The Expression and Characterization of Disulfide-bond Stabilized Amyloid-β peptides

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Research gap: Aβ oligomers are challenging to study

- Amyloid-β (Aβ)
  - Central to the pathogenesis of Alzheimer’s disease

- Aβ oligomers
  - Toxic
  - Aggregation-prone

http://pdb101.rcsb.org/motm/189
Inspiration: β-hairpin is required for Aβ oligomerization

- β-hairpin → Aβ oligomers
- Conformational change → Aβ fibrils

PDB ID: 2OTK
White: affibody
Green: Aβ β-hairpin

Research goal: study effects of β-hairpin alignment of Aβ
Mutant Aβ plasmids were generated through molecular cloning.
Disulfide-bond stabilized Aβ were expressed and purified

Representative HPLC: Aβ (M1–42/A21C–I32C) (mutant 2)

Representative mass spec: Aβ (M1–42/A21C–I32C) (mutant 2)
Disulfide-bond stabilized mutants like to form oligomers

SDS-PAGE results of Aβ (M1–42) wild-type and disulfide-stabilized peptides (at 31.25 µM)
Disulfide-bond stabilized mutants do not form fibrils

ThT assay results of Aβ (M1–42) wild-type and disulfide-stabilized peptides (at 10 µM)
Reduction of the disulfide-bond induced the formation of the fibrils

ThT assay results of Aβ (M1–42/A21C–I32C) in the absence or presence of TCEP reducing agent (at 10 µM)

- Red line: 10 µM Aβ(M1–42/A21C–I32C) (mut 2) + 5 mM TCEP
- Blue line: 10 µM Aβ(M1–42/A21C–I32C) (mut 2) + water
Disulfide-bond stabilized mutants – circular dichroism
Disulfide-bond stabilized mutants – ATR-FTIR

β-turn

β-sheet

α-helix
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