CHAPERONE ASSISTED FOLDING OF YEAST MITOCHONDRIAL ACONITASE IN *ESCHERICHIA COLI*

By

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DEDICATION

to my dear parents, Brother, Bhabhi and Tishya

for their everlasting love and patience....

CERTIFICATE

This is to certify that the thesis entitled "Chaperone assisted protein folding of yeast mitochondrial aconitase in *Escherichia coli*" being submitted by Ms. Parul Gupta to the Indian Institute of Technology Delhi, for the award of the degree of 'Doctor of Philosophy', is a record of the bonafide research work carried out by her, which has been prepared under our supervision in conformity with the rules and regulations of the "Indian Institute of Technology Delhi". The research reports and the results presented in this thesis have not been submitted for any degree or diploma in any other University or Institute.

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Dr. Tapan Kumar Chaudhuri

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Parul Gupta

Abstract

Over last two decades many researchers have demonstrated the mechanisms of how the *Escherichia coli* chaperonin GroEL and GroES work in the binding and folding of different aggregation prone substrate proteins both *in vivo* and *in vitro*. However, preliminary aspects, such as influence of co-expressing GroEL and GroES on the over expression of other recombinant proteins in *E. coli* cells and subsequent growth aspects, as well as the conditions for optimum production of recombinant proteins in presence of recombinant chaperones, which significantly affect the yields of recombinant proteins, have not been properly investigated. In the present study we have demonstrated the temperature dependent growth characteristics of *E. coli* cells, which are over expressing recombinant aconitase and *E. coli* chaperonin GroEL and GroES. For the aconitase over-expressing cells the changes in the growth rate indicated the participation of endogenous chaperonin in the folding of a fraction of over expressed aconitase. However, in presence of co-expressed GroEL and GroES the growth rate of aconitase producing cells confirmed the assistance of exogenous chaperone system for the folding of recombinant aconitase. *In vivo* folding of aconitase required co-production of complete *E. coli* chaperonin machinery GroEL and GroES, together.

Apo-aconitase, the Fe_4S_4 cluster free form of Tri carboxylic acid cycle enzyme aconitase, binds with GroEL and dissociates itself, upon maturation, through the insertion of the cluster. It is not quite clear why the apo-protein binds with GroEL. In order to explore the possibility, that the stability is a factor responsible for the aggregation of apo-form and hence, the non-native protein associates with GroEL to avoid the unfavorable event, we carried out the equilibrium and kinetic unfolding studies with holo- and apo-

aconitase. By probing the unfolding process through the changes in secondary structural element, exposed surface hydrophobicity, and the microenvironment around tryptophan residues, we were able to calculate various relevant parameters associated with the event. Results indicate that the lower ground state stability and higher solvent exposed hydrophobic surface make the apo-form aggregation prone. Based on the present observation and earlier findings, we propose that the binding of apo-aconitase to GroEL, not only rescues it from the aggregation, but also assists in the final stage of maturation by orienting the cluster insertion site on GroEL bound apo-protein. This information sheds new light on the potential role of GroEL in the biosynthetic pathway of the metallo proteins.

Osmolytes are known to stabilize proteins against aggregation. The present study also investigates, the *in vitro* chemical assisted activity restoration of aconitase. Our study suggests a strategy to enhance the percentage of correct aconitase refolding through the addition of some chemical folding aids into the refolding buffer. As is commonly recognized, the primary refolding problem is aggregation, either *in vitro* or *in vivo*. The simple treatments with added osmolytes applied in this study provide strong evidence that some osmolytes acting as folding aid reagents can effectively aid aconitase on the path to correct folding.

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