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Different effects of Alzheimer's peptide Aβ(1–40) oligomers and fibrils on supported lipid membranes

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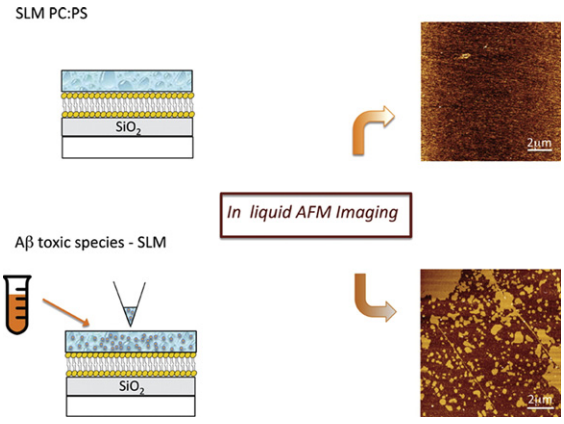
HIGHLIGHTS

- The supported lipid bilayer is a membrane model system sensitive to Aβ aggregates.
- The approach allows to point on interaction effects without any aggregation.
- Aβ oligomers produce large holes in the double layer.

GRAPHICAL ABSTRACT



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ABSTRACT

Beta-amyloid (1–40) is one of the two most abundant species of amyloid-beta peptides present as fibrils in the extracellular senile plaques in the brain of Alzheimer's patients. Recently, the molecular aggregates constituting the early stage of fibril formation, i.e., oligomers and protofibrils, have been investigated as the main responsible for amyloid-beta cytotoxic effect. The molecular mechanism leading to neurodegeneration is still under debate, and it is common opinion that it may reside in the interaction between amyloid species and the neural membrane. In this investigation Atomic Force Microscopy and spectroscopy have been used to understand how structural (and mechanical) properties of POPC/POPS lipid bilayers, simulating the phospholipid composition and negative net charge of neuritic cell membranes, are influenced by the interaction with Aβ(1–40), in different stages of the peptide aggregation. Substantial differences in the damage caused to the lipid bilayers have been observed, confirming the toxic effect exerted especially by Aβ(1–40) prefibrillar oligomers.

1. Introduction

Alzheimer's disease (AD) is a chronic and progressive syndrome, which affects about 5% of the population over age 65. It represents the most common cause of dementia in the elderly population. From a molecular point of view, AD is characterized by the accumulation of a 39–42 amino acid peptide, the amyloid-beta peptide (Aβ), in insoluble cerebral plaques, known as amyloid fibrils [1]. The amyloid aggregation process in solution follows typical nucleation-polymerization kinetics, characterized, in each phase, by structural intermediates presenting

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specific dimensions, morphologies and cytotoxic activity. Substantial evidence suggests that small, prefibrillar oligomers that form at the beginning of the aggregation path represent the most toxic amyloid species [2–4]. Experimental evidences connect the interaction between Aβ peptides and the neural membranes as the trigger of the neurotoxic mechanism [5–7]. Nevertheless, the specific molecular mechanisms underlying Aβ/membrane interaction remain to be elucidated. The reciprocal action of Aβ and membranes can be analyzed in two different perspectives. On one hand, Aβ species have been reported to damage the membrane structure [8,9] and to perturb its ionic balance [10] as well as its mechanical stability [11]. On the other hand, the membrane surface itself, depending on its chemistry, may locally act as a catalyzer for the peptide misfolding, producing dangerous intermediates and triggering the fibrillogenesis [12]: the membrane acts as a bidimensional template for the aggregation steps of the Aβ peptide. In this scenario, the use of supported lipid bilayers (SLBs), obtained through Langmuir–Blodgett deposition or through fusion of unilamellar vesicles on a flat surface [13,14], and mimicking the composition of natural neural membranes is of particular appeal in order to select and elucidate particular aspects of this interaction at a molecular level. In particular, Atomic Force Microscopy (AFM) is an ideal tool to study the effect of exogenous molecules on lipid bilayers, especially because it allows the in situ investigation in physiological conditions; AFM has therefore become a well-established technique for imaging SLBs at the nanoscope resolution and, in the spectroscopy mode, for the analysis of the membrane nanomechanics; this latter is analyzed through the acquisition of force–distance curves and the determination of the breakthrough force F_b for SLBs [15,16] and is directly related to lipid packing and lipid order in the membrane.

In this investigation we have studied the effects of different Aβ(1–40) intermediates on SLBs by AFM imaging and force spectroscopy. As model membrane, SLBs made of a mixture of 9:1 mol/mol of 1-palmitoyl-2-oleoyl-phosphatidylcholine (POPC) and 1-palmitoyl-2-oleoyl-phosphatidylserine (POPS) were used, in order to mimic, as closely as possible, the composition and the negative charge state of neuritic cell membranes without increasing the complexity of the system too much. The same lipid mixture was used in our preliminary studies showing that the interaction and intercalation of different Aβ species are very dependent on anionic state and composition of the membrane [17,18].

The different Aβ species at the various instants of the aggregation kinetics have been found to produce different effects not only on the structure but also on the mechanical properties of the SLBs. A peculiar instant of transition between membrane active and membrane-nonactive Aβ peptides has been also evidenced.

2. Materials and methods

2.1. Aβ aggregation by fluorescence spectroscopy

The synthetic peptide Aβ(1–40) (PolyPeptide Laboratories, Strasbourg, France) was pretreated according to the procedure of Fezoui et al. [19] for improving the reliability of experiments at neutral pH. Stock aliquots (200 μg each) were stored at −80 °C.

Aβ(1–40) samples were prepared by dissolving the lyophilized peptide in 50 mM phosphate buffer, pH 7.4, at a concentration of about 70 μM. The solution was serially filtered through 0.22 μm (Millex-LG) and 20 nm (Anotop-Whatman) filters into a fluorescence quartz cuvette containing a small magnetic stirring bar. Aβ(1–40) concentration was determined by tyrosine absorption at 276 nm using an extinction coefficient of 1390 cm^{-1} M^{-1} [20]. The sample was then diluted to the working Aβ(1–40)concentration of 48 μM, by adding the appropriate amount of buffer and tiny amount of a concentrated solution of Thioflavin T (ThT) to have a final ThT concentration of 12 μM.

The change in ThT fluorescence emission during the kinetics of Aβ aggregation was monitored by using a JASCO FP-6500 spectrometer.

The excitation and emission wavelengths were 450 and 485 nm, respectively and the slit width was set to 3 nm both in excitation and emission. The sample was placed in the cell compartment thermostated at 37 °C, and continuously sheared at 200 rpm by using a magnetic stirrer (mod. 300, Rank Brothers Ltd., Cambridge). Each experiment was repeated at least three times. Aliquots of 100 μL of solution were taken away at different times to be used for AFM characterization and bilayer interaction studies.

2.2. Supported lipid bilayers

The phospholipids used for vesicle preparation were the zwitterionic 1-palmitoyl-2-oleoyl-phosphatidylcholine (POPC) and the negatively charged 1-palmitoyl-2-oleoyl-phospatidylserine (POPS) from Avanti Polar Lipids (Alabaster, Alabama, US). The lipids were dissolved in chloroform/methanol (Sigma-Aldrich) 2:1, mixed in a ratio POPC/POPS 9:1 mol/mol and gently dried under a nitrogen flux. Solvent traces were removed under vacuum, overnight. The lipid mixtures were then resuspended in Phosphate Buffer Saline (PBS 1×), in a concentration of 1 mg/ml, vortexed and let to swell for at least 30 min. The resulting opalescent suspension was extruded at least 11 times through polycarbonate membranes with 100 nm pores using a commercial extruder (Avanti Polar Lipids). The obtained large unilamellar vesicles (LUVs) were then diluted 10 fold and 50 μL of the vesicle suspension was administered to the solid support for AFM investigation. Square silicon supports 5 mm × 5 mm were used as substrates; before use, the Si surface was cleaned with a sodium dodecyl sulfate (SDS) solution under sonication (30 min), rinsed with Milli-Q water several times and put in an UV/Ozone chamber (Bioforce, US) for at least 30 min to remove any organic contamination. The vesicles were let in incubation overnight at ambient temperature at high relative humidity to allow vesicle fusion and to achieve uniform lipid bilayer formation. The substrate was then gently rinsed with Milli-Q water to remove the vesicle in excess.

2.3. Aβ peptide

After imaging the lipid bilayer to acquire a topographical view of the surface and collecting force curve maps in at least 5 different areas, so to ensure homogeneity of the sample, fresh solutions of Aβ were prepared by suspending in phosphate buffer the aliquots, collected at the different stages of fibrillization. The final concentration was 1 μM. Thereafter, 40 μL of 1 μM Aβ solutions was administered to the liquid subphase in contact to the lipid bilayer and let to incubate 20 min and carefully rinsed. The sample was then placed again under the AFM for topographical inspection.

2.4. AFM imaging

All AFM measurements were performed by using a Nanowizard III (JPK Instruments, Germany) mounted on an Axio Observer D1 (Carl Zeiss, Germany) inverted optical microscope. V-shaped silicon nitride cantilevers (SNL, USA), with a nominal spring constant ranging from 0.12 N/m to 0.48 N/m, with a resonance frequency in air ranging from 40 kHz to 75 kHz and tip with typical curvature radius of 2–12 nm were used. The actual spring constant of each cantilever was determined in situ, using the thermal noise method [21].

Aliquots of protein solutions (1 μM) were deposited onto freshly cleaved mica surfaces (Agar Scientific, Assing, Italy) and incubated for up to 20 min before rinsing with deionized water and drying under a low pressure nitrogen flow. Imaging of the protein was carried out in intermittent contact mode in air.

To image the supported lipid bilayers, the silicon substrates were fixed to a glass coverslip by using a two-component epoxy glue. The samples were always kept in an aqueous subphase. AFM images were collected using Quantitative Imaging mode (QI mode, JPK), working in liquid environment. In QI mode the height information is extracted