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**Title:** Natural and non-natural factors influencing Alzheimer’s Aβ  
**Issue Date:** 2014-06-18
Chapter 7

Non-chaperone proteins can inhibit aggregation and cytotoxicity of Alzheimer’s Aβ Peptide

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JBC(under revision)
7.1 Abstract

Many factors are known to influence the oligomerisation, fibrillation and amyloid formation of the Aβ peptide that is associated with Alzheimer’s Disease. Other proteins that are present when Aβ peptides deposit in vivo, are likely to have an effect on these aggregation processes. In order to separate specific vs. broad-spectrum effects of proteins on Aβ aggregation, we tested a series of proteins not reported to have chaperone activity: catalase, pyruvate kinase, albumin, lysozyme, α-lactalbumin, and β-lactoglobulin. All tested proteins suppressed the fibrillation of Alzheimer’s Aβ(1-40) peptide at sub-stoichiometric ratios, albeit some more effectively than others. All proteins bound non-specifically to Aβ, stabilised its random coils and reduced its cytotoxicity. Surprisingly, pyruvate kinase and catalase were at least as effective as known chaperones in inhibiting Aβ aggregation. On the basis of our results, we propose a general mechanism for the broad-spectrum inhibition Aβ fibrillation by proteins. The mechanisms we discuss are significant for prognostics and perhaps even for prevention and treatment of Alzheimer’s Disease.

7.2 Introduction

The correlation between Alzheimer’s disease (AD) and the occurrence of extracellular amyloid β (Aβ) peptide plaques (oligomers/fibrils) in brain has been well established since the pioneering work of Glenner and Wong in 1984 (1). Aβ oligomers that precede the formation of fibrils and amyloid have been shown to be the most toxic form of Aβ. These pre-fibrillar oligomers are now considered to be a prime link between cognitive impairment / neurodegeneration and Aβ plaques (2). Although the Aβ fibrils have been recognized as a less toxic structural variant of Aβ, these fibrils may drive the formation of oligomers by secondary nucleation (3).

Several small molecules are known to interfere with oligomerisation and fibrillation of Aβ, for instance mecloxycline sulfosaliclate, hemin, and hematinit (4). Also certain specifically engineered cyclic peptides have similar inhibitory effects on fibrillation (5, 6). However, these organic compounds are potentially toxic. Even more effective was an engineered affibody that ceased the aggregation of Aβ (7) and stabilized the structure of the Aβ oligomers (8). Also chaperone proteins, which are the body’s own defense against protein misfolding, have been recently found to interfere with Aβ aggregation. AlphaB-crystallin (9), clusterin (10) and heat shock protein (Hsp)B8 (11) have all been associated with pathological lesions of AD. They also inhibit fibrillation and reduce cytotoxicity of Aβ in vitro (12).
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Amyloid aggregates can sequester other proteins, which may disrupt essential cellular functions(13). As promiscuous binder of (disordered) proteins, Aβ in its aggregated state may therefore also function as a molecular hub in protein interaction networks(14). For instance, Aβ binds catalase with high affinity and inhibits its hydrogen peroxide breakdown(15). Catalase is an enzyme associated with senile plaques. Aβ also inhibits key enzymes of mitochondrial respiration(16). These results indicate that, besides chaperone proteins, other proteins interact with Aβ and may therefore interfere with its fibrillation. Indeed, in vitro studies revealed that albumin, membrane related Brichos domains and lysozyme efficiently delay the fibrillation of Aβ(17–19). It is unknown to what extent these aggregation inhibiting activities are specific functionalities of these proteins, or whether there is a more generic, broad-spectrum mechanism at work.

Here, we report that proteins not known for chaperone activity can inhibit Aβ(1–40) aggregation with surprisingly high efficiency. Catalase and pyruvate kinase completely suppressed Aβ fibrillation at a molar ratio of 1:100 (protein: Aβ). But also albumin was effective, albeit at a lower molecular ratio of 1:10 (protein: Aβ). Even the whey proteins β-lactoglobulin and α-lactalbumin inhibited Aβ aggregation (but at a 1:1 molar ratio). As these whey proteins are not present in brain, they cannot have a biological function in inhibiting Aβ aggregation. In the presence of all tested proteins, the Aβ peptides retained their secondary random coiled structure. In an earlier, preliminary study, we already reported that lysozyme inhibits fibrillation at a 1:1 molar ratio15. The tested proteins also suppressed the toxicity of Aβ aggregates. HSQC NMR spectroscopy indicated that the interactions between the tested proteins and monomeric Aβ peptide were non-specific. These results prompt us to propose a common, broad-spectrum, protein-based inhibition mechanism of Aβ aggregation.

7.3 Materials & Methods

7.3.1 Materials

Human lysozyme (cat: L1667), human serum albumin (cat: A3782), α-lactalbumin from bovine milk (cat: L6010), Catalase from bovine liver (cat:C9327) and β-lactoglobulin from bovine milk (cat: L3908) were purchased from Sigma Inc. Pyruvate kinase (cat: 10836821) from rabbit muscle was bought from Roche Diagnosis GmbH. The Aβ(1–40) peptides (either unlabeled or 15N-labeled) were purchased from AlexoTech AB (Umeå, Sweden) and prepared according to previously described protocols. The Aβ peptides
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were dissolved in 10 mM NaOH to a peptide concentration of 2 mg/mL-1 and then sonicated for 1 min in an ice bath before dilution in the appropriate buffer. The preparations were kept over ice.

7.3.2 ThT fluorescence assay

A 10 mM ThT stock solution was prepared in dH2O. The proteins were added in desired amounts to aliquots of this solution. Then freshly prepared Aβ(1–40) peptide was added, yielding final samples containing 5 µM ThT, 10 µM Aβ(1–40), 50 mM Tris buffer, and protein in desired concentrations. All buffers and samples were prepared on ice in order to avoid the aggregation. The samples were pipetted into a well-plate with 384 wells, holding 45 µl each. Fluorescence measurements were recorded with an Infinite M1000 PRO microplate reader every 15 min, using excitation and emission wavelengths of 446 and 490 nm, respectively. The plate was thermostrated at +37 °C, and the wells were automatically shaken 30s before each measurement.

Each sample was prepared in duplicate, and average fluorescence signals were calculated after subtracting the baseline fluorescence of control samples without Aβ(1–40) peptide. The fluorescence intensity (I) data were fitted to a sigmoid curve with a sloping baseline, using the equation: , where the parameter k1 describes the sloping baseline, A is the amplitude, k2 is the elongation rate constant, and t1/2 is the time of half completion of the aggregation process.

For the disaggregation assay, a stock of Aβ(1-40) peptide aggregates was obtained by incubating 50 µM Aβ(1-40) peptide in 50 mM Tris, pH 7.4 at 200 rpm for 18h at 30°C. Then, 5 µM of the aggregates were diluted and mixed with the test proteins and ThT for the fluorescence assay. The protocol and buffer conditions were the same as described above. Fluorescence was measured after 0 h, 5h, 10h, and 15h.

7.3.3 CD spectroscopy

A Chirascan CD unit from Applied Photophysics was used to monitor the kinetics of a 10 µM solution of Aβ(1–40) peptide dissolved in 5 mM sodium phosphate buffer at pH 7.3, in the presence and absence of test proteins. CD spectra were recorded between 190–260 nm using a step size of 2 nm and a slit size providing 1 nm resolution at 30 min intervals. The sample was thermostrated at 37 °C in a 10 mm path length quartz cuvette (lot: 119.004-QS, Hellma Analytics), and mechanical rotary stirring with a magnet was used to speed up the aggregation process.
7.3.4 Cell viability assay

Neuroblastoma SH-SY5Y cells were used with a maximum passage number of 15. Cells were cultured in Dulbecco’s modified eagle medium, a 1:1 mixture of DMEM and Ham’s F12 medium and 10% supplemental fetal bovine serum, containing 1% (vol/vol) penicillin/streptomycin at 37°C, 5% CO2 in a 75 cm2 flask (Greiner Bio-one, cat. 658170). In order to avoid using trypsin, cells were detached by 5 mM EDTA/PBS for 5 min in 37°C. Then cells were resuspended at a concentration of 200 000 cells/ml in DMEM/F12, containing 1% (vol/vol) penicillin/streptomycin. The resuspended cells were plated at a volume of 50 µl and a cell density of 20 000 cells/well in a 96-well plate. The plated cells were incubated for 48 h at 37°C at 5% CO2. Aβ40 oligomer-enriched fractions were prepared at a concentration of 100 µM in the presence of the test proteins at 25°C for 100 min in PBS with 1mM EDTA. The Aβ oligomer solutions were diluted to final concentrations of 30 µM in wells in the presence of the different proteins. As a control, the PBS-dissolved test proteins in 50 µl medium were added to control wells and left to incubate for 48 h. After 48 h, the plate was equilibrated at room temperature for approximately 30 minutes. CellTiter-Glo® Luminescent Cell Viability Assay (Promega, cat. G7571) compound was added to each well and then the contents in the plate were mixed using an orbital shaker for 2 minutes to induce cell lysis. Luminescent intensity was measured (1000 ms integration time) with the Infinite M1000 PRO 384-well microplate reader. Measurements from three independent experiments were analysed statistically to calculate average values and standard deviations.

7.3.5 NMR spectroscopy

A Bruker Avance 500 MHz spectrometer was used to record 1H–15N-HSQC spectra at +5°C of 100 µM 15N-labeled Aβ(1–40) peptide in 20 mM sodium phosphate buffer at pH 7.3 (90/10 H2O/D2O), both in the absence and presence of the different proteins. The spectrometer was equipped with a triple-resonance cryogenically cooled probe head, and the spectra were referenced to the water signal. All NMR measurements were done at +5°C to slow down the aggregation process. The assignment of the amide peaks for the Aβ(1–40) peptide is known from previous work.

7.3.6 Bioinformatics

The molecular weight and theoretical PI, amyloidogenic regions of proteins were calculated by ExPASy server and Waltz software(20), respectively. Total solvent excluded
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surface area and total solvent accessible surface area were calculated using the Chimera software suite(21).

7.4 Results

7.4.1 All test proteins suppress Aβ peptide fibrillation

We monitored the effect of various concentrations of catalase, pyruvate kinase, albumin, lysozyme, α-lactalbumin, and β-lactoglobulin on the fibrillation of Aβ (at 10 µM concentration) using a ThT assay (fig.1). All test proteins inhibited Aβ fibrillation. The fluorescence increase characteristic of Aβ fibrillation significantly dropped in the presence of 25 nM of pyruvate kinase and did not occur at all in 100 nM pyruvate kinase (fig.1.a). Fibrillation of Aβ was hardly observed in 100 nM catalase (fig.1.e).

Although α-lactalbumin, albumin and β-lactoglobulin delayed the fibrillation of Aβ at low concentrations, their inhibition efficiencies were weaker compared to catalase and pyruvate kinase. Albumin and α-lactalbumin completely prevented Aβ fibrillation over 24 hours at 1 µM and 10 µM, respectively. And β-lactoglobulin decreased fibrillation of Aβ at concentrations over 1µM. We included the results we obtained earlier with lysozyme (using a different batch of Aβ) in fig. 1d(17). As a control, we ascertained that ThT fluorescence was not affected by the test proteins in the absence of Aβ (red curves in fig.1).

We investigated whether the test proteins could re-dissolve Aβ-aggregates by incubating them with pre-formed Aβ fibrils. Only catalase reduced the fluorescence intensity of Aβ (fig. S1), suggesting that catalase interacts with Aβ fibrils and reverses the amyloid fibrillation. In contrast, pyruvate kinase and α-lactoalbumin slightly increased the fluorescence intensity of pre-prepared Aβ fibrils. This might indicate that these proteins could also condense and (partially) unfold onto Aβ fibrils. Alternatively, pre-incubated Aβ fibril could nucleating the fibrillation of these test proteins. Neither lysozyme, albumin nor β-lactoglobulin affected the fluorescence intensity of pre-formed Aβ fibrils.

7.4.2 The test proteins stabilised the random coil structure of the monomeric Aβ peptide

We investigated the effect of the test proteins on the structural transition of Aβ by CD spectroscopy. The mean residue ellipticity of Aβ was obtained after subtracting the signal of the test protein from the measured spectra (fig.2). As expected, Aβ in
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**Figure 1:** ThT aggregation kinetics of 10 µM Aβ peptide in the presence of the test proteins pyruvate kinase(a), α-lactalbumin(b), albumin(c), lysozyme(d) catalase(e), β-lactoglobulin(f). For pyruvate kinase (panel a), which was most active in preventing fibrillation, we tested five different concentrations: 0 nM (black), 25 nM (olive), 100 nM (magenta), 500 nM (cyan) and 1000 nM (blue). For the other test proteins we used five slightly higher concentrations: 0 nM (black), 100 nM (olive), 500 nM (magenta), 1 µM (cyan) and 10 µM (blue). Red curves (all coinciding with the horizontal axis) are controls in which no Aβ was added to 20 µM test proteins. The curves show the average of 3 independent experiments.

The absence of the test proteins converted its secondary structure from random coil to β-sheet structure in 3 hours (fig.2a). However, in the presence of sub-stochiometric concentrations of test protein, Aβ peptide retained its random coil secondary structure (fig. 2b-g). We observed subtle differences in secondary structure changes of Aβ in the presence of the different proteins. The CD amplitudes at 200nm increased slightly during incubation with albumin, alpha-albumin, β-lactoglobulin, catalase and pyruvate kinase. In the presence of lysozyme, there was a CD up-shift at 220nm during the incubation (fig.2.f).

### 7.4.3 All test proteins reduce cytotoxicity of Aβ aggregates

The effect of the test proteins on the cytotoxicity of the aggregates of Aβ was measured in an *in vitro* cell assay. We first incubated 100µM of Aβ with the proteins at various concentrations for 2 hours and added the aggregates to neuronal SH-SY5Y cells up to a concentration of 30 µM. After 2 days incubation with Aβ aggregates, 20% of the cells survived but survival significantly increased with Aβ aggregates that had formed in the
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Figure 2: The secondary structure transition of 10 μM amyloid β peptide in the absence and presence of the test proteins: 0.5 μM alpha-albumin, 1.25 μM albumin, 1 μM β-lactoglobulin, 0.25 μM catalase, 1 μM lysozyme and 0.05 μM pyruvate kinase by CD spectroscopy. In the assay, we recorded the kinetics of structural transition in the time interval of 0.5h, black (0h), red (0.5h), green (1h), blue (1.5h), cyan (2h), magenta (2.5h), dark yellow (3h), navy (3.5h), purple (4h) and orange (4.5h).

presence of the test proteins. Cell survival was around 100% in the aggregates of Aβ and pyruvate kinase/catalase, which was much higher than the cell survival rate after incubation with the aggregates in the presence of Aβ and α-lactalbumin/albumin/lysozyme/β-lactoglobulin. But even for these latter test proteins, a positive effect on cell survival was observed.

7.4.4 The test proteins bind Aβ in a non-specific fashion

To explore the molecular interaction between Aβ and proteins, we used NMR 1H–15N-HSQC spectroscopy (fig 4). The results from NMR spectroscopy allow us to identify the chemical shifts of each detectable residue after the addition of the test proteins. The amide chemical shifts of Aβ moved to slightly after addition of α-lactalbumin (fig 4a), accompanied with a decreased of the cross-peak intensity (fig 5a). Although no amide chemical shift of Aβ was observed after addition of albumin or lysozyme (fig 4b,c), the cross-peak intensities of Aβ increased significantly (fig 5b,c). Upon addition of β-lactoglobulin or catalase, there were no noticeable changes in chemical shifts (fig 4d,e) and the cross peak intensity of most Aβ residues was reduced (fig 5d,e). Lysozyme and pyruvate kinase increased the cross-peak intensity of Aβ by a factor of 2 (fig 5 c,f),
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Figure 3: The cell survival after the incubation with Aβ aggregates that had formed in the presence and absence of the test proteins. In sample 1, the cell survival of 30 µM Aβ aggregates that had formed in the absence of test proteins is in light gray column and the cell survival of the control is in white. The samples 2, 3, 4, 5, 6, 7 are in the presence of pyruvate kinase, α-lactalbumin, albumin, lysozyme, catalase and β-lactoglobulin, respectively. The white column represents the cell survival of 1 µM of protein without Aβ aggregates in sample 2, and 2.5 µM of protein in the sample 3-7. The dark gray columns are the cell survival after incubation with 1 µM protein-30 µM of Aβ aggregates in sample 2 and 3 µM test protein-30 µM of Aβ aggregates in sample 3-7. The light gray columns are cell survival in the presence of 0.2 µM test protein, 30 µM of Aβ aggregates in sample 2 and 0.6 µM test protein, 30 µM of Aβ in sample 3-7.

but there was no change in chemical shifts (fig 4c, f). These results suggest that the protein interacted with Aβ in a non-specific fashion.

7.5 Discussions & Conclusions

Our results indicate that the non-chaperone proteins that we tested, suppressed Aβ fibrillation by stabilising the random coil structure of Aβ. Pyruvate kinase and catalase were the most efficient inhibitors of Aβ fibrillation, suppressing Aβ fibrillation effectively at a molar ratio of 1:100. Only catalase could reverse Aβ fibrillation. All test proteins also reduced the cytotoxicity of Aβ oligomeric aggregates. But again, pyruvate kinase and catalase had the strongest effect. Pyruvate kinase increased the NMR cross-peak intensity of Aβ residues on average by a factor of about 2, but catalase reduced the cross-peak intensity. The CD spectra of Aβ, which indicate that all test proteins stabilised the random coil structure of Aβ, suggest that pyruvate kinase and catalase binding to monomeric Aβ or oligomeric Aβ. Since the NMR spectra suggest that there is no specific interaction between Aβ and the tested proteins, and since strong effects were observed at sub-stoichiometric amounts of test protein, we propose that the proteins may also interact with Aβ prefibrillar aggregates.
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Figure 4: NMR 1H–15N-HSQC spectra of 90 μM 15N-labeled Aβ(1–40) peptides in 20 mM sodium phosphate buffer at pH 7.3, +5 °C, before (red) and after (blue) the addition of 18 μM α-lactalbumin(a), 5.4 μM albumin(b), 200 μM lysozyme(c), 50 μM β-lactoglobulin(d), 9 μM catalase(e), 4.5 μM pyruvate kinase(f).
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**Figure 5:** The relative change in intensity of the HSQC-NMR cross peaks induced by the addition of the test proteins. It was calculated using the spectra in figure 4.

### 7.5.1 The correlation between AD and the test proteins

Catalase, pyruvate kinase and albumin can be found in the brain. To some extent, they are related to the progression of AD. As a peroxidase enzyme, catalase may mediate the cellular levels of reactive oxygen species (ROS), which is relevant for the hypothesis of oxidative stress in AD. The activity of pyruvate kinase is significantly increased in frontal and temporal cortex of AD brains(22), but direct interaction between them remains to be investigated. The level of Aβ-albumin complex in serum is decreased in AD’s brain(23). There is no direct relationship between lysozyme and AD, but lysozyme increases upon inflammation reactions, which are also triggered in the Alzheimer brain.

### 7.5.2 A comparison with chaperones against the Aβ fibrillation

Extracellular chaperones and chaperone-like proteins have been reported to act as disposal mediators in the proteostasis of Aβ aggregation(24, 25). For instance, clusterin, a potent sHsp-like chaperone, sequesters prefibrillar oligomers and influences Aβ fibrillation: at a molecular ratio of 1:10(clusterin:Aβ), suppression of Aβ fibrillation was reported(10). However, clusterin in vitro does not affect the cytotoxicity of Aβ aggregates at a molar ratio of 1:500 and increases the cytotoxicity of Aβ aggregates at a molar ratio
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of 1:10 (26). Like clusterin, haptoglobin and α2-macroglobulin (found in extracellular fluid), completely suppressed Aβ fibrillation at a molecular ratio of 1:10 by interacting with prefibrillar Aβ oligomers(27). In addition to extracellular proteins, intracellular chaperones, like heat shock proteins hsp70 and hsp90, inhibit phenotypes related to Aβ aggregation in a model of Alzheimer’s disease(28). Hsp70 and hsp90, interact with oligomeric aggregates and suppress Aβ fibrillation effectively at sub-stoichiometric molecular ratio (1:50)(12). Similarly, hsp104 targets on the oligomeric intermediates and inhibits the elongation of the Aβ fibrils(29).

Besides extracellular and intracellular chaperones, lysosome and albumin have been shown to bind to Aβ peptide and suppress Aβ fibrillation. Human serum albumin binds to Aβ oligomers/protofibrils in prevailing stoichiometry and affinity (1–100 nM range), and inhibits Aβ(1–42) fibril growth at substoichiometric concentrations (molecular ratio 1:18)(30, 31). Human lysozyme inhibits Aβ fibrillation at a molecular ratio of 1:1. Furthermore, the BRICHOS domain, associated with familial British dementia, interferes with Aβ aggregation before the formation of fibrils at a molecular ratio of 1:10 (BRICHOS domain:Aβ)(18).

In order to identify which aspects of these proteins – and the test protein on which we report here – contribute to their apparent biological activity, we summarized some of their important characteristics in table 1.

### 7.5.3 Size - and surface dependent interaction between Aβ and folded proteins

As shown in table 1, both non-chaperone and chaperone proteins, are assumed to bind Aβ oligomer aggregates rather than Aβ fibrils and monomers. Aβ oligomeric aggregates have been proposed to compete with the Aβ monomers for binding to the same domain target of other proteins, like human serum albumin(30). Oligomeric Aβ is not stable, and its size and shape evolves during oligomerisation. Hence, binding of Aβ oligomers to protein surfaces might be dependent on the aggregation state. Still, there is no clear indication of a very specific interaction from table 1 (and neither from the experimental results reported here).

Table 1 does suggest a positive correlation between molecular weight and inhibition efficiency. For instance, catalase, pyruvate kinase and Hsps with molecular weight over 50 kDa, inhibit the fibrillation of Aβ at a molecular ratio of 1:50. We also investigated the effect of the hydrophobicity / hydrophylicity of the accessible surface area and found that the most effective inhibition of the Aβ fibrillation can be observed for proteins with a ratio of (solvent excluded surface area: solvent accessible surface area) of about 1:1.
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<table>
<thead>
<tr>
<th>Protein</th>
<th>MW (kDa)</th>
<th>State</th>
<th>PI</th>
<th>Molar effective inhibition (a)</th>
<th>Weight effective inhibition (b)</th>
<th>Interacting targets of Aβ peptide</th>
<th>Amylogenic regions</th>
<th>SESA/SASA (c)</th>
</tr>
</thead>
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<tr>
<td>Catalase</td>
<td>69</td>
<td>Hexamer</td>
<td>6.90</td>
<td>~1:100</td>
<td>1:7:1</td>
<td>Oligomeric Aβ aggregates</td>
<td>53-58; 81-86; 132-137; 194-202; 265-278; 277-295; 323-329; 459-464; 469-474</td>
<td>1.25</td>
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<td>Pyruvate kinase</td>
<td>237</td>
<td>Monomer</td>
<td>7.96</td>
<td>~1:100</td>
<td>~1:1</td>
<td>Oligomeric Aβ aggregates</td>
<td>144-148; 391-395; 506-514</td>
<td>1.00</td>
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<tr>
<td>Lysozyme</td>
<td>14</td>
<td>Monomer</td>
<td>9.38</td>
<td>~1:1</td>
<td>1:0:3</td>
<td>Oligomeric Aβ aggregates</td>
<td>73-79</td>
<td>0.89</td>
</tr>
<tr>
<td>α-lactoalbumin</td>
<td>14</td>
<td>Dimer</td>
<td>4.92</td>
<td>~1:1</td>
<td>1:0:3</td>
<td>Oligomeric Aβ aggregates</td>
<td>70-78; 90-95; 105-125</td>
<td>0.84</td>
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<td>20</td>
<td>Monomer</td>
<td>4.93</td>
<td>~2:1</td>
<td>1:0:1</td>
<td>Oligomeric Aβ aggregates</td>
<td>14:21; 96:101; 117:122</td>
<td>0.91</td>
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<td>Human Serum Albumin</td>
<td>67</td>
<td>Monomer</td>
<td>5.85</td>
<td>~1:10</td>
<td>1:0:6</td>
<td>Oligomeric Aβ aggregates</td>
<td>9-18; 45-57; 177-182; 352-357; 574-578</td>
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<tr>
<td>Pro-SP-C BRICSHS domain</td>
<td>19</td>
<td>Monomer</td>
<td>6.19</td>
<td>~1:30</td>
<td>~1:6</td>
<td>Oligomeric Aβ aggregates</td>
<td>42-51; 100-114; 119-127</td>
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<td>200</td>
<td>Monomer</td>
<td>—</td>
<td>~1:10</td>
<td>1:0:2</td>
<td>Oligomeric Aβ aggregates</td>
<td>13-19; 188-209; 220-225</td>
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<td>α2-macroglobulin</td>
<td>73</td>
<td>Dimer</td>
<td>6.24</td>
<td>~1:10</td>
<td>1:0:59</td>
<td>Oligomeric Aβ aggregates</td>
<td></td>
<td></td>
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<tr>
<td>Clusterin</td>
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<td>Dimer</td>
<td>5.88</td>
<td>~1:10</td>
<td>1:0:61</td>
<td>Oligomeric Aβ aggregates</td>
<td>4-16; 364-370</td>
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<td>HSP 70</td>
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<td>5.47</td>
<td>~1:50</td>
<td>1:3:1</td>
<td>Oligomeric Aβ aggregates</td>
<td>25-30; 40-46; 163-188; 141-153; 169-174; 179-184; 193-199; 205-210; 367-372; 375-381; 424-432; 438-445</td>
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<td>90</td>
<td>Dimer</td>
<td>4.94</td>
<td>~1:50</td>
<td>1:2:4</td>
<td>Oligomeric Aβ aggregates</td>
<td>30-53; 135-144; 338-342; 360-365; 490-496; 516-523; 660-674</td>
<td>0.90</td>
</tr>
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<td>HSP 104</td>
<td>100</td>
<td>Hexamer</td>
<td>5.31</td>
<td>~1:100</td>
<td>1:4:3</td>
<td>Oligomeric Aβ aggregates</td>
<td>107-113; 278-284; 347-352; 429-429; 698-615; 628-633; 736-737; 768-775</td>
<td></td>
</tr>
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<td>Affibody ZAβ</td>
<td>10</td>
<td>Monomer</td>
<td>6.01</td>
<td>1:100</td>
<td>1:4:3</td>
<td>Monomeric Aβ</td>
<td></td>
<td></td>
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<td>α-synuclein</td>
<td>14</td>
<td>Disordered</td>
<td>4.67</td>
<td>—</td>
<td>—</td>
<td>Monomeric or Oligomeric Aβ</td>
<td></td>
<td>35-40</td>
</tr>
</tbody>
</table>

(a) SESA/SASA: Total solvent excluded surface area/Total solvent accessible surface area  
(b) Weight effective inhibition: the weight ratio(mg/mg) of the effective inhibition of the Aβ fibrillation in 24 hours(protein:Aβ)  
(c) Molar effective inhibition: The molecular ratio(M/M) of the effective inhibition of the Aβ fibrillation in 24 hours(protein:Aβ)  
Amylogenic regions is predicted by Waltz program

Table 1: The comparison of properties of non-chaperone and chaperone proteins on the inhibition of the Aβ fibrillation
7.5.4 Potential amyloid cross interactions between Aβ and (non-)folded proteins

Amyloid proteins also affect Aβ fibrillation and toxicity. For instance, α-synuclein oligomers interact with Aβ monomers and or oligomers and affect its fibrillation(32). Such amyloid cross protein interactions with Aβ may promote the progression of AD(33). This interaction may result from conformational transition of Aβ and α-synuclein(34) where the flexible amylogenic regions with more hydrophobic residues interact with each other and electrostatic interactions form in the flexible regions. To investigate if such interactions between Aβ and the test proteins could occur, we searched for their potential amylogenic regions using the Waltz algorithm (table 1). In spite of a cluster of amylogenic regions predicted on the surface of proteins, the folded proteins, lacking a flexible hydrophobic surface, interacted with the Aβ monomer only weakly as we observed by NMR (which is in agreement with a previous report(18)). So potential amyloid cross interactions cannot explain the effects on Aβ fibrillation that we report.

7.5.5 A model for broad-spectrum inhibition of Aβ fibrillation

The structure of cylindrins has been proposed to be similar to that of the most toxic amyloid oligomer(35). One of the hypotheses is that once such an oligomer contacts the cell membrane, its hydrophilic regions may interact with the head group of lipids and then hydrophobic residues disrupt the tail groups, inducing weakening of the membrane, potential leaking of the cell and eventually apoptosis. We investigated the hydrophobic and hydrophilic surface of the test proteins and of the cylindrin model (fig.6). Based on this surface charge distribution, we assume that, once the proteins interact with Aβ oligomers, the hydrophobic and hydrophilic groups in the protein may act as the tail and head groups of cellular lipids, respectively. However, unlike the lipidic cell membrane or the amyloid oligomers, the proteins are relatively stable. The proteins may therefore retain their structure but amyloid oligomer may be disrupted. Eventually, the interaction affects the amyloid fibrillation and reduces the toxicity of aggregates.

In summary, we discovered that a series of non-chaperone proteins hinder the Aβ fibrillation and reduce the toxicity of Aβ aggregates. We propose that the proteins interfere with the stability of the Aβ oligomer by hydrophobic and hydrophilic interactions of their surface. There is a positive correlation between protein surface size and Aβ neutralizing activity, but this does not fully explain why some proteins are more active in inhibiting Aβ than others. Our results would for instance predict that high levels of catalase in the brain would not only reduce damage by neutralizing reactive oxygen species, but also by reducing the toxicity of Aβ oligomers. There may be other proteins...
Non-chaperone proteins can inhibit aggregation and cytotoxicity of Alzheimer’s $\mathrm{A\beta}$ Peptide

Figure 6: Schematic diagram of the inhibition mechanism of intracellular chaperones and extracellular proteins (non-chaperone proteins) in the prevention of $\mathrm{A\beta}$ fibrillation. The hydrophobic and hydrophilic regions are represented in orange and gray, respectively. $\mathrm{A\beta}$ is produced from APP by $\gamma$-secretase. Toxic oligomers of $\mathrm{A\beta}$ can be sequestered by intracellular chaperone and extracellular non-chaperone proteins.

in extracellular cerebral fluids that similarly reduce $\mathrm{A\beta}$ toxicity to a significant extent and monitoring or even influencing their levels in AD-prone individuals may be relevant for preventive strategies.

7.6 References

Non-chaperone proteins can inhibit aggregation and cytotoxicity of Alzheimer’s Aβ peptide


Non-chaperone proteins can inhibit aggregation and cytotoxicity of Alzheimer’s Aβ Peptide

7.7 Supplementary information

**Figure S1:** The ThT disaggregation assay of Aβ peptide in the presence of alpha-helical proteins. 50 μM of amyloid β peptide was incubated for 18 hours at 30°C, in a 200 rpm shaker (50 mM tris buffer, pH7.4) and then 5 μM aggregates (after diluting the 50 μM stock in 50 mM tris buffer, pH7.4) were incubated with different test proteins, at 37 °C. The protocol for ThT disaggregation assay is same as for the ThT aggregation assay.