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One Milimolar Is One Micromole per Milliliter, Which Is . . .

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Beckman Scholar 2003-04

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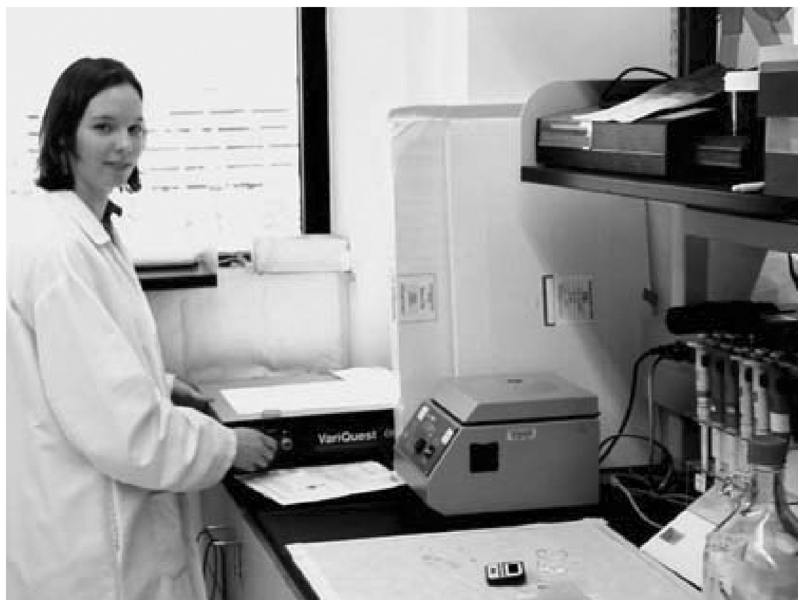

Background

"One milimolar is one micromole per milliliter, which is one nmole per microliter… if you take the K_m of AdoMet for LSMT and you are in a pre-steady state system…"

When I began research in Dr. Robert Houtz's lab in January, 2002, I heard a lot of this… very quick, concise information… but to me, mumbling that didn't quite make sense where some words rang a bell. I felt that my year and half of college training was quite insufficient for successful discernment of Dr. Houtz's instructions and suggestions. However, after acquiring explicit *written* directions of what he was trying to communicate and a little trial and error, I began to understand and flourish with scientific research.

Up to that point academically, I was one of numerous undecided college students. I had started in the Biosystems Engineering program at UK, but found this to be unaligned with my interests. Also, I knew I wasn't "pre-med/vet/dental/pharmacy/P.A./P.T., etc.," which almost everyone else who studies biological sciences seems to be. However, after being fully immersed in my research and experiencing the joy of a successful, data-producing experiment, I decided that a Ph.D. followed by a teaching/research position at a large university might be the right path.

My first significant contribution to my lab's work was in the summer of 2002. Up to that time I was learning basic skills and deciding what type of project I wanted to devote myself to. The enzyme that I work with, Rubisco Large Subunit Methyltransferase (LSMT), had been classified as containing a newly identified protein fold, the SET domain. This fold is ubiquitous to almost all organisms and is of great interest, because it is found in some proteins that influence gene silencing, chromatin formation, and certain cancers. Consequently, National Institutes of Health crystallographer, Dr. Jim Hurley, became interested in our enzyme and solved its 3-D structure. I was then able to learn first hand the difficulty and the beauty of enzyme kinetics. The lab's efforts turned into a publication in the prestigious journal *Cell,* with two other crystal structures of SET domain containing proteins in the issue. A précis of the three articles clearly stated that our paper was the best (and made the cover!) due to the resolution of the structure and the extensive functional data.

There are two types of people as far as enzyme kinetics are concerned: those who understand them and those who don't. The learning curve is steep, mainly because after fully understanding kinetic constants, which are usually denoted by k and some subscript (k_{cat}, k_m, k_a, k_d, K_D, k_{ia}, k_i, etc.), you comprehend the majority of enzyme kinetics. Second, you have to learn how to interpret the complex data and be able to know when the values you obtain are reasonable or are not, a skill that develops with time. I am proud to say that I have become proficient in enzyme kinetics, which has not only trained me to be very precise, but has also helped make me become a better experimentalist.

Undergraduate research is extremely rewarding. Not only do I know a little bit about enzyme kinetics, I have been published, and have become well versed in other areas of experimental biology. I appreciate the evolution I have made as a student due to undergraduate research — now I am more analytical, critical, and capable of designing and implementing independent thought. I feel that I have gotten the most out of my education and have truly prepared myself for the next step — graduate school at the University of California at Berkeley.

Now, new students in the lab look at me in a similar, puzzled way as I did to Dr. Houtz two years ago. I take great pride in being a legitimate source of information and a person who can analyze data and teach certain skills. Furthermore, I feel lucky and humble to be on a career path that I feel confident about and drawn to; as well, I am grateful for all of the help I have received along the way.

Being awarded the Beckman scholarship has been a critical part of my growth intellectually and scientifically while an undergraduate. Receiving such a prestigious award serves as an affirmation of my abilities and a source of encouragement to achieve more, and is a great reward

for all of my efforts. Furthermore, at the Beckman Symposium in the summer, 2003, I interacted with incredibly talented scientists. This provided another source of inspiration to become more proficient myself.

Although research may seem esoteric and narrow to some, all contributions of information are used to solve larger problems. I am pleased to take this realization away and to have developed into a good scientist during my undergraduate career.

My Research

Protein methylase III enzymes are a unique class of post-translational processing enzymes responsible for the mono-, di-, or tri-methylation of the e-amino groups of internal lysyl residues in a diverse group of proteins. Many S-adenosyl-L-methionine dependent protein methyltransferases contain a highly evolutionary conserved secondary structural domain composed of 110 amino acids known as the SET (Suppressor of variagation 3-9, Enhancer of zeste, Trithorax) domain. During my time as a Beckman scholar, I have investigated and largely characterized specific functions of Rubisco LSMT, a member of this group, using structure as a guide.

Rubisco LSMT: investigation of successive methyl transfers

First, I have focused on the trimethylation by Rubisco LSMT on Lysine 14 of large subunits. Kinetic analysis of LSMT reveals characteristics of a ping-pong reaction mechanism; however, similar analysis of SET7/9, a histone methyltransferase that catalyzes the mono-methylation of histone H3 Lys-4, is random order. Because the disassociation constant (K_D) for the Rubisco LSMT complex is approximately 50,000 times lower than the Michaelis constant (K_M) of the enzyme for Rubisco, the kinetic plots may be distorted from the actual reaction mechanism.

Alternatively, the bi-polar arrangement of substrate binding sites in the SET domain are geometrically ideal for successive methyltransfers without disassociation of the protein substrate, and this phenomenon could be responsible for the appearance of a ping-pong like reaction mechanism. A SET7/9 mutant, Y305F, performs both mono- and di-methylation of histone H3, and its kinetics are altered in a manner that resembles the distorted plots of the reaction mechanism of LSMT. The similarity in the kinetic plots of LSMT and the SET7/9 Y305F mutant suggests that each enzyme obeys the same reaction mechanism and that the protein substrate remains bound during consecutive methyltransfers.

Through product analysis, I have found that Rubisco LSMT is a processive enzyme. That is, three methyl transfers occur before the enzyme dissociates from the protein substrate Rubsico. This finding corroborates structural data that would suggest a successive mechanism of methylation.

Rubisco LSMT: a structurally unique enzyme

Rubisco LSMT is the only SET domain containing protein with a structurally unique C-terminal domain, while the N-terminal domain of the enzyme contains the functional SET motif. Thus, to examine the role that the C-terminal domain plays in catalysis as well as substrate binding, I have expressed truncated clones of the LSMT enzyme. These attempts may help determine if the C-terminal lobe of LSMT has a structural or biochemical importance.

Based on models mimicking the binding between Rubisco LSMT and Rubisco, the C-terminal domain may contribute to the specificity and interaction between these two proteins. Also, expression of N-terminally truncated forms of Rubisco and subsequent binding analysis using ELISA suggest that the C-terminal domain can interact with Rubisco in the absence of the N-terminal catalytic SET domain. I am investigating homologous Open Reading Frames (ORFs) from humans and mice for analysis of protein methyltransferase activity and the role, if any, that the C-terminal domain assumes in these proteins. The function of the C-termini in the interaction of Rubisco and Rubisco LSMT may have important implications for other protein methyltransferases given that this sequence is found with 80% consensus in *Neurospora crassa*, *Drosophila melongaster*, *Bos Taurus*, and *Sus scrofa*.

Conclusion

My work in the Houtz lab has helped reveal more functional/mechanistic data about Rubisco LSMT, a SET domain-containing protein methyltransferase. I have found that it is a processive enzyme, which helps explain its distorted reaction mechanism kinetics. Furthermore, I have explored the structurally unique C-terminal domain of the enzyme and found that it is essential for binding to the protein substrate Rubsico and truncated forms of the enzyme possess no enzymatic activity. This information is of great significance because it reveals more information about a class of proteins with SET domains. SET domain-containing proteins are found in many organisms, including human histone methyltransferases that are linked to human cancers, chromatin compaction, and gene silencing.