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α-Aminoisobutyric acid modified protected analogues of β-amyloid residue 17–20: a change from sheet to helix

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Abstract—The strong intermolecular interactions mediated by short hydrophobic sequences (e.g., 17–20, -L-Leu-L-Val-L-Phe-L-Phe-) in the middle of Aβ are known to play a crucial role in the neuropathology of Alzheimer's disease. FTIR, TEM and Congo red binding studies indicated that a series of L-Ala substituted terminally protected peptides related to the sequence 17–20 of the β-amyloid peptide, adopted β-sheet conformations. However, the Aib-modified analogues disrupt the β-sheet structure and switch over to a 3_{10} helix with increasing number of Aib residues. X-ray crystallography shed some light on the change from sheet to helix at atomic resolution.

1. Introduction

The molecular self-assembly of synthetic peptides demands special interest in advanced medicine because such molecules can serve as bioactive extra cellular materials. Self-aggregation of proteins or protein fragments is particularly important for studying pathogenesis of certain age-related diseases.[1] Though there is no similarity in native structures, sequences, length and composition of different proteins or peptides, subsequent formation of insoluble amyloid plaque is the key factor for several neurodegenerative diseases including Alzheimer's disease,[2] Huntington's disease[3] and prion protein diseases.[4] The fibrillar deposits of amyloid plaque with diameters ranging from 60 to 120 Å appeared in vivo by the transition of a misfolded protein or a peptide from its native structure to a supramolecular β-sheets arrangement.[5] For Alzheimer's disease, the Aβ peptide is generated by the proteolytic processing of a type-1 glycoprotein APP by successive β-cleavage at the N-terminus of Aβ and γ-cleavage (in the trans membrane domain) either at position 40 or 42. Moreover, APP is more frequently cleaved between amino acid 16 and 17 of the Aβ region (α-cleavage) (Fig. 1).[6] The fibrillar aggregation due to the strong intermolecular interaction of the resultant hydrophobic peptide fragments may be the direct or indirect cause of the pathological conditions associated with the amyloid diseases.[7] Some recent results demonstrate that not the matured fibrils but their precursor is pathogenic.[8] Hence, the therapeutic target is to prohibit the fibrillogenesis process.

In order to design therapeutic drugs against amyloid diseases, one of the popular approaches is the modification of amyloidogenic proteins to prevent their ability to adopt a β-sheet conformation. Previously, numerous studies have been performed using β-sheet breaking elements into short recognition sequence of amyloid protein to develop inhibitory drugs.[9] Proline and α-aminoisobutyric acid (Aib)[10] have been widely used for this purpose. Gazit et al. have

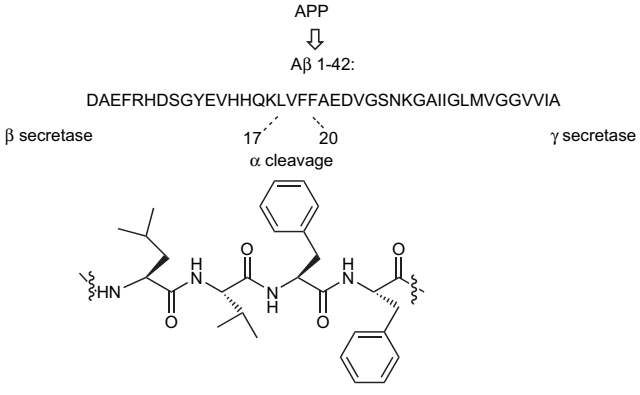

Figure 1. The sequence of amyloid β-peptide ($Aβ^{42}$) and schematic presentation of the residue 17–20 of $Aβ^{42}$. The proteolytic processing of a type-1 gycloprotein APP to generate Aβ peptide. APP is first cleaved at the N-terminus of Aβ (β-cleavage) and then in the trans membrane domain (γ-cleavage), either at position 40 or 42. Moreover, APP is more frequently cleaved between amino acid 16 and 17 of the Aβ region (α-cleavage).

Keywords: Amyloid β-peptide; Amyloid-like fibril; Aib; Supramolecular β-sheet; β-Turn; 3_{10} Helix.
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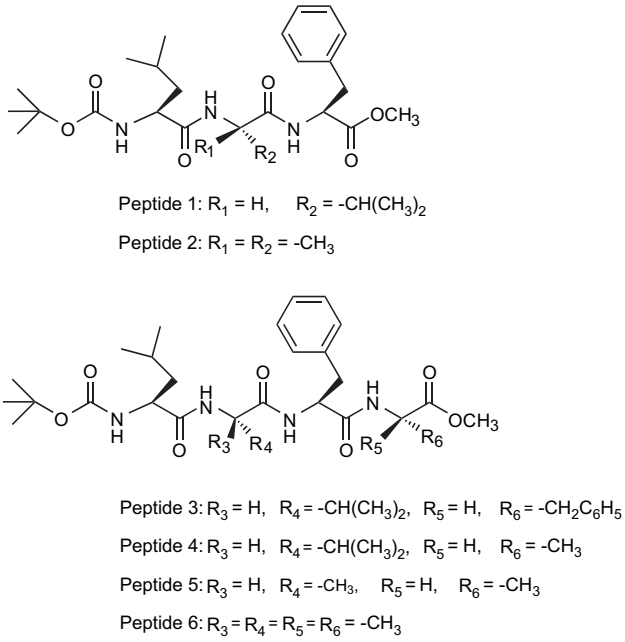
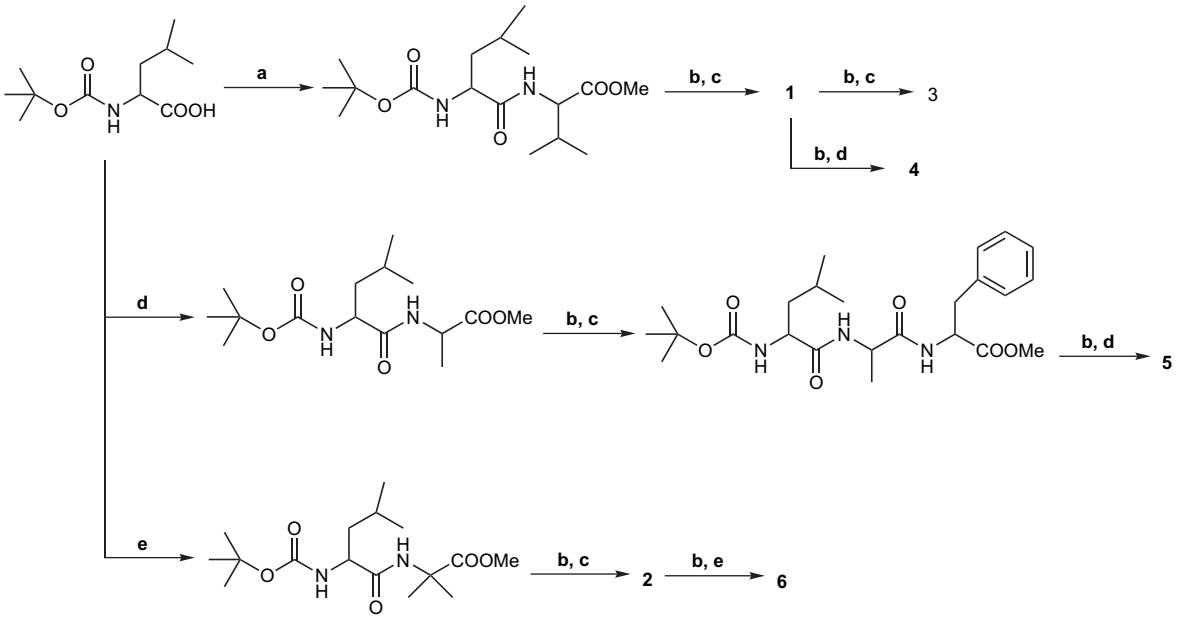


Figure 2. The schematic presentation of peptides **1**–**6**.

reported that the Aib modification of 13–20 residue of human islet amyloid polypeptide (hIPP) generates significant inhibition.[11] Formaggio and co-workers have demonstrated that the incorporation of an Aib residue into a fully protected Alzheimer's β-amyloid fragment (Aβ 17–21) can change the backbone conformation in organic solvents.[12] But all these studies depend on conventional methods like TEM and FTIR and the finer structural detail at the atomic level is still elusive.[11,12] In this context, we have synthesized some terminally protected L-Ala and Aib[13] modified analogs of Aβ 17–20 (LVFF) peptide sequences (Fig. 2). The L-Ala substituted peptides (peptides **4** and **5**) LVFA and LAFA which also have existence in the β-sheet region of corresponding protein structures (NCBI GI 5915735 (60–63) and GI 23019621 (300–303), Protein Data Bank) retain their β-sheet conformations and form amyloid-like fibrils. However, from X-ray crystallography, the Aib-modified analogues (peptide **2** and peptide **6**) disrupt the β-sheet structure and switch over to a 3_{10} helix conformation in the solid state.

2. Results and discussion

The peptides reported in this study have been synthesized using conventional solution phase methodology (Scheme 1).[14] FTIR spectra were recorded to determine the internal conformation of the reported peptides in the solid state (KBr matrix). The most informative frequency ranges are (i) 3500–3200 cm^{-1}, corresponding to the N–H stretching vibrations of the peptide and (ii) 1800–1500 cm^{-1}, corresponding to the stretching band of amide I and bending peak of amide II.[15] Figure 3 shows that molecules of the fully protected Aβ 17–20 peptides Boc-Leu-Val-Phe-OMe (**1**) and Boc-Leu-Val-Phe-Phe-OMe (**3**) have strong intermolecular H-bonds in the solid state. An intense band at 3295 cm^{-1} (peptide **1**) and 3291 cm^{-1} (peptide **3**) and a shoulder at 3328 cm^{-1} were observed for the reported peptides, indicating the presence of strongly hydrogen-bonded NH groups.[16] No band was observed at around 3400 cm^{-1}, indicating that all NH groups are involved in intermolecular hydrogen bonding.[16] The CO stretching band at 1628, 1648 cm^{-1} (amide I) and the NH bending peak at 1554, 1544 cm^{-1} (amide II) corresponding to peptides **1** and **3** suggest the presence of a β-sheet conformation in the solid state.[17] Modification of the native peptides with L-Ala produced no significant change in the infrared spectra. Peptides **4** (Boc-Leu-Val-Phe-Ala-OMe) and **5** (Boc-Leu-Ala-Phe-Ala-OMe) have peaks at 3316 and 3289 cm^{-1}, which might indicate the presence of intermolecular hydrogen-bonded NH groups (Fig. 4). Moreover, the characteristic IR absorption bands at about 1642 and 1640 cm^{-1} (amide I)



Scheme 1. Reagents and conditions: (a) DMF, H-Val-OMe, DCC, HOBt, 0 °C, 90% yield; (b) MeOH, 2 M NaOH, 85% yield; (c) DMF, H-Phe-OMe, DCC, HOBt, 0 °C, 80% yield; (d) DMF, H-Ala-OMe, DCC, HOBt, 0 °C, 80% yield; (e) DMF, H-Aib-OMe, DCC, HOBt, 0 °C, 90% yield.