Membrane-disordering effects of β-amyloid peptides

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Abstract

β-Amyloid (Aβ) protein is the major constituent of senile plaques and cerebrovascular deposits characteristic of Alzheimer's disease (AD). The causal relationship between Aβ and AD-specific lesions like neurodegeneration and atrophy is still not known. The present article summarizes our studies indicating that rather low concentrations of Aβ significantly alter the fluidity of cell membranes and subcellular fractions from different tissues and different species including humans, as a possible initial step of its biological effects. Using different fluorescent probes our data show clearly that Aβ peptides specifically disturb the acyl-chain layer of cell membranes in a very distinct

Membrane Active Peptides

These parameters, which can be determined separately using other techniques along with estimates of the two-dimensional density of the membrane components, provide a prediction of the sensor performance. The steps in the detection mechanism are shown in Figure 5. Experimental responses are shown in Figure 6.

Discussion

Sensitivity

Sensitivity is dependent on the antibody 'on rates' and affinity. For an antibody with 10^4 M^{-1}.s^{-1} 'on rate' and an affinity > 1 nM^{-1}, detection to sub-picomolar levels is possible in < 5 min.

Stability and variability

The stability of the tethered membrane has been enhanced by replacing ester with ether linkers. A test of the sealed membrane was successfully extended beyond 3 months when fully hydrated. It has also been shown that the sensing function returns on re-hydrating a fully assembled sensor. Long-term storage will be of dried sensors.

Depending on the concentration range, coefficients of variation for the sensor performance are in the range 6–15 %.

Chip arrays

A major advantage of this approach is the potential for miniaturization. A silicon chip array is under development. An optical technique permits specific receptors to be tethered to chosen sites within the array.

Optical patterning

The patterning of the electrode arrays is by optical masks that permit selective deprotection of biotin sites attached to either the membrane lipid or the ion channels in the membrane.

References


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Introduction

β-Amyloid (Aβ) protein is the major constituent of senile plaques and cerebrovascular deposits characteristic of Alzheimer's disease (AD). The causal relationship between Aβ deposition and AD-specific neuropathological lesions like neurodegeneration and cortical atrophy is still not known. Observations that Aβ has neurotoxic properties [1,2] represented the first link between Aβ formation and the AD-specific neuropathological lesions. Subsequent studies have confirmed the neurotoxic effect of Aβ [3,4] and have further suggested that its mechanism is probably related to the amplifying effect of Aβ on cellular calcium signalling and/or the induction of oxidative stress [3–8,40]. Again, the molecular mechanism of the Ca²⁺-amplifying and the oxidative-stress-inducing effects are not known. At low concentrations, Aβ alone does not affect baseline Ca²⁺, but only enhances Ca²⁺ influx after various stimuli [6], suggesting the activation of endogenous Ca²⁺-conductive mechanisms. On the other hand, observations that Aβ at high concentrations produces Ca²⁺ fluxes through artificial planar lipid bilayers suggest that Aβ may also disrupt or disturb membrane structure or integrity, leading to channel-like alterations of the membranes [9].

Membrane fluidity was determined using different fluorescent probes. When added to cellular or even artificial membranes, the fluorescent dye DPH (1,6-diphenylhexa-1,2,3-triene) is localized preferentially in the hydrocarbon core, while its cationic trimethylamino derivative TMA-DPH remains at the level of the polar heads of the phospholipid bilayer [12–15]. DPH intercalates preferentially axial between the acyl chains of membrane fatty acids (Figure 2). Accordingly, its mobility predominantly depends on acyl-chain flexibility that is restricted by the physico-chemical environment of the membrane. By contrast, excimer formation of the fluorescent probe pyrene represents predominantly the lateral motion of the probe within the lipid bilayer (bulk fluidity), while annular fluidity as measured by energy transfer from trytophan residues to pyrene gives information about the fluidity of lipids close to membrane proteins [16] (also see figure 2).

Aβ peptides alter membrane fluidity in mouse and rat brain membranes

The addition of Aβ25–35, which represents Aβ's neurotoxic sequence [1,2], to mouse brain membranes enhanced the fluorescence polarization (anisotropy) of membrane-bound DPH, which is inversely correlated with membrane fluidity [12]. The effect of Aβ25–35 was stable about 45 min after addition and was dependent on peptide concentration. In membranes of young animals, the effect was nearly maximal at 1 μM. Increasing the Aβ25–35 concentration 10-fold had little further effect on anisotropy. By contrast, anisotropy of aged mouse brain membranes was less affected by Aβ25–35 concentrations up to 1 μM, but showed some changes at a peptide concentration of 10 μM [12]. The effect of Aβ25–35 on the fluidity of mouse brain membranes was specific for the neurotoxic Aβ sequence, since a scrambled peptide (Aβ35–25) showed no effect when investigated under similar conditions [12].

In rat cortex, baseline anisotropy values were about the same as in the mouse brain [12]. However, rat cortex membranes were less sensitive to the fluidity-decreasing effects of Aβ25–35 [12]. While Aβ25–35 had no effect at 1 μM, it elevated anisotropy at 10 μM to about the same values as in the mouse brain. The same pattern was found for the rat striatum, while in the rat hippocampus 1 μM Aβ25–35 already showed a significant effect on anisotropy that was further increased by a 10-fold higher concentration. Membranes of rat cerebellum were the least sensitive [12].

High sensitivity of human cortex membranes

The addition of Aβ25–35 similarly decreased the fluidity of human frontal cortex membranes as indicated by the increased anisotropy of DPH (Figure 1). The effect of Aβ25–35 was concentration-dependent and could be seen already at concentrations as low as 100 nM. Although this concentration is still above the physiological Aβ level in human brain [17], it is substantially lower.
than the in vitro concentrations usually employed
to demonstrate Aβ's neurotoxic properties [1-4].
The pathologically relevant fragments Aβ₁-₄₂ and
Aβ₁-₄₃ affected the membrane in the same manner,
but were much more active than the neurotoxic
sequence Aβ₂₅-₃₅ (Figure 1), confirming data from
mouse brain membranes [12].

Other Aβ fragments showed qualitatively
similar but quantitatively different effects on
membrane fluidity (Figure 1). Aβ₁₋₂₈ had only a
weak effect on the fluidity of human frontal cortex
membranes, even at the highest concentration
investigated (10 μM). By contrast, incubation with
10 μM Aβ₂₅₋₃₅ or Aβ₁₋₄₀ gave a pronounced
increase in membrane anisotropy (Figure 1).
While 1 μM or 5 μM Aβ₁₋₄₀ and Aβ₁₋₄₃ were about
equally effective, 10 μM Aβ₁₋₄₀ was more active
than the same concentration of Aβ₂₅₋₃₅. The
scrambled peptide Aβ₂₅₋₃₅ was not active under the
same experimental conditions up to 10 μM (Figure
1). Aβ₂₅₋₃₅ affected membranes from frontal and
parietal human cortex in the same fashion [13].

The effects of Aβ on the fluidity of human
cortex membranes at the lipid/water interface
(TMA-DPH anisotropy) were qualitatively similar
to that of the membrane hydrocarbon core, but
were quantitatively less, indicating weaker effects
at this membrane layer [13]. The effects of Aβ
were not restricted to brain membranes, but could
also be observed with human lymphocyte mem-
branes [12,18].

Aβ sensitivity of different membrane
layers

Using the different fluorescent probes already
mentioned, we also investigated the sensitivity of
the different layers of mouse brain membranes
for the perturbing effects of Aβ₂₅₋₃₅ and Aβ₁₋₄₂
(Table 1). The data clearly indicate that the
flexibility of the acyl chains is most sensitive and
that this part of the membrane structure (Figure 2)
becomes less fluid, as indicated by increased DPH
anisotropy. Very interestingly, lateral motion
within the same region is increased (bulk fluidity

![Figure 1](http://example.com/figure1.png)

**Figure 1**

Effect of four different Aβ peptides on the anisotropy (DPH) of human frontal cortex membranes

Inset: the effect of increasing concentrations of three Aβ peptides on the anisotropy (DPH) of human frontal cortex membranes. All data are means±S.D. of six experiments, each representing an individual brain sample. Data are adapted from [1 3]. ***P < 0.001 when compared with the anisotropy without Aβ (paired Student's t test).
Table I

<table>
<thead>
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<tr>
<td><strong>Effects of aggregated Aβ peptides Aβ_{25-35} (5 μM) and Aβ_{1-42} (5 μM) on the biophysical properties of crude brain membranes from aged mice (22 months)</strong></td>
</tr>
</tbody>
</table>

Membrane fluidity of the hydrocarbon core and of the region of phospholipid head groups was determined with polarized fluorescence spectroscopy using the dyes DPH and TMA-DPH, respectively. Bulk and annular fluidity were assessed using pyrene excimer formation at the fluorescence emission wavelengths of 334 and 286 nm, respectively. Means±S.D. are shown (n = 8). *P < 0.05, **P < 0.01 and ***P < 0.001 when compared with the respective control (paired Student’s t test).

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Aβ_{25-35} (5 μM)</th>
<th>Δ</th>
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</thead>
<tbody>
<tr>
<td>Anisotropy (DPH)</td>
<td>0.1954±0.0008</td>
<td>0.2095±0.0080***</td>
<td>+7.2%</td>
</tr>
<tr>
<td>Anisotropy (TMA-DPH)</td>
<td>0.2101±0.0018</td>
<td>0.2217±0.0019**</td>
<td>+5.5%</td>
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<td>Bulk fluidity</td>
<td>0.4413±0.0371</td>
<td>0.4772±0.0487</td>
<td>+8.1%</td>
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<tr>
<td>Annular fluidity</td>
<td>0.6503±0.0331</td>
<td>0.6535±0.0430</td>
<td>+0.5%</td>
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</table>

<table>
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<th>Aβ_{1-42} (5 μM)</th>
<th>Δ</th>
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<tr>
<td>Anisotropy (DPH)</td>
<td>0.1954±0.0008</td>
<td>0.2434±0.0188***</td>
<td>+24.6%</td>
</tr>
<tr>
<td>Anisotropy (TMA-DPH)</td>
<td>0.2101±0.0018</td>
<td>0.2246±0.0065***</td>
<td>+6.9%</td>
</tr>
<tr>
<td>Bulk fluidity</td>
<td>0.4413±0.0371</td>
<td>0.4827±0.0440</td>
<td>+9.4%</td>
</tr>
<tr>
<td>Annular fluidity</td>
<td>0.6503±0.0331</td>
<td>0.6541±0.0425</td>
<td>+0.6%</td>
</tr>
</tbody>
</table>

measured with pyrene). The latter observation is usually explained by enhanced fluidity. This interesting observation can be partially explained by different effects on different subcellular membranes (see below). Fluidity close to the interface with membrane proteins (annular fluidity) was not affected by both Aβ peptides (Table 1).

**Figure 2**

**Determination of membrane dynamics**

Fluorescent dyes represent a prominent tool for the determination of membrane dynamics. DPH intercalates predominantly between the acyl chains of fatty acids in the membrane hydrocarbon core. TMA-DPH remains at the level of the hydrophilic head groups of membrane phospholipids. Because TMA-DPH is positively charged it labels predominantly the negatively charged cytoplasmic leaflet. Pyrene diffuses along the hydrocarbon core and mainly labels the interphase between both membrane leaflets.

**Different sensitivity of synaptosomal and mitochondrial membranes**

On the basis of our findings that Aβ peptides decrease the membrane fluidity of rather crude membrane homogenates from rodent and human brain [12,13], we extended our investigations to the effects of Aβ on fluidity parameters of purified synaptosomal plasma membranes (SPM) and mitochondrial membranes [39]. These structures are linked to neuronal signal-transduction pathways and the intracellular generation of free radicals respectively. The processes of brain aging and AD affect both. We examined the effects of Aβ on subcellular brain membranes of different age groups. This seems to be particularly important since it has been shown that brain aging enhances Aβ neurotoxicity in vivo [19].

Both brain-membrane fractions from aged mice (22 months) showed increased DPH anisotropy, indicating reduced flexibility of the membrane acyl chains in the hydrocarbon core (Figure 3). By contrast, SPM from aged mice brain showed increased pyrene excimer formation, suggesting enhanced lateral motion, whereas the mobility of pyrene in mitochondrial membranes was unchanged. The results indicate that aging leads to opposite effects on the bulk fluidity in different membrane areas of the two subcellular membrane fractions investigated: the acyl-chain flexibility of membrane fatty acids decreases, whereas the lateral mobility within the bilayer increases. This is remarkable, since it shows for the first time that...
Membrane Active Peptides

Brain aging leads to complex alterations in different membrane regions and in different cell compartments.

\( \text{\textit{A}}\beta \) peptides decreased the fluidity in the region of acyl chains of membrane fatty acids, as indicated by increased DPH anisotropy in SPM and mitochondrial membranes in a similar fashion as shown for the effects of \( \text{\textit{A}}\beta_{1-40} \) in Figure 1. For both brain-membrane fractions from young and aged mice we always found the same order of potency: \( \text{\textit{A}}\beta_{1-42} > \text{\textit{A}}\beta_{1-35} > \text{\textit{A}}\beta_{1-40} \). Both investigated brain-membrane fractions from aged mice were less susceptible to the effects of \( \text{\textit{A}}\beta \) peptides than similar fractions from young mice with regard to fatty acid chain flexibility [39].

In contrast, \( \text{\textit{A}}\beta \) peptides increased the lateral mobility of pyrene in both membrane fractions from young and aged mice only weakly [39], with the exception of much stronger effects of \( \text{\textit{A}}\beta_{1-42} \) on the bulk fluidity of mitochondrial membranes (Figure 2). Thus, the differential effects of \( \text{\textit{A}}\beta \) on the two dyes located in the fatty acid chain layer (decreased fluidity as indicated by DPH anisotropy and increased fluidity by pyrene eximer formation) has to be attributed mainly to the mitochondrial membrane fraction.

Nevertheless, our data show that \( \text{\textit{A}}\beta \) peptides profoundly alter similar parameters of SPM as well as mitochondrial membranes. This might suggest that not only \( \text{\textit{A}}\beta \) effects on signal trans-
duction [20] but also its effects on the activity of respiration-chain enzymes, as found in isolated rat brain mitochondria as well as in PC12 cells [21,22], might be initially mediated by its membrane-disordering properties. If this effect contributes to the observed mitochondrial dysfunction in AD [23–26] it needs further clarification.

The relevance of cholesterol
Since DPH anisotropy is usually related to the cholesterol content of brain membranes [27,28], we investigated whether enhanced cholesterol might explain our observation of increased DPH anisotropy in both aged membrane fractions. However, in agreement with other findings [28], cholesterol content was enhanced in aged SPM fractions, but not in aged mitochondrial membranes.

We have shown previously that enhanced membrane cholesterol reduces the membrane disordering effects of Aβ in vitro [11] and that endogenous membrane cholesterol differently modulates the effects of Aβ on brain membranes from AD patients [29]. Moreover, cholesterol protects PC12 cells from Aβ toxicity in vitro [30] and inhibits the effects of Aβ on cellular calcium signalling [11]. Since several recent studies suggest brain cholesterol alterations as one important factor in AD neurobiology [31–33,41], our recent findings of enhanced cholesterol levels and reduced disordering effects of Aβ peptides in aged SPM membranes may increase the understanding of the role of cholesterol in AD pathology.

Because mitochondrial membrane cholesterol levels were unchanged during aging, we assume that Aβ effects were additionally modified by oxidative membrane alterations in aged mice brain [34,35].

Aβ localization inside membranes
Our observation of a pronounced effect of Aβ peptides on the hydrocarbon core of membranes is supported by X-ray diffraction analysis [36,37] and fluorescent probe techniques [38] using artificial membranes. Membrane insertion is independent of oxidative reactions [38,39].

Final conclusion
In summary, Aβ disturbs membrane properties not only of SPM, but also of mitochondrial membranes. Brain aging also differently modifies both Aβ effects and itself modifies the fluidity of intracellular membranes. These findings are of particular interest in view of the neurotoxic properties of Aβ [18] and its intracellular biology [36]. Moreover, our data point to membrane alterations as a possible therapeutic target in AD brains [37].

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References

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Influenza fusion peptides
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Abstract
The 'fusion peptides' of a group of enveloped viruses that includes influenza, paramyxoviruses, retroviruses and filoviruses are N-terminal regions of their membrane fusion proteins generated by cleavage of non-functional precursors. For the influenza membrane fusion protein, haemagglutinin (HA), the three-dimensional structures of precursor HA, cleaved HA and fusion-activated HA show that the fusion peptides are located in different positions in all three forms and adopt different structures. Analyses of mutant HAs with changes in fusion peptide sequence indicate the importance of specific residues for membrane-fusion activity and suggest a structure for the fusion peptide in a fusion-active molecule.

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Abbreviations used: HA, haemagglutinin.

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In contrast to cellular membranes, for influenza, the haemagglutinin (HA) surface glycoprotein is both a receptor-binding and membrane fusion protein [1]. It is a trimer of identical subunits, each containing two disulphide-linked polypeptides, HA₁ and HA₂, that are derived by proteolytic cleavage of a precursor, HA₀, that has a signal sequence at its N-terminus and a membrane anchor sequence at its C-terminus. Cleavage to form HA₁ and HA₂ generates the N-terminus of a smaller polypeptide, HA₁, which has the membrane anchor sequence at its C-terminus. Cleavage is required for membrane fusion activity. The HA₀ N-terminal sequence is called the 'fusion peptide' because cleavage at similar hydrophobic sequences is also required for the activity of other virus fusion proteins, and because 20-residue synthetic peptide analogues of the sequence fuse membranes in vitro.

Comparison of the three-dimensional structures of cleaved HA and uncleaved HA₀ indicates their close similarity except for 19 residues at the site of cleavage (Figure 1). The site is an almost circular surface loop with the membrane-proximal