

INVESTIGATION OF CHAPERONE-MEDIATED FOLDING OF MALTODEXTRIN GLUCOSIDASE

By

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Submitted

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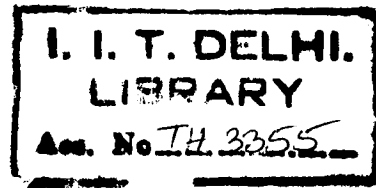
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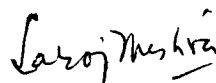
DEDICATION

*This thesis is dedicated with affection to
my dear parents, brother and wife whose everlasting love, courage and patience
have been an inspiration to me.*

CERTIFICATE

This is to certify that the thesis entitled “**INVESTIGATION OF CHAPERONE-MEDIATED FOLDING OF MALTODEXTRIN GLUCOSIDASE**” being submitted by **Mr. Subhankar Paul** 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 him, 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.


Dr. Tapan K. Chaudhuri


Prof. Saroj Mishra

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Abstract

In this study, the investigation of the GroEL and GroES assisted *in vivo* and *in vitro* folding of *E.coli* Maltodextrin glucosidase (MalZ) has been carried out. MalZ is a 69 kDa monomeric protein, responsible for degradation of maltodextrins to maltose by eliminating one glucose residue from the reducing end.

It was observed that recombinant MalZ could fold to a smaller extent in absence of any over-expressed GroEL and GroES in *E.coli*. However, there was a four-fold enhancement in the folding of MalZ in presence of exogenous bacterial chaperones GroEL and GroES. GroEL forms stable binary complex with non-native MalZ. Unfolded MalZ, when refolded without any chaperonin in the refolding buffer, gave very poor yield of folded protein (~7%). However, GroEL- GroES mediated refolding study with guanidinium hydrochloride denatured MalZ revealed that GroEL or GroES alone, even in the presence of nucleotides ATP or ADP, were not capable of refolding denatured MalZ. The efficient (~50%) *in vitro* refolding of MalZ required the complete chaperonin system GroEL and GroES in presence of ATP. In order to verify the type of mechanism GroEL followed, proteinase K protection studies on GroEL-MalZ-GroES-ADP complex were carried out. No undigested MalZ was recovered suggesting that MalZ was not encapsulated by GroES. On the other hand, it was observed that GroES was essential for the productive folding of MalZ. Hence, GroES must bind to GroEL during the nucleotide dependent folding process of MalZ. Considering these two points, it can be assumed that GroES binds to the ring not occupied by MalZ, the *trans* ring. In order to further verify the mechanism of GroEL-GroES assisted folding, a folding experiment was also performed

with the single ring version of GroEL, SR1. The philosophy behind the experiment was that, SR1 being a single ring version of GroEL can only allow the GroES to bind on its apical domain, hence if any binary complex of SR1-MalZ folds by addition of GroES and ATP, GroES must bind on top of bound MalZ, an indication of *cis* sided folding. The results on SR1-GroES-ATP mediated folding of denatured MalZ did not yield any advantage over the spontaneous folding of MalZ. Hence, the proposed mechanism of GroEL-GroES mediated folding of MalZ is a *trans* sided one. This is the first report of detailed mechanistic study of a large *E.coli* protein (>60 kDa) to be folded by GroEL and GroES both *in vivo* and *in vitro* using a *trans* mechanism. The study also indicated the necessity of studying the GroEL-GroES assisted folding of other *E.coli* proteins having molecular mass between 50 to 68 kDa to understand the molecular weight cut-off for GroEL-GroES assisted *cis* sided folding.

The thermal denaturation of MalZ was also studied to understand the stability of the protein and reversibility of the unfolding process. The outcome of the thermal denaturation studies revealed that the process was irreversible. It also signified the need of GroEL and GroES for the correct folding of MalZ both *in vivo* and *in vitro*. Conformational studies of MalZ were carried out using intrinsic and extrinsic fluorescence spectroscopy. It was observed that the Tryptophan residues in MalZ were in the negatively charged environment and accessible to the non-ionic quenchers. From the ANS-MalZ interaction studies, it was found that the protein belonged to the low surface hydrophobicity group. However, the chemical unfolding process for MalZ can be monitored by observing the substantial change of surface hydrophobicity during the process. Presence of stable non-native intermediate during the unfolding pathway was

observed from monitoring the change of surface hydrophobicity during the process. Through these sets of unfolding experiments, it was demonstrated that the unfolding process of MalZ is a three-state process involving Native, Intermediate and Denatured State.

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