β_2-microglobulin amyloid-lipid interactions

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Introduction

Amyloid fibril accumulation is the pathological hallmark of a number of devastating human diseases including type II diabetes mellitus, Alzheimer's disease and Parkinson's disease. Due to their prevalence, diversity and generally debilitating nature, amyloid diseases present one of the most important and costly challenges for modern medicine. Currently, one of the major stumbling blocks in stimulating the design of amyloid disease therapeutics is a lack of understanding of the mechanisms of cell death and tissue damage associated with amyloid disease pathologies.

While the molecular mechanism of amyloid disease is not yet well understood, interaction between amyloid proteins and cell membranes has been implicated in cytotoxicity. Hence, the membrane lipid composition and chemical environment in which amyloid-lipid interactions occur *in vivo* may play an important role in amyloid disease physiology. In this report we have investigated the interaction between β_2-microglobulin (β_2m), the precursor of amyloid fibrils formed in dialysis related amyloidosis, and lipid vesicles of differing anionic lipid composition at varying pH using a series of biophysical experiments including: tryptophan fluorescence quenching and a sucrose-gradient liposome flotation assay to measure β_2m-membrane interactions; and liposome leakage to measure membrane damage conferred by β_2m. Combined with visualization of membrane disruption by β_2m amyloid fibrils using cryo-electron tomography, these studies provide important insights into the molecular mechanism of amyloid-lipid interactions and the molecular basis of cytoxicity.

Results

β_2m monomer was expressed recombinantly and purified before being assembled into *long* straight fibrils by incubating quiescently at pH 2 for 48 h. *Long* β_2m fibrils were subsequently fragmented by agitation for 48 h to form *short* fibrils (Figure 1a). Despite different molecular architectures, upon addition of liposome vesicles a decrease in tryptophan

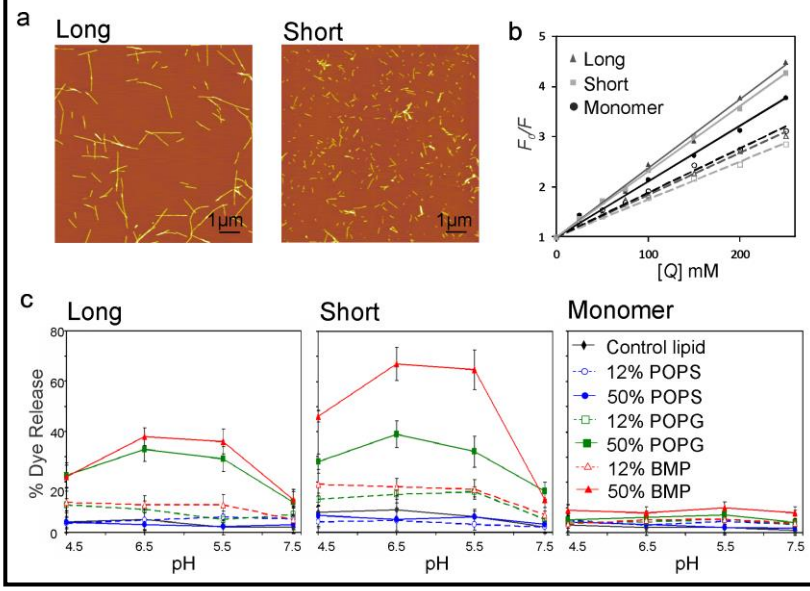

Figure 1: biophysical investigations of β_2m-lipid interactions. **(a)** AFM images of *short* (0.30 ± 0.01 μm) and *long* (1.30 ± 0.05 μm) β2m fibrils. **(b)** Stern-Volmer plots of Trp quenching by acrylamide for β_2m monomer, *short* and *long* fibrils in both the absence (*dashed line*) or presence of liposomes containing 12 % BMP (*solid line*), pH 4.5. **(c)** Relative CF dye release from LUVs containing 36 POPC: 20 POPE: 7 sphingomyleiln: 25 cholesterol (control) plus 12 or 50% POPS, POPG or BMP at pH 4.5-7.4. Errors: SD from n=3.

fluorescence quenching by aqueous acrylamide, corresponding to a decrease in tryptophan solvent accessibility, is seen for both β_2m monomer and fibrils (Figure 1b). This result indicates that both β_2m monomer and fibrils interact with the lipid bilayer. Indeed, physical

separation of membrane-associated β_2m, using a discontinuous 10:60:80% sucrose-gradient liposome-flotation centrifugation assay indicates that ~40-60% of β_2m protein associates with the lipid bilayer regardless of the β_2m species present. In addition, no significant correlation between β_2m membrane affinity and lipid composition or pH was observed (*data not shown*).

Previous studies have indicated that amyloid fibrils can cause membrane leakage. A carboxyflorescein (CF) dye release assay was used to investigate membrane damage resulting from interaction with β_2m. Briefly, β_2m was incubated with lipid vesicles loaded with 50 mM CF. The fluorescence of CF encapsulated in the lipid vesicle is self-quenched. The increase in CF fluorescence upon leakage from the liposomes was monitored and the extent of dye release was measured as the ratio of CF fluorescence due to dye release and CF fluorescence arising when the lipid vesicles were disintegrated by the addition Triton X-100 to 2%. Despite all species interacting with the liposomes, only β_2m fibrils, and not monomer, cause significant membrane damage (Figure 1c). Membrane damage conferred by fibrils primarily occurs at acidic pH, is inversely proportional to fibril length and requires an anionic lipid mix, but varies depending on the identity of anionic lipid component present (Figure 1c). Most strikingly, greatest membrane disruption was observed for lipids containing 50% bis(monoacylglycero)phosphate (BMP) liposomes at acidic pH (5.5-6.5), conditions likely to be encountered in the endocytotic pathway.

Membrane damage by specific interaction between β_2m fibrils and liposomes was also visualised using cryoelectron tomography. We demonstrate that *short* β_2m fibrils interact strongly with liposomes resulting in distortion of the normally spherical vesicles into pointed teardrop-like shapes with the fibril ends seen in proximity of the membrane disortions. Moreover, the tomograms indicated that the fibrils extract lipid from the membranes at these points of distortion by removal or blebbing of the outer membrane leaflet. Small (15-25 nm) vesicles, presumably formed from the extracted lipids are observed decorating the fibrils; a previously undescribed class of lipid-protein interactions in membrane remodelling (Figure 2).

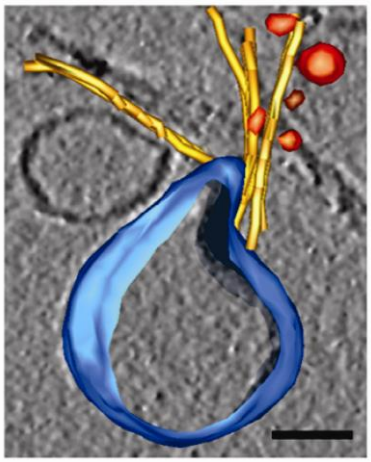

Figure 2: a rendered 3D model based on cryoelectron tomography of a distorted liposome (4 PC: 1 PG) (*blue*), surrounding fibrils (*yellow*) and adjacent small vesicles (*orange*). Scale bar: 50nm.

Together, these findings highlight a potential role of fibrils, and particularly fibril ends, in amyloid pathology, where specific disruption of vesicles of endosomic origin may potentially govern the physiology of β_2m amyloidosis.

Publications

Milanesi, L., Sheynis, T., Xue, W.-F., Orlova, E., Hellewell, A., Jelinek, R., Hewitt, E., Radford, S. & Saibil, H. (2012) Direct three-dimensional visualization of membrane disruption by amyloid fibrils. *Proc. Natl. Acad. Sci. U.S.A.* **109:** 20455-20460.

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Collaborators

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