ROSETTA-informed design of structurally stabilized cyclic anti-amyloid peptides

Chandler Est, Parth Mangrolia and Regina Murphy
Department of Chemical and Biological Engineering
University of Wisconsin
Madison, WI, 53706

Introduction

- Alzheimer's disease pathology is characterized by deposition of beta-amyloid (A β) fibrils into insoluble plaques
- Homo-tetrameric transthyretin (TTR) is an endogenous transport protein in the blood and CSF that is also a sequestering agent of A β , thereby inhibiting fibrillogenesis
- A monomeric TTR mutant (mTTR) cannot fulfill TTR's physiological transport functions and serves solely as an ideal anti-A β therapeutic candidate; however, mTTR is susceptible to proteolysis, and binding to A β is diminished in the presence of nonspecific proteins

- Cyclic peptide sequence mimics of the A β binding domain on TTR resist proteolysis and retain specificity for A β in more realistic environments, but show disordered structure and weaker binding affinity than mTTR

- We propose these therapeutics might be improved by rationally designing cyclic peptides that more closely mimic the structure of the A β binding site by including disulfide and/or backbone stabilizing motifs

Methodology

- In silico*, rational design of stabilized cyclic peptide structures using the Simple Cyclic Peptide Application (SCPA) of the ROSETTA software suite
- Validation of SCPA predicted secondary structure by circular dichroic spectroscopy
- Characterization of anti-amyloid efficacy by Thioflavin T (ThT) assay

Acknowledgements & References

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ROSETTA SCPA Simulation Results

cG8 (black – original cyclic peptide); cG8_{ss} (blue – putative disulfide and cyclic constrained peptide); cG8_{ss/dProPro} (red – disulfide and further cyclically constrained peptide); cG8_{dProPro} (green – further cyclically constrained peptide).

A) Comparison of energy values and atomic RMSD for 10,000 independent completed closures. **B)** Cartoon overlay showing residue side chains of the five lowest energy structures from the full simulation. **C)** Cartoons showing residue side chains of each of the five lowest energy structures individually. **D)** Cartoon overlay of the five lowest energy structures with residue side chains hidden.

Experimental Validation

cG8 (black – original cyclic peptide); cG8_{ss} (blue – putative disulfide and cyclic constrained peptide); cG8_{ss/dProPro} (red – disulfide and further cyclically constrained peptide); cG8_{dProPro} (green – further cyclically constrained peptide).

CD spectra at a peptide concentration of 45 μ M in 10 mM sodium phosphate, 140 mM NaF, pH 7.4 buffer.

A β fibril formation, as measured by ThT fluorescence intensity, alone or in the presence of designed therapeutics at a 20:1 (A β :therapeutic) molar ratio, at 1, 24 or 48 h. Data were taken at 37°C. All data are background subtracted, and error bars indicate SEM. (*) indicates different vs. A β alone ($P < 0.05$); (θ) indicates different vs. cG8 ($P < 0.05$). All peptides were ThT-negative in the absence of A β . (Gray, no therapeutic; white, mTTR)

Conclusions

- Disulfide constraint of the peptide backbone has minimal effect on stability or anti-amyloid efficacy
- Incorporation of an additional ¹³ProPro β -turn template increases structural homogeneity, but has little effect on anti-amyloid efficacy
- Regardless, cyclization, β -sheet structure and conformational homogeneity are preferable design features of anti-amyloid peptides
- Lack of enhanced anti-amyloid efficacy may suggest cG8_{dProPro} does not adopt the proper conformation for maximal A β binding
- Recent solution phase NMR spectra (right, adapted from Oroz et al) indicate the structure of mTTR in solution may not be as rigid as previously believed, suggesting next generation peptides may need design against a different backbone structure template