

Electronic Supporting Information

Direct Electrochemical and AFM Detection of Amyloid- β Peptide Aggregation on Basal Plane HOPG

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Materials and Methods

Materials. A β 42 (purity, 95.2%) was purchased as a lyophilized solid from Biosynthesis Biotechnology Co. LTD (China). L-tyrosine (Tyr) and the components of buffer solutions were purchased from Sigma-Aldrich (Germany). All reagents were of analytical grade or of ultra-high purity and used as received. All solutions were prepared with de-ionized Biocell Milli-Q water (18.2 M Ω , Millipore, Bedford, MA, USA). Highly oriented basal plane pyrolytic graphite, HOPG (ZYB Grade, 10x10x1.2 mm³) was from NT-MDT Co, Russia.

Fresh peptide stock solution and aggregation. A β 42 stock solution was prepared by dissolving 1 mg ml⁻¹ of peptide in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP), shaken for 12h and stored at -20°C, to prevent aggregation. The HFIP was evaporated under vacuum and the remaining A β 42 dissolved in buffer solution with 2% of dimethyl sulfoxide (DMSO) and sonicated for 20 min before using. The A β peptide aggregation was induced by 48 h incubation 500 μ l of a 125 μ M A β 42 solution in 20 mM phosphate buffer, pH 7.4, at 37°C under constant orbital shaking at 150 rpm. During the fibrillization process, aliquots of different-stage aggregating A β 42 solution were collected in triplicates every 12 hours and immediately stored at -20°C prior AFM and electrochemical measurements. All samples were analyzed within two days.

Preparation of electrodes. A β 42 in different stages of aggregation was adsorbed onto hydrophobic highly oriented pyrolytic graphite (HOPG). Prior to modification the smooth, shiny, and clean HOPG electrode surface was produced by exfoliating a thin HOPG layer with a Scotch adhesive tape. The Scotch tape-adhered piece of HOPG was then placed in a home-made Teflon holder and fixed in it by the O-ring and assembling top part, exposing the HOPG surface of 0.11 cm² to the electrolyte solution and avoiding any electrolyte contact with the rest of the HOPG. The HOPG was electrochemically connected to the stainless steel solid rod contact of the holder by the internal flexible stainless wire. The electrode was rinsed with Millipore water and dried in a stream of N₂ before modified. For the peptide film formation, 3 μ l of the A β 42 aqueous solution in PBS, pH 7.0, (0.5, 0.56, 1, 1.5, 2, 3, 4 and 5 mg ml⁻¹) were placed onto the electrodes surface and dried in a low stream of N₂. Then the A β 42-modified electrodes were rinsed with a 20 mM phosphate buffer solution, containing 0.15 M NaCl (PBS), pH 7.0, and mounted in the cell.

Instrumentation and procedure. Differential pulse voltammetry (DPV) measurements were performed at 22 \pm 1°C in a standard three-electrode cell connected to the potentiostat AUTOLAB PGSTAT 30 (Eco Chemie B. V., Utrecht, Netherlands) equipped with GPES 4.9.007 software. An Ag/AgCl (3M KCl) (Metrohm) electrode was the reference and a Pt wire was used as the auxiliary

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electrode. The working electrodes were the HOPG pieces, fitted into Teflon holders (3.96 mm diameter disks exposed to the solution). DPV parameters were as follows: potential step=10 mV, pulse amplitude=25 mV, pulse time=0.05 s and the apparent potential scan rate was 20 mV s⁻¹. All the potential are quoted with respect to Ag/AgCl (3M KCl). The reproducibility of the data was verified by measurements with at least four equivalently prepared electrodes. The surface coverage of A β 42 at HOPG electrodes has been estimated according to (Ferapontova E., Ruzgas T., Gorton L. *Anal. Chem.* 75 **2003**, 4841-4850):

$$\Gamma = 4RT(I_p)_{\max}\tau e / (\Delta E n^2 F^2) \quad (1)$$

where $(I_p)_{\max}$ is the detected DPV peak current maximum at a fixed sampling time, τ is the pulse width, ΔE the pulse amplitude and e equals to 2.718.

The 125 μ M A β 42 concentration was chosen as an intermediate concentration to further characterize A β 42 fibrillization since it allowed both a distinct electrooxidation signal and not too dense loading of the HOPG surface with pre-fibrils and fibrils, providing distinct images and their unambiguous analysis. All studies were performed in a 20 mM phosphate buffer solution, containing 0.15 M NaCl (PBS), pH 7.0, as most relevant/corresponding to the the physiological conditions of fibrillization.

AFM. A β 42 peptide was adsorbed onto freshly produced hydrophobic basal plane HOPG surface, prepared by the Scotch tape exfoliation. More specifically, the fresh HOPG surface was prepared by pressing the Scotch tape on one side of the 1x1x0.2 cm piece of graphite. The tape was then removed to reveal a smooth, shiny, and clean surface used form protein adsorption and imaging. The A β 42 samples were diluted to 20 μ M and a droplet of 10 μ L was placed on the freshly exposed substrate and incubated at room temperature for approximately 5 minutes. The excess solution was then removed and air dried. HOPG samples with A β 42-modified surface exposed to the top were then placed onto a 15 mm metal specimen disc (16218, Ted Pella Inc.) with double-backed tape. AFM image data were generated in situ at room temperature using silicon cantilevers with nominal resonance frequency of 330 kHz and nominal spring constant 35 N/m in tapping mode in a Nano-scope IIIa SPM system (Veeco, USA). In all presented Figures the height information is represented by contrast difference, with the lighter shades corresponding to the higher features.

HOPG as an inert substrate: Based on the value of the equilibrium constant K reflecting the strength the A β 42 binding to the surface HOPG, the HOPG substrate is not expected to affect the conformational state of the peptide (weak binding) as well as the fibril state, as can be followed from the AFM images, and can be considered as a chemically inert substrate, most appropriate to both visualize and simultaneously monitor the conformational changes of A β 42 after exposure to fibrillization.

Figure 4, Inset. Dependence of the extent of the A β 42 fibril formation on the fibrillization time was estimated from the variation of the Tyr oxidation signal recorded from the monomer A β 42 molecules adsorbed onto HOPG, which population decreased in the course of fibril formation until all A β 42 monomers become aggregated (no Tyr oxidation was detected). Specifically, the % decrease of the redox signal from freshly dissolved monomer A β 42 versus the redox signals from A β 42 incubated for different periods of time was correlated with the extent of fibrillization. An assumption was made that the surface relation between the electrochemically active monomers and electrochemically mute aggregates/proto-fibrils/fibrils reflected the one existing in solution. Data were deduced from the Table 1 data on the electrochemically active monomer surface coverage.