Biophysical aspects of amyloid fibrils

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Abstract:

Introduction: Amyloid fibrils are associated with lots of human diseases such as **Alzheimer, Parkinson, spongiform encephalopathies and diabetes**. Various protofibrils assemblies together and form ordered packed B-parallel structures. Their crucial role in initiation of toxic process in neurodegenerative disorders in well known. Divers sequences of amino acids can form the amyloidic state of proteins.

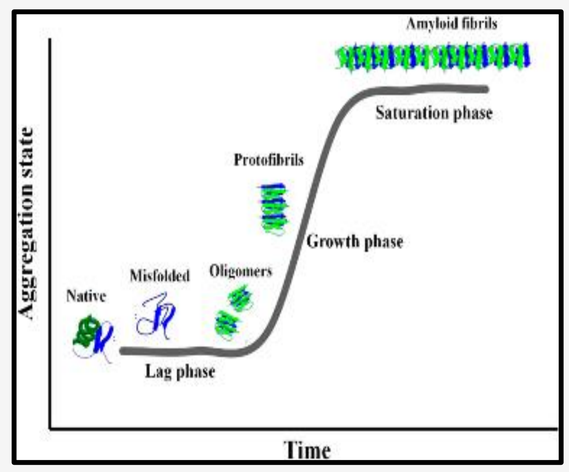
Methods: In this study, the spectroscopy methods were **NMR** spectroscopy, **CD** spectroscopy and Infrared spectroscopy. Kinetic measurements of amyloid fibril elongation were done using Thioflavin-T **(ThT)** and quartz crystal microbalance (QCM). Solution small angle x-ray scattering **(SAXS)** and differential scanning calorimetry **(DSC)** combined with simulated annealing of the protein were applied. Computational studies help to understand the application and analysing the obtained results.

Results and discussion: Experimental kinetic measurements and theoretical analysis, showed that **electrostatic effects control protein aggregation.** Furthermore, the magnitude of binding of a variety of ions to protein molecules was determined. Our results suggest that **longer amyloid fibrils are more stable**. Our spectroscopy techniques confirmed that the formation of amyloid fibrils are a **generic property** of polypeptide chains, and for different peptides and proteins, the mechanism of formation is similar.

Conclusion: amyloid fibrils cause **neurotoxic effects** in neurodegenerative disorder. Detailed biophysical studies of amyloid fibrils can elucidate new aspects and features of this diseases and help our investigating on **the prevention of these fibril formation** and treatment of these diseases to be more effective.

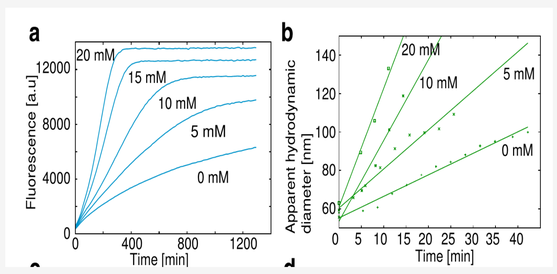
1.Buell, A.K., et al., *Electrostatic effects in filamentous protein aggregation.* Biophysical journal, 2013. 104(5): p. 1116-1126.
2.Choo, L.-P.i., et al., *In situ characterization of beta-amyloid in Alzheimer's diseased tissue by synchrotron Fourier transform infrared microspectroscopy.* Biophysical Journal, 1996. 71(4): p. 1672-1679.
3.Shen, C.-L., et al., *Light scattering analysis of fibril growth from the amino-terminal fragment beta (1–28) of beta-amyloid peptide.* Biophysical journal, 19a93. 65(6): p.۲۳۹۵-۲۳۸۳ .
4.Wilkins, D.K., C.M. Dobson, and M. Groß, *Biophysical studies of the development of amyloid fibrils from aa peptide fragment of cold shock protein B.* European journal of biochemistry, 2000. 267(9): p. 2609-2616.
5.Xu, Z., R. Paparcone, and M.J. Buehler, *Alzheimer's Aβ (1-40) Amyloid Fibrils Feature Size-Dependent Mechanical Properties.* Biophysical journal, 2010. 98(10): p. 2053-2062.

❖Amyloid fibrils are highly ordered protein aggregates.
❖ They play a crucial role in the initiation and progression of various neurodegenerative disorders, including Alzheimer's disease, Parkinson's disease, type 2 diabetes.
❖ Under certain conditions these disease proteins can undergo structural rearrangements resulting in misfolded proteins that can lead to the formation of aggregates with a fibrillar amyloid-like structure



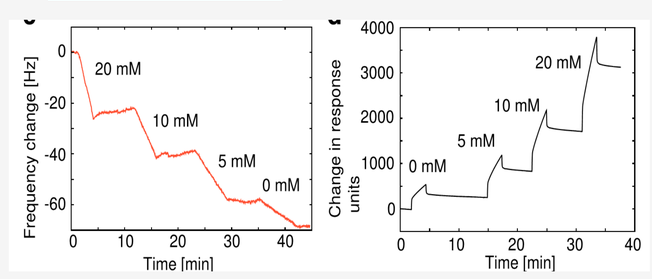
Thioflavin T fluorescence 40°C

dynamic light scattering 45°C

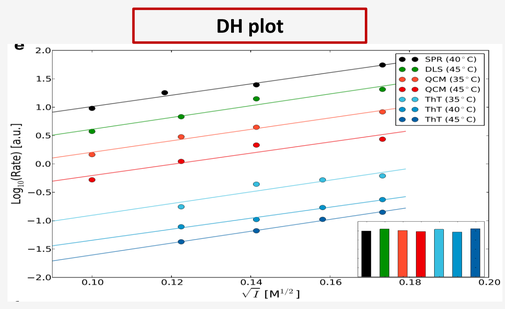


quartz crystal Microbalance 35°C

surface plasmon resonance 40°C



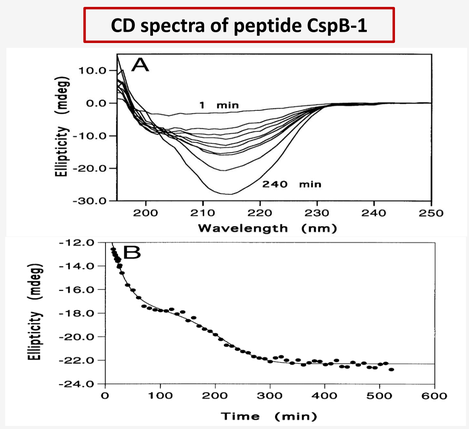
DH plot



The experiments were done at Different NaCl concenterations

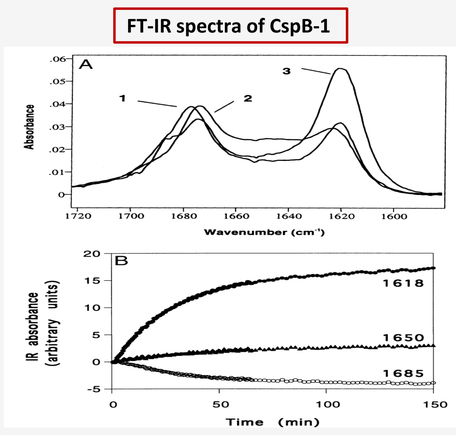
❖ The excellent agreement between the four techniques confirms that the process of fibril elongation along a surface is equivalent to the elongation in bulk solution
❖ the plots illustrate that the observed screening effects have no detectable temperature dependence in the investigated temperature rang

CD spectra of peptide CspB-1



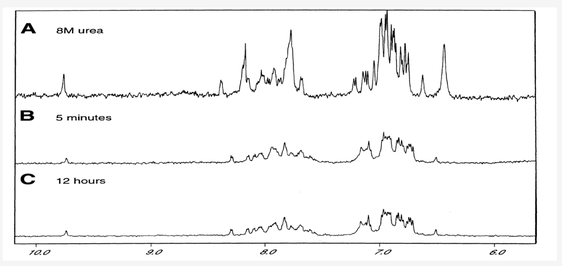
(A) a kinetic CD experiment over 4 h
(B) typical timecourse of a CD experiment recording the ellipticity at 215 nm over 5 h

FT-IR spectra of CspB-1



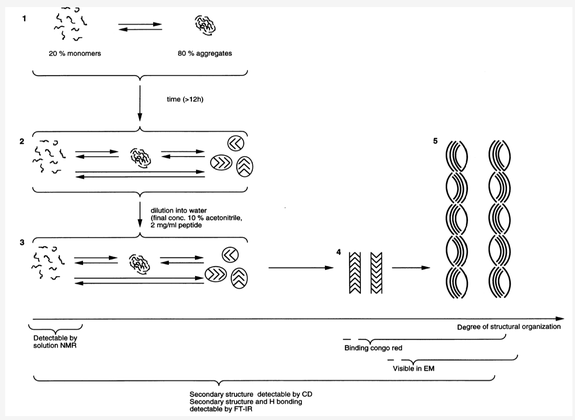
(A) FT-IR spectra of CspB-1
(B) timecourses derived from the FT-IR intensity changes at three different wavelengths

NMR spectra of CspB-1 in denaturant and in a fibril formation experiment



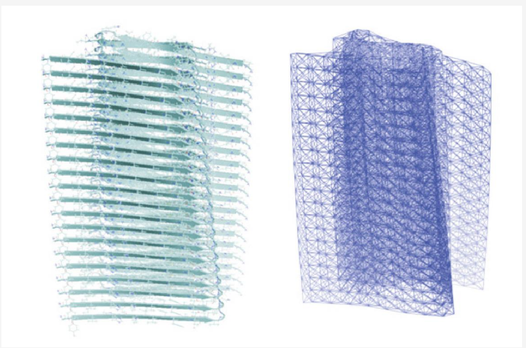
(A) Spectrum of CspB-1 in 8murea
(B)spectrum recorded 5 min after initiation of fibril Formation
(C) spectrum from the same experiment after 12 h

Schematic model for the mechanism of fibril formation

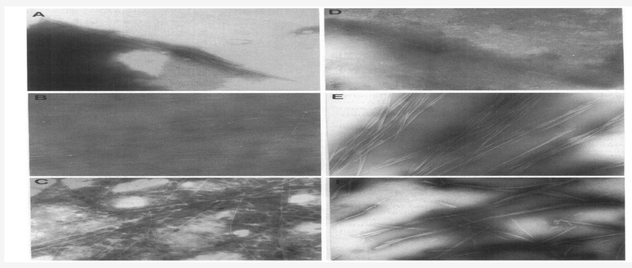


(1) Unstructured monomers and disordered aggregates are present in solution afterdissolving the peptide in 50% CH3CN.
(2) After the 12 h ageing step, the species initially present coexist with ordered aggregates.
(3) Following the solvent shift to 10% CH3CN, some of the ordered aggregates redissolve, but
there are still the same three species in solution
(4) complete fibrils.

Full-atomistic and corresponding ENM representations of a twofold symmetric amyloid fibril

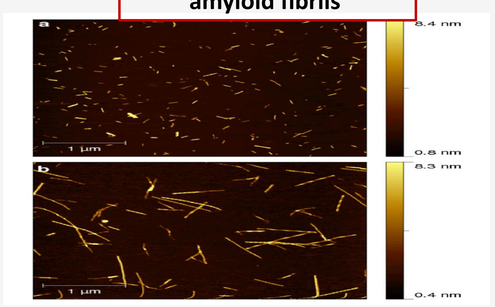


Electron micrographs of B(1-28)



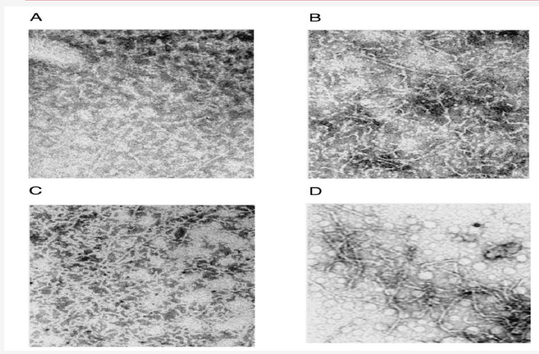
(A)1 hour phosphate buffer , (B)21 hours phosphate buffer
(C) 3 month phosphate buffer , (D)3 hours in PBS
(E) 7 weeks in PBS , (F) pH= 4.4

AFM images of insulin amyloid fibrils



dees detacinoS (a) fibrils
(b) Elongatd fibrils

Electron micrographs of CspB-1 amyloid fibrils observed



(A)30 min
(B)67 min
(C) 137 min
(D)1 week

Discussion

❖ electrostatic effects control protein aggregation
❖ longer amyloid fibrils are more stable
❖ the formation of amyloid fibrils are a generic property of polypeptide chains
❖ magnitude of binding of a variety of ions to protein molecules