Methods for ultra-pure preparation of Amyloid-beta monomers and oligomers and their detection in body fluids.

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Introduction

CSF concentration of total β-amyloid protein (Aβ) is the main hallmark and biomarker of Alzheimer's disease (AD) pathology. Aβ, however, may exists in different aggregation states: monomer, oligomer, and insoluble fibril. Based on the amyloid cascade hypothesis, these distinct aggregates play different key pathological roles. Notwithstanding, no reliable methods to detect the different aggregation states of Aβ currently exist, mainly because of the lack of rigorous protocols for ultra-pure preparations of the different aggregates. The availability of such methods might be pivotal for a better understanding of the AD pathology and for the design of both diagnostic tools and targeted therapies, i.e. give advice on current monoclonal antibodies in immunotherapy clinical trials.

Methods

First, Aβ has been dissolved in HFIP before lyophilisation and stored at -80°C. Aliquots have been re-suspended in different buffers and ultracentrifuged at different times and speeds. The Aβ-monomer purity has been verified by Surface Plasmon Resonance, with different specific antibodies against the different aggregation states. The same procedure applied for ultra-pure oligomer preparations, followed by incubation at different times and temperatures of the monomers generated as aforementioned.

Results

Ultra-pure monomers, w/o contaminations of aggregates, should be obtained from the supernatant of o.n. ultracentrifuged (300.000xg) Aβ dissolved in NH_4OH (*Fig.1*). Ultracentrifugation step is essential to remove oligomeric impurities (*Fig.2*). Re-test of such monomeric preparations demonstrated to be stable (monomeric purity) up to one month.

Aβ oligomers (>90% purity) should be produced starting from the same monomeric solution as above, followed by 1h incubation at 37°C and subsequent 5h ultracentrifugation at 300.000xg. The higher concentration of oligomers should be obtained by the recovery of 15% of the subnatant (*Fig.3*), in order to remove contamination from monomers (*Fig.4*).

Conclusions and Discussion

Our protocol allows generating ultra-pure monomers without any detectable contamination from Aβ aggregates, which remain stable up to one month at -80°C. The production of ultra-pure oligomers showed a residual contamination of monomers, probably due to the known equilibrium existing between monomers and oligomers.

We are now attempting to generate innovative immunoenzymatic assays for the detection and characterization of auto-antibodies against the different aggregates of Aβ in body fluids of CAA and AD patients.[1,2,3]

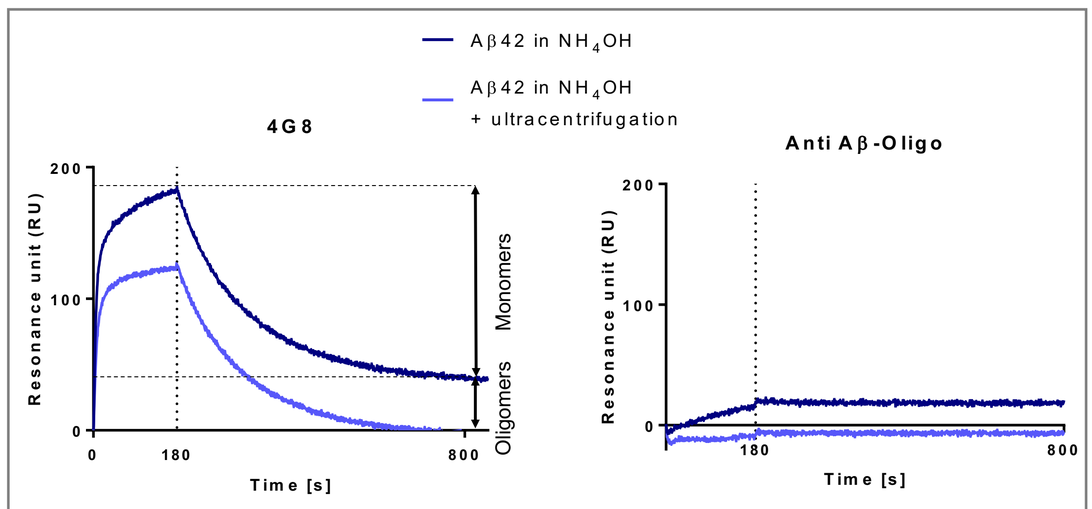

Fig. 1 *– SPR analysis of Aβ42 monomers dissolved in NH_4OH injected on different antibodies with and w/o ultracentrifugation.*

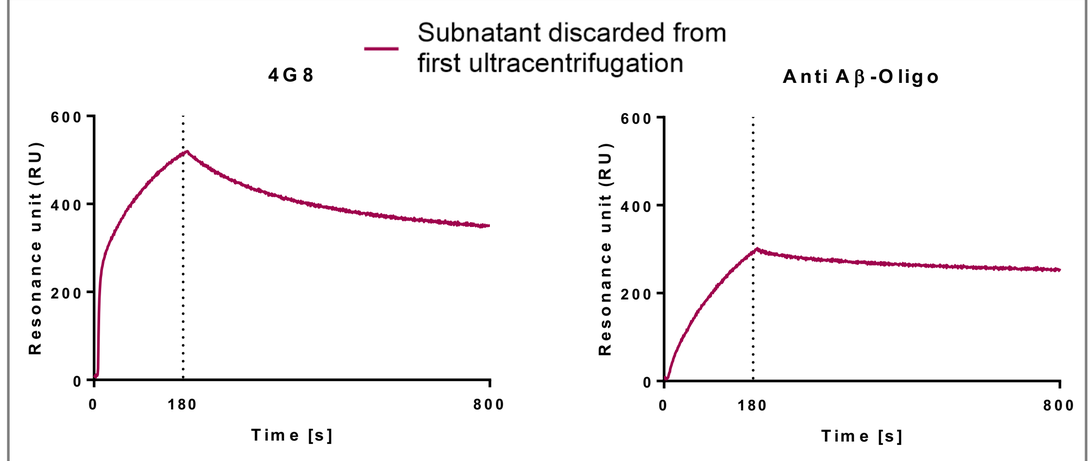

Fig. 2 *– SPR analysis on the subnatant from the ultracentrifugation o.n. 300.000xg.*

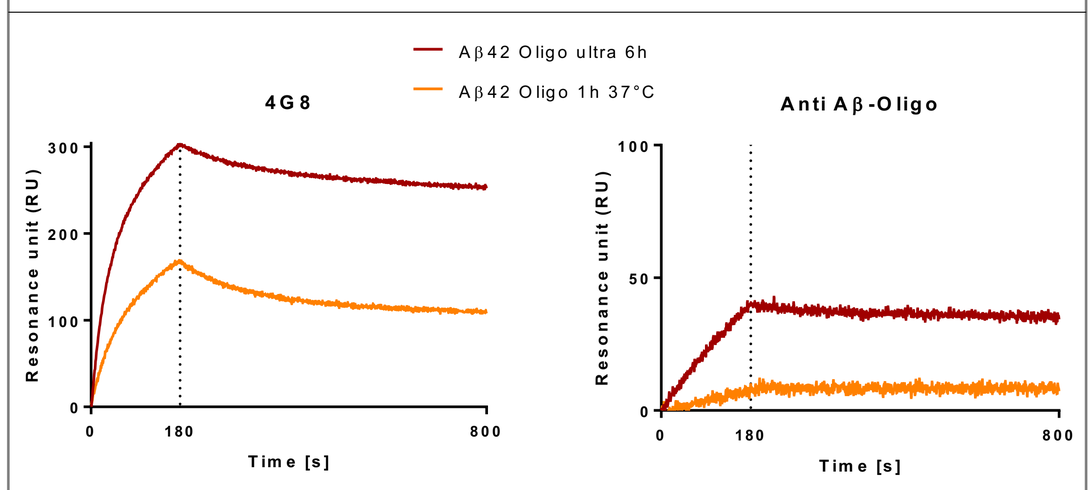

Fig. 3 *– SPR analysis of Aβ42 Oligomers (1h, 37° C) before and after 6h ultracentrifugation at 300.000xg.*

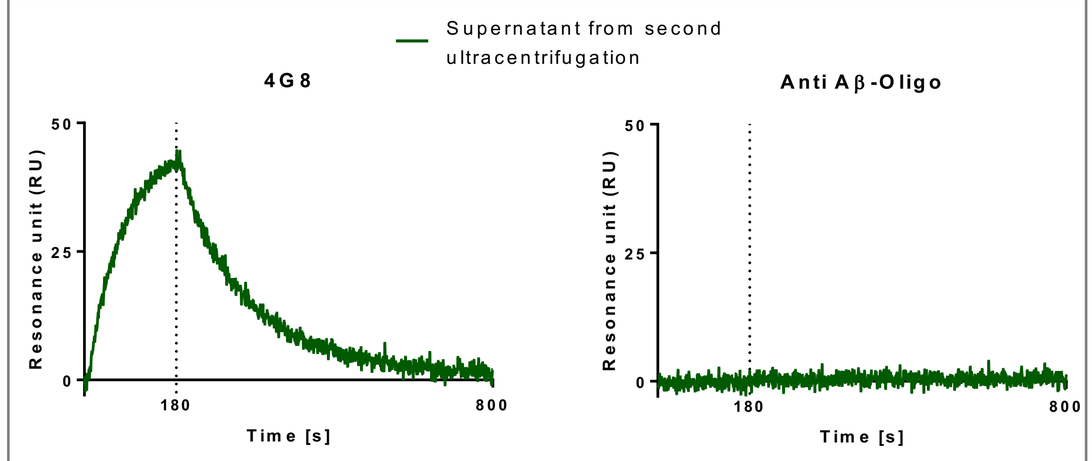

Fig. 4 *– SPR analysis on supernatant discarded form the 2nd ultracentrifugation.*

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