Membrane interactions and the effect of metal ions of the amyloidogenic fragment Aβ(25–35) in comparison to Aβ(1–42)

Tong-Lay Laua,b, John D. Gehman a, John D. Waded, Keyla Perezb,d, Colin L. Masters b, Kevin J. Barnham b, Frances Separovic a,*

a School of Chemistry, Bio21 Institute, The University of Melbourne, VIC 3010, Australia
b Department of Pathology, The University of Melbourne and the Mental Health Research Institute of Victoria, Melbourne, VIC 3010, Australia
c Howard Florey Institute, University of Melbourne, VIC 3010, Australia
d Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, VIC 3010, Australia

Received 17 February 2007; received in revised form 2 May 2007; accepted 3 May 2007
Available online 22 May 2007

Abstract

Aβ(1–42) peptide, found as aggregated species in Alzheimer’s disease brain, is linked to the onset of Alzheimer’s disease. Many reports have linked metals to inducing Aβ aggregation and amyloid plaque formation. Aβ(25–35), a fragment from the C-terminal end of Aβ(1–42), lacks the metal coordinating sites found in the full-length peptide and is neurotoxic to cortical cell cultures. We report solid-state NMR studies of Aβ(25–35) in model lipid membrane systems of anionic phospholipids and cholesterol, and compare structural changes to those of Aβ(1–42). When added after vesicle formation, Aβ(25–35) was found to interact with the lipid headgroups and slightly perturb the lipid acyl-chain region; when Aβ(25–35) was included during vesicle formation, it inserted deeper into the bilayer. While Aβ(25–35) retained the same β-sheet structure irrespective of the mode of addition, the longer Aβ(1–42) appeared to have an increase in β-sheet structure at the C-terminus when added to phospholipid liposomes after vesicle formation. Since the Aβ(25–35) fragment is also neurotoxic, the full-length peptide may have more than one pathway for toxicity.

© 2007 Elsevier B.V. All rights reserved.

Keywords: Amyloid Aβ; Peptide–lipid interactions; Phospholipid membranes; Solid-state NMR; Structure; Metal interactions

1. Introduction

The mechanisms that govern the progression of Alzheimer’s disease (AD), marked by the accumulation of neuronal plaques consisting mainly of aggregated 39–42 residue amyloid-β peptides (Aβ), have yet to be established. Aβ peptides are a product of cleavage by the β- and γ-secretases of the larger transmembrane amyloid precursor protein (APP) [1–3] and aggregate to form amyloid fibrils (reviewed in [4]). While structural models for Aβ(1–40) fibrils based on solid-state NMR data have been presented [5], it is likely that soluble oligomers of Aβ peptides are principally responsible for cytotoxicity [4]. Both Aβ(1–40) and Aβ(1–42) have been extensively studied for their cytotoxic effect, for which several interrelated mechanisms are suggested: metal coordination by histidines 6, 13 and 14 is implicated in oligomer formation [6], and inducing Aβ insertion into phospholipids membrane bilayers [6,7]. Copper and zinc ions (Cu2+ and Zn2+), found at increased levels in neuronal plaques [8,9], can also undergo Fenton-like chemistry and produce free radicals that may result in both the observed dityrosine crosslinks between intermembrane Tyr10 residues [6,10,11], lipid peroxidation [12], and the extensive oxidative stress observed in post mortem examination of AD patient brain [13–15].

Significantly, the largely hydrophobic Aβ(25–35) fragment of naturally occurring Aβ peptides does not have any known direct metal binding or cross-linking potential, yet aggregates much more readily than other fragments and is toxic toward cultured cortical cells as well as neuronal cells [4,16] via membrane interaction and bilayer disruption similar to the full-length Aβ(1–42) [17–19]. Further investigation of this frag-
ment is needed due to the potential role Aβ(25–35) may play in cytotoxicity of the longer Aβ peptides.

We, therefore, examine the effect of Aβ(25–35) on mixed phospholipid bilayers in the presence of metal ions and compare structural changes of Aβ(25–35) to those of Aβ(1–42) in the presence of phospholipids. Particular attention is directed toward lipid membrane composition, which can influence the presence of phospholipids. Preferential interactions of Aβ peptides with charged phospholipids as important regulators of membrane disruption have been implicated in our previous studies of Aβ(1–42) [20], together with other NMR results [21], fluorescence and atomic force microscopy [22]. Consequently, varying the lipid composition of membranes may aid in understanding the progression of AD.

Cholesterol represents another important membrane component, as sterols in general decrease membrane fluidity (or conversely increase acyl chain order) [23]. The role of cholesterol in modulating Aβ/lipid interactions, however, remains controversial: cultured PC12 cells enriched with cholesterol-lowering statin conversely increase acyl chain order) [23]. Therefore, we examined the effect of Aβ(1–42) peptides with charged phospholipids as important regulators of membrane fluidity (or conversely increase acyl chain order) [23].

In addition to metals and lipid composition, the manner in which the peptide is added to model membranes has bearing. The distinction of peptide addition made in our previous studies of Aβ(1–42) demonstrated that Aβ(1–42) disrupts model membranes when incorporated within the lipid vesicles, while peptide added after vesicle formation binds preferentially to the phospholipid headgroups [19,20]. These modes of peptide addition may sample different points in the kinetics of Aβ interactions and AD progression. Associated peptide may represent the toxic soluble Aβ oligomer interacting with a membrane surface; alternatively, the “soluble” (non-amyloidic) oligomer may actually preferentially partition into membranes and be better represented by incorporated peptide, while the associated peptide readily aggregates toward the insoluble, amyloidic species.

The lipid charge, membrane order or ‘fluidity’ and peptide–lipid interactions, together with metal ions, may play an important role in inducing peptide toxicity and aggregation. Therefore, we examined the effect of Aβ(25–35) on mixed phospholipid bilayers together with metal ions and compared structural changes of both Aβ(1–42) and Aβ(25–35) in the presence of phospholipids.

2. Materials and methods

2.1. Materials

Synthetic deuterated 1-palmitoyl(di2) 2-oleoyl-sn-glycero-3-phosphocholine (d2POPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-L-serine (POPS), brain total lipid extract and brain polar lipid extract (porcine) were purchased from Avanti Polar Lipids (Alabaster, USA) and used without further purification. Cholesterol was purchased from Sigma Aldrich (St. Louis, USA). Unlabelled Aβ(25–35) peptide, with sequence GSNKAIAGLGM was purchased through Auspep (Melbourne, Australia). Specifically labelled Aβ(25–35) and Aβ(1–42) peptides were made using standard FMOC-solid phase peptide synthesis [6], purified by reverse phase HPLC and the products identified by mass spectrometry with m/z of 1064.29 for Aβ(25–35) and m/z of 4519.11 for Aβ(1–42). The peptides were 13C labelled with sequence: G SNK[13C1]G [13C1,1A IG[13C1]LM for Aβ(25–35), and DAEFR HDSGY EVHQQ KLVFF AEDYV SNK[13C1]G[13C1]A IG[13C1]LM VGGV IA for Aβ(1–42).

Note: residues of Aβ(25–35) are indexed herein to be consistent with that of Aβ(1–42).

2.2. FMOC-13C-labelled amino acid synthesis

Na2CO3 solution (1 M, 30 ml) was added to 1 g each of 13C1-Leu, 13C1-Ala and 13C1-Gly at room temperature. A 12% mol excess of Fmoc-OSu in MeCN (40 ml) was added to each reaction mixture drop-wise and the reaction stirred, with white precipitate forming overnight. De-ionised water (40 ml) was added to each solution and stirred until precipitate was dissolved. The aqueous phases were extracted with diethyl ether (1×50 ml, 1×60 ml, 1×70 ml) and washed with de-ionised water (10 ml) with each extraction. The aqueous phase was acidified to approximately pH 2.0 with HCl (3 M) and extracted with dichloromethane (3×70 ml). Organic phases were combined, dried over Na2SO4 and filtered. Solvent was removed under vacuum, and the white crude product was recrystallised from dichloromethane/hexane, ethyl acetate/hexane and chloroform/hexane for leucine, alanine and glycine, respectively. Crystallised product was dried under vacuum [28]. The yields of Fmoc-amino acids were 2.54 g (94%) and 0.17 g (6%) from first and second recrystallisations, respectively, for 13C1-Leu; 3.24 g (93%) for 13C1-Ala; and 3.68 g (93%) and 0.27 g (7%) from first and second recrystallisations, respectively, for 13C1-Gly.

2.3. NMR sample preparation

For solid-state NMR studies, multimamellar vesicles (MLV) were used rather than LUV preparations. LUV typically have diameter of 100–200 nm and hence, due to motional averaging, do not reveal the full axially symmetric 13P chemical shift anisotropy (CSA) and 1H quadrupolar splittings (∆ νQ) of lipid lamellar-phase lipid membranes. MLV were prepared by co-dissolving equal amounts of d2POPC and POPS in chloroform and methanol (9:1) solution in a round bottom flask, removing solvent under vacuum, re-dissolving the resulting lipid film in 3 ml 0.05 M Tris–HCl buffer (pH 7) and, freeze–thawed several times using liquid N2 and warm water. Typically 30 mg of lipid and 1.5 mL of solvent was used to make several samples. For the sterol containing samples, d2POPC, POPs and cholesterol (1:1:1 molar) were co-dissolved in solvent to prepare the MLVs as described above. Experiments were also conducted at pH 5 with no obvious difference in the 13P lineshape and 1H quadrupolar splittings. Hence only spectra at pH 7 are shown.

2.4. Incorporated Aβ

d2POPC/POPS (1:1) or d2POPC/POPS/cholesterol (1:1:1) were dissolved in chloroform and methanol (9:1) solution in a round bottom flask, removing solvent under vacuum, re-dissolving the resulting lipid film in 3 ml 0.05 M Tris–HCl buffer (pH 7) and, freeze–thawed several times using liquid N2 and warm water. Typically 30 mg of lipid and 1.5 mL of solvent was used to make several samples. For the sterol containing samples, d2POPC, POPS and cholesterol (1:1:1 molar) were co-dissolved in solvent to prepare the MLVs as described above. Experiments were also conducted at pH 5 with no obvious difference in the 13P lineshape and 1H quadrupolar splittings. Hence only spectra at pH 7 are shown.

2.5. Associated Aβ

Aβ(25–35) or Aβ(1–42) was solubilized in HFIP and dried into a thin film. MLV preparations were subsequently added at P/L of [1:30], followed by
several freeze–thaw cycles. Each sample typically contained 1 mg of peptide in 5 mg of lipid. Labelled peptide used for $^{13}$C NMR studies was also freeze-dried from HFIP.

2.6. Addition of metal ions

Copper and zinc ions were prepared using 0.1 M copper glycinate (CuGly$_2$) solution or 0.1 M zinc glycinate solution (ZnGly$_2$), respectively, by dissolving 1:2 mole ratio of metal chloride with glycine in de-ionised water. Copper and zinc glycinate solution was added to the samples to give 1:1 mole ratio of metal ions to peptide.

2.7. Solid-state NMR

NMR experiments were conducted on a Varian Inova 300 MHz spectrometer (Palo Alto, USA) operating at a resonance frequency of 300 MHz for $^1$H. Static spectra were acquired at 28 °C, using a Doty (Colombia, USA) 5 mm double resonance probe. $^{31}$P broad-line spectra were collected between 1.5 s recycle delays with a solid echo pulse sequence using a 6 kHz spectral width, and processed with 50 Hz exponential line broadening. $^{13}$C chemical shifts were referenced using 80% phosphoric acid (H$_3$PO$_4$) at 0 ppm.

2.8. $^2$H spectra dePaking, and order parameters of lipid acyl chains

$^2$H spectra were collected with shortened pre-acquisition delays to permit post hoc selection of the echo maximum. Data to be used for subsequent dePaking were processed in NMRpipe [32] to DC-correct, left-shift to the echo-maximum, and apodize with a Gaussian function (300 Hz decay constant) beginning at 1.2 ms (and un-attenuated at earlier time), zero-fill to 2048 points, Fourier-transform and only zero-order phase corrected to generate static, unoriented power spectra. Oriented spectra were generated by numerically “dePaking” using Single Value Decomposition (SVD) [33] as well as a straightforward nonlinear least squares approach. The computer code was written in C and used functions freely available from the GNU Scientific Library (GSL) v1.7 [34] to handle the SVD and least squares fitting mechanics. Order parameters were calculated simply by measuring the quadrupolar splitting in the calculated 0°-oriented spectrum for each palmitoyl-CD$_n$ acyl chain position and divided by 255 kHz, the static coupling constant [35,36].

3. Results

3.1. Effect of $\text{A}^\beta(25–35)$ and metals on cholesterol–phospholipid bilayers

The association of $\text{A}^\beta(25–35)$ with the phospholipid bilayer resulted in a small but significant reduction in the $^2$H quadrupolar splitting but little effect on the $^{31}$P CSA. These results indicated that the addition of $\text{A}^\beta(25–35)$ to the lipid bilayer resulted in more ‘disorder’ in the deuterated acyl region of the membrane and less effect on the phosphate headgroups (Fig. 1a–d, Table 1). Subsequent addition of Cu$^{2+}$ after association of $\text{A}^\beta(25–35)$ with the bilayer resulted in a slight increase in the $^2$H quadrupolar splitting and a decrease in $^{31}$P CSA together with $T_2$ broadening (due to the presence of the paramagnetic copper) of the lineshape. Although the effect of changes in $T_2$ on the lineshape are complicated since $T_2$ is anisotropic across the $^{31}$P powder pattern [37,38], the changes indicate disordering in the lipid headgroup region and restriction in phospholipid acyl chain motions (Fig. 1e, f). Similar results were observed with the addition of Zn$^{2+}$ ions (Fig. 1g, h).

The presence of cholesterol in membrane bilayers is known to promote lipid domain or ‘raft’ formation and an increase in lipid order (or decrease in lipid ‘fluidity’) with increasing cholesterol level. If membrane disruption or destabilization is
related to the toxic mechanism of Aβ, the presence of cholesterol may play a role in influencing membrane integrity. 31P and 2H NMR spectra showed that cholesterol resulted in a significantly greater 2H quadrupolar splitting and narrower 31P CSA (Table 2a) compared to pure dPOPC/POPS bilayers (Table 1a) [20]. This result is consistent with the understanding in the literature of the effects of cholesterol on lipid bilayers, and in particular on the 31P powder patterns, arising from the separation of lipid headgroups by intercalation of cholesterol between the lipids [36].

When Aβ(25–35) was associated, an increase in 2H splitting but not in the 31P CSA was observed (Table 2). Following subsequent addition of Cu2+ ions, a significant decrease in 31P CSA (together with $T_2$ broadening due to the strong paramagnetic effect of the copper) was observed, with only a slight increase in the quadrupolar splittings (Table 2); while Zn2+ addition also had a slight increase in quadrupolar splitting but no significant effect on the phosphorus headgroup. Addition of either Cu2+ or Zn2+ ions to the cholesterol-phospholipid bilayer in the absence of the peptide resulted in a slight increase in the quadrupolar splitting and very little effect on the 31P CSA (Table 2). A decrease in signal to noise ratio was also observed for the phosphorus headgroup in the presence of Cu2+ ions in comparison to the 2H spectra due to the paramagnetic relaxation properties of copper, indicative of a preferential interaction of Cu2+ for the lipid headgroup.

### 3.2. NMR studies of Aβ(25–35) with brain lipids

Since studies have shown a preference for Aβ(1–42) to interact with charged phospholipids [39,40], total extract and the polar extract of brain lipids were used to probe the effect of lipid composition with respect to peptide interaction and membrane stability. The experiments carried out using natural brain extracts were limited to 31P NMR experiments and did not include 2H NMR experiments due to the absence of deuterium labels in the lipid acyl chains. The results indicated that no significant changes were seen in the 31P line shape upon addition of Aβ(25–35) to either total brain lipid extract and the polar lipid extract. While subsequent addition of Zn2+ ions also had no effect, Cu2+ ions added after the association of Aβ(25–35) caused a decrease in bulk 31P CSA together with the appearance of an isotropic peak in the polar brain lipid extract (as seen in our earlier spectra [20]). Since the presence of Cu2+ did not induce membrane disruption for the total brain lipid extract but did for the polar extract, it is evident that lipid composition is critical in maintaining membrane integrity in the presence of Aβ(25–35) and Cu2+ ions.

### 3.3. CP-MAS NMR of membrane-associated 13C specifically labelled Aβ(25–35)

Structure-related information was obtained for Aβ(25–35) in a phospholipid bilayer using peptide specifically labelled in the middle of the sequence and near the C-terminus. 13C spectra of labelled Aβ(25–35) peptide as a dry powder prior to incorporation or association of the peptide into model lipid membranes are shown in Fig. 2a. Peptide structural changes as suggested by changes in carbon chemical shift were compared with reference to polypeptide conformational studies of 13C carbonyl and C(α) chemical shifts using solid-state NMR [41,42]. The chemical shift of specific labelled sites of the incorporated peptide (Fig. 2b) when compared to the labelled Aβ(25–35) peptide powder indicated an upfield shift for the

---

### Table 1

<table>
<thead>
<tr>
<th>Observed nuclei</th>
<th>2H (±0.3) kHz</th>
<th>31P (±2) ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>a Lipid+Cholesterol</td>
<td>CD2 46 (±0.3)</td>
<td>CD3 42 (±0.1)</td>
</tr>
<tr>
<td>b Lipid+Cholesterol+Aβ(25–35)</td>
<td>CD2 48 (±0.3)</td>
<td>CD3 46 (±0.1)</td>
</tr>
<tr>
<td>c Lipid+Cholesterol+Aβ(25–35)+Cu2+</td>
<td>CD2 49 (±0.3)</td>
<td>CD3 49 (±0.1)</td>
</tr>
<tr>
<td>d Lipid+Cholesterol+Aβ(25–35)+Zn2+</td>
<td>CD2 49 (±0.3)</td>
<td>CD3 49 (±0.1)</td>
</tr>
<tr>
<td>e Lipid+Cholesterol+Cu2+</td>
<td>CD2 49 (±0.3)</td>
<td>CD3 49 (±0.1)</td>
</tr>
<tr>
<td>f Lipid+Cholesterol+Zn2+</td>
<td>CD2 49 (±0.3)</td>
<td>CD3 49 (±0.1)</td>
</tr>
</tbody>
</table>

CD2 and CD3 quadrupolar splittings ($\Delta \nu_Q$) are taken from the 90° edges of the powder pattern.

### Table 2

<table>
<thead>
<tr>
<th>dPOPC/POPS/cholesterol (1:1:1)</th>
<th>2H ($\Delta \nu_Q$) kHz</th>
<th>31P ($\Delta \sigma$) ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>a Lipid+Cholesterol</td>
<td>CD2 46 (±0.3)</td>
<td>CD3 42 (±0.1)</td>
</tr>
<tr>
<td>b Lipid+Cholesterol+Aβ(25–35)</td>
<td>CD2 48 (±0.3)</td>
<td>CD3 46 (±0.1)</td>
</tr>
<tr>
<td>c Lipid+Cholesterol+Aβ(25–35)+Cu2+</td>
<td>CD2 49 (±0.3)</td>
<td>CD3 49 (±0.1)</td>
</tr>
<tr>
<td>d Lipid+Cholesterol+Aβ(25–35)+Zn2+</td>
<td>CD2 49 (±0.3)</td>
<td>CD3 49 (±0.1)</td>
</tr>
<tr>
<td>e Lipid+Cholesterol+Cu2+</td>
<td>CD2 49 (±0.3)</td>
<td>CD3 49 (±0.1)</td>
</tr>
<tr>
<td>f Lipid+Cholesterol+Zn2+</td>
<td>CD2 49 (±0.3)</td>
<td>CD3 49 (±0.1)</td>
</tr>
</tbody>
</table>

CD2 and CD3 quadrupolar splittings ($\Delta \nu_Q$) are taken from the 90° edges of the powder pattern.
carbonyl resonances (β-sheet, [41,42]) of Ala30 and Leu34 (from 174 to 173 ppm) and no significant change for Gly29 (43 ppm) from the initial random coil shift [41,42] of the dry peptide. Associated peptide (Fig. 2c) had a similar effect when added to the PC/PS membrane with a slightly more upfield shift for the carbonyls (171 ppm) when compared to the incorporated peptide. Although carbon chemical shifts were similar for both the associated and incorporated peptides, T2 measurements of the 31P headgroups indicated that the phospholipid headgroup interactions for the associated species was stronger, as observed by the reduction in T2 relaxation time from ~6.2 ms (lipid alone) to ~1.8 ms, whereas incorporated Aβ(25–35) gave a T2 of ~7 ms similar to that of pure lipids. As a result of the anisotropic nature of T2 [37,38], MAS techniques were used to measure T2 in order to determine the gross effect of the peptide on the phospholipids rather than a detailed study, which would require measurement of that anisotropy to explain the lineshape change. Interestingly, only one peak was seen by 31P, suggesting that the two phospholipids were in a similar environment, although the linewidth as a result of the short NMR T2 would preclude resolving the resonances. Incorporated Aβ(25–35) also caused a significant increase in the 2H quadrupolar splittings and the 31P CSA, indicating perturbation of both the acyl region as well as the phosphorus headgroup of the bilayer. The 2H acyl chain order parameter from dePaked spectra showed an increase in order at the CD2 positions nearer to the lipid headgroups with less effect in the CD2 position toward the end of the chain and the terminal CD3 when the peptide was incorporated in lipid vesicles compared to the associated species (Fig. 3).

3.4. CP-MAS NMR of 13C specifically labelled Aβ(1–42)

Structure-related information was obtained for Aβ(1–42) in phospholipid bilayers. 13C chemical shifts of labelled Aβ(1–42) were obtained as: (i) a dry powder, (ii) when incorporated in lipid bilayers, and (iii) when associated with lipid bilayers. The Aβ(1–42) peptide was labelled at the C-terminal end region, at the same residues as for the Aβ(25–35) fragment used in the studies. Chemical shift comparison of both the incorporated and the associated peptide relative to the peptide as a dry powder (HFIP pre-treated as discussed in Materials and methods) indicated an upfield shift, consistent with a change from α-helical to β-sheet for the carbonyl resonances (Fig. 4, Table 3) [41,42].

From our experiments, the dry Aβ(1–42) peptide displayed β-sheet propensity at Gly29 and helical propensity at both Ala30 and Leu34. We were unable to resolve Gly29 C(α) chemical shift due to overlapping acyl-lipid peaks when the peptide was in the presence of phospholipids. However, the 13C=O resonances shifted from 178 ppm, indicative of α-helical structure [41,42], to 173 ppm (for incorporated) and 171 ppm (for associated), indicative of β-sheet structure [41,42], for the Aβ(1–42) peptide with POPC/POPS bilayers (Fig. 4, Table 3). The changes in 13C chemical shift as seen by CP-MAS experiments upon association of Aβ(1–42) with the lipid bilayer suggest the peptide adopted a β-sheet conformation [41,42] toward the C-terminus where the labelled residues (29, 30 and 34) are located. This result is complementary to circular dichroism (CD) data published in earlier studies [20], where an increase in β-sheet conformation was also observed when Aβ(1–42) was associated with membrane vesicles. The carbonyl resonances of associated peptide have a higher upfield shift than the incorporated peptide, which may suggest Aβ(1–42) adopts a more β-sheet-like structure when associated.

4. Discussion

The importance of metal–peptide interactions mediated through binding to histidine residues and promoting structural changes for both full-length Aβ(1–40) and Aβ(1–42) has been correlated to the neurotoxic properties of Aβ peptides. However, the shorter Aβ(25–35) peptide fragment also exhibits neurotoxicity, but does not contain the metal coordinating histidine residues. This suggests Aβ can exhibit neurotoxicity through a variety of alternative pathways [16]. Lipid composition of the membrane has been shown to be important for seeding peptide aggregation, with Aβ appearing to bind preferentially to negatively charged over neutral lipids. Lipid charge distribution has been shown to be involved in signalling cell death, for example, phosphatidyserine (PS) lipid translocation from the inner to the outer membrane leaflet has been shown to occur as an apoptotic trigger in cells [43]. Also, aggregation of the peptide is affected when membrane fluidity or order is altered, for example, in the presence of cholesterol. We have examined the effect of associated or incorporated Aβ(25–35) in lipid membranes of different fluidity and changes in peptide structure when added to lipid membranes using solid-state NMR. For comparison to our earlier studies of Aβ(1–42) in phospholipid bilayers [20], we used a L/P ratio of 30:1. Aβ(25–35) structural change was subsequently compared to full-length Aβ(1–42) in phospholipid membranes. Associated peptide could represent ‘soluble’ Aβ interacting with a membrane surface, whereas incorporated peptide could represent Aβ already within a membrane bilayer.
4.1. Effect of associated and incorporated Aβ(25–35) and metals on membrane bilayer

Our observations of the effect of Zn\(^{2+}\) and Cu\(^{2+}\) ions on the membrane integrity showed the formation of smaller lipid structures indicative of membrane disruption by the ions [9]. Metal coordination of these ions to the histidine residues of the full-length Aβ\((1–42)\) peptide has been shown to be important for Aβ toxicity where a decrease in peptide toxicity was seen using methylated histidine mutant peptides that bind metals with an altered coordination mode compared to wild-type peptide [6]. The Aβ\((25–35)\) fragment contains no histidine residues, thus excluding obvious potential for direct metal binding, although the effect of Aβ\((25–35)\) and metal ions on bilayer integrity could still be studied. Our data suggest that Aβ\((25–35)\) inserts less deeply into the bilayer when associated with phosphatidylcholine/phosphatidylserine (PC/PS) membranes as a dry peptide film than when incorporating peptide during vesicle formation. The association with peptide results in a decrease in 2H NMR quadrupolar splitting, increase in 31P CSA and a significant decrease in 31P \(T_2\) relaxation time from 6.2 to 1.8 ms, which indicates decreases in lipid acyl chain order and direct perturbations of phospholipids headgroup consistent with peptide located more at the interface between the membrane and aqueous solution. Previous neutron diffraction studies of similarly associated Aβ\((25–35)\) [44–46] also indicate at least a partial preference for the more hydrophobic membrane environment over the aqueous membrane interface, and positions the penultimate Leu34 residue \(\sim 15\ \text{Å}\) from the centre of the \(\sim 30\ \text{Å}\) hydrophobic distance between glycerol backbones [47]. These results together with our present study indicate the Aβ\((25–35)\) is partially inserted into the bilayer, while still interacting with the 31P headgroups. Addition of Cu\(^{2+}\) ions subsequent to peptide addition only affected the 31P headgroups and reduced the 31P CSA with an increase in \(T_2\) broadening, consistent with paramagnetic relaxation. Addition of Zn\(^{2+}\) after Aβ\((25–35)\) peptide did not lead to significant changes.

In contrast, incorporated Aβ\((25–35)\) increased 2H NMR quadrupolar splitting, increase in 31P CSA and a significant decrease in 31P \(T_2\) relaxation time from 6.2 to 1.8 ms, which indicates decreases in lipid acyl chain order and direct perturbations of phospholipids headgroup consistent with peptide located more at the interface between the membrane and aqueous solution. Previous neutron diffraction studies of

### Table 3
Comparison of \(^{13}\text{C}-\text{CPMAS}\) chemical shifts of Aβ\((1–42)\) specifically enriched at \(^{13}\text{C}\) of Gly29 and \(^{13}\text{C}==\text{O}\) of Ala30 and Leu34 as a dry peptide powder and with dPOP/POPS lipid bilayers at 1:30 peptide/lipid ratio in Tris–HCl pH=7.2 at 28 °C.

<table>
<thead>
<tr>
<th></th>
<th>(^{13}\text{C}\alpha) (ppm)</th>
<th>(^{13}\text{C}==\text{O}) (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aβ((1–42)) (dry powder)</td>
<td>44</td>
<td>178</td>
</tr>
<tr>
<td>Incorporated Aβ((1–42)) in lipid</td>
<td>43 (broad peak)</td>
<td>173</td>
</tr>
<tr>
<td>Associated Aβ((1–42)) in lipid</td>
<td>43 (broad peak)</td>
<td>171</td>
</tr>
</tbody>
</table>

4.2. Effect of cholesterol on Aβ\((25–35)\) insertion into membrane bilayer

The presence of cholesterol in a membrane bilayer orders the lipid acyl region while ‘disordering’ the headgroup due to incorporation of the sterol into the membrane. Cholesterol increased the lipid 2H quadrupolar splitting of the chains and decreased the 31P CSA of the headgroup as seen by Davis et al. [36]. The effect of Aβ\((25–35)\) on the lipid bilayer (Table 1c) was reduced by the presence of cholesterol (Table 2b), which suggested that cholesterol could prevent bilayer insertion of Aβ\((25–35)\). Since cholesterol decreases membrane fluidity, the increase in lipid acyl-chain order may reduce peptide insertion. Cholesterol also tends to preserve membrane integrity and, unsurprisingly, the effect of Aβ\((25–35)\) on both the total brain lipid extract (with cholesterol) and the polar brain lipid extract.
(without cholesterol) was different. For the total brain lipid extract there was no significant perturbation of the $^{31}$P lipid headgroup while for the polar brain extract, there was a significant increase in $^{31}$P isotropic phase with the addition of Cu$^{2+}$ ions. The appearance of this isotropic phase, representative of smaller lipid structures as seen in earlier studies [20] indicated that the membrane integrity was destabilized for the more polar lipid in the presence of Aβ(25–35) and Cu$^{2+}$ ions. These data suggest that, in the presence of Aβ, cholesterol may have a stabilizing role on membrane structure.

4.3. Structural characteristics of membrane-associated and -incorporated Aβ

CP-MAS data using specifically $^{13}$C labelled peptide provides information on the peptide structure in association with lipid vesicles. The data indicated that both Ala30 and Leu34 carbonyl carbons had more β-sheet structure than the peptide powder, more so for associated than incorporated Aβ(25–35) in PC/PS bilayers, but with little such differential effect at the α-carbon of Gly29 in both cases. The interaction of the native Alzheimer’s peptide C-terminal fragment Aβ(29–42) with neutral phospholipid bilayers by solid-state NMR has been reported [48]. The results for this longer peptide also support a β-sheet oligomeric association of the peptide at the bilayer interface. Interestingly, although we expected the hydrophobic transmembrane region of Aβ(1–42) to exhibit greater preference for helical structure upon membrane interaction, the CD studies reported earlier [20] together with our $^{13}$C CP-MAS results from specific labels in the hydrophobic region of Aβ(1–42) showed a change from α-helical as a dry powder to β-sheet structure when both associated and incorporated with phospholipid vesicles. Although not in association with lipid, in amyloidic fibril form, and significantly two residues shorter, the structural model from Tycko and coworkers based on solid-state NMR data [5] also show β-strand conformations for residues 12–24 and 30–40 of Aβ(1–40).

4.4. Comparison of Aβ(25–35) with Aβ(1–42)

We have shown previously that Aβ(1–42) behaves similarly to our present observation on Aβ(25–35) when associated with phospholipid bilayers, as it binds to the phosphorus headgroup causing a decrease in spin–spin ($T_2$) relaxation time; phospholipid headgroup binding of Aβ(1–42) was further confirmed by $^{31}$P magic angle spinning SS-NMR with the appearance of a lipid–Aβ bound phase together with a peptide-free lipid phase in large unilamellar vesicles [49]. This commonality suggests that the Aβ(25–35) region within the full-length peptide may be involved in phospholipid interaction. Incorporation of Aβ(1–42) and Aβ(25–35) in phospholipid membranes, however, yielded diverse results. Aβ(1–42) when incorporated destabilizes the bilayer, as reported previously [20], while Aβ(25–35) inserted into the hydrophobic acyl-chain region of the phospholipid membrane and enhanced membrane stability, as indicated by the absence of an isotropic peak in the $^{31}$P NMR spectra.

Although more membrane associated Aβ peptide structural detail is necessary before probable toxicity mechanisms can be developed, the weaker but similar shift toward β-sheet structure of Aβ(25–35) as Aβ(1–42), together with evidence of headgroup interactions, may reflect partial insertion of the shorter peptide and aggregation of the full-length peptide largely on the membrane surface, respectively [21], suggesting that the toxic form of Aβ may reside on the bilayer surface with β-sheet structural propensity driven somewhat by the Aβ(25–35) fragment.

5. Conclusion

Aβ(25–35) appeared to promote Cu$^{2+}$ destabilization of the membrane of polar lipid brain extracts. Although metal ions could adhere non-specifically to amyloid oligomers, our earlier NMR results [20] also showed that Cu$^{2+}$ alone but not Zn$^{2+}$ destabilized PC/PS bilayers without peptide. Aβ(25–35), if inserted through to the hydrocarbon–water interface of the bilayer, could allow more accessibility for Cu$^{2+}$ binding for the polar lipid extract than for the less fluid or more ordered membrane system, such as total brain lipid extract with cholesterol. Structural comparison of Aβ(1–42) and Aβ(25–35) at similar residues suggested that Aβ(1–42) undergoes an increase in β-sheet structure in the C-terminal region while Aβ(25–35) maintained its conformation in the presence of phospholipids. Since both peptides are cytotoxic to cell cultures, the conformational change towards β-sheet may be a crucial change required for Aβ(1–42) to exhibit cytotoxicity. Interestingly, Aβ(25–35) has been shown to mediate the effects of the full-length peptide and to be homologous to the tachykinin neuropeptide family [50]. Our previous studies showed alterations to the metal coordination by Aβ histidine residues attenuated peptide toxicity [6]. It seems Aβ(25–35), which lacks the histidine residues required for metal coordination, adopts a different toxic pathway that does not involve metal coordination [51]. This pathway can also be engaged by Aβ(1–42) as an alternative to metal dependent toxicity since Aβ(25–35) is embedded in the Aβ(1–42) sequence. However, the methionine is C-terminal in Aβ(25–35), which may play a crucial role [51,52] and, intriguingly, Aβ(25–36) with one additional residue is not toxic to cultured neurons [51]. If the cytotoxic pathway of Aβ(25–35) represents an alternate toxic pathway for the full-length peptide, and the composition of the membrane plays a role in the advancement of dementia, then the disease could be amplified by a deficiency in maintaining ‘normal’ lipid composition crucial for preserving membrane order or fluidity.

Acknowledgements

T.L.L. thanks the University of Melbourne for award of a PhD scholarship and David Hays Postgraduate Writing-up Award. K.J.B. is funded by the NHMRC. F.S. is grateful to the ARC for financial support (grant # DP0664601).

References


