide	Melting point (°C)	Recrystallization solvent[a]	$[\alpha]_D^{20}$ (°)[b]	TLC			IR[c] (cm^{-1})
				R_{fI}	R_{fII}	R_{fIII}	
Z-L-Phe-L-Asp(O*t*Bu)-O*t*Bu	49–51	EtOAc/LP	−14.9	0.95	0.95	0.80	3302, 1728, 1693, 1656, 1537
Z-(L-Phe)$_2$-L-Asp(O*t*Bu)-O*t*Bu	85–86	EtOAc/LP	−26.6	0.95	0.95	0.80	3302, 1728, 1701, 1642, 1532
Z-L-Val-(L-Phe)$_2$-L-Asp(O*t*Bu)-O*t*Bu	191–193	CH_2Cl_2/LP	−31.5	0.95	0.95	0.65	3293, 1727, 1638, 1526
Z-Aib-(L-Phe)$_2$-L-Asp(O*t*Bu)-O*t*Bu	155–157	$CHCl_3$/LP	−30.0	0.95	0.95	0.55	3295, 1732, 1696, 1647, 1528
Z-L-(αMe)Val-(L-Phe)$_2$-L-Asp(O*t*Bu)-O*t*Bu	76–77	$CHCl_3$/LP	−44.0	0.95	0.95	0.55	3307, 1724, 1695, 1655, 1532
Z-Ac$_6$c-(L-Phe)$_2$-L-Asp(O*t*Bu)-O*t*Bu	97–99	$CHCl_3$/LP	−28.9	0.95	0.95	0.70	3314, 1728, 1693, 1654, 1523
Z-L-Leu-L-Val-(L-Phe)$_2$-L-Asp(O*t*Bu)-O*t*Bu	218–220	CH_2Cl_2/LP	−30.2	0.95	0.95	0.60	3291, 1729, 1702, 1639, 1533
Z-L-Leu-Aib-(L-Phe)$_2$-L-Asp(O*t*Bu)-O*t*Bu	166–168	CH_2Cl_2/LP	−33.4	0.95	0.95	0.50	3300, 1728, 1698, 1655, 1528
Z-L-Leu-L-(αMe)Val-(L-Phe)$_2$-L-Asp(O*t*Bu)-O*t*Bu	138–140	CH_2Cl_2/LP	−33.4	0.95	0.95	0.50	3307, 1729, 1697, 1656, 1526
Z-L-Leu-Ac$_6$c-(L-Phe)$_2$-L-Asp(O*t*Bu)-O*t*Bu	185–187	$CHCl_3$/LP	−28.1	0.95	0.95	0.65	3292, 1724, 1694, 1653, 1528

[a] EtOAc, ethyl acetate; LP, light petroleum.
[b] $c = 0.5$, methanol.
[c] The IR absorption spectra were obtained in KBr pellets (only significant bands in the 3500–3200 and 1800–1500 cm^{-1} regions are reported).

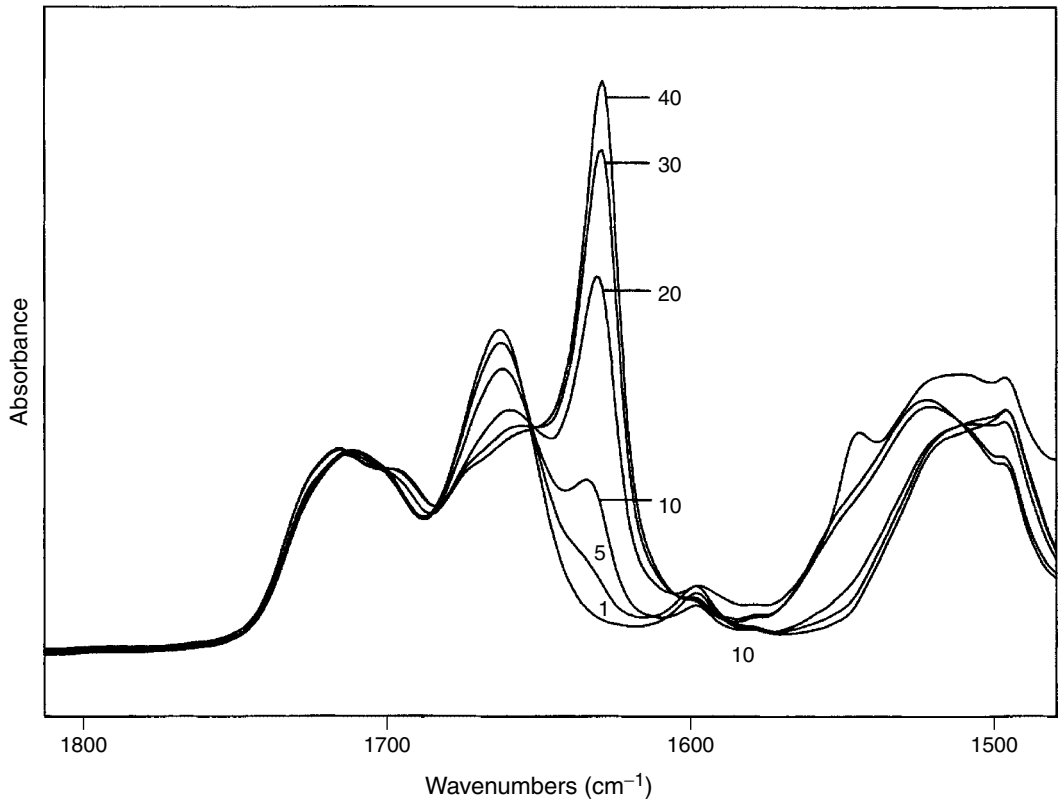

Figure 1 FT-IR absorption spectra (1800–1500 cm^{-1} region) of the protected pentapeptide Z-L-Leu-L-Val-(L-Phe)$_2$-L-Asp-(O*t*Bu)-O*t*Bu in CH_2Cl_2 solution at various concentrations (given in the Figure as mM concentrations).

particular, 10% DMSO and 5% TFE or HFIP are sufficient to suppress completely the 1635 cm^{-1} band. Closely comparable results were obtained with the $CHCl_3$/DMSO solvent mixture. The dissociative effect brought about by the so-called 'chemical chaperones' (e.g. DMSO and fluorinated alcohols) [18] on the intermolecular β-sheet structure was reported in model and biologically active peptides, including Aβ peptides and the scrapie prion protein [1,2,5–7,18,19].

The effect of a single amino acid replacement (L-Val at position 2 of the pentapeptide) by the helicogenic C$^{\alpha}$-tetrasubstituted Aib, (αMe)Val, and Ac_6c residues is illustrated in Figure 2. Even at 40 mM concentration in CH_2Cl_2 solution there was no evidence of the 1630 cm^{-1} band in the IR absorption spectrum of any of the three pentapeptide analogues. Again, a similar trend was observed for the 3290 cm^{-1} band (not shown). The strong C=O stretching absorption of these peptides at 1672 cm^{-1} is most probably associated with the onset of a 3_{10}-helical structure [20]. Dilution from 40 to 0.1 mM concentration did not change the spectral patterns of the three analogues.

The 3_{10}-helical structure of the L-(αMe)Val analogue was confirmed by ^{1}H NMR experiments in $CDCl_3$ solution at 1 mM concentration by adding increasing amounts of either DMSO [21] or the paramagnetic nitroxide TEMPO [22]. Indeed, the results of these titrations clearly indicate that exclusively the N(1)H and N(2)H protons of the pentapeptide are sensitive to the addition of the perturbing agents (not shown), a signature characteristic of the 3_{10}-helix [23]. Conversely, the NH proton signals in the ^{1}H NMR spectrum of the L-Val reference peptide are remarkably broadened at 40 mM concentration in $CDCl_3$ solution, a phenomenon indicative of self-aggregation. Addition of 10% DMSO makes all NH proton signals sharp, as they appear in the spectrum of the L-(αMe)Val peptide in neat $CDCl_3$.

In summary, it was unambiguously demonstrated that the β-sheet structure of a fully protected pentapeptide related to the sequence 17–21 of Aβ in weakly polar organic solvents can be completely disrupted by replacing a single residue with a

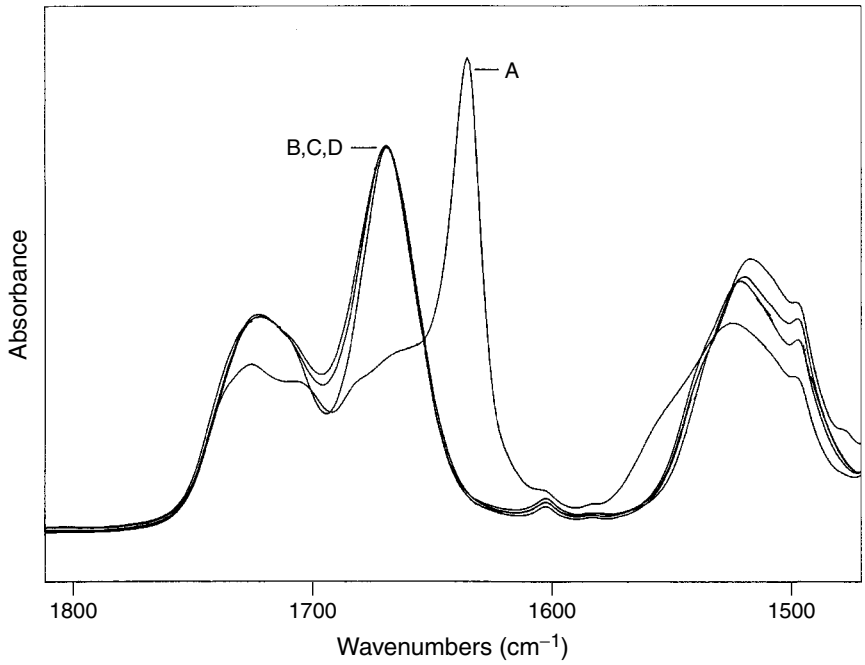

Figure 2 FT-IR absorption spectra (1800–1500 cm^{-1} region) of the protected pentapeptides Z-L-Leu-Xxx-(L-Phe)$_2$-L-Asp-(O*t*Bu)-O*t*Bu, where Xxx is L-Val (A), L-(α-Me)Val (B), Aib (C) or Ac$_6$c (D), in CH_2Cl_2 solution at 40 mM concentration.

helix supporting C$^\alpha$-tetrasubstituted α-amino acid. As these residues are known to be much more efficient β-sheet breakers than L-Pro or D-amino acids [7], it our contention that this approach would be promising for an extension to the related fully deprotected peptides in aqueous solution. This work is currently in progress in our laboratory.

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