INVESTIGATION OF CHAPERONE-MEDIATED FOLDING OF MALTODEXTRIN GLUCOSIDASE

By

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Submitted
In fulfillment of the requirement of the degree of

DOCTOR OF PHILOSOPHY

To the

INDIAN INSTITUTE OF TECHNOLOGY, DELHI

SEPTEMBER 2006

Proteins - Maltodextrin glucosidase
Protein chemistry - Molecular chaperone
Protein misfolding

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.

Tapan Kumar Chaudhuri
Dr. Tapan K. Chaudhuri

Saroj Mishra
Prof. Saroj Mishra

ACKNOWLEDGEMENT

A journey is easier when you travel together. Interdependence is certainly more valuable than independence. This thesis is the result of three years of work whereby I have been accompanied and supported by many people. It is a pleasant aspect that I have now the opportunity to express my gratitude for all of them. This thesis has been for a long time work in progress. An important reason why it ceased to be so is that, during the years, I have received a lot of help from many people – probably more than those to whom I have space to thank here. To them I owe much of what I know about care as well as the ability to express it.

My supervisor, Dr. Tapan K. Chaudhuri, has carefully read, over the years, many drafts of this thesis. I am grateful for his comments – often unique and thus even more valuable – encouragement and thoughts for further development of my ideas. But I also owe to him the ambition to put my arguments in a wider perspective and to relate them to work in moral and political philosophy of which I would have otherwise remained ignorant. Prof. Saroj Mishra, who has co-supervised my Ph.D. thesis on the same topic, has kindly read various parts of my work and has given me valuable praise and criticism.

I would like to thank Prof. V. S. Bisaria, Dr. Prashant Mishra and Dr. Nalin Pant who monitored my work and took effort in reading and providing me with valuable comments on earlier versions of this thesis. I am thankful to all other faculty members for their help and support. I would also like to thank Neera mam for helping me finding many important books and journals whenever required. I would also like to thank the office staff, Mrs. Meena Mathur, Mr. D.S. Tarzan, Mrs. Sunita Verma, Babulalji and Mr. Rajeev for their kind cooperation.

I am really grateful to my labmate Parul who was another Ph.D. student in my laboratory. I had the pleasure to work with several students who did their MTP project in our Structural Biology Laboratory Chanpreet, Pravin, Nidhi, Asif, Ruchi, Shekhar, Saurabh, Nishtha, Pragya and project students Unnati, Bhawna, Jhyoti, Mridula, Sashikala. I thank all of them for always cheering me up.

The invaluable help extended by Aswini on various stages of my Ph.D. made him a very good friend. I am also indebted to Anjali, Anand, Ashish, Asif, Bhawna, Alok, Raju, Rupali, Usha, Mili, Shipi and Shilpa for their untiring help during my stay at IIT Delhi. I received good cooperation from my juniors Roohi, Ziauddin, Ramesh and Mukesh.

I would like to thank Mr. V. K. Ghosh for teaching me few important microbiological techniques when I was joined merely a fresh Research Scholar in this department. I would like to thank Kishanji for helping me finding the right things during experiments and Ram Gopal for his willingness for cleaning up the lab instruments everyday. I would like to thank Mukeshji, Ranaji, Yadavji, Bagbhan Singhji and Swapan Patra for helping me whenever required.

Finally, there are a few people who have constantly been on my side throughout the often-disquieting moments of planning and writing this thesis. It has been the greatest blessing ever to have my parents and my brother Soumen who always stood behind me and motivated me. I am greatly indebted to them for their constant encouragement and emotional support provided throughout my Ph.D. program. My beloved wife, Atasi, has unconditionally given me her trust and encouragement and has proofread every single page of my work, never letting me down. Without their support, I may never have found the minimum energy and balance necessary to see my thesis to an end.

At last a million thanks to the grace of almighty God and all of my well-wishers.

Subhankar Paul.

Subhankar

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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