SOLUTION TO MEMBRANE CONFORMATIONAL CHANGE OF BCL-$X_L\Delta$TM

by

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A dissertation submitted to Johns Hopkins University in conformity with the requirements for the degree of Doctor of Philosophy

Baltimore, Maryland
October, 2005

Abstract

The central question that this thesis project addresses is the pH-dependent solution-to-membrane conformational change of Bcl-X_LΔTM *in vitro*. Evidence exists for both solution and membrane-inserted conformations playing important roles in mediating the pro-survival activity of Bcl-X_L, a Bcl-2 family protein. Our hypotheses were that acidic pH conditions mediate the solution-to-membrane conformational change (i) by destabilizing the soluble conformation and form a "molten-globule" intermediate that favorably inserts into membranes and/or (ii) by favoring the oligomerization of the solution conformation resulting in the insertion of Bcl-X_L into the membrane and/or (iii) by altering the electrostatic surface of the profile and enhancing an electrostatic attraction between the protein and the surface of the membrane.

Biophysical characterization under neutral and acidic pH conditions in solution indicated that there were no significant changes in the secondary, tertiary or quarternary structure of the protein suggesting that there is no formation of an "acid-induced molten-globule" or an "acid-induced oligomeric" state. However, we observed interesting changes in the dynamics of the C-terminal end of the protein suggesting a role for protein dynamics in mediating this conformational change. The requirement for anionic lipids in the membrane suggested that pH-modulated electrostatic interactions between the protein and membrane mediated the conformational change and was confirmed by a salt-dependence study. Though there were no significant positively charged surfaces on the protein that could interact electrostatically with the membrane surface, we identified a surface on the protein capable of an electrostatic interaction with the membrane. Relative

to wild-type, a mutant, E153Q/D156N, showed altered pH-dependence of binding to lipid vesicles, altered membrane insertion properties and an enhanced ability to inhibit Bax-induced release of dextran from lipid vesicles indicating that the membrane-inserted form might play a critical role in mediating the pro-survival activity of Bcl-X_L. The protonation of histidines and the presence of Ca^{2+} were shown not to play a significant role in the conformational change. These findings have led to the development of a simple thermodynamic model coupling protonation of ionizable groups to a partitioning into the membrane that explains this pH dependence.

Thesis Advisor and Primary Reader: Dr. Blake Hill

Secondary Reader: Dr. Bertrand Garcia-Moreno

Thesis committee members: Dr. David Shortle, Dr. Joel Tolman

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Acknowledgements

Besides dedicating the thesis to my parents, I want to acknowledge them from the bottom of my heart for making me what I am. They have played the most important role so far in my life and I want to thank them over and over again. My mother for being the constant source of strength and inspiration instilling in me the courage to plow ahead in the face of difficulties. And my father, for being my friend, philosopher and guide, giving me the freedom to become my own.

As the Tamil dedication usually goes "Maadhaa, Pidhaa, Guru, Theivam" translating to "Mother, Father, Teacher and then God", I want to now thank the efforts of all my teachers and professors throughout my academic life. Every one of them played their part in molding my intelligence and creativity. However, some of them have played an integral part in shaping my personality and enthusiasm. A special mention has to be made for Blake since he has been a mentor to me in many ways than one. First of all, for the freedom that he gave me, to design and execute my thesis research project, which has helped me mature as a scientist. Secondly, for teaching me how scientific research works, mentoring me on life as a graduate student and beyond, and instilling in me the confidence for a brighter future. Besides Blake, the members of my thesis committee, Dr. Bertrand Garcia-Moreno, Dr. David Shortle and Dr. Joel Tolman have made immense contributions to the direction and success of my project. Thank you for having been very supportive of my attempting to tackle a difficult question, for constantly encouraging me and for consistently challenging me to take the right course and make the best decisions.

Dr. Lenny Brand and Dr. Dima Toptygin have been instrumental in my studies using fluorescence spectroscopy and have always lent a valuable helping hand whenever I needed one. Dr. Ernesto Freire and Victoria Kholodenko are acknowledged for their help with the calorimetry experiments. Dr. Adrian Velazquez-Campoy deserves special mention for his support and advice regarding calorimetry experiments and teaching me a lot about thermodynamics. Dr. Himat Patel and Dr. Mike Rodgers helped me with the analytical ultracentrifugation experiments and their advice has been instrumental with my data interpretation. I have to say special thanks to Dr. Michael Edidin, Dr. Ernesto Freire and Dr. Sarah Woodson for the wonderful research environment they provided when I rotated through their labs and those experiences though short will be cherished forever. I also wish to thank Dr. Mark van Doren, Dr. Maurice Bessman and Dr. Doug Koshland for their show of support for my research project. And special thanks to Dr. Ananya Majumdar for being a wonderful mentor, always ready to help and for being a wonderful teacher, teaching me all about NMR. And it takes a special person to be your mentor and your friend, and thanks Ananya for being both.

I want to take this moment to thank all the members of the department who I interacted with and especially all members, past and present, of the Hill lab for making this stay a wonderfully memorable one. Especially, Salva and Fred, who I spent the most time talking to, for being wonderful buddies and for teaching me a number of things and bringing in unique perspectives. For all the pointless arguments and the ego clashes, here is to them... Salud! I hope the arguments don't stop here... And thanks Salva for teaching me how to say, "Tres billetes, co**, por favor!"

I wish to thank the other members of the “current” lab (or as I remember it), Lora, Robbie, Jon, Ann, Sarah, Eddie and Jeffrey (the sophisticated version), for bringing in a wonderful, friendly environment and a fresh perspective on collaborative research. Of all previous members, I would like to acknowledge Michelle Dallapiazza (soon to be Dr.) for being a good friend and for the support she provided during my troubled days. Oh! and for the insights into Mali... thank you!

The members of my class, Jed, Abby, Tony, Sarah (W and B), Matt, Michelle, Steve, Ky, Julie, Diane and Jay for being a wonderful bunch. I still can’t forget the good time we had putting up a skit during our second year retreat. I should say special thanks to Jed for being a wonderful buddy, and for proving to me that completely different people could be really good friends. Well, we certainly do have some things in common and I should thank him (and Michelle D.) for introducing me to the pleasures of “middle-distance running”, and for running with me (or for me) these past five years of the Baltimore marathon relay.

Now, I have to thank two important people who I remain forever indebted to for keeping me sane during the insanity that prevailed for a while here in Baltimore, Prashanth and Kate. Prashanth, for affecting my life more than I affected his (although he might disagree), for being a great human being, and for surprising me again and again (not really, I am not surprised by surprises anymore ‘coz I started expecting them from you, Prashanth). And Kate, especially after Prashanth left Baltimore. Thanks for taking care of me, little sister. Not surprising, but most of what I said for Prashanth, applies to Kate. To both of you, thanks for being you, because you are wonderful the way you are.

My friends from college and high school, Sriram, Vipra, Senthil, Sid, Saravanan and Nishanth from Anna University, Srihari, Kota, Koka, Ramya, Dan and Jen from UMN and Kannan, Sathish, Deepak and Prem from Anna Nagar, you guys for sticking with me, for trusting in me and for encouraging me all the way through. Most of all, believing in me and letting me be the person I am. And the Silver Spring crew, my brother (for teaching me many a million things and for being a good friend), Vijay and Shashi for being my family here, helping me get through the tough times. Thank you, words can't say any more...

Before I thank God, I should acknowledge all my friends whose names I haven't mentioned for their roles in shaping me into who I am. And for the people in my family who have constantly believed in me and wished well for my future, thanks to all of you.

I want to finally thank the Almighty, who I still don't understand, nor wish to, for placing me in the midst of these wonderful people and for making things possible.

Guru Thuduppathy.

October 2005.

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S u ± A û] Y Ú d Ï m"

G u ß m A u × P u ,

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Preface

Cell death – a genetically programmed pathway – why, when and how?

Albert Einstein believed that the universe could be explained in simple elegant terms. His theories which have been revolutionary and have propelled the progress of modern scientific thought resulted from his belief that the universe in itself is beautiful, elegant and simple. Life, in the biological realm, is also beautifully constructed at the morphological level. One often marvels at the diversity in morphology of life that we see around us, from the icosahedral viral morphology (whether viruses qualify as life still remains controversial) to the spiral morphology of the bacterial pathogen, *T. pallidum*, to more complicated forms of life, the five pointed star fish, plants and the human body. Even at the molecular level, despite the chaotic sense that is portrayed, there is an underlying harmony that is carefully built and controlled. The elucidation of how this organized form was built, block by block over the very long time scale of evolution of life would be a fantastic journey to undertake. Somewhere along this journey, we come across the very significant (at least to us humans) step of the origins of multicellularity. For the world of single celled organisms, multicellularity was a very significant step, progressing towards existence as a community rather than as an individual, to an extent that any individuality is overwhelmed in the overbearing importance of the community.

Why did multicellularity evolve? Simply put, what was so unique about multicellular organisms and what kind of advantage did they provide over single celled versions in certain niches (single celled organisms still dominate most of the planet at least in numbers) that they came to stay? One possible answer would be that there was

an advantage for the synergy between the individuals each of which had a specialized function they were tailored for. The assembly line of individual cells and tissues which kept the entire organism alive was simply designed well enough to function in a very streamlined way and any of the broken down versions were quickly removed from the population and the best designs were selected for. Also with multicellularity came the problem of what happens to a runaway rogue cell in the whole organism.

In an organism like ours built from 10-100 trillion cells, the probability of some cells turning over to the "dark side" is very possible and an elimination of these organisms as a whole from the population would be an inefficient way of coping with the event. However, a mechanism that would coax the "bad" cells to commit suicide would prove beneficial for the population. Evolutionarily, the design of these "bad" cells committing suicide was pretty successful that it has been coded in almost all metazoans as a genetic program that could control cell survival. Although, a genetically programmed cell death pathway can be reasoned as resulting from multicellularity, whether such a process is fundamentally limited to only a multicellular organism or is present in some unicellular species as a means to control a community of individual cells is still under debate. The attempts to reveal relics of a programmed cell death pathway in the unicellular eukaryote, *S. cerevisiae* have proved to be unsuccessful though its presence still remains controversial. However, programmed cell death has been described in unicellular organisms like the kinetoplastid parasites *Trypanosoma cruzi* and the free living slime mold *Dictyostelium discoideum* that arose between 1 and 2 billion years ago and much before the evolution of the yeasts. Moving beyond the evolutionary

standpoint, the organization of a genetically controlled programmed cell death pathway would be a fascinating work of art in itself.

How would one go about building a genetically controlled pathway for cell death?

Integrating the cell death pathway to cellular processes that could sense stress or damage would be a good way to design it. In fact, stress signals like DNA damage, oxidative species and specific death ligands, nutrient deprivation, addition of cell damaging drugs etc. all seem to cause an induction of cell death. In a setup where the cell death pathway is integrated into the network of cellular homeostasis pathways, a perturbation in homeostasis could be sensed by the cell death pathway and a response could be made to maintain the healthy state of the organism as a whole. This gives rise to the hypothesis that some if not all of the proteins involved in the regulation of cell death have functions other than in cell death. It has been observed that a key regulatory step in promoting cell death in mammals is the release of cytochrome c into the cytosol. However, cytochrome c is known to be essential for carrying out oxidative phosphorylation. Cytochrome c is an integral part of the electron transport chain that eventually helps create the proton gradient and thereby drives ATP production not only to maintain cellular homeostasis but also to drive growth and cell division. Linking the energy generation processes at the mitochondria with programmed cell death, one could think of cell death as a process that regulates cellular homeostasis.

This has been an evolutionary perspective on the origins, relevance and importance of programmed cell death. I believe that this perspective sets the stage for the relevance of studying programmed cell death in detail. In my thesis project, I have

attempted to improve the understanding of the biophysical forces involved in the regulation of the process of programmed cell death.

Table of contents

List of Tables

List of Figures