Proteomics Course

LECTURE-5
Proteins: Folding and misfolding

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Primary Structure
Secondary Structure
Tertiary Structure
Quaternary Structure

Amino acid residues

α Helix
Polypeptide chain
Assembled subunits
Lecture outline

- Amino acid sequence determines 3-D structure
- Protein folding
- Thermodynamics of protein folding
- Molecular chaperone for protein folding
- Protein misfolding; diseases

Amino acid sequence determines 3-D structure
Anfinsen experiment

Proof for relation between amino acid sequence and its conformation

- Anfinsen's experiment
  - Denaturation
  - Refolding
Anfinsen’s experiment

- Ribonuclease A
- Urea, guanidine HCl
  - denaturants
- β-mercaptoethanol
  - breaks disulfide bonds

Ribonuclease

4 disulfide bonds
Role of Urea and Guanidinium Chloride

Effectively disrupts non-covalent bonds of proteins

Role of $\beta$-mercaptoethanol

- Cleavage of disulfide bonds
- Large excess converts disulfides to sulfhydryls
Anfinsen’s experiment

- 8 M urea and β-mercaptoethanol treatment
  - converted native protein to fully reduced, randomly coiled polypeptide “denatured”
  - lacked enzymatic activity
Anfinsen’s experiment (3)

Disulphide bonds
Noncovalent interaction
Native ribonuclease A

β-mercaptoethanol
6M urea

Denatured ribonuclease A

Remove urea & β-mercaptoethanol
Broken disulphide linkages

Anfinsen’s experiment (4)

Denatured ribonuclease A

Removed urea and β-mercaptoethanol
Native state ribonuclease A

Remove β-mercaptoethanol only
Inactive ribonuclease A
Anfinsen’s experiment (5)

- Scrambled – wrong pairings, 104
- Trace amount of β-mercaptoethanol catalyzed rearrangement of disulfide pairing

Anfinsen’s experiment (6)

- Urea and β-mercaptoethanol removed by dialysis
- Denatured ribonuclease regained activity
- Enzyme refolded into active form
- Sulphydryl groups became oxidized by air
Protein folding

Horwich et al. 2002

- Amino acid sequence determines 3D structure
**Protein folding**

- Polar amino acid side chains tend to gather on outside of the protein
- Non-polar amino acid side chains are buried inside

**Polar and non-polar amino acids**

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<th>Amino Acid</th>
<th>Abbreviation</th>
<th>Symbol</th>
<th>Hydrophobicity/Charge</th>
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Protein folding

Overall increase in an entropy drives the folding process

- Polar side chains
- Non-polar side chains
- Hydrophilic and hydrophobic interactions
- Folded conformation in aqueous environment

Protein unfolding by denaturation

- Protein denaturation by chemical and heat

  - Expose to high concentration of Urea
  - Remove Urea

  - Purified Protein isolated from cells
  - Denatured Protein
  - Original conformation of protein re-forms
Cooperative transition from folding to unfolding

- A sharp transition from native (folded) to denatured (unfolded) form
Folding from many conformations to just one?

Many unstable conformations

One stable conformation

Progressive stabilization of intermediates

• Folding is a cooperative process
• In general, any protein adopts only one conformation
• Or, few very closely related characteristic functional conformations “native state”
• Amino acid sequence dictates protein structure
• knowledge-based and *Ab initio* “from the beginning” prediction to predict protein structure
Thermodynamics of protein folding

Folding of proteins into their native conformations occurs spontaneously under physiological conditions and is dictated by the primary structure of the protein.

• Hydrophobic amino acids are driven to associate-hydrophobic collapse
• Thus, overall increase in an entropy drives the folding process
Thermodynamics of protein folding

Unfolded polypeptide chain

Partially folded polypeptide

Native state α-helix

Molten globule state

Unfolded polypeptide chain, high free energy & entropy

Helix formation commences, free energy & entropy decrease

Beginning of helix formation
Thermodynamics of protein folding

Molecular chaperone for protein folding
Molecular chaperone systems

Protein misfolding & diseases
Protein misfolding

- Protein folds into a single, energetically favorable conformation, specified by its amino acid sequence
- A protein may fold into alternative 3D structure due to mutations, inappropriate covalent modifications
Protein misfolding

• Accumulation of misfolded protein or proteolytic fragments results into few degenerative diseases
• Characterized by presence of insoluble protein plaques in organs such as brain and liver
Summary

- Anfinsen experiment
- Protein folding
- Thermodynamics of protein folding
- Molecular chaperons
- Protein mis-folding and diseases

REFERENCES