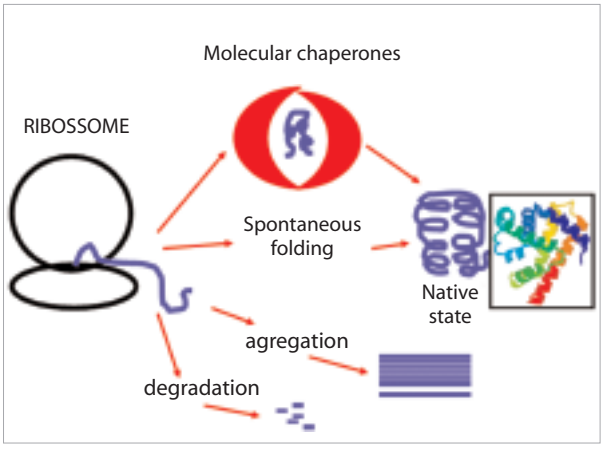
PROTEIN FOLDING, STABILITY AND STRUCTURE

Carlos Henrique Inácio RAMOS

Chemistry Institute / State University of Campinas (Unicamp)



The fate of protein in the cell. Protein is produced by the ribossome as an extended polypetide that usually folds spontaneously to its native state. However, in certain conditions, proteins fold only partially generating aggregates which are unproductive because the function of most proteins is ordinarily related to its native conformation (some researchers think that misfolded proteins are the origin of, as much as, half of the human diseases!). Protein aggregation is worsened in stress conditions and molecular chaperones are the major factors that had enhanced expression during stress. Proteins that escape the initial action of chaperones and precipitate can be ressolubilized by other chaperones. Therefore, molecular chaperones constitute the central cellular defense against protein misfolding and aggregation that have major pathological consequences

The conversion of a polypeptide backbone into a native protein is a key element in the translation of the genetic information of an organism. As the organism ages, folding seems to deviate, which signals for several diseases (mainly neurodegenerative ones). Protein misfolding causes its deposition in the cell in the form of aggregates or amyloid fibrils, both of which have toxic effects. Molecules, that play an important role in cell protection, are molecular chaperones, which help protein folding and protein disaggregation. Therefore, chaperones seem to have a fundamental role in the organism by increasing the success of physiological functions and protecting cells from becoming ill. Our proposal has the main objective of understanding protein folding by: 1) studying the folding pathway and the stability of proteins, mainly globins; 2) characterizing the forces and the mechanisms of amyloid fibril formation; 3) structurally and functionally characterizing chaperones; and 4) studying the mechanisms by which chaperones help folding, stop aggregation, resolubilize aggregates, and interact with proteins involved in cell malignization.
Our goal is to understand protein folding inside the cell: such knowledge will generate important new ways of thinking, and may help lead to new therapies.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Human chaperones have been cloned and purified in their folded conformation, as measured by circular dichroism and fluorescence spectroscopy. The hydrodynamic properties of the proteins are under investigation by hydrodynamic techniques (analytical ultracentrifugation, gel filtration chromatography and dynamic light scattering). *Ab initio* calculations are underway and will give further insights on quaternary structure.

Xanthomonas secretion chaperone and target: two sets of secretion chaperones and their respective targets were selected by two-hybrid studies. Proteins were purified and their interaction measure by *in vitro* techniques.

Sugarcane chaperones: we initiated further characterization of chaperones that are part of the HSP70-HSP90 complex (involved in abiotic and biotic stress in plants – a pathogen causes biotic stress).

Results on protein folding showed that the information that is present on the amino acid sequence is also important to avoid aggregation. Permutation mutants may still exhibit native structure and function, but aggregate easily.

MAIN PUBLICATIONS

1. Ramos CHI, Ferreira ST. 2005. Protein folding, misfolding and aggregation: evolving concepts and conformational diseases. (Review). *Protein Pept. Lett*. **12:**213-222.

2. Borges JC, Ramos CHI. 2005. Protein folding assisted by chaperones. *Protein Pept. Lett*. **12**: 257-261.

3. Borges JC, Hannes F, Craievich AF, Ramos, CHI. 2005. Low-resolution structural study of two human HSP40 chaperones in solution. HJA1 from subfamily A and HJB4 from subfamily B, have different quaternary structures. *J. Biol. Chem.* **280**:13671-13681.

4. Ribeiro-Jr EA, Ramos CHI. 2005. Circular permutation and deletion studies of myoglobin indicate that the correct position of its N-terminus is required for native stability and solubility but not for native-like heme binding and folding. *Biochemistry.* **44**:4699-4709.

5. Oliveira C, Borges JC, Torriani I, Ramos CHI. 2006. Low resolution structure and stability studies of human GRPE#2, a mitochondrial nucleotide exchange factor. Arch. *Biochem. Biophys*. **449**:77-86.

6. Borges JC, Ramos CHI. 2006. Spectroscopic and thermodynamic measurements of nucleotide-induced changes in the human 70-kDa heat shock cognate protein. Arch. *Biochem. Biophys.* **452**:46-54.

7. Tiroli AO, Ramos CHI. 2007. Biochemical and biophysical characterization of small heat shock proteins from sugarcane. Involvement of a specific region located at the N-terminus with substrate specificity. Int. J. *Biochem. Cell Biol*. **39**:818-831.

8. Tasic L, Paula FL Borin, Khater L, Ramos CHI. 2007. Cloning and characterization of three hypothetical secretion chaperone proteins from *Xanthomonas axonopodis pv. citri*. *Prot. Expr. Pur.* **53**:363-369.

9. Ramos CHI, Weisbuch S, Jamin M. 2007. Diffusive motions control the folding and unfolding kinetics of apomyoglobin pH 4 molten globule intermediate. *Biochemistry*. **46**:4379-4389.

10. Khater L, Alegria MC, Paula FL Borin, Santos TM, Docena C, Tasic L, Farah SC, Ramos CHI. 2007. Identification of the flagellar chaperone FLGN in the phytopathogen *Xanthomonas axonopodis* pathovar *citri* via characterization of its interaction with hook-associated FLGK. Arch. *Microbiol*. **188**:243-250.

Carlos Henrique Inácio RAMOS

Instituto de Química
Universidade Estadual de Campinas (Unicamp)
Caixa Postal 6154 - Cidade Universitária
13084-862 – Campinas, SP – Brasil

+55-19-3521-3144
cramos@iqm.unicamp.br