Abstract

Multimeric protein deposits play a central role in the pathogenesis of neurodegenerative diseases. Using biophysical and biochemical methods, we established that purified, filamentous bacteriophage N13 (NPT002) directly and potently binds Aβ42 dodecamers and dimers at a broad range of amyloids, including Aβ, α-syn, and synuclein. We also showed that NPT002 mediates lowering of multimerized protein aggregates following single intracranial administrations to transgenic mouse models with Aβ, α-syn, and tau deposits, respectively. Recently, we isolated the phage protein domain from NPT002 responsible for the amyloid interacting activities, which we call the General Amyloid Inhibitor Motif (GAIM). Here, we demonstrate that GAIM mediates potent binding to amyloid fibrils, ameliorates oligomer induced cytotoxicity, and potently inhibits amyloid fiber assembly. An immunoglobulin (Ig) fusion of GAIM mediates amyloid fiber disassembly with nanomolar affinity, and remodels them into non-amyloidogenic, non-toxic conformers in vitro. Multimeric GAIM competes for binding Aβ42 conformers with different affinities. GAIM-based molecules recapitulate activities of whole phage (NPT001/002).

Introduction

NPT002 is a filamentous bacteriophage (F13) for the treatment of neurodegenerative diseases involving protein misfolding and aggregation, including Alzheimer’s disease and Parkinson’s disease. NPT002 is composed of 21 protein domains per virion, which is 100 times more than other single-protein phages, making it an attractive option for a range of amyloidogenic diseases, such as Alzheimer’s disease. (See posters Gannon, et al/MPI-287 and Levenson, et al/MPI-288 for more supporting data)

NPT001 binds Aβ42 fibers with high affinity

Aim: To determine binding properties of NPT001 with Aβ42 fibrils. The binding was exploited to develop a fluorescence based binding assay. a) Binding competition study with phage (Kdd) lacking GAIM motifs. b) SPR binding studies showing NPT001 does not bind single Aβ monomers.

NPT001 disaggregates a wide array of amyloids

a) ThT binding studies of NPT001 compared with NPT002. b) GAIM multimers show binding of NPT001 and NPT002 to Aβ42 alone (see Supplementary data).

NPT002 reduces Aβ plaque loads in aged male Tg2576 mice

Aged (15-19 mo) male Tg2576 mice were intracranially injected with 2µl of 2x10⁹ or 2x10¹¹ particles of NPT002 7 days after injection mice were sacrificed and brains were examined and fixed. 50µm sections were cut and Aβ plaque load was determined using a monoclonal antibody for Aβ (AbE12). Significant decrease in Aβ plaque load in Tg2576 model was observed with NPT002. n = 7, p < 0.01. Values are mean ± SEM. *p < 0.01, all comparisons to Tg PBS (Dunnett’s test). Representative images of IBA1 immunoreactivity are shown below summary graph.

GAIM: A Novel Virus Motif That Targets Misfolded Protein Assemblies

Gaim inhibits Aβ42 assembly

a) GAIM monomers bind Aβ42 conformers with different affinities. b) EM images showing inhibition of Aβ42 assembly in the presence of GAIM. c) The products of inhibition were also analyzed by circular dichroism and d) ThT binding. GAIM-bound Aβ42 is multimeric in nature as analyzed by Spectra Densitometry. Gel Electrophoresis (data not shown).

Conclusions

- NPT001/002 bind a wide variety of amyloid fibers with high affinity, which leads to fiber remodeling and disaggregation
- Binding is conformation-specific and not sequence-specific. NPT001/002 do not bind monomers of amyloid-forming proteins
- Binding is mediated by a 25KD General Amyloid Inhibiting Motif (GAIM). Inactivation or deletion of this motif abolishes GAIM activity
- GAIM-based molecules are being developed for clinical trials and preclinical studies.