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Influence of Alzheimer's β-amyloid peptide on the lateral diffusion of lipids in raft-forming bilayers

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The effect of added Alzheimer's Aβ(1-40) peptide of the wild type on the lateral diffusion of lipids in macroscopically oriented bilayers of 'raft' compositions [a mixture of dioleoylphosphatidylcholine (DOPC), sphingomyelin (SM) and cholesterol (CHOL)] was studied by an NMR-diffusion technique. In homogeneous bilayers, diffusion coefficients decrease, while they change differently in liquid ordered and in liquid disordered phases at varied concentrations of CHOL; this was explained by the decreased maximum solubility of CHOL in phospholipids in the presence of Aβ(1-40) peptide.

Experimental data[1,2] demonstrated that Alzheimer's Aβ amyloid peptide interacts with biomembranes, and this interaction modifies the properties of lipid bilayers, further disrupting the fluidity and functions of the membranes. Sphingomyelin (SM), phosphatidylcholine (PC) and cholesterol (CHOL) are important lipids of eukaryotic cellular membranes and neuronal tissues, and they presumably participate in the formation of membrane domains, known as 'rafts', through intermolecular interaction, lateral microphase decomposition and formation of SM-depleted (liquid disordered, ld) and SM-enriched (liquid ordered, lo) liquid crystalline phases.[3–5] Aβ peptide induces a strong membrane destabilization in these lipid bilayers[2,6] and binds to both phases but preferably to the lo phase. Lateral diffusion of lipids is an essential dynamic factor in the two-dimensional lipid membrane system,[7] which can be indicative of interlipid interactions and decomposition processes, particularly in 'raft'-forming membranes.[4,5] In this work, we employed pulsed field gradient NMR to study the lateral diffusion of lipids in the macroscopically oriented bilayers of raft compositions [containing SM, dioleoylPC (DOPC) and CHOL] with and without added Aβ(1-40) peptide.

Human wild-type Alzheimer's Aβ(1-40) peptide with an amino acid sequence (DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVV) was synthesized by a standard solid-phase FMOC procedure with HBTU activation and subsequently purified by HPLC as described previously.[8] The purity of the final product was 95%, as found by MALDI-TOF mass spectrometry. Commercial DOPC, egg yolk SM and CHOL (Avanti Polar Lipids, Alabaster, AL) were used. Macroscopically oriented multibilayers were prepared following reported procedure.[9] The Aβ(1-40) peptide was initially used in monomeric form. The lipid/peptide molar ratio was 700:1. Hydration (D_2O) was performed during five days, and the final water content of the test samples was 50 wt%; pH 4.4–5.2. To elucidate how the lipid composition influences the secondary structure of the peptide, UV circular dichroism (CD) spectra were measured in the same systems at a 5000-fold dilution because of too high absorptivity, and the final concentration of Aβ(1-40) was 12.5 μmol dm^{-3}. A Jasco J-810 spectropolarimeter equipped with a quartz cuvette having a sample volume of 370 μl and an optical path length of 1 mm was used in the CD measurements. The experiments were carried out in a wavelength range of 190–250 nm. Each sample was measured eight times, and the CD spectra were averaged; the spectrum of a buffer was subtracted from the original spectrum (Figure 1). These spectra are identical to the CD spectra of Aβ(1-40) in similar lipid/peptide systems.[1] A rather strong spread of experimental points at low angles (190–205 nm) conditioned by an increased absorption in this wavelength range.[10] The shapes of the spectra better correspond to the β-sheet structure (Figure 1). To analyze CD spectra quantitatively, we employed the SDSSTR program from the DICHROWEB interactive software,[11] which is a modification of the Varslc software.[12] The analysis gave a set of the following Aβ(1-40) secondary structure components: 0.07, helix; 0.41, β-strand; 0.21, β-turns; and 0.31, unordered structure.

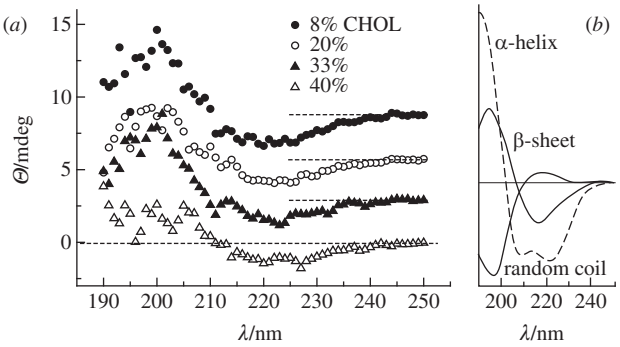

Figure 1 (*a*) CD spectra of 12.5 μM Aβ(1-40) in DOPC/SM/CHOL lipid vesicles with DOPC:SM = 1.1 and indicated CHOL concentration (mol%). T = 20 °C. Spectra for 8, 20 and 33 mol% CHOL were shifted along the Θ-axis for convenience. Dashed lines show zero degree angle. (*b*) CD spectra characteristic of three different peptide conformations.[24]

A Chemagnetic InfinityPlus NMR spectrometer (Agilent) operating at proton frequencies of 359.2 MHz was used. A square cross-section sample tube containing macroscopically oriented lipid multibilayers was placed in a specifically designed goniometer probe that enabled the bilayers to be oriented with the bilayer normal at the magic angle (54.7°) with respect to the constant magnetic field of the spectrometer.[4] This causes the dipolar interactions to vanish, resulting in a significant reduction of the line width. The sample preparation and NMR PFG method for measuring lipid lateral diffusion on macroscopically oriented bilayers were described in the literature.[4,5,9,13] The stimulated echo pulse sequence was applied to diffusion measurements.