

Alzheimer's Disease: Soluble Oligomeric A β (1–40) Peptide in Membrane Mimic Environment from Solution NMR and Circular Dichroism Studies

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Amyloid beta peptide (A β) is a small peptide present in normal cells and aggregated A β is the main constituent of the extracellular amyloid plaques found in Alzheimer's disease (AD) brain. Recent studies suggest that soluble A β oligomers are neurotoxic rather than amyloid fibrils found in amyloid plaques. This study using multidimensional NMR spectroscopy and circular dichroism (CD) provides the first direct evidence that alterations in membrane structure can trigger the conversion of soluble α -helical monomeric A β into oligomeric A β in a β -sheet conformation.

KEY WORDS: A β peptide; Alzheimer's disease; CD; micelle; NMR; oligomer.

INTRODUCTION

Alzheimer's disease (AD) is a devastating neurodegenerative disease affecting up to 15 million individuals worldwide and is associated with progressive brain deposits of amyloid plaques composed of fibrillar aggregates of amyloid beta peptide (A β) (1). The initial hypothesis was that the fibrillar amyloid deposits were neurotoxic; however, another possibility is that soluble A β monomers give rise to soluble β -sheet oligomers (2–6) which are neurotoxic. A β can be of varied length (1–40, 1–42), but in this report A β corresponds to the 1–40 amino acid form unless noted otherwise (Fig. 1).

Various studies have demonstrated that A β can alter membrane structure (7,8). *In vitro* and *in vivo* ³¹P magnetic resonance spectroscopy (MRS) studies have demonstrated alterations in membrane phospholipid metabolism such as reductions in the phosphomonoesters (PME), i.e., phosphocholine, phosphoethanolamine, and inositol-1 phosphate; elevations of phosphodiester (PDE), i.e., glycerophosphocholine and glycerophosphoethanolamine; and elevations in *myo*-inositol (9–18). The PME are precursors of membrane phospholipids and the PDE are breakdown products of membrane phospholipids (19,20). It also was shown that the phospholipids phosphatidylcholine (PtdCho), phosphatidylethanolamine (PtdEtn), and phosphatidylinositol (PtdIns) are reduced in AD brain (14) with elevations in sphingomyelin and phosphatidylethanolamine plasmalogen. Alterations in membrane phospholipid mole fractions in AD brain are likely to cause alterations in membrane molecular structure as has been suggested (14). Taken together, these studies demonstrate an alteration of membrane phospholipid metabolism in AD with

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| | | | |
|---|----|----|----|
| 1 | 5 | 10 | 15 |
| Asp -Ala- Glu -Phe- <u>Arg</u> - <u>His</u> - Asp -Ser-Gly-Tyr- Glu -Val- <u>His</u> -His-Gln | | | |
| 16 | 20 | 25 | 30 |
| <u>Lys</u> -Leu-Val-Phe-Phe-Ala- Glu - Asp -Val-Gly-Ser- <u>Asn</u> - <u>Lys</u> -Gly-Ala | | | |
| 31 | 35 | 40 | |
| Ile-Ile-Gly-Leu-Met-Val-Gly-Gly-Val-Val | | | |

Fig. 1. Amino acid sequence of A β . Amphipathic A β contains six negatively charged (bold face) and six positively charged (underlined) amino acid residues.

proposed alterations in brain membrane molecular structure. If membrane molecular structure is altered due to alteration in membrane phospholipid mole fraction, could membrane structure alterations give rise to alterations in the structure of proteins or peptide associated with the membrane?

This study tests the hypothesis that alterations in membrane molecular structure can alter A β conformation. To test this hypothesis, A β conformation was studied by CD and NMR spectroscopy in a well defined membrane-mimic model system [dodecylphosphocholine, (DPC)] with different amounts of DPC. Molecular dynamics calculations suggest that differing amounts of DPC alter the phospholipid packing and structure of membrane micelles (21). Our results demonstrate that in a highly organized neutral micelle environment (20 mM DPC) A β is in an α -helical configuration; in a less organized neutral micelle environment (5.5 mM DPC) a soluble β -sheet conformation is induced in A β . In both cases DPC concentration was well above the critical micelle concentration (CMC) for DPC (1.2 mM) (22). An appealing hypothesis is that alterations in neuronal membrane phospholipid metabolism leads to alterations in membrane molecular structure which is an important trigger to initiate a cascade of events leading to the formation of toxic, soluble β -sheet oligomers of A β .

EXPERIMENTAL PROCEDURE

Materials. Uniformly ^{15}N -labeled A β was purchased from Recombinant Peptide Technologies (Atlanta, GA, USA); deuterated dodecylphosphocholine (DPC_{d28}) was purchased from Cambridge Isotope Laboratories.

Preparation of A β Solution in DPC. Uniformly ^{15}N -labeled A β peptide was dissolved in either 5.5 mM or 20 mM DPC_{d28} solutions and sonicated for 30 s, yielding a clear solution. The final molar concentration of A β peptide was 0.8 mM in both cases. The pH of the solution was 5.6.

Circular Dichroism (CD) Measurement and Analysis. CD spectra were recorded on an Aviv CD spectrometer at 25 °C over the wavelength range of 190 to 290 nm with a resolution of 0.2 nm and a bandwidth of 1 nm. A scan speed of 1 nm/s with a 2 s response time was employed. A 1 mm cell was used. The overall A β peptide concentration was 0.03 mM and DPC concentration were 5.5 mM or 20 mM. CD data were analyzed using the CONTINLL program, part of a web based software package (<http://www.cryst.bbk.ac.uk/cdweb/html/home.html>) (23,24).

NMR Experiments and Data Analysis. All 2D NMR experiments were carried out on a Bruker DRX spectrometer at a proton frequency of 500 MHz using a 5 mM TXI probe (Bruker, Germany). The ^{31}P NMR spectra of the DPC micelles were acquired with an Aspect 500 MHz Bruker NMR spectrometer under proton decoupling (40 kHz) using a ^{31}P pulse width of 9 μs duration.

Homo-nuclear Overhauser enhancement spectroscopy (homo-NOESY), total correlation spectroscopy (TOCSY), hetero-nuclear single quantum spectroscopy (HSQC) (25) and ^{15}N filtered 3D NOESY experiments were performed in a DPC (20 mM or 5.5 mM) micelle environment for sequence-specific assignment and structural studies. Homo-NOESY (mixing time 150 ms), TOCSY (mixing time 60 ms), HSQC and ^{15}N filtered 3D NOESY (mixing time 150 ms) were performed for sequence specific assignments and structural studies. NMR data were processed using nmrPipe (26) and analyzed using PIPP (27) and SPARKY (28) programs.

RESULTS

Figure 1 shows the amino acid sequence of A β (1–40). A β consists of both positively and negatively charged amino acid residues within the 1–28th position and hydrophobic residues from the 29 to 40th position, which is the transmembrane domain. Figure 2 gives the overlay of CD spectra of A β in 5.5 mM DPC (magenta) and 20 mM DPC (blue). A β in 20 mM DPC shows double minima at 222nm and 208 nm, characteristic of α -helical secondary structure. A β in 5.5 mM DPC has a minimum around 218 nm indicating the presence of β -sheet secondary structure. Quantitative analysis of the A β CD spectra (23,24) in 20 mM DPC micelles yields 68% α -helix with the remainder unordered; A β in 5.5 mM DPC micelles contains 55% β -sheet structure and the rest is unordered. The CD spectra do not show the characteristic large positive band at 195 nm for α -helix (repeated 3 times) and similar pattern for broken α -helix has been observed by others (29).

Figure 3 demonstrates ^{31}P spectra of 5.5 mM DPC and 20 mM DPC micelles. The ^{31}P PDE line-width of 5.5 mM DPC micelles is narrow compared

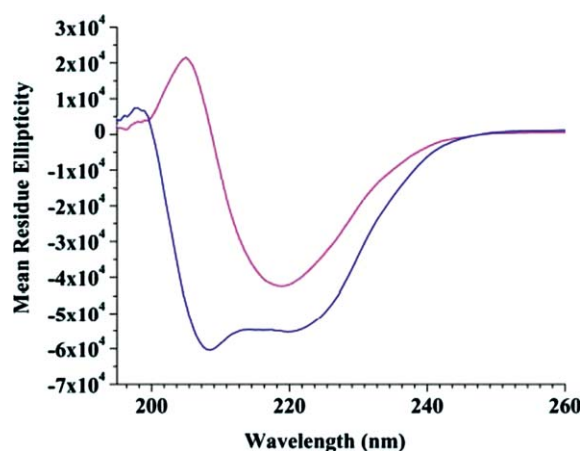


Fig. 2. Overlay of CD spectra of A β in 5.5 mM DPC (magenta) and 20 mM DPC (blue), pH 5.6, 25 °C. CD spectra of A β peptide in 5.5 mM DPC (magenta) shows a characteristic minima around 218 nm indicative of a β -sheet conformation while A β in 20 mM DPC (blue) shows a double minima around 222 nm and 208 nm indicating an α -helical conformation. Based on the mean residue ellipticity analysis, A β contains 68% β -sheet in 5.5 mM DPC (magenta) and 58% α -helix in 20 mM DPC. All spectra were averages of five scans recorded at 25°C after subtraction of the reference spectra of the media. The critical micelle concentration (CMC) of DPC is 1.5 mM.

to 20 mM DPC micelles indicating the PDE correlation time in 20 mM DPC is longer due to increased PDE packing compared to PDE packing in 5.5 mM DPC. DPC packing density increases from 5.5 mM DPC to 20 mM DPC as recently shown by molecular dynamics studies (21). The linewidths of ^{31}P spectra also change with environments. In 5.5 mM DPC solution, there is no A β peptide influence on ^{31}P linewidth which is solely determined by the fast tumbling of the micelles. However, in 20 mM DPC solution, ^{31}P linewidth is broader due to slow molecular tumbling only. Our NMR relaxation measurements in 20 mM DPC solution suggest (data not shown) that A β peptide remains in the micelle-aqueous phase only.

Fig. 4 shows the overlay of HSQC spectra for A β in 20 mM DPC (blue) and 5.5 mM DPC (magenta). Amide peaks for A β in 20 mM DPC are identified based on ^{15}N filtered 3D NOESY, TOCSY experiments and all sequence specific assignment are complete except for the N-terminal amide proton, Asp1. A β in 5.5 mM DPC micelles generates 53 amide peaks (magenta), even though A β has only 40 amino acid residues. The additional 13 amide peaks (Fig. 4) in 5.5 mM DPC probably indicate oligomerization. The broad and well dispersed amide peaks (magenta) between 9.6 ppm and 7.5

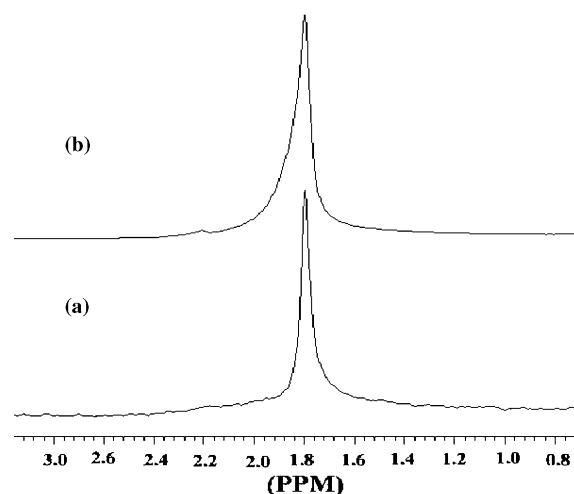


Fig. 3. 1D ^{31}P NMR spectra of DPC micelle (a) in 5.5 mM DPC and (b) in 20 mM DPC, pH 5.4, 25 °C. ^{31}P linewidth (22Hz) of 20 mM DPC is larger by 9 Hz compared to 5.5 mM DPC. The critical micelle concentration (CMC) of DPC is 1.5 mM.

ppm (Fig. 4) indicate the existence of β -sheet secondary structure (30) which is independently supported by CD spectra (31) (Fig. 4). It is worthwhile to note that A β in 20 mM DPC micelles gives rise

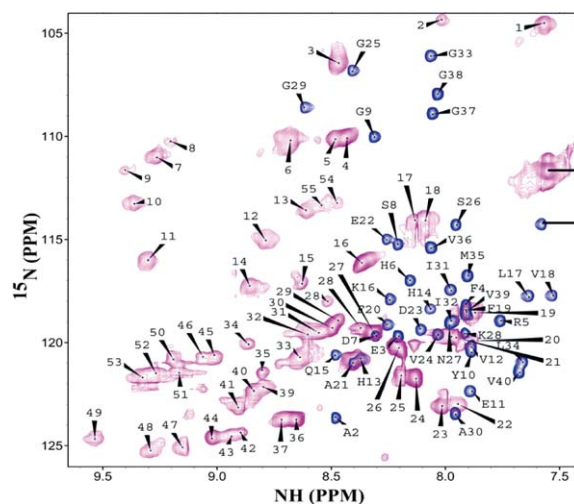


Fig. 4. Overlay of the ^{15}N -HSQC spectra of A β (0.8 mM) in 20 mM DPC (blue) and in 5.5 mM DPC (magenta) at 303 K. The A β concentration is 0.8 mM in both cases (pH 5.6). The critical micelle concentration (CMC) of DPC is 1.5 mM. Backbone amide protons for 39 out of 40 residues were assigned unambiguously in 20 mM DPC (blue). A β peptide in 5.5 mM DPC (magenta) shows 53 clearly resolved backbone amide proton peaks, some of which are broadened.

to an HSQC spectral signal-to-noise ratio (S/N) 10 times larger than A β in 5.5 mM DPC micelles even though A β peptide concentration is the same in both cases. The 13 additional amide peaks (magenta, Fig. 4) as well as the overall broad linewidth of the amide ^1H peaks are probably due to slow tumbling of higher mass entities arising from oligomer formation. Our earlier studies (data not shown) show that A β peptide in aqueous medium (pH 7.4) remains a monomer for several days before precipitation. In aqueous medium, NMR derived structural studies of A β peptide indicate that A β peptide is monomeric and unstructured which rules out any possibility of A β being converted to a oligomer in an aqueous medium before addition of DPC micelle. Assignment of the 53 amide peaks (magenta, Fig. 4) and structural analysis for the oligomeric A β peptide is in progress using transverse relaxation optimized spectroscopy (TROSY) pulse sequences (25,32) at a higher (900 MHz) magnetic field.

Figure 5 describes the NOE connectivities of A β in DPC (20 mM). These NOEs are characteristic of α -helical structure from residue Val12 through Met35. Some expected NOE connections are missing due to resonance overlap.

DISCUSSION

The most important information used in determining secondary structure of a protein by NMR are the characteristic short-range NOEs (33). Alpha-helical secondary structure is characterized by sequential NN ($i, i+1$) and short-range αN (i to

$i+3$) NOEs. The survey of sequential and short-range NOEs is presented in Fig. 5 along with the secondary structural elements derived for A β in 20 mM DPC micelles. A β in 20 mM DPC micelles adopts an α -helical conformation starting from Val12 to Met35 as evident from NOE connectivity (Fig. 5) and structure calculations (34). NMR experiments at pH 7.4 in DPC (20 mM) also were conducted at two higher peptide concentrations (1.0 mM and 1.5 mM). The chemical shift position and linewidth of the different amide peaks remain the same in HSQC spectra. Thioflavin assay can only detect the insoluble fibrils and is not suitable for detecting soluble oligomer. HSQC is a powerful NMR experiment to determine the monomer or oligomer (dimer, tetramer etc) of peptide/proteins. If the 40 amino acid residue peptide is monomeric in nature in 20 mM DPC, then it is expected to show only 40 amide peaks in the HSQC spectra at different peptide concentrations without any differences noted in chemical shift or lineshapes.

Structural studies of A β (1–28) with 120 mM DPC solution revealed a random coil conformation at pH 5.6 (35). The existence of hydrophobic residues at the C-terminal is crucial for an α -helical conformation in 20 mM DPC micelles. CD data indicate characteristic β -sheet curve (minima at 218 nm) and analysis of the CD data of A β peptide in 5.5 mM DPC indicates 68% β -sheet content and the rest is random coil. HSQC spectra of A β peptide in 5.5 mM DPC solution shows 53 amide peaks with wide dispersion (9.6–7.5 ppm) of amide peaks, which is an important characteristic of β -sheet. Hence, the existence of β -sheet is independently verified by two spectroscopic techniques. The identifi-



Fig. 5. Summary of the observed inter-residue NOE connectivities for A β in the DPC (20 mM) micelle-bound form. The thickness of the horizontal bars approximately corresponds to the size of the NOE. These NOEs suggest the presence of α -helix structure from residues Val12 through Met35.

cation of all the amide peaks in 5.5 mM DPC and structure calculation is in progress, which will reveal the type of oligomer.

An antibody which specifically recognizes soluble oligomers among all other types of amyloidogenic proteins and peptides has been reported (4). These soluble oligomers have been implicated as one of the possible reasons for neurotoxicity in AD. It is of considerable interest that the A β inhibitors are "micelle like" which strongly suggest that inhibitors are providing an artificial membrane to which A β can bind to reduce aggregation which is enhanced in aqueous medium.

The results presented herein demonstrate that in an ordered model system such as 20 mM DPC micelles, A β adopts a single α -helical conformation. However, in a disordered membrane system such as 5.5 mM DPC micelles A β can adopt soluble β -sheet oligomeric conformations. In summary, in addition to previous studies that demonstrate that A β can interact with membranes and alter the molecular structure of specific membrane regions, this study demonstrates by CD and NMR methods that molecular structural characteristics of DPC model membranes can greatly influence the conformation and conformational mobility of A β peptide embedded in the DPC micellar membrane. Therefore, membrane phospholipid alterations which have been demonstrated in AD brain could give rise to membrane molecular structural alterations resulting in conformational changes in normal soluble α -helical A β generating soluble β -sheet conformations leading to A β aggregation (2,4,36,37).

Future studies to investigate the conversion of the monomer to soluble β -sheet oligomers, the role of different metabolites such as glycerophosphocholine as well as gangliosides (30,38) on this conversion need careful and thorough investigation.

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