Folding and interaction studies of subunits in protein complexes

Ximena Aguilar Iturri

Akademisk avhandling

som med vederbörligt tillstånd av Rektor vid Umeå universitet för avläggande av filosofie doktorsexamen framläggs till offentligt försvar i sal KB3A9, KBC, fredagen den **14:e februari, kl. 9:00**.
Avhandlingen kommer att försvaras på engelska.

Fakultetsopponent: Dr. Alexis Verger
French National Centre for Scientific Research
Université de Lille, France.

Department of Chemistry
Umeå University, Sweden
Umeå 2014

Organization
Umeå University
Department of Chemistry

Document type
Doctoral thesis

Date of publication
24 January 2014

Author
Ximena Aguilar Iturri

Title
Folding and interaction studies of subunits in protein complexes.

Abstract
Proteins function as worker molecules in the cell and their natural environment is crowded. How they fold in a cell-like environment and how they recognize their interacting partners in such conditions, are questions that underlie the work of this thesis.

Two distinct subjects were investigated using a combination of biochemical- and biophysical methods. *First*, the unfolding/dissociation of a heptameric protein (cpn10) in the presence of the crowding agent Ficoll 70. Ficoll 70 was used to mimic the crowded environment in the cell and it has been used previously to study macromolecular crowding effects, or excluded volume effects, in protein folding studies. *Second*, the conformational changes upon interaction between the Mediator subunit Med25 and the transcription factor Dreb2a from *Arabidopsis thaliana*. Mediator is a transcriptional co-regulator complex which is conserved from yeast to humans. The molecular mechanisms of its action are however not entirely understood. It has been proposed that the Mediator complex conveys regulatory signals from promoter-bound transcription factors (activators/repressors) to the RNA polymerase II machinery through conformational rearrangements.

The results from the folding study showed that cpn10 was stabilized in the presence of Ficoll 70 during thermal- and chemical induced unfolding (GuHCl). The thermal transition midpoint increased by 4°C, and the chemical midpoint by 0.5 M GuHCl as compared to buffer conditions. Also the heptamer-monomer dissociation was affected in the presence of Ficoll 70, the transition midpoint was lower in Ficoll 70 (3.1 μM) compared to in buffer (8.1 μM) thus indicating tighter binding in crowded conditions. The coupled unfolding/dissociation free energy for the heptamer increased by about 36 kJ/mol in Ficoll. Altogether, the results revealed that the stability effect on cpn10 due to macromolecular crowding was larger in the individual monomers (33%) than at the monomer-monomer interfaces (8%).

The results from the interaction study indicated conformational changes upon interaction between the *A. thaliana* Med25 ACtivator Interaction Domain (ACID) and Dreb2a. Structural changes were probed to originate from unstructured Dreb2a and not from the Med25-ACID. Human Med25-ACID was also found to interact with the plant-specific Dreb2a, even though the ACIDs from human and *A. thaliana* share low sequence homology. Moreover, the human Med25-interacting transcription factor VP16 was found to interact with *A. thaliana* Med25. Finally, NMR, ITC and pull-down experiments showed that the unrelated transcription factors Dreb2a and VP16 interact with overlapping regions in the ACIDs of *A. thaliana* and human Med25.

The results presented in this thesis contribute to previous reports in two different aspects. *Firstly*, they lend support to the findings that the intracellular environment affects the biophysical properties of proteins. It will therefore be important to continue comparing results between *in vitro* and cell-like conditions to measure the magnitude of such effects and to improve the understanding of protein folding and thereby misfolding of proteins in cells. Better knowledge of protein misfolding mechanisms is critical since they are associated to several neurodegenerative diseases such as Alzheimer's and Parkinson's. *Secondly*, our results substantiate the notion that transcription factors are able to bind multiple targets and that they gain structure upon binding. They also show that subunits of the conserved Mediator complex, despite low sequence homologies, retain a conserved structure and function when comparing evolutionary diverged species.

Keywords: Macromolecular crowding, cpn10, Mediator, Med25, Dreb2a, VP16, conformational changes, NMR, ITC.

Language
English

ISBN
978-91-7459-795-0

Number of pages
57 + 3 papers