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# Effect of D-amino acids at $Asp^{23}$ and $Ser^{26}$ residues on the conformational preference of $A\beta_{20-29}$ peptides

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**Abstract**

The effects of D-amino acids at $Asp^{23}$ and $Ser^{26}$ residues on the conformational preference of β-amyloid (Aβ) peptide fragment ($A\beta_{20-29}$) have been studied using different spectroscopic techniques, namely vibrational circular dichroism (VCD), vibrational absorption, and electronic circular dichroism. To study the structure of the $A\beta_{20-29}$, [D-$Asp^{23}$]$A\beta_{20-29}$, and [D-$Ser^{26}$]$A\beta_{20-29}$ peptides under different conditions, the spectra were measured in 10 mM acetate buffer (pH 3) and in 2,2,2-trifluoroethanol (TFE). The spectroscopic results indicated that at pH 3, $A\beta_{20-29}$ peptide takes random coil with β-turn structure, while [D-$Ser^{26}$]$A\beta_{20-29}$ peptide adopts significant amount of polyproline II (PPII) type structure along with β-turn contribution and D-Asp-substituted peptide ([D-$Asp^{23}$]$A\beta_{20-29}$) adopts predominantly PPII type structure. The increased propensity for PPII conformation upon D-amino acid substitution, in acidic medium, has important biological implications. In TFE, $A\beta_{20-29}$, [D-$Asp^{23}$]$A\beta_{20-29}$, and [D-$Ser^{26}$]$A\beta_{20-29}$ peptides adopt $3_{10}$-helix, α-helix, and random coil with some β-turn structures, respectively. The VCD data obtained for the Aβ peptide films suggested that the secondary structures for the peptide films are not the same as those for corresponding solution and are also different among the Aβ peptides studied here. This observation suggests that dehydration can have a significant influence on the structural preferences of these peptides.


*Keywords:* β-Amyloid; β-sheet; β-turn; α-helix; Peptide; Aggregation; Circular dichroism; D-Amino acid; Racemization

Protein folding plays an important role in the aggregation of proteins that leads to many diseases such as Alzheimer's (AD), Huntington's, prion, and Parkinson's diseases. It has been suggested by several researchers that the formation of senile plaques resulting from aggregation of long β-amyloid (Aβ) peptides (39–43 residues long) is the major hallmark of the AD patients [1,2]. The Aβ peptide is generated by proteolytic cleavage of the transmembrane amyloid precursor protein (APP) [3]. Aggregation of amyloid peptides is caused by antiparallel β-sheet conformation. It is believed that the conformational transition from random coil and/or α-helix to antiparallel β-sheet structure is the onset of amyloid aggregate formation, which leads to the formation of insoluble fibrils and toxicity to the neuronal cells. Pike et al. [4–6] have demonstrated that the Aβ peptides that exist in an aggregated state are directly toxic to cultured neurons, whereas soluble Aβ peptides lack direct toxicity. Based on the previous reports, the conformational transition from random coil and/or helix to β-sheet, which is the driving force for the toxic fibril formation, depends on several factors which include concentration [7,8], pH [7,8], temperature [9], salt concentration [10], and solvent composition [11]. It should be noted that the exact mechanism of Aβ aggregate

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formation is not yet clear at the molecular level. However, it has been shown that the tendency of Aβ peptide to form aggregates that are stable at neutral pH appears to require a portion of C-terminal hydrophobic part [6]. Specifically, it was found that the synthetic Aβ peptides containing the 29–35 residues exhibit both the formation of stable aggregates and induction of neurotoxicity. This observation has suggested that the 29–35 region of Aβ peptide is vital to Aβ deposition and bioactivity. Several other groups have also reported that the C-terminal fragments showed β-sheet structure and/or fibril [12–14] formation that are similar to those of full-length Aβ peptides. However, a variety of peptide fragments corresponding to the relatively hydrophilic N-terminal [15–19] regions of Aβ can also exhibit β-sheet conformation and/or fibril formation. It has also been shown that co-incubation of some of the N- and C-terminal Aβ peptide fragments with full-length Aβ peptide enhanced the aggregation of $Aβ_{1–40}$ [20]. However, $Aβ_{1–28}$ peptide in acidic solution was recently reported to be in a monomeric form [21]. In this context, we became interested to know about the conformational and aggregating propensity of the central part of the full-length Aβ peptide and have chosen the 20–29 residue sequence, FAEDVGSNKG, of full-length Aβ peptide.

It has been suggested that the presence of D-amino acids, especially for Ser and Asp residues, in Aβ in senile plaques is a typical age-dependent process [22]. It should be noted that proteins of living mammalian cells are synthesized almost entirely from L-amino acids. However, a progressive increase in D-amino acids with age has been observed in proteins of longevity such as tooth enamel, dentin, eye lens, and myelin-rich cerebral white matter [23–25]. In the case of AD, β proteins extracted from amyloid cores of neuritic plaques have been found to contain D-amino acids for Ser and Asp residues [23,26]. Tomiyama et al. [27] showed, using the peptide sequence containing 1–35 residues by means of spectrophotometry, sodium dodecyl sulfate–polyacrylamide gel electrophoresis, and electron microscopic studies, that D-$Asp^{23}$ residue significantly affects the aggregation properties of Alzheimer amyloid β protein analogues. It has also been reported [22] that [D-$Ser^{26}$]$Aβ_{1–40}$ was non-toxic to PC12 cells and did not exhibit significant fibril formation. However, [D-$Ser^{26}$]$Aβ_{1–40}$, but not $Aβ_{1–40}$, was converted in vitro to a potent neurotoxic and truncated peptide, [D-$Ser^{26}$]$Aβ_{25–35}$ or [D-$Ser^{26}$]-$Aβ_{25–40}$ [22].

The central region of Aβ has been implicated in various biological functions. For example, the interaction between apolipoprotein E and Aβ has been shown to depend on the secondary structure of the amyloid peptide and most likely involves residues 12–28 [28]. In the present work, we have focused on the conformational preference of the central part of the Aβ peptide fragment containing 20–29 residues ($Aβ_{20–29}$) and also the effect of D-amino acids [29] at $Asp^{23}$ and $Ser^{26}$ residues on the conformational preference of $Aβ_{20–29}$ peptide. The list of peptides and corresponding abbreviations used in the present work is given in Table 1. The studies were carried out using different conformationally sensitive spectroscopic techniques, namely vibrational circular dichroism (VCD), [30–33] vibrational absorption (VA), and electronic circular dichroism (ECD) [34]. ECD has been widely used to study the secondary structures of proteins and peptides in solution state. However, interpretation of ECD can sometimes be complicated due to the overlap of transitions from backbone amide and side-chain aromatic chromophores [35]. This difficulty is particularly relevant for the study of peptides containing Phe, Tyr, and Trp residues that contribute significantly to electronic absorption in the near UV region (190–230 nm), which otherwise is dominated by backbone amide transitions. Furthermore, ECD measurements are limited to clear protein or peptide solution due to the problem with light scattering; in addition, ECD cannot be used to study the films, without special instrumentation, due to spectral artifacts [36,37]. VCD is a relatively new technique that has been finding increasing applications in conformational analysis of proteins and peptides in organic and aqueous solutions. Characteristic VCD spectra have been reported for different types of secondary structures, such as α-helices, β-sheets, random coil, β-turns, polyproline II (PPII), etc., for peptides and proteins in solution [38–41]. We have recently demonstrated that VCD can be used to study the structure of proteins and peptide films derived from aqueous solutions [42,43]. Thus, a combined ECD and VCD study could eliminate the ambiguities about the secondary structures of proteins and peptides under different conditions. In the present study, the structures of $Aβ_{20–29}$ and its D-amino acid-substituted analogues have

Table 1
List of peptides studied in the present work

| Abbreviation | Peptide sequence | Peptide mass | |
|---|---|---|---|
| | | $[M + H]^+_{exp}$ | $[M]_{theor}$ |
| $Aβ_{20–29}$ | Ac-FAEDVGSNKG-$NH_2$ | 1064.4 | 1063.5 |
| [D-$Asp^{23}$]$Aβ_{20–29}$ | Ac-FAE-dD[a]-VGSNKG-$NH_2$ | 1064.1 | 1063.5 |
| [D-$Ser^{26}$]$Aβ_{20–29}$ | Ac-FAEDVG-dS[a] -NKG-$NH_2$ | 1064.3 | 1063.5 |

$[M + H]^+_{exp}$ and $[M]_{theor}$ are the experimental and theoretical molecular weights, respectively.
[a] dD and dS represent the D-Asp and D-Ser amino acids.