Membrane Interaction of Alzheimer's Beta Peptides

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Background

A number of extracellular proteins can misfold spontaneously and aggregate to form β-sheet rich amyloid deposits characteristic of a variety of “protein misfolding” diseases, most notably Alzheimer’s disease (Bucciantini et al., 2002; Ellis et al., 2002). While the precise molecular basis of such conditions is unclear, strong arguments are emerging to implicate organ-specific amyloidogenic proteins, particularly oligomeric intermediates on the pathway to amyloid fibril formation, in disease pathogenesis (e.g. Walsh et al., 2002).

Aβ is a 39-43 amino acid peptide produced by proteolytic cleavage of the amyloid precursor protein by γ-secretase (Haass and De Strooper, 1999). Aβ’s toxicity to various types of cells is well-documented, but it remains unclear whether it is the spontaneously-forming amyloid fibrils, or some other intermediate aggregate, that is the cytotoxic form. A number of papers report that Aβ neurotoxicity may be mediated, at least in part, by direct interactions between the peptide and the membrane lipids, while other observations suggest that extracellular Aβ fibrils and plaques are relatively inert (Hardy et al., 2002), like those formed by another amyloidogenic peptide, IAPP (Janson et al., 1999), raising questions about the precise mechanism of Aβ peptides.

These neutron measurements form part of a larger study in which we are investigating the structural basis of the interaction between Aβ peptides and phospholipid membranes. The peptide used was the 11-residue fragment Aβ (25–35), which is known to possess much of the biological activity of the full-length peptide (Dante et al., 2002). We were studying the kinetics of membrane interaction, using mixtures of two types of Aβ (25–35), one comprising all L-amino acids and one comprising all-D. Preliminary data show that each peptide, on its own, interacts with phospholipids in the same way, but the behaviour of mixtures is very different.

Neutron Measurements

The 11 amino acid L- and D-Aβ (25–35) peptide was synthesized by Almac Sciences Ltd. (20 Castle Terrace, Edinburgh, EH1 2EN, Scotland, U.K.) using solid-phase synthesis. Their purities were > 95%, as determined by analytical HPLC, MALDI-TOF mass spectrometry, and amino acid analysis. 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) was purchased from Avanti Polar Lipids Inc., USA, and used without further purification. Chloroform, methanol and buffers are all graded AR and purchased from Sigma-Aldrich (Irvine, UK).

Neutral lipid samples were measured on the N5 spectrometer at Canadian Neutron Beam Centre (CNBC), Chalk River Laboratories, Ontario, Canada. 20 mg of DOPC was dissolved with 1 mol % peptide in Milli-Q water, sonicated for 10 minutes and evenly spread onto silicon wafer slides. Then the slides were dried for 24 hours at room relative humidity in a dust-free environment and subsequently placed in a vacuum desiccator for over 12 hours in order to remove all traces of the solvent. Samples were hydrated at a relative humidity using saturated salt solutions of KCl, KNO3 or K2SO4 in 8.06 % or 25 % (v/v) D2O. The salt solutions set the relative humidity to 84%, 94% or 98%, respectively. All samples were run at a temperature of 25°C with a circulating water bath. Sample equilibration was determined after sequential θ-2θ scans, which showed no change in the position of the Bragg peaks. The mosaic spread of the first order of diffraction was also determined for each sample using standard procedures. Every data set, comprising five orders of diffraction, was finally collected. The background was subtracted and Bragg peaks were fitted with Gaussian functions using Origin Pro 8.5 software. Gaussian distributions were then fitted to the Bragg peaks and the angular position, width and intensity of each peak were recorded. Raw data corrections and the calculation of structure factor amplitudes proceeded as previously described (Davies et al., 2003).
A series of experiments have been conducted to compare the peptide distribution at 1 mol% L-Aβ peptide in the DOPC lipid bilayer. Appropriate raw neutron diffraction data were phased and placed on a relative absolute scale to reconstruct the SLD profiles by Fourier synthesis. In above figure, the blue line is the SLD profile of pure DOPC and the red line is the SLD profile of DOPC lipid with L-Aβ peptide at 8.06% D₂O. The green line is the difference between two lipid SLD profiles under 8.06% D₂O in the presence and absence of peptide, representing the transbilayer peptide distribution. The observed difference profile can be fitted as one Gaussian shaped peaks in reciprocal space following the procedure already described (Ashley et al, 2006), revealing the position, width and area of transbilayer peptide distribution. The best fit to observed peptide profile clearly shows that the interaction between L-Aβ peptide and neutral DOPC lipid bilayer takes place at a single position at 1 mol% peptide concentration. A fraction of the peptides were deeply inserted into the hydrophobic core of lipid bilayer at around 9.8 Å from the center of the bilayer.

Figure 1 Preliminary data analysis, showing difference profile (green) calculated by subtracting the bilayer distribution of DOPC from the corresponding profile of DOPC plus 1 mol% Aβ (25-35) peptide.

References


