The Role of Native Lens α-crystallin in Amyloid Suppression Using β-amyloid as a Model Amyloid Client

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ABSTRACT

Cataracts are a result of age-related protein aggregate formation in the eye lens, and the leading cause of blindness worldwide. α-crystallin acts a molecular chaperone that serves as the primary defense mechanism against protein aggregate formation in the lens. Recent evidence suggest amyloid formation in the lens may contribute to cataract formation, and that β-amyloid is present in lens epithelia of age-related cataracts. Previous studies have shown that αB-crystallin in other parts of the body, such as the brain, is protective against neurodegenerative diseases such as Alzheimer's disease that are associated with plaque deposits containing β-amyloid. *In vitro,* αB-crystallin inhibits fibril elongation of β-amyloid. However, the capacity of native lens crystallin protection against β-amyloid formation is still unclear. The aim of this thesis was to determine if the native lens specific αL-crystallin, a 3:1 ratio of the isoforms αA- and αB-crystallin, would prevent the fibril elongation of β-amyloid comparatively to αB-crystallin.

Thioflavin T (ThT) based chaperone assays, biochemical analysis by SDS-PAGE, and structural characterization by electron microscopy were used to determine if αL-crystallin can prevent fibril elongation of the amyloidogenic peptide Aβ 1-42. Detection of β-amyloid formation by ThT assay showed that αL-crystallin prevented fibril nucleation/elongation but was less effectively than αB-crystallin. However, negative stain electron micrographs and halt-times showed that αL-crystallin interacted effectively with β-amyloid fibrils under varying stoichiometric ratios. From these data, it was found that αL-crystallin was an effective chaperone for β-amyloid *in vitro.* Further experimentation could help understand the mechanism behind αL-crystallin function against preventing the formation of amyloid species in the eye lens.

OBJECTIVE

How does structure and function of α-crystallin and the structural changes that the chaperone undergoes to prevent aggregation of amyloid clients?
How does age-related changes in the structure of α-crystallin lead to cataract formation?
How does native lens α-crystallin, αL-crystallin, interact with β-amyloid *in vitro* and can this be used to determine if αL-crystallin suppresses β-amyloid fibril formation and elongation?

INTRODUCTION

Cataract is the leading cause of blindness worldwide, representing 51% of the world blindness. In developed countries, cataracts may be treated with a simple surgery; however, in many countries there are barriers that prevent access to this surgery such as cost and resources, leading to eventual loss of vision and blindness. Therefore, there is a need to better understand the molecular basis of this disease, so that we may develop more effective forms of treatment that can be widely distributed to a variety of populations.

At the molecular level, cataract formation is the result of age-related protein aggregation within the eye lens. The eye lens is saturated with a class of proteins, known as crystallins, which are proteins within lens fiber cells that are never turned over, meaning that they are subjected to a lifetime of environmental stress. Ultimately, this damage leads to protein degradation and aggregation that form light-scattering opacities, known as cataracts.

α-crystallin, is a member of the small heat shock protein (sHsp) family and the major protein in the eye lens. It acts as a molecular chaperone that sequesters misfolded proteins, a function that serves to maintain the transparency of the lens. αA-crystallin is specific to the lens, αB-crystallin is constitutively expressed throughout the body, including the brain, cardiac and skeletal muscle, and the lungs. In the eye lens, these two isoforms co-assemble into a large polydisperse oligomeric structures, which together form the primary defense mechanism against age-related aggregation.

In brains of deceased Alzheimer's disease patients, sHsps, mostly αB-crystallin, have been shown to co-localize with plaque deposits and are present in increased concentration levels. The formation of these plaque deposits is primarily due to aggregation of the β-amyloid and the tau protein tangles, but also contain small heat shock proteins (sHsps) such as αB-crystallin, suggesting a co-aggregation of the chaperone/filament complexes. The structure of β-amyloid is characteristic of other amyloids, it contains aggregates that form highly ordered heterogeneous fibrils. *In vitro,* αB-crystallin has been shown to interact with β-amyloid and prevent fibril elongation.

While the interaction between αB-crystallin and β-amyloid has been well-established, the interaction between β-amyloid and the lens-specific αA-crystallin and/or the native αA/αB-crystallin (αL) have not yet been characterized. Therefore, it is unclear if this lens chaperone can interact with and/or prevent β-amyloid nucleation or elongation using a similar mechanism. This may also help to elucidate the reason that, despite conditions in the human eye lens that should favor amyloid formation, amyloids are in low abundance in the lens.

METHODS

- Negative Stain Electron Microscopy (NSEM) analysis with a Tecnai T12 and Amylofit
- Thioflavin T Fluorescence
- SDS-PAGE

RESULTS

Table 1. *The half-times ($T_{1/2}$) for β-amyloid formation under varying experimental conditions for αL-crystallin and αB-crystallin. $T_{1/2}$ values reported in hours obtained from AmyloFit software (Meisl et. al. 2016) (N.D. = not determined). (ns indicates P > 0.05, * indicates P ≤ 0.05)*

CONDITION		$T_{1/2}$ (hours)
Aβ 1-42 (control; 10 mM)		5.972 +/- 1.850
αL-crystallin (0.1 – 1.0 mM)	1:10 Aβ 1-42	11.81 +/- 0.017*
	1:20 Aβ 1-42	10.68 +/- 0.476^{ns}
	1:50 Aβ 1-42	9.033 +/- 0.540^{ns}
	1:100 Aβ 1-42	8.749 +/- 1.499^{ns}
αB-crystallin (0.1 – 1.0 mM)	1:10 Aβ 1-42	N.D.
	1:20 Aβ 1-42	17.18 +/- 1.879*
	1:50 Aβ 1-42	10.99 +/- 0.084^{ns}
	1:100 Aβ 1-42	8.503 +/- 1.914^{ns}

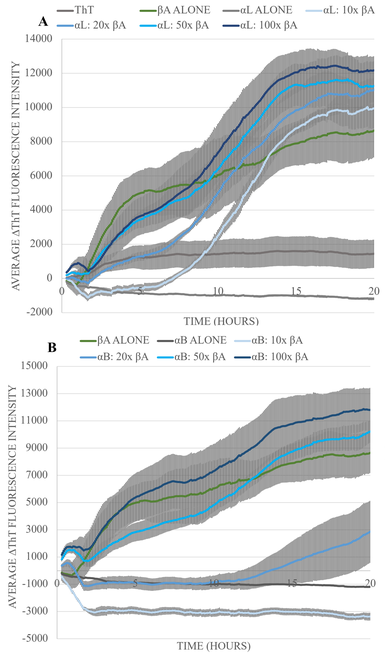

Figure 1*. Average change in Thioflavin T fluorescence for 10 μM Aδ 1-42 alone, or in 10 – 100x molar excess of αL-crystallin (A) and αB-crystallin (B). A gain of 100 was used for the experiment and windowed averaging of 10 values was used. A 4-minute cycle time was used with 360 kinetic cycles. Each condition was run in triplicate and standard error of the mean was plotted for each condition.*

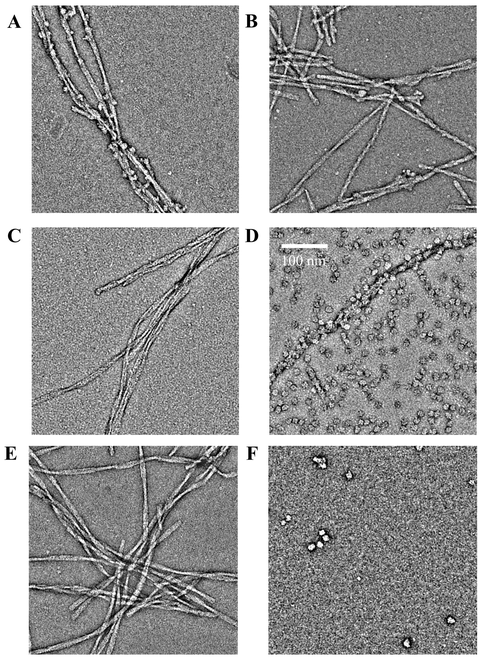

Figure 2. *Negative stain electron micrographs of experimental conditions and control conditions. αL-crystallin in varying concentrations and 10 μM δ-amyloid were represented: 1:10 αL-crystallin in (A), 1:50 αL-crystallin in (B), 1:100 αL-crystallin in (C), and 1:10 αB-crystallin in (D). Control conditions were δ-amyloid in (E) and αL-crystallin in (F). Electron micrographs were captured by Russell McFarland in the Reichow Laboratory, Department of Chemistry, Portland State University.*

DISCUSSION

In the first 8 hours of the fluorescence assay (Figure 1), each ratio of αL suppressed the fluorescence increase compared to the β-amyloid alone. However, after the first eight hours of the assay there was a second increase in fluorescence, where even the 10x αL-crystallin became overwhelmed and had similar values to that of β-amyloid alone. In contrast, the 10:1 of αB-crystallin showed complete suppression of β-amyloid formation. However, results for both αB-crystallin and αL-crystallin at 1:50 and 1:100 Aβ1-42: α-crystallin showed similar kinetics as the chaperones became overwhelmed.

To quantify the efficiency of αL-crystallin as a molecular chaperone for β-amyloid compared to αB-crystallin, the half-times ($T_{1/2}$) of fibril formation for each of the experimental conditions was determined using the AmyloFit program. The $T_{1/2}$ values for both αL- and αB-crystallin showed a dose-dependent response leading to $T_{1/2}$ values that were all longer than the β-amyloid control. The chaperone αB-crystallin showed a longer half-time than αL-crystallin for the 20x and 50x conditions, and at 10x completely suppressed fibril formation. Thus, both chaperones were effective at reducing the kinetics of fibril nucleation/elongation. However, αL-crystallin was not as effective of a chaperone as αB-crystallin.

NSEM was used to directly visualize the interactions between αL-crystallin and β-amyloid. Electron micrographs from the two control conditions (Figure 2E and F) showed the difference between the two species: β-amyloid as a helical fibril and αL-crystallin as a polydisperse oligomer. The experimental conditions showed that αL-crystallin specifically interacted with β-amyloid and decreased the fibril size as the concentration of αL-crystallin to β-amyloid increased. This was shown by αL-crystallin aligned along the fibril and significantly smaller fibrils than that of the positive control (Figure 2A). As the αL-crystallin concentration decreased, the prevention of amyloid elongation decreased (Figure 2B and C). Some αL-crystallin was still present on the amyloid fibril at 50x and 100x, but these effects were significantly diminished. Higher ordered fibril elongation occurs primarily in the 100x condition. This indicated that even in small concentrations, αL-crystallin can have a preventive effect to the elongation of highly ordered β-amyloid fibrils. Notably, in the context of the eye lens, αL-crystallin would be expected to be in large excess over amyloidogenic peptides and would therefore be effective at preventing/delaying fiber formation.

The 10x αB-crystallin condition also showed interaction with β-amyloid as monitored by NSEM (Figure 2D). The chaperone was present in greater number than in the 10:1 αL-crystallin, and free αB-crystallin was also present in solution. These micrographs in addition to the fluorescence assays validate that in the 10x αB-crystallin condition, the elongation of β-amyloid was completely suppressed. This indicated that αB-crystallin was a more potent inhibitor of β-amyloid than αL-crystallin *in vitro*.

CONCLUSION

The sHsp, αB-crystallin, has been previously shown to provide cytoprotective effects in neurodegenerative disease pathways by forming stable associations with amyloid species, specifically β-amyloid. Since β-amyloid has also been found to be present in age related cataracts, it is necessary to determine whether the lens specific αL-crystallin interacted with β-amyloid as a molecular chaperone in a similar manner to αB-crystallin. Using ThT fluorescence-based chaperone assays, biochemical analysis by SDS-PAGE and structural characterization by electron microscopy, it was observed that αL-crystallin did directly interact with β-amyloid fibrils and prevent the elongation of β-amyloid *in vitro*; however, the suppression effects of αL-crystallin were not as strong as what was found for αB-crystallin. This indicated that αL-crystallin may indeed be an effective chaperone against β-amyloid formation, but not be as potent as αB-crystallin.

Moving forward, it would be beneficial to observe the chaperone abilities of αA-crystallin with β-amyloid by similar methodologies. Since a decrease of chaperone activity was observed for αL-crystallin compared to αB-crystallin, it would be worthwhile to discern whether this decrease was due to the αA-crystallin present in αL-crystallin, or if there was another factor that could not be distinguished from these experiments.

Overall, this work has expanded on the knowledge of chaperones in the eye lens and their role in protein aggregation disease. This increased the understanding of the chaperone ability of αL-crystallin compared to αB-crystallin *in vitro* and had important implications for amyloid deposition and elongation and its role in progression in disease. Further progression of the knowledge of crystallins in the eye lens could lead to effective drug therapeutics and less invasive methodologies for intervention of cataracts.

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