Biophysical Chemistry 229 (2017) 11–18

Contents lists available at ScienceDirect

Biophysical Chemistry

journal homepage: www.elsevier.com/locate/biophyschem

Pathogenic Aβ A2V versus protective Aβ A2T mutation: Early stage aggregation and membrane interaction

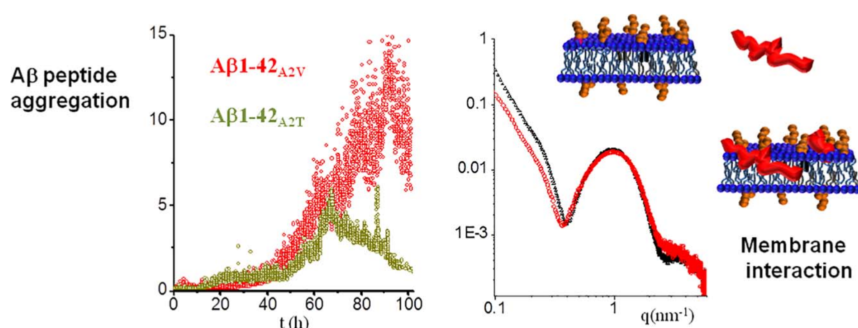
Laura Colombo[a], Alessio Gamba[b], Laura Cantù[b], Mario Salmona[a], Fabrizio Tagliavini[c], Valeria Rondelli[b], Elena Del Favero[b,*], Paola Brocca[b]

[a] Department of Molecular Biochemistry and Pharmacology, IRCCS Istituto di Ricerche Farmacologiche "Mario Negri", Via La Masa 19, 20156 Milan, Italy
[b] Department of Medical Biotechnology and Translational Medicine, University of Milan, V.le F.lli Cervi 93, 20090 Segrate, Italy
[c] Neurology V & Neuropathology, IRCCS Foundation "Carlo Besta" Neurological Institute, Via Celoria 11, 20133 Milan, Italy

HIGHLIGHTS

- Following the aggregation process of Aβ1–42 peptides by scattering techniques.
- Punctual mutations in Aβ1–42 give rise to aggregates different in size and number.
- Interaction of Aβ1–42 A2V and A2T peptides with membranes and cells.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Aβ1–42 peptide
A2V
A2T
Aβ aggregation
Aβ membrane interaction
Laser light scattering
X-ray scattering

ABSTRACT

We investigated the effects of punctual A-to-V and A-to-T mutations in the amyloid precursor protein APP, corresponding to position 2 of Aβ1–42. Those mutations had opposite effects on the onset and progression of Alzheimer disease, the former inducing early AD pathology and the latter protecting against the onset of the disease. We applied Static and Dynamic Light Scattering and Circular Dichroism, to study the different mutants in the early stages of the aggregation process, essential for the disease. Comparative results showed that the aggregation pathways differ in the kinetics and extent of the process, in the size of the aggregates and in the evolution of the secondary structure, resulting in fibrils of different morphology, as seen by AFM. Mutated peptides had comparable toxic effects on N2a cells. Moreover, as assessed by X-ray scattering, all of them displayed disordering effects on the internal structure of mixed phospholipids-gangliosides model membranes.

1. Introduction

The mechanisms underlying Alzheimer disease (AD) are not yet completely clear but genetic, pathological and biochemical aspects are at the basis of the onset and development of this pathology. In particular, the generation and accumulation of β-amyloid (Aβ) peptides, proteolytic fragments of the membrane-associated amyloid precursor protein (APP), represent a crucial aspect in the manifestation of this pathology [1]. Aβ peptides are extracellularly released in the brain as soluble macromolecules, then showing the tendency to form oligomeric, multimeric and fibrillar aggregates, thus triggering neurodegeneration. Soluble oligomers are now considered the main responsible

* Corresponding author at: Dept. of Medical Biotechnologies and Translational Medicine, University of Milano, LITA, Via F.lli Cervi 93, 20090 Segrate, Italy.
E-mail address: elena.delfavero@unimi.it (E. Del Favero).

http://dx.doi.org/10.1016/j.bpc.2017.05.001
Received 13 April 2017; Received in revised form 5 May 2017; Accepted 5 May 2017
Available online 06 May 2017
0301-4622/ © 2017 Elsevier B.V. All rights reserved.

for cognitive dysfunction, especially in the very early phases of the disease [2]. According to the amyloid cascade theory [3], the aggregation and accumulation processes of Aβ peptides end up with the formation of extracellular plaques that are considered the hallmarks of the disease. We first described a missense mutation in the alanine 673 residue of the amyloid precursor protein (APP), which corresponds to the second alanine of the amyloid Aβ A2V sequence, with dramatic impact on homozygous carriers [4]. More recently, Jonsson et al. (2012) [5] reported a mutation on the same alanine residue (A673T) that protects against the onset and development of Alzheimer disease and age-related cognitive impairment. The complete mechanism of this protective effect is not yet understood, although some studies on different cell models suggest that this last variant reduces the BACE1-mediated processing of APP, then lowering the levels of Aβ production [5,6]. Differences in the fibrillogenic propensity of the mutated peptides have also been claimed, but results on Aβ A2T (both 1–40 and 1–42) are controversial, reporting either an effect on Aβ1–40_{A2T} kinetics of aggregation [7], or a reduced level in Aβ1–42_{A2T} aggregation but not in Aβ1–40_{A2T} [6]. We reported that the punctual mutation (A2V) increased the fibrillogenic properties of both Aβ1–40_{A2V} and Aβ1–42_{A2} [4,8]. These observations contribute to underlining the critical role played by the N-terminal Aβ region, which seems to affect the kinetics of oligomerization of peptides.

In the present work we were interested in clarifying whether a single amino acid substitution A-to-T or A-to-V in position 2 could cause the global reorganization of the peptide structure. To this regard, we observe that the controversial results for Aβ A2T were essentially obtained by the thioflavin T fluorescent dye method [6–7,9] that detects the presence of β-sheet structures then inferring fibrillation. Conversely, in this work we applied a more direct and multi-technique approach, using Static and Dynamic laser Light Scattering and Circular Dichroism. We also used the so called “depsi peptide method” for Aβ peptide synthesis, that enables obtaining seed-free batches of monomeric peptides [10–12].

We were able to compare in great detail the evolution from monomers towards fibrils of wild type Aβ WT sequence and of the mutated Aβ A2V and Aβ A2T, and to unravel the different nature of the oligomeric structures of Aβ peptides in the early stages of aggregation. We also compared the toxicity in vitro, on N2a cell lines, of the wild type peptide and the variants.

Finally, we also studied the interaction of Aβ A2V and A2T oligomers with model membranes using X-ray Scattering. With this technique one can observe the structural response of membranes to external stimulation. Soluble wild type Aβ oligomers are “membrane-active” species that can facilitate membrane puncturing and increase its permeability [13–15]. After interaction with the Aβ peptide, model membranes show structural rearrangement, with an expansion of the surface area and an alteration of their microviscosity [16–18]. We also recently studied the interaction of the Aβ WT peptide with complex biomimetic membranes by Neutron Reflectometry [19] assessing how different stages of aggregation of the peptide result in different extent of interaction. Here we report that both mutated peptides induced changes in the structure of model membranes, revealing the Aβ interaction with the hydrophobic core of the lipid membranes.

The multi-technique approach may help in understanding how the various phenomena involved in Aβ production and aggregation concur in determining the occurrence and timing of the pathological or protecting route.

2. Materials and methods

2.1. Peptide synthesis and samples preparation

Aβ1–42 peptides were synthetized using depsipetide method as previously described [10–12]. Aβ1–42_{WT}, Aβ1–42_{A2V} and Aβ1–42_{A2T} (sequences in Supplementary material) were stored in acidic solution (water: trifluoroacetic acid, 0.02%) at a concentration of ~200 μM. The depsi-peptide method allows obtaining a seed free batch, as much as possible near to monomeric condition. The method consists in introducing an *O*-acyl isopeptide structure between the Gly25–Ser26 residues, stable at acidic pH and able to inhibit the self-aggregation. On changing to basic pH (switching procedure), the peptide is converted into the Aβ1–42 native sequence. Before the switching procedure, to minimize the pre-aggregated species and to obtain the best reproducibility, peptides were dissolved in acidic solution (water: trifluoroacetic acid, 0.02%) at 1 mg/ml and ultracentrifuged o/n (at 55.000 rpm, 4 °C) to obtain a seeds-free samples, filtered on a Microcon (centrifugal filter devices, c.o. 10 kDa, Millipore) and, finally, concentrated on a Microcon (centrifugal filter devices, c.o. 3 kDa, Millipore) up to a concentration ≥ 200 μM. The switching procedure of depsi-Aβ was carried out at basic pH, in particular a mix of sodium hydroxide (NaOH) and ammonium hydroxide (NH_4OH) (ratio 3:1) was added to the peptide solutions to obtain final concentration of hydroxyl group of 10 mM (final pH of ~10) and incubated on ice for 10–15 min.

Mixed model membranes for X-ray scattering investigation were prepared by dissolution of the desired lipids, dipalmitoylphoshatidylcholine (DPPC) and ganglioside (GM1), in appropriate organic solvent. After complete solvent evaporation under rotation, the lipid thin film was humidified and rehydrated (c = 20 mg/ml). Unilamellar vesicles formed spontaneously (DPPC:GM1 = 10:1.5 mol:mol).

2.2. BACE activity assay

BACE activity was carried out according to Ghosh & al. 2000 using as substrates the following three sequences: EVKMDAE (WT), EVKMDVE (A2V) and EVKMDTE (A2T) [20,21]. The BACE1 substrates were synthesized by solid-phase peptide synthesis on an automated synthesizer (Applied Biosystems 433A) using Fmoc-protected L-amino acid derivatives. The substrates were linked to a fluorescent dye (EDANS) on the glutammic acid in position 2 and to a quenching group (DABACYL) on the lysine in position 11. All peptides were purified in HPLC and their purity and identity were determined by MALDI-TOF analysis (model Reflex III, Bruker). BACE1 assay was carried out at 37 °C using 0,5 U of human recombinant BACE1 enzyme and different concentrations of substrate, 3.3, 6.6 and 10 μM, in 50 mM sodium acetate buffer (pH 4.5) in a final volume of 300 μl. Fluorescence was measured using a spectrophotofluorimeter (Perkin Elmer, Waltham, MA). Wavelengths of excitation and emission were 336 nm and 493 nm, respectively. The kinetics was followed for 90 min.

2.3. Laser light scattering

Both Dynamic and Static Laser Light Scattering (DLS and SLS) techniques were used. Experiments were carried out on a non-commercial apparatus equipped with a laser (λ = 532 nm), a digital correlator, and a thermostated cell [22]. High sensitivity is reached with four optical channels at 90°, displaced 5° above or below the scattering plane, that allow independent parallel measurements of the intensity scattered from the same very dilute sample (about 0.1 mg/ml in the present work). Data reported in the Results were obtained by averaging the signal collected by all of the four independent optical channels.

The three peptides (Aβ1–42_{WT}, Aβ1–42_{A2V} and Aβ1–42_{A2T}) were analyzed immediately after the switching procedure, described in 2.1, followed by dilution to the final concentration of 25 μM in phosphate buffer 50 mM, pH 7.4 at 22 °C, by parallel and independent SLS and DLS. SLS average intensity depends on the molecular mass of particles in solution. So, the kinetics of aggregation of peptides was detected by acquiring the scattered intensity (SLS) for several days. The concentration being the same for all the samples and the measurements performed at the same temperature (22 °C) with the same apparatus, differences in the scattered intensity could be directly related to differences in the number and molecular mass of the aggregates. Parallel

Download English Version:

https://daneshyari.com/en/article/5370602

Download Persian Version:

https://daneshyari.com/article/5370602

Daneshyari.com