

RICE UNIVERSITY

Crystallographic and Computational Studies of the Metal Ion Binding
Properties of Parvalbumin

by

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A THESIS SUBMITTED
IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE

Doctor of Philosophy

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April, 2000

Abstract

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An astonishing number of important physiological processes are regulated by the small alkaline earth metal, calcium. Regulatory Ca^{2+} -binding proteins must be able to distinguish Ca^{2+} ions in the presence of greater concentrations of other metal cations, such as Mg^{2+} , Na^+ and K^+ . The EF-hand family is a large class of Ca^{2+} -binding proteins that displays this sort of preferential Ca^{2+} -binding. The secondary and tertiary structure of the EF-hand metal ion binding site is highly conserved from one member of the family to the next. Because of this conservation, we can use the small, amenable, EF-hand protein, parvalbumin, as a model system to study the mechanisms that define the metal ion affinities and specificities of EF-hand Ca^{2+} -binding sites in general.

Our collaborator, Dr. James Potter, has designed a mutant to test directly the role of the last coordinating residue in the EF-hand binding site, the PVEF-E101D parvalbumin mutant. The crystal structures of both the Ca^{2+} - and Mg^{2+} -bound complexes of PVEF-E101D have been determined. The PVEF-E101D mutant displayed a 100-fold decrease in the binding affinity for Ca^{2+} , and the Mg^{2+} -binding affinity was increased 10-fold. Moreover, the Ca^{2+} off-rate escalated from 1 s^{-1} in wild-type parvalbumin to 600 s^{-1} in the PVEF-E101D mutant. The conformation of the

mutated EF-hand in the PVEF-E101D/Mg²⁺ structure was typical of a Mg²⁺-bound EF-hand, with the exception of an F helix movement of ~1 Å toward the bound cation that allowed the shorter aspartate residue to coordinate the Mg²⁺ ion. The PVEF-E101D/Ca²⁺ structure showed that the aspartate residue is unable to bind Ca²⁺ in the bidentate mode normally adopted by the wild type glutamate. The resulting sixfold Ca²⁺ coordination in the mutant is usually characteristic of Mg²⁺-bound EF-hands, and this finding indicates that the binding loop is not sufficiently flexible to allow the aspartate residue to move in far enough to offer bidentate ligation of the Ca²⁺ ion.

Two MD simulations were used to further investigate the relationship between the last coordinating residue of the EF-hand binding loop and the overall plasticity and flexibility of the loop region. The first simulation, called Alchemy, simulated the transition from Ca²⁺ to Mg²⁺ coordination through varying the van der Waals parameters for the bound metal ions. The glutamate at position 12 was accurately and reversibly predicted to be the source of bidentate ligation of Ca²⁺ in our simulations. A second simulation, the Aspartate simulation, produced results that correlated well with the experimental result that an E101D substitution at EF loop position 12 resulted in monodentate Ca²⁺ coordination. The F helix was able to move in to the binding cavity during the simulation to allow one aspartate oxygen to bind the Ca²⁺ ion, but the aspartate was unable to achieve a favorable orientation for bidentate Ca²⁺ coordination. The findings indicate that the interplay between the last coordinating residue of the loop, and the plasticity, or flexibility, of the binding loop, to a great extent determines the species of cations that are allowed to bind in a particular EF-hand site.

Acknowledgements

I owe many thanks to my advisor, Dr. George Phillips, for providing me with a project that was extraordinarily well suited to my talents and interests. I have also appreciated his receptiveness to my ideas and thoughts, and his benevolence regarding my pursuit of tangential avenues, that were sometimes scientifically productive, and sometimes not, but I have always valued that I was given the opportunity to pursue them. The members of my thesis committee, Dr. Ed Nikonowicz, Dr. Kathy Matthews, Dr. Seiichi Matsuda, and Dr. Gustavo Scuseria have offered many helpful suggestions, for which I have also been grateful. Additionally, I wish to thank Dr. John Olson, who has always been both supportive and available, even though he was not a member of my committee.

This body of work has been a collaborative effort, and I would like to thank each of my collaborators. First, I owe a great debt of gratitude to Miguel Teodoro, who has collaborated with me on all of the NAMD molecular dynamics simulations. He has been very gracious about sharing his hard-won knowledge of the NAMD program with me, and has thereby made the learning process a gentler one for me. Also, I want to thank Andrew Daniels and Dr. Gustavo Scuseria for introducing me to the Gaussian software and for their contribution and cooperation in the computational aspect of my graduate studies. Dr. James D. Potter, our collaborator from the University of Miami Medical School, designed the parvalbumin mutants contained in this study, and Dr. Qi Li in the Potter Laboratory expressed the mutant protein and determined the binding

properties. Finally, Mike Berry and Emai Ho, who worked on this project before I joined the lab, determined the structure of the F102W/Ca²⁺ mutant complex, and performed all but the final few rounds of refinement on the PVEF-E101D/Mg²⁺ complex.

I have very much appreciated the support and friendship of my fellow graduate students during my time at Rice University. In particular, I want to mention Nicole Magnasco and Dave Maillett, who have both been willing to read more parvalbumin papers and hear more parvalbumin presentations than anyone should be expected to endure. Also, I will always remember Eric DeJong and Elaine Liong for having survived in the trenches together. Many thanks to Diane Wycuff and Nicole for walking me through my first protein expression, and to Tod Romo for being the infinite source of information and assistance on both graphics rendering and computational issues. I would also like to express special appreciation for Wil Radding, who possesses an unbelievable store of scientific information and is always willing to patiently discuss it with a novice.

Finally, and most importantly, I would like to thank my family for their support and encouragement. My parents and my brother have always expressed pride and enthusiasm for my pursuit of higher education. My children, Catherine and John, have tolerated having a graduate student for a mother admirably; they are immense sources of joy in my life. Even so, the person without whom I could not have done this is my husband, John. Sometimes people have asked me how I have managed graduate school

with a husband and children at home, but I wonder how I could have possibly managed it without them.

This body of work would not have been possible without financial support from the Keck Center for Computational Biology, National Library of Medicine Medical Informatics Training Grant No. 1T15LM07093, from the Robert A. Welch Foundation grant C-1142, and also from the NIH.

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