Protein Folding Intermediates, Their Analysis and Role in Diseased States

Jahnabi Roy
Burke Group Literature Seminar
4/20/13
Protein Folding - An Introduction

Diagram showing the process of protein folding, from DNA transcription to protein synthesis, transport, and folding in the endoplasmic reticulum (ER), with degradation and modification pathways.
Why Study Protein Folding?

Effective Analysis of Mutation Experiments

Analysis of Diseased States
Studying Intermediates in Protein Folding

Vendruscolo and Dobson *Nat. Chem. Biol.* 2013, 9, 216-217
Analytical Methods to Study Protein Folding

Protein NMR
1. amino acid sequence
2. assign $^1$H signals
3. identify pairs of $^1$H close in space
4. final structure solved

Circular Dichroism

Dual Polarisation Interferometry

Optical Tweezers

Graphical Real Time Display
Full Data Processing

Force (pN)

Extension (μm)
NMR Methods to Study Protein Folding

Equilibrium NMR Experiments

- direct NMR studies on unfolded proteins
- peptide studies

Kinetic NMR Experiments

Fully Unfolded Polypeptide

- HX pulse labeling

Local Elements of Secondary Structure

- Compact Molten Globule States
  - HX trapping
  - magnetization transfer

- Folded, Non-Native State
  - real-time NMR fast mixing T-jump

Folded, Native Protein

Time Scale

- ns - µs
- ms
- sec

Dyson and Wright Annu. Rev. Phys. Chem. 1996. 47:369–95
Dynamic Ensemble Refinement - NOE & Molecular Dynamics Simulation

Backbone trace of ubiquitin

Molecular Dynamics Simulations

Cold Denaturation and NMR Spectroscopy

CylR2 protein and buried hydrophobic residues

Amyloid Fibrils and Neurodegenerative Disorders

Mechanism of Amyloidogenic Aggregation

Aggregation with normal proteins

Interefering with chaperone machinery

Mechanism of Amyloidogenic Aggregation

Disruption of lipid membranes

Tillement et. al. Mitochondrion 2011, 11, 13-21
Intermediate Structure of Amyloid Fibril Formation

Structure of the low-populated intermediate.

A39V/N53P/V55L Fyn SH3 domain

Surface Aggregation Propensities

Native State

Intermediate

Decay in HSQC signals of the amide couplings

Intermediate Structure of Amyloid Fibril Formation

Solid State NMR to Study Amyloid Fibrils

MAS solid-state NMR experiments recorded for a perdeuterated fibril sample of the Alzheimer’s disease peptide Aβ.
Summary of Structural Features of Amyloid Fibrils

- Strong ordered structures
- Highly insoluble
- Present in form of β-pleated sheets
- High tensile strength
- Usually contain glutamine repeats

Questions that still remain...

- Do disease progressions correlate with any structural variations?
- How are functional amyloids different from disease associated amyloids?