Chapter 19

by Vede J. Ramdass

Topics to be discussed:

1) Meaning of the terms *in vivo* and *in vitro*
2) Mechanisms of folding *in vivo*
3) Metastable folding intermediates

Meaning of the terms *in vivo* and *in vitro*

In vivo and *in vitro* have different meanings for Physicists and Biologists.

In vivo

Physicists:

Protein folding in a cell free system (with all its ribosomes, initiation factors, chaperones etc.) is considered *in vivo.*

Biologists:

The whole organism intact.

In vitro:

Physicist:

- A separate protein in solution, ‘as to the cell-free system . . it contains too many complicating life realities.’

Biologists:

- This is when a procedure is performed in a controlled environment such a test tube and not in an organism.

Mechanisms of folding *in vivo*

Machinery of Protein folding In a Cell

1) Ribosomes
2) Enzymes
3) Chaperones
4) Co-translational Folding

1) Ribosomes

Makes a protein residue by residue from its N- to C end.
Key points about process:
- It is not uniform: temporary pauses at the"rare" codons.
  - It is assumed that pauses may correspond to boundaries in the structural domains, that can help a quiet maturation of the domain structures.

    What is a domain?
  - It is part of the protein structure that can exist and function independently to the rest of the protein!

2)Enzymes

The accelerate the process of *in vivo* folding.
Examples:

1) Prolyl Peptide catalyzes the trans to cis conversions of prolines. This can sometimes be the rate-limiting step in *in vivo* folding.
2) disulfide isomerase catalyzes the formation and decay of S-S bonds.

3)Chaperones

-These are protective proteins and are considered the cell's trouble shooters.
-Act as incubators for protein folding.
-Act as 'freezers' by postponing folding at the point when it is to be folded into quaternary structure or the protein is to be transported to another part of the cell.
-Their main task is to fight the consequences of aggregation (which is the aggregation of mis-folded proteins).
The types of Chaperones are ;
1) Small Chaperones
2) Big Chaperones

3) Chaperones *cont'd*

1) 'Small' Chaperones
- binds to a nascent protein to prevent aggregation
- after it has prevented aggregation it dissociates from the nascent protein (this process consumes 1 ATP)

2) 'Large' Chaperones
- work mainly with multi-domain proteins, and especially with proteins whose domains are composed of remote chain regions.

3) Chaperones *cont'd*

Examples of 'Large' Proteins

1) GroEL/GroES

GroEL:

- Forms a 'test tube' and the nascent proteins comes into the 'test tube'. The GroEL then protects the nascent protein from aggregation and the proteases (enzymes that act against proteins) within the cells.
- The GroEL undergoes conformational changes which causes it hydrophobicity to change and therefore 'shaking' the 'test tube'.

3) Chaperones *cont'd*

continuation of GroEL/GroES
GroES:
- *This acts as a lid and therefore opens and closes the 'test tube'*

<u>Final word about chaperone</u>
Thus the chaperone lets the protein go only when it is already folded and has ceased sticking to the “tube”. Protein aggregation in a cell often leads to the formation of “inclusion bodies” where the proteins do not have their native structures

4) Co-translational Folding

Definition: The folding of a nascent chain while it is still attached to the ribosome

Why process occurs?

Folding occurs in a highly crowded molecular environment and as a result co-translation folding and even chaperones act as protective mechanisms.

How does it work?

- Folding occurs in ribosomal “tunnels”, where they are protected from aggregation and degradation.
- Ribosomes have molecular chaperones themselves to mediate the refolding of denatured proteins.

Key Points about Bio-machinery of Cell;

1. Ensures that Protein synthesis occurs.
2. It acts as an incubator for protein synthesis.
3. Does not determine the protein structure.

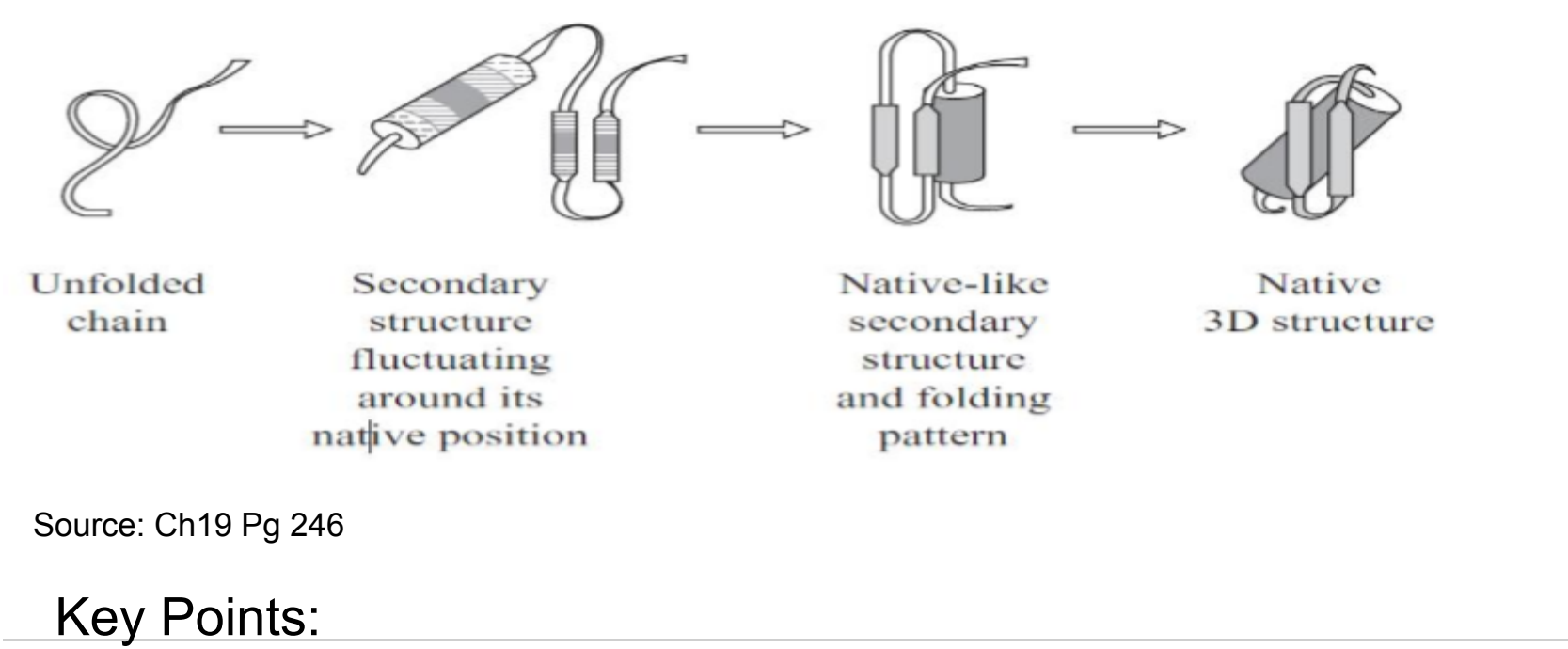
Metastable folding Intermediates

Introduction:

How does the protein achieve the stable conformation in "biological time"?

Solution: There is a folding pathway and the native state is the end of this process rather than the most stable state. In other words the native structure is under kinetic control rather than thermodynamic control. (~Levinthal)

"Framework Model“ by O.B.P



Source: Ch19 Pg 246

Key Points:

- Stimulated investigation of intermediates.
- Stressed the importance of rapidly folded α-helices and β-hairpins in the initial folding steps.
- Showed the gluing of helices and hairpins into molten globule.
- Then crystallization of the molten globule into the native structure.

Molten Globule

This is a metastable kinetic intermediate.

- The metastable terms means it is quasi-stable because lives long but then disappears.

It is called Molten Globule because:

- Its secondary structure and compactness are close to the native state.
- But it does not have the side chain packing or enzymatic activity of the solid 'native' protein.

Experiment Performed to Prove the existence of an Intermediate State

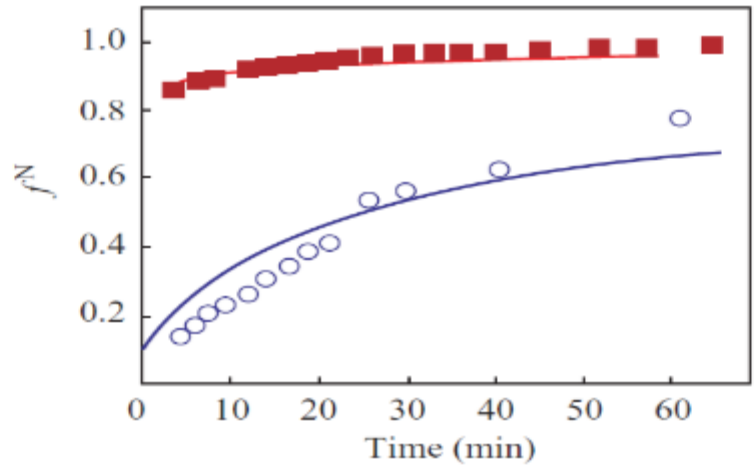


Figure 19.3. Kinetics of recovery of a "degree of nativity" (f_N) in the process of carbonic anhydrase B renaturation. Transition of the protein from its fully unfolded state (existing at 5.45 M GuHCl concentration) to the native state (at 0.97 M GuHCl) is followed by intrinsic viscosity (■■■), by ellipticity at 222 nm (—) and at 270 nm (—), and by enzymatic activity (○ ○ ○). Adapted from Dolgikh D.A., Kolomiets A.P., Bolotina I.A., Ptitsyn O.B., *FEBS Lett.* (1984) **164**: 88–92.

<u>WHAT DOES IT SHOW?</u>

-It shows that the "native" values of intrinsic viscosity (characteristic of globule's volume) and ellipticity at 222 nm (characteristic of the secondary structure) are restored, or, rather, nearly restored in the course of renaturation much faster than the ellipticity at 270 nm (characteristic of side-chain packing) or protein activity.

Key points about Molten Globule:

- This intermediate takes a few milliseconds to form.
- The rate-limiting folding step is the formation of the native "solid" protein from the molten globule.
- Is an intermediate in *in vitro* folding but can be observed under physiological conditions.
- The Molten Globule is not the only intermediate state and there is a state with a partially formed secondary structure and a partially condensed chain, that precede the Molten Globule.

Effects on Folding:

They help trace the folding pathway in a process that is called 'chemical logic'. This can be compared to intermediates in a biochemical reaction trace the pathway of a reaction.