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Adsorption of β-amyloid oligomers on octadecanethiol monolayers

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ABSTRACT

Hypothesis: β-Amyloid oligomers of different aggregation and physiological functions exhibit distinct adsorption behavior allowing them to be discriminated in preparations.
Experiments: Two forms of amyloid oligomers, small 1–4 nm and large 5–10 nm were formulated using synthetic 42 amino acids β-amyloid peptide. Forms differ in their size and physiological function. A systematic study of adsorption of these amyloid species on self-assembled monolayers of octadecanethiol on gold was performed. Structural changes upon adsorption of oligomers were interrogated by the reflection absorption infrared spectroscopy.
Findings: The amount of adsorbed peptide material, as detected by surface plasmon resonance spectroscopy, is similar in case of both small and large oligomers. However, adsorption of small oligomers leads to a transformation from beta sheet rich to beta sheet depleted secondary structure. These changes were accompanied by the unique morphology patterns detectable by atomic force microscopy (AFM), while the quartz microbalance with dissipation indicated a formation of a compact adsorbate layer in case of small oligomers. These effects may be integrated and utilized in bioanalytical systems for sensing and detection of Alzheimer's disease related peptide forms in artificial, and possibly, real preparations.

1. Introduction

Interaction of amyloid-β (Aβ) peptides and their oligomer forms with surfaces of various hydrophobicity/hydrophylicity attracted considerable attention of different research groups. Interest in adsorption properties of amyloidogenic proteins stems from the presumed role of interfaces in the formation of various Aβ species implicated in Alzheimer's pathogenesis. Some of those species may exert adverse physiological effects both in cell cultures and tissues, and, consequently, trigger pathological changes. Interfaces may alter oligomerization pathways both in vivo and in vitro conditions. In case of interaction of artificial biocompatible materials with plasma and/or cerebral spinal fluid containing amyloidogenic proteins may affect oligomerization and result in unwanted long term effects in organisms [1]. On the other hand, as recently reported [2], the affinity of amyloid species towards the hydrophobic surfaces may provide a therapeutic platform for the removal of toxic amyloid species from blood.

According to various research groups, surface-induced nucleation and oligomerization, though being different at the molecular level, seems to be a general property of proteins and peptides prone to forming supramolecular species associated with various physiological processes and pathologies in organisms [1,3–7]. Amyloid peptides and, in particular, its fragment Aβ(10–35) was shown to adsorb and oligomerize upon contact with surfaces of the self-assembled monolayers (SAMs) [8]. Adsorption of amyloid species were observed for both hydroxyl- and methyl-terminated as well as charged SAMs [9,10]. Aβ(1–42) peptide was found to form spherical amorphous clusters on hydrophobic SAMs, while charged surfaces promoted the formation of protofibril entities [9]. It was pointed out that most of the surfaces exhibit accelerating effect on β-sheet rich structure and amyloid fibril formation [10].

Adsorption and interaction of Aβ(1–42) with surfaces were studied using methods of molecular dynamic (MD) simulations [11–13]. Theoretical analysis of the energy of interaction of low molecular weight oligomers (dimers through hexamers) with methyl-terminated self-assembled monolayers revealed domi-

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nance of hydrophobic interaction as a driving force of adsorption [13]. Interestingly, MD simulations indicate extensive secondary structure changes when dimers of a fragment Aβ(17–42) adsorbs on methyl-terminated SAMs, while the higher oligomerization degree species retained their predominantly β-strand-loop-β-strand structural motif [13].

So far, in most Aβ(1–42) adsorption studies, a comparison of adsorption properties on different surfaces (hydrophilic/hydrophobic, charges/uncharged) were carried out [8,9,14]. In this work, we look at the Aβ(1–42) adsorption phenomenon from the different perspective. Our objective was to compare the adsorption behavior of different size and morphology Aβ(1–42) oligomers, which, as some of our group demonstrated earlier [15], exhibit quite different physiological activities. Small, 1–4 nm z-height (as measured by an AFM) spherical oligomers trigger necrosis in cell cultures, while larger 5–10 nm Aβ(1–42) species does not exhibit cytotoxic effect [15]. In this study, we investigated an interaction of those two different biological activity Aβ(1–42) oligomer forms with the hydrophobic octadecanethiol SAMs on gold. Different physical signatures of interaction of Aβ(1–42) oligomers with the hydrophobic surfaces may be utilized in the bioanalytical detection and separation systems that utilize the adsorption phenomenon. The detailed mechanism of the adsorption of these species also may contribute to understanding of complex mechanisms of oligomerization, which is believed being involved in the Alzheimer's pathogenesis.

2. Experimental

2.1. Atomic force microscopy

The morphology of Aβ(1–42) oligomer preparations were characterized using the atomic force microscope (AFM) Dimension Icon (Bruker, Germany) scanning probe work station, operating in the tapping mode. Model FESP (Veeco, Plainview, NY) (f = 75–100 kHz, k = 2.8–5 N/m) micro cantilevers were used in this work. 20 μL of 1 μM Aβ(1–42) sample solution was spotted on octadecanethiol (ODT) – modified gold surface incubated at room temperature for 10 min, rinsed with deionized water, Milli-Q system (EMD Millipore, USA) and blown dry with filtered (<0.2 μm) nitrogen stream. Images data were acquired at scan rates between 0.5 and 1 Hz at 512 × 512 pixel resolution. The morphology of the surfaces was estimated by measuring the profile of the sample along the x–y plane of the AFM images. The mean z-height was estimated by using the plane correction module of the Gwyddion software and determining by the step-height graphs.

2.2. Reflection absorption IR spectroscopy (RAIRS) measurements

RAIRS spectra were recorded on FTIR spectrometer Vertex 80v (Bruker, Germany) equipped with the liquid nitrogen cooled MCT narrow band detector and the horizontal reflection accessory. The spectral resolution was set at 2 cm^{-1}. Spectra were acquired by 400 scans at a grazing angle of 80° by using p-polarized light. The sample chamber and the spectrometer were evacuated during the measurements. The spectrum of ODT SAM on Au was used as a reference. Parameters of the bands were determined by fitting the experimental contour to Gaussian–Lorentzian form components using the GRAMS/AI 8.0 (Thermo Scientific, USA) software.

2.3. Preparation of Aβ(1–42) oligomers

Preparation of oligomers was carried out according to protocols described in detail earlier [15]. Briefly, according to protocol I, soluble oligomers were prepared by dissolving 1 mg of peptide (American Peptide, Inc., USA) in 400 μL of hexafluoroisopropanol (HFIP) (Sigma–Aldrich, Germany) for 30–60 min at room temperature under sonication. About 100 μL of the resulting solution was added to 900 μL of H_2O in a siliconized Eppendorf tube. After 10–20 min incubation at room temperature, the samples were centrifuged for 15 min at 12,000 rpm, the supernatant was transferred to a new siliconized tube and HFIP was evaporated on the 25 °C water bath until no FTIR spectra line at 1192 cm^{-1} of the asymmetric CF_3 stretching vibration was visible. Then, sample was incubated in closed vial for 24 h at 20 °C. Protocol I yields particles with predominantly 1–2 nm z-height as measured by the AFM. Occasionally this preparation contained some (typically below 20%) fraction of oligomer particles up to 4 nm. Hereinafter, such preparation (1–4 nm) is referred to as "small oligomers". These preparations exhibited strong cytotoxic effect [15]. Large oligomers with the 5–10 nm z-height were obtained by transferring the supernatant after the centrifugation to a non-siliconized Eppendorf tube and then, gently purging the solution with nitrogen for 7 min. The solution was then stirred in the same vial at ~500 rpm for 24 h using a magnetic Teflon-coated stirring bar. Such a protocol will be further referred to as protocol II. These oligomers are further referred to as "large oligomers". Morphology of oligomers prepared according to described protocols can be found in Ref. [15]. Concentration of Aβ(1–42) material is presented as peptide monomer concentration.

2.4. Gold substrates and formation of self-assembled monolayers

Gold substrates for AFM were prepared on V-4 quality mica (SPI Supplies, USA). For RAIRS, as well as for an electrochemical impedance spectroscopy (EIS) 25 by 75 mm glass slides from ThermoFischer Scientific (UK) were used. For quartz crystal microbalance with dissipation (QCM-D) 14 mm diameter quartz plates with 5 MHz fundamental resonance frequency were used. BK7 glass slides for SPR (25 mm diameter, 1 mm thickness) were from Autolab (Methorm, The Netherlands). Gold layers were deposited by the magnetron sputtering using PVD75 (Kurt J. Lesker Company, USA) vacuum deposition system. Cr adhesion and gold film layers were 2 ± 0.5 nm and 50 ± 2 nm respectively. For RAIRS and QCM measurements gold thickness was 100 ± 10 nm. Sputtering parameters for 2 in. diameter metal targets were the flowing: Cr – power 200 W, sputtering current 0.50 A at 4.5 mTorr argon pressure; Au – power 120 W, sputtering current 0.26 A at 4.2 mTorr argon pressure. Prior to coating the deposition chamber was evacuated to <10^{-7} mbar the residual pressure. Ultrahigh purity, scientific grade argon (AGA, Sweden) was used for sputtering. Self-assembled monolayer were formed by incubation of freshly coated gold films in an ethanolic 0.2 mM solution of ODT. Incubations was carried out for 12–18 h with a subsequent hand-washing in pure ethanol followed by a brief 8–12 sonication in ethanol.

2.5. Electrochemical impedance spectroscopy

Electrochemical impedance (EI) spectroscopy measurements were carried out using Zennium electrochemical workstation (Zahner GmbH, Germany). The EI spectra were recorded in a potentiostatic mode with an 10 mV ac perturbation voltage at 0 V vs. Ag|AgCl|NaCl(sat), with the potential +197 mV respective to the standard hydrogen electrode. Measurements were carried out in a 6-vial measurement block-cell described earlier (for details see Supporting information section of Ref. [16]). Each cell had 32 mm^2 working electrode surface at the bottom exposed to a solution. All measurements were carried out in the frequency range from 0.1 to 50,000 Hz.