Human Albumin Impairs Amyloid β-peptide Fibrillation Through its C-terminus: From docking Modeling to Protection Against Neurotoxicity in Alzheimer’s disease

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Abbreviations: AD, Alzheimer’s disease; APP, amyloid precursor protein; Aβ, Amyloid-β peptide; CD, Circular dichroism; CSF, cerebrospinal fluid; CTerm, albumin C-terminus; fAβ(1–42), HiLyte Fluor488 labelled human Aβ1–42; HPLC, high performance liquid chromatography; LC-MS, Liquid chromatography-mass spectrometry; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NMR, nuclear magnetic resonance; PBS, phosphate-buffered saline; PDB, Protein Data Bank; PPI, protein-protein interactions; SDS, sodium dodecyl sulfate; TEM, transmission electron microscopy; TFA, trifluoroacetic acid; UV, ultraviolet.

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https://doi.org/10.1016/j.csbj.2019.06.017
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1. Introduction

Alzheimer’s disease (AD) histopathological hallmarks are the extracellular aggregation of amyloid β-peptide (Aβ) in the brain, and the intraneuronal aggregation of hyperphosphorylated tau protein [1,2]. The amyloid cascade hypothesis proposes that Aβ aggregation into oligomers and fibrils induces synaptotoxicity and ultimately neuronal death [3–5].

The Aβ1–42 peptide is a ~4 kDa peptide released from the amyloid precursor protein (APP) [6] by the sequential action of the enzymes β- and γ-secretases [7,8]. Aβ is produced throughout the life of an individual, being physiologically degraded in the brain by different enzymes or cleared through the blood brain barrier into the blood [9]. During aging there is an increase in Aβ production and an impairment of Aβ clearance from brain to blood [10], favoring its aggregation into β-sheets. Then, Aβ forms oligomers known to be highly neurotoxic [3,4], and they progress into forming amyloid fibrils that are packed into senile plaques.

At present, AD prevalence is increasing as the world population ages [11] and has become a major health and social problem expected to reach pandemic status by 2050 [12]. Unfortunately, despite the current knowledge on APP processing, there are no treatments that effectively prevent AD development or its progression. On the other hand, the use of antibodies against Aβ to disassemble cerebral Aβ aggregates [13–15] has not yielded positive results in clinical trials.

Interestingly, clusterin (also termed apolipoprotein J) and albumin are cerebral proteins that have been reported to bind Aβ under physiological conditions [16–18]. In fact, clusterin impairs Aβ aggregation in the brain [16] and some clusterin polymorphisms are major risk factors for late onset AD [19]. Albumin has also been demonstrated to inhibit Aβ aggregation [20–23]. These findings make the study of clusterin and albumin of high relevance to understand AD etiology and to elucidate mechanisms of neuroprotection in AD.

Albumin is the most abundant protein in the cerebrospinal fluid (CSF) produced mostly by ultrafiltrate of plasma proteins [24] and minimally by the microglia [25]. It is a single peptide chain of 585 amino acids with a secondary structure containing 67% α-helix, 23% extended chain and 10% β-sheet [26–28]. Albumin is a flexible molecule, with the ability to change its structure depending on environmental conditions such as temperature, pH or ionic strength [29,30]. It can bind a plethora of different molecules [30–33], and it can even act as a free radical scavenger [34]. More specifically, albumin can bind Aβ [20,35,36] and is responsible for 95% of its transport in the blood [18,37,38], thereby regulating the amount of circulating Aβ.

Previously, we used the Protein-Protein Interface Prediction Server (iFrag) to predict the interaction for short protein fragments and found that clusterin shares a common sequence with the C-terminus domain of human albumin (CTerm; Fig. S1) [39]. Here, we address the effect of its CTerm in Aβ aggregation using in silico and in vitro techniques.

Computational methods to predict and evaluate protein-protein interactions (PPIs) represent a feasible alternative to experimental approaches. Although docking experiments are currently used to predict the conformation of PPIs [40], it has been shown that the distribution of scores of different docking populations could be used to discern between interacting and non-interacting protein pairs. This is valid even when the native conformation cannot be identified among the poses [41] and the population of docking poses can be used to predict the binding energy if the structure of the complex is unknown [42]. In this work, we used similar principles to assess the binding potential of clusterin, albumin and the CTerm to the alpha and beta conformations of the Aβ1–42 peptide. We focused our efforts on elucidating the physiological relevance of the CTerm and its interaction with Aβ1–42 peptide by in vitro experiments using different techniques and cell cultures.

2. Methodology

2.1. Modeling of the Clusterin Peptide

We found the four homologous sequences between clusterin and albumin in a previous work through sequence alignments [39]. Due to the lack of available structures for clusterin or the peptide region of interest, 5 decoys of the most promising peptide according to iFrag [39] were built with MODELLER [43–45] following the alignment shown in Fig. 1A. The five decoys were minimized and scored with Rosetta [46] and the best-scored decoy was selected as the structural representative of the clusterin-peptide (Fig. 1B).

2.2. Aβ1–42 -Docking Analyses

With the exception of the clusterin-peptide model, structures for all the different elements required for the docking analyses were obtained from the Protein Data Bank (PDB) [47]. The clusterin-peptide and the CTerm structure (crystal structure PDB ID: 5FUO [48], residues 504–538) were docked with two different target binders: (1) the Aβ1–42 α conformation (nuclear magnetic resonance (NMR) ensemble PDB ID: 1IYT [48]), (2) the Aβ1–42 β conformation (cryo-EM structure PDB ID: 5OQV [50]). Additionally, CTerm was also docked to the most terminal region of albumin (PDB ID: 5FUO, residues 539–582), to which is normally bound (Cterm-ld). This region of the wild-type albumin hides the putative recognition site between CTerm and Aβ1–42 (Fig. 2A).

From the two only available NMR structures in the PDB [47] presenting the full 42 residues of the Aβ1–42 peptide, we selected 1IYT over 1ZQO [doi:https://doi.org/10.1002/ch2.200500223] (see alignment of resolved residue densities for all Aβ1–42 peptides in the PDB at https://github.com/structuralbioinformatics/amylloids/blob/master/albumin/source/alpha_amyloids/alignment.highlight.aln) for two main reasons. On the one hand, most of the standard PDB metric qualities (namely clashscore, Ramachandran outliers and sidechain outliers) are far better in 1IYT than in 1ZQO. On the other hand, 1ZQO is described as an intermediate state between the α and β conformations of Aβ1–42, while 1IYT aims to represent the stable alpha state of the peptide.

For each pair, a total of 8000 decoys were generated with Rosetta’s docking protocol [51] and optimized with Rosetta’s FastRelax [52]. Decoys were analyzed in terms of their dG, shape complementarity and structural variation of the target binder.

2.3. CTerm Synthesis

The CTerm peptide, AETFTFHDADICLTSEKERQJKQTALVELVKHPKamide, containing the hydrophobic domains reported to be Aβ binding sites by García et al. [39] was produced in C-terminal carboxamide form by Fmoc solid phase synthesis in a peptide synthesizer Prelude (Gyros Protein Technologies). It was purified to near homogeneity (97%) by reversed-phase high performance liquid chromatography (HPLC).

2.4. CTerm Analysis

Analytical reversed-phase HPLC was performed on C18 columns (4.6 × 50 mm, 3 μm; Phenomenex) in a liquid chromatograph (LC-1010A; Shimadzu). Solvent A was 0.045% trifluoroacetic acid (TFA) in H2O; solvent B was 0.036% TFA in acetonitrile. Elution was carried out with linear 15–50% gradients of solvent B into A over 15 min at 1 mL/min flow rate, with UV detection at 220 nm. LC-mass spectrometry (MS) was performed in a LC-MS 2010EV instrument (Shimadzu) fitted with an XBridge column (4.6 × 150 mm, 3.5 μm; Waters), eluted with
a 15–50% linear gradient of B into A (A = 0.1% formic acid in H2O; B = 0.08% formic acid in acetonitrile) over 15 min at a flow rate of 1 mL/min, with UV detection at 220 nm.

2.5. CTerm Purification

Preparative HPLC runs were performed on a Luna C18 column (21.2 mm × 250 mm, 10 μm; Phenomenex), using linear 15–50% gradients of solvent B (0.1% in acetonitrile) into A (0.1% TFA in H2O), as required, with a flow rate of 25 mL/min. Fractions of high (>95%) HPLC homogeneity were further characterized by electrospray mass spectrometry using a XBridge column C18 (Waters) and a gradient at 1 mL/min of A (0.1% formic acid in H2O) into B (0.08% formic acid in CH3CN). Detection was performed at 220 nm. Fractions of adequate homogeneity and with the expected mass (4067.77 Da) were combined, lyophilized, and used in subsequent experiments. See Figs. S2 and S3 and Table S1 for additional details.

2.6. Circular Dichroism (CD)

CTerm was dissolved in 5 mM NaOH at a stock concentration of 1 mg/mL. The peptide was further dissolved to yield a 50 mM concentration in 10 mM phosphate buffer at pH 7.2 in the presence or absence of 20 mM SDS. The CD was performed as follows. The spectral region was recorded from 190 to 250 nm, with a 0.5 nm step resolution, on a Jasco J-715 CD spectropolarimeter (Jasco Inc.) using a quartz cell of 1.0 mm optical path length at room temperature. The scanning speed was 100 nm/min with 2 s time response, and the spectra were collected and averaged over 10 scans. Secondary structure estimation derived from CD data was assessed using K2D3 [53].

2.7. Amyloid Preparation

Lyophilized Aβ1–42 (Anaspec) was solubilized as previously described [54]. Briefly, 1 mg Aβ was dissolved in 250 μL of MilliQ water and pH was adjusted to ≥10.5 using 1 M NaOH solution to avoid the isoelectric point of Aβ disassembling and aggregates that may be present. Peptides were diluted in 250 μL of 20 mM phosphate buffer (pH 7.4). Furthermore, the solutions were sonicated for 1 min in a bath-type sonicator (Bioruptor, Diagenode). The preparations were immediately used for aggregation or stored in 25 μL aliquots at −20 °C until used.

2.8. Reversed-Phase Chromatography Analysis of CTerm Effect on Aβ Aggregation

Soluble and pre-aggregated (24 h at 37 °C) 1 μM Aβ1–42 was dissolved in Ham’s F12 medium and incubated in the presence/absence of 5 μM CTerm at 37 °C during 24 h for assembly and disassemble assays. Samples were quenched in 2% trifluoroacetic acid and injected in a Waters 2690 HPLC coupled to a UV detector set to 214 nm. A linear gradient of 25%–45% of 0.1% trifluoroacetic acid in acetonitrile was applied for 90 min into a 250 × 4.6 mm (5 μm) C4 column at a flow rate of 0.75 mL/min.

2.9. Reversed-Phase Chromatography Analysis of Control Peptide Effect on Aβ Aggregation

Random peptides with the same size that CTerm were synthetized: peptides X, Y and Z. Soluble and pre-aggregated (24 h at 37 °C) 1 μM Aβ1–42 was dissolved in Ham’s F12 medium and incubated in the presence/absence of 5 μM peptides at 37 °C during 24 h for assembly and
disassemble assays. Analytical reversed-phase HPLC was performed on C18 columns (4.6 × 50 mm, 3 μm, Phenomenex) in a model LC-2010A system (Shimadzu). Solvent A was 0.045% TFA in H2O; solvent B was 0.036% TFA in acetonitrile. Elution was carried out with linear 5–95% gradients of solvent B into A over 15 min at 1 mL/min flow rate, with UV detection at 220 nm.

2.10. Transmission Electron Microscopy (TEM)

Soluble and pre-aggregated (24 h at 37 °C) 1 μM Aβ1–42 was dissolved in Ham's F12 medium and incubated in the presence/absence of 5 μM CTerm at 37 °C during 24 h for assembly and disassemble assays. Samples of aggregated peptides obtained as described above were placed onto carbon-coated 200 mesh copper grids and incubated for 5 min. The grids were washed with distilled water and negatively stained with 2% (w/v) uranyl acetate for 2 min. Micrographs were obtained in a JEM-1010 (JEOL) transmission electron microscope operated at 80 kV accelerating voltage and equipped with an Orius CCD camera.

2.11. Cell Viability Assay by MTT Reduction

A human neuroblastoma cell line (SH-SYSY cells) was seeded in poly-l-lysine coated 96-well plates at a concentration of 2 × 10^4 cells/well. Soluble and pre-aggregated (24 h at 37 °C) 1 μM Aβ1–42 was dissolved in Ham's F12 medium and incubated in the presence/absence of 5 μM CTerm at 37 °C during 24 h for assembly and disassemble assays. After they were ready the samples were added. Cells were treated with the different samples during 24 h at 37 °C. Cell viability was tested by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction. 10% v/v of MTT stock solution (5 mg/mL) was added. After 2 h the media was replaced with 100 μL of dimethylsulfoxide. MTT absorbance was determined in an Infinite 200 multiplate reader.
at A540 nm and corrected by A650 nm. Data are shown compared to untreated controls (100%).

2.12. Aβ_{1–42} Binding to Neuronal Processes

Primary cultures were carried out as previously reported [4]. The procedure was previously approved by the Ethics Committee of the Institut Municipal d’Investigacions Mèdiques-Universitat Pompeu Fabra (EC-IMIM-UPF). Cortical neurons were isolated from 18-day-old OF1 mouse embryos. The brain was removed and the cortex aseptically dissected on ice-cold Hanks’ balanced salt solution supplemented with 4.5 g/L glucose and trypsinized for 17 min at 37 °C. In order to eliminate rests of the trypsinization medium and to disaggregate the cells, the cell solution was washed thrice in Hanks’ balanced salt solution + glucose and mechanically dissociated. Then, cells were seeded on poly-D-lysine coated coverslips in 24 well-plates with DMEM medium plus 10% horse serum at 10^5 cells/well. Once neurons were attached to the polylysinated wells (2 h), the seeding medium was removed and Neurobasal medium was added containing 2% B27 supplement, 1% GlutaMAX and 1% penicillin/streptomycin. On day 3 of in vitro culture, cells were treated with 2 μM 1-β-D-arabinofuranosylcytosine for 24 h to eliminate glia. Primary cortical neurons were cultured at day 10 [15]. Cells were treated for 10 min at room temperature in mild agitation with 10 μM HILyte Fluor488 labelled human Aβ_{1–42} (FA1–42) preformed in the presence/absence of 20 μM CTerm. After three washes with phosphate-buffered saline (PBS) cells were incubated on ice with 75 μg/mL red concanavaline in neurobasal medium for 20 min. Then cells were washed again in cold PBS and fixed in 4% paraformaldehyde in PBS before mounting. Images were obtained in a SP5 Leica confocal microscope using 40× objective. Image fluorescence analysis was performed using Image J software.

2.13. Statistical Analysis

Data are expressed as the mean ± SEM of the values from the number of experiments as indicated in the corresponding figures. Student’s t-test or one-way analysis of variance (ANOVA) with Bonferroni multiple comparisons test were used for statistical analyses.

3. Results

3.1. Albumin Binds Aβ With Higher Affinity Than Clusterin

Clusterin is known to bind and to inhibit Aβ aggregation [16], in fact some polymorphisms that cause the dysfunction of this protein are a major risk factor for late onset AD [19]. We used Aβ_{1–42} in in silico and in vitro experiments because it is the most aggregation-prone species of Aβ peptide and, therefore, the most neurotoxic in humans [55]. The comparison of the binding affinities of the clusterin peptides and the CTerm to both the alpha and beta conformations of the Aβ_{1–42} (Fig. 1C) shows that the four combinations have relatively acceptable ΔG values. Still, albumin’s CTerm peptide systematically obtained significantly better scores (p < .001) for the global distribution of all poses. This observation suggests that the CTerm peptide can bind to the Aβ_{1–42} Peptide with an affinity equal or possibly higher than clusterin.

3.2. In silico Analysis of Aβ_{1–42}-CTerm Interaction

A peptide region of human albumin that had been previously identified with iFRAG [39], was predicted to bind to Aβ_{1–42}. The iFRAG method predicts putative fragments that participate in the interface of an interaction through the search for similar sequences of known interactions. We run iFRAG with the albumin and clusterin sequences interacting with the sequence of the Aβ_{1–42} peptide. We obtained several fragments of clusterin and human albumin that would putatively interact with Aβ_{1–42}. We selected the high score fragments from iFRAG and aligned the sequences with CLUSTAL [56]. The alignment showed very high similarity and >25% identity, which increased to >70% on a short stretch of 7 amino-acids [39]. The analysis showed that both proteins share a common sequence located at the albumin’s CTerm [39]. The CTerm domain has 35 amino acids (AETFTHADICLTSEKQRKQLALVEILVHKPK) and shows high sequence similarity to clusterin (QNAVGKVQKJTEKTNEEELTILSNLEAAKKK) [39].

Here, the binding affinity of the CTerm peptide was computationally evaluated against its regular partner (CTerm-lid) and Aβ_{1–42} in its α and β conformations (Fig. 2A). The score distributions obtained from the generated decoys (Fig. 2B) suggest a slightly higher affinity for the α conformation of Aβ_{1–42} than for the β, albeit far from the affinity to its normal molecular partner. Interestingly, the CTerm peptide seems to trigger strong conformational changes when binding to the Aβ_{1–42} peptide. While binding to the peptide in the α configuration translates into changes between the relative position of the two α-helices, binding to the peptide in the β configuration seems to result in a break of the β configuration that might affect the pairing with other Aβ_{1–42} peptides. The evaluation of the individual contribution of each residue of the CTerm peptide to the binding with Aβ_{1–42} (Fig. 2C) shows the involvement of residues located throughout the entire peptide, suggesting the need for the full peptide for the recognition of its binding partners. When we analyzed the top 5 best scored decoys (Fig. 2D), we observed a very similar binding configuration. These configurations compete directly with the natural helix-loop-helix segment (CTerm-lid) to which the CTerm binds. All the data concerning the docking analysis can be found at https://github.com/structuralbioinformatics/amyloid/tree/master/albumina. A video showing the docking of the CTerm with Aβ_{1–42} is available at https://github.com/structuralbioinformatics/amyloid/raw/master/albumina/images/albumina_top5_overlap.mp4.

To further confirm the different behaviour of the α and β conformations, we docked CTerm and clusterin to the described α-β transition state structure (PDB ID: 1Z0Q) and compare it with the results obtained for the α (PDB ID: 1IYT) and the β (PDB ID: 5OQV) conformation (Fig. S4). This comparison also allows to compensate for the difference in crystallization solvents used between 1IYT and 1Z0Q. As expected from its described condition as transition state, 1Z0Q mostly behaves as an intermediate between the two conformations, the only exception being the RosettaScore evaluation. This fact is not surprising considering that some of the same metrics that define entry quality in the PDB do affect the scoring provide by Rosetta. The differences in ddG between the binding to albumin or clusterin in 1Z0Q are lower than those we obtained for 1IYT or the β conformation. However, it is still within the range to support that albumin can bind to the Aβ peptide with at least the same or better affinity than clusterin.

3.3. In vitro Analysis of Aβ_{1–42}-CTerm Interaction

We synthesized the CTerm as explained in the Methodology Section. The CD mean residue molar ellipticity of the CTerm is shown in Fig. S5. In solution, the peptide shows α-helical distinct features (minima at 204 and 223 nm), with α-helix secondary structure content of 56%. SDS micelles (20 mM detergent) used as helical inducer, increases the propensity of the CTerm secondary structure to 90%, with minima at 206 and 223 nm and a maximum at 192 nm.

Next, we showed the anti-aggregatory properties of the CTerm on Aβ_{1–42}. CTerm was used at 5 μM, which is the physiological concentration found in human CSF. The hydrophobicity analysis of the species present in several mixtures by reversed-phase chromatography (Fig. 3A) reveals several species. At early elution times (left of the chromatograph), the specific peaks due to the presence of Aβ_{1–42} oligomers are absent when the CTerm is present. In addition, we observed that the CTerm shifts the elution profile to the left, towards more hydrophilic species in the presence of Aβ_{1–42} oligomers when compared with the elution profile of Aβ_{1–42} alone (Fig. 3A,a). This behavior indicates that the CTerm prevents the assembly of oligomeric species. Moreover, the
CTerm was also able to disassemble preformed Aβ1–42 aggregates of high (fibrils) and low (oligomers) molecular weights (Fig. 3A,b).

Three peptides of the same size as the CTerm (termed X, Y, Z) were randomly generated to use as controls. We found that the three peptides favour Aβ aggregation and stabilize the pre-existing oligomers and fibrils. The results obtained with Peptide Y, as a representative experiment, are shown in Fig. S6. This pattern of interaction between Aβ and different peptides is expected since it has been reported that the binding of Aβ to proteins can act as seeds for amyloid aggregation and stabilization of fibrils [57–60].

Furthermore, the anti-aggregant properties of the CTerm were studied by TEM (Fig. 3B). When Aβ1–42 was incubated alone for 24 h, we observed the presence of the typical amyloid unbranched fibrils (Fig. 3B,a), oligomers and protofibrils (inset in Fig. 3B,a). Upon co-incubation of Aβ1–42 with the CTerm, amorphous aggregates were the only structures present (Fig. 3B,b). To study the disassemble ability of the CTerm, when Aβ1–42 was prepared as in Fig. 3B, and was incubated for 24 h with the CTerm, small fibrils and amorphous aggregates were observed (Fig. 3B,c). The interference of CTerm in the analysis was discarded because no aggregates were observed when the CTerm was incubated alone (Fig. 3B,d). These results are in agreement with those obtained in the experiment presented in Fig. 3A and Fig. S7.

3.4. CTerm Protects Neurons against Amyloid Neurotoxicity

We also studied the effect of the CTerm on Aβ-induced neurotoxicity on a human neuroblastoma cell line. These studies have been performed with soluble Aβ1–42 incubated in the presence/absence of the CTerm for 24 h in vitro. Later, the oligomers were added to cells for 24 h (Fig. 4A). A significant protection was observed when Aβ1–42 aggregates were preformed in the presence of the CTerm (p < .05). Since Aβ aggregates, particularly oligomers, bind to the neuronal membrane impairing its

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**Fig. 3.** Effect of the CTerm on Aβ1–42 assembly and disassemble in vitro. Soluble or aggregated 1 μM Aβ1–42 and 1 μM Aβ1–42 + 5 μM CTerm were incubated for 24 h to allow soluble Aβ assembly or fibrillar Aβ disassemble. A) Fractions eluted by reverse-phase chromatography. In the central part of the graph are the peaks of the high molecular weight fibrils and on the left the peaks that correspond to low molecular weight oligomers. Representative peaks in terms of hydrophobicity: 1, peak corresponding to Aβ1–42 fibrils; 2, 3, and 4, peaks that shift their hydrophobicity in the presence/absence of Aβ1–42 fibrils. A,a) Aggregation assay performed with soluble Aβ1–42. A,b) Disassemble assay performed with aggregated Aβ1–42. Data are from a representative experiment. B) TEM images of aggregation assays. B,a) 1 μM Aβ1–42 aggregated for 24 h. Insets correspond to oligomers and protofibrils. B,b) 1 μM Aβ1–42 + 5 μM CTerm aggregated for 24 h. B,c) Aggregated 1 μM Aβ1–42 + 5 μM CTerm incubated for 24 h. B,d) CTerm alone incubated for 24 h. Representative images are shown.
normal function, we studied the binding of Aβ1–42 aggregates formed in the absence/presence of the CTerm to primary cultures of cortical neurons. We used these cell cultures to study the binding of the Aβ1–42 aggregates to the surface of neurites mirroring what happens in vivo.

Experiments were run with concanavalin A, a membrane marker, and a fluorescent fAβ1–42 peptide to study their colocalization (Fig. 4B). Cortical neurons treated with fAβ1–42 aggregates (at 10 μM to allow its visualization) showed higher binding to the membrane throughout the neurite network than oligomers formed in the presence of the CTerm. Fluorescence was quantified and the aggregates of fAβ1–42 formed in the presence of the CTerm showed a 25% reduction in binding to neuronal membranes (p < .001; Fig. 4B) while the staining for concanavalin was not modified.

Finally, we prepared Aβ1–42 aggregates for 24 h in vitro. Then, they were incubated with the CTerm in vitro for 24 h in order to disassemble the oligomers. Later, human neuroblastoma cells were challenged with these samples for 24 h (Fig. 4C). Aβ1–42 aggregate-induced neurotoxicity was significantly impaired when the Aβ1–42 aggregates were pre-incubated with the CTerm (p < .01; Fig. 4C). The CTerm alone had no effect on cell viability (Fig. 4D).

4. Discussion

In order to elucidate the albumin domain where the anti aggregant Aβ property is located, we determined the regions of human albumin that could interact with Aβ using the iFRAG procedure [39]. In our previous work we had compared the sequence of clusterin, a well-known Aβ binding protein [16] whose polymorphisms are risk factors for late onset AD [19] with that of albumin. We found that the albumin CTerm has >40% similarity (70% identity on a short 7-residue stretch) with...
clusterin, and therefore we focused our study on the properties of this fragment. In the present work we show in silico evidence that the CTerm peptide produces conformational changes on Aβ1-42 upon binding. This interaction might impair Aβ1-42 conformation into β sheets preventing oligomer and fibril formation. These predictions were confirmed in vitro by reversed-phase chromatography and electron microscopy. Moreover, the in silico results that suggest a change of the β-sheet Aβ1-42 structure fit with the ability of the CTerm to induce disassembly of aggregated Aβ1-42, data that we obtained by reverse phase chromatography and electron microscopy.

As expected, the CTerm at physiological CSF concentration was able to protect neurons against Aβ1-42 neurotoxicity. These results are in agreement with our findings of decreased binding of Aβ aggregates to neuronal membranes when the CTerm is co-incubated with Aβ. Since AD is proposed to begin with the insidious interference of Aβ neuronal membranes when the CTerm is co-incubated with Aβ, preventing oligomer and fibril formation also favours disassembly of already fibril states. J Neurosci 2007;27:8195–8. doi.org/10.1523/JNEUROSCI.5161-07.2007.

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