PEW RESEARCH PROPOSAL

Project title:

Investigating the role of a salt bridge in PRMT1 dimerization and enzymatic activity.

Submitted by
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I. Statement of the end product(s)

This research will be presented by Chemistry and/or Biochemistry students at the annual American Chemical Society meeting in 2023.These results could be published in peer-reviewed scientific journals. Additionally, this research would help me advance my scientific career at Union University.

II. Statement of the scholarly activity.

My research project focuses on proteins that catalyze the modification of other proteins via methylation called Protein Arginine Methyltransferases or PRMTs. Proteins are large, complex molecules that play a key role in virtually all of the cell's operations. Arginine methylation is an important modification of proteins, involved in many cellular processes like cell signaling, cell division, and even viral replication. In recent years, the significance of PRMTs in human diseases has been increasingly studied, especially in cancer and cardiovascular disease. Although the importance of these enzymes is recognized, the understanding of exactly how PRMTs function is still limited. My aim is to better understand the mechanisms by which these functions and to elucidate how they are regulated. By studying the crystal structure of PRMT1, the major isoform of PRMTs in humans, we have found two oppositely charged amino acids that seem to be interacting and might play a role in stabilizing the active conformation of the protein, and I would like to test this hypothesis. This knowledge is crucial for the design of new drugs that would help us target these proteins in the diseases they are involved in. This grant would help me purchase some of the expensive equipment needed for the mutation and purification of these enzymes.

III. Goals of the research project:

- Perform site-directed mutagenesis to modify PRMT1 at R353 and D37 to amino acids that would change their native charges.
- Express and purify the protein variants.
- Perform methyltransferase assays of the purified protein variants to assay if the activity has been altered.
- Run the protein samples on native gels and size exclusion chromatography to determine its oligomeric state.

IV. Background and literature review.

The post-translational modification of proteins is a tool of signal transduction used by cells to react to changes or events in their environment. These marks expand the structural and functional diversity of the proteome. One such post-translational modification, methylation, can occur on amino acids such as lysine, arginine, histidine, or proline, and has also been found on carboxy groups (1). Arginine methylation is a common post-translational modification which functions as an epigenetic regulator of transcription and plays key roles in mRNA processing, DNA damage repair, mRNA translation, cell signaling, and cell fate decision (2-4). This modification is carried out by the family members of the protein arginine methyltransferases (PRMTs). The PRMTs transfer a methyl group from the donor molecule S-adenosyl-L-methionine (AdoMet) to the basic amino acid arginine in the substrate protein. The substrate arginine residue can be methylated in three distinct ways on the guanidino group, forming three different products, according to which the PRMTs can be classified into three types. Type I, type II and type III enzymes all catalyze the formation of monomethyl arginine (MMA). Type I PRMTs (PRMT1, 2, 3, 4, 6 and 8) further catalyze the production of asymmetric dimethylarginine (ADMA), and type II PRMTs (PRMT5, PRMT9) further catalyze the formation of symmetric dimethylarginine (SDMA) (5). PRMT7 is only capable of forming MMA, and it is the only known type III PRMT (6-9). The main result of arginine methylation is the alteration of protein-protein or protein-nucleic acid interactions. This alteration arises primarily from changes in hydrogen bonding to the methylated arginine residue (10).

PRMT1 was the first mammalian protein arginine methyltransferase to be cloned and characterized (11). This enzyme is responsible for the majority (about 85%) of total protein arginine methylation activity in mammals (12). PRMT1 methylates histone H4 at arginine 3, depositing an ADMA mark, activating transcription and thus contributing to the histone code (13). In prostate cancer, the dimethylation of histone H4R3 catalyzed by PRMT1 has been associated with transcriptional activation and positively correlates with prostate tumor grade, which is associated with increased abnormality and an increased likelihood of tumor growth. As a consequence, this mark can be used to predict the risk of prostate cancer recurrence in patients (14). Additionally, when H4 arginine 3 is asymmetrically dimethylated, the methylarginine recognizing molecule Tudor domain-containing protein (TDRD3) interacts with this mark. TDRD3 has been identified as one of the candidate genes for a scoring system of breast cancer as higher expression of this gene is linked to lower survival rates of patients with this condition (15,16).

It has been shown that a null allele of PRMT1 in mice leads to embryonic lethality. This loss leads to spontaneous DNA damage, cell cycle progression delay, checkpoint defects, aneuploidy, and polyploidy. These data show that PRMT1 is required for genome integrity and cell proliferation, and that arginine methylation is a key posttranslational modification in the DNA damage response pathway in proliferating mammalian cells (17). Endothelium-derived nitric oxide (NO) is a potent vasodilator that plays a critical role in maintaining vascular homeostasis. Altered NO biosynthesis has been implicated in cardiovascular disease (18). Evidence from animal models and clinical studies suggests that asymmetric dimethylarginine (ADMA) and monomethyl arginine (MMA) are nitric oxide synthase inhibitors. These products contribute to reduce NO generation and to disease pathogenesis (19). In pathological conditions such as pulmonary hypertension, coronary artery disease, diabetes and

hypertension, plasma ADMA levels have been shown to increase above average concentrations (20-23). Moreover, it has been shown that ADMA levels are a good prediction of cardiovascular mortality in patients who have coronary heart disease (24). PRMT1 plays a critical role in these cases because it is the primary enzyme in the formation of ADMA-containing proteins. Therefore, understanding all of the intrinsic and extrinsic factors that contribute to the regulation of PRMT1 activity and the production of ADMA by PRMT1 is crucial.

The significant role that protein arginine methyltransferases play in biological pathways and diseases has made the PRMTs prominent targets for the development of inhibitors that could serve as therapeutic agents. This surge towards inhibitor design (10,25) has reinforced the need for a complete understanding of the specific mechanisms by which the different PRMTs operate. Importantly, proteins harboring each of the modified arginines can be biologically distinct (26,27). Another layer of complexity exists when a substrate has more than one target arginine, which could lead to a combinatorial set of modified proteins. Thus, characterizing the kinetic mechanism of the PRMTs and deciphering how the final methylation state of the substrate arginine is determined, are crucial in order to fully understand how this family of proteins functions and can be controlled or inhibited.

In a study intending to provide knowledge about amino acid residues that are key for PRMT1 activity, the Hevel lab at Utah State University previously reported the automethylation characteristics of the M48L PRMT1 mutant (*15*). After this study, it was hypothesized that Arg-353 was the site of automethylation. To confirm these findings, the M48L-R353K PRMT1 variant was generated. The enzymatic activity of this variant was first tested with a peptide substrate, and showed lower methylation rates compared to M48L. Automethylation assays were also performed and the methyltransferase activity was greatly reduced indicating that Arg-353 was likely the automethylation site for M48L-PRMT1.

Because the overall methylation rates were decreased in the double variant M48LR353K when compared to the single M48L variant, it was hypothesized that residue R353 was also important for PRMT1 catalysis. To test this hypothesis a single a single mutant R353K-PRMT1 was also generated. This mutation severely affected the activity of PRMT1, as seen by the significantly decreased methylation rate.

Later, Dr. Orlando Acevedo a computational chemist at the University of Miami modeled the N-terminus helix, which is not seen in the PRMT1 structure (PDB 1OR8), using the PRMT3 structure as a guide. Using this modeled structure, the observation was made that the R353 of one PRMT1 monomer seems to be forming an ionic bond (salt bridge) with D37 of another monomer .We hypothesized that the disruption of this bond might be affecting PRMT1 dimerization, which is necessary for activity, therefore, causing the low activity that we see in the R353K mutant(29, 30). To test this both R353 and D37 can be mutated to amino acids that would change their inherent charges. Size-exclusion chromatography and native gel assays can be used to test if the dimerization of the enzyme is affected by changing these charges and if that correlates with the decrease in methyltransferase activity. It is important to highlight that R353 may be one of the first residues identified to control activity outside of the active site, pointing to a novel mechanism of controlling PRMT1 catalysis. A recent study using molecular dynamics to study the communication pathways and allostery between PRMT1 monomers,

suggested that R353 might have an effect in long-range communication. Additionally, this study suggested that this residue may play an important role in the binding of positively charged substrates, which also underscores the potential value of studying this residue (30).

Understanding the key residues that regulate the activity of these proteins can help in the development of new drugs to target those diseases. The funds obtained through this grant would also help me mentor undergraduate research students at Union University who plan to enter health professions and scientific careers. This research will be later presented at the national American Chemical Society conference by students in the Chemistry department.

References:

1. Eden, S., and Cedar, H. (1994) Role of DNA methylation in the regulation of transcription. *Current opinion in genetics & development* **4**, 255-259

2. Auclair, Y., and Richard, S. (2013) The role of arginine methylation in the DNA damage response. *DNA Repair (Amst)* **12**, 459-465

3. Yu, M. C., Bachand, F., McBride, A. E., Komili, S., Casolari, J. M., and Silver, P. A. (2004) Arginine methyltransferase affects interactions and recruitment of mRNA processing and export factors. *Genes Dev* **18**, 2024-2035

4. Cha, B., and Jho, E. H. (2012) Protein arginine methyltransferases (PRMTs) as therapeutic targets. *Expert Opin Ther Targets* **16**, 651-664

5. MT, B. (2007) Arginine methylation at a glance. *Journal of Cell Science* **120**, 4243-4246

6. Miranda T. B., M. M., Frankel A., Clarke S. (2004) PRMT7 is a member of the protein arginine methyltransferase family with a distinct substrate specificity. *Journal of Biological Chemistry* **279**, 22902-22907

7. Lee J. H., C. J. R., Yang Z. H., Mirochnitchenko O., Gunderson S. I., Felix A. M., Herth N., Hoffmann R., Pestka S. (2005) PRMT7, a new protein arginine methyltransferase that synthesizes symmetric dimethylarginine. *Journal of Biological Chemistry* **280**, 3656-3664

8. Zurita-Lopez, C. I., Sandberg, T., Kelly, R., and Clarke, S. G. (2012) Human protein arginine methyltransferase 7 (PRMT7) is a type III enzyme forming omega-NG-monomethylated arginine residues. *J Biol Chem* **287**, 7859-7870

9. Fisk, J. C., Sayegh, J., Zurita-Lopez, C., Menon, S., Presnyak, V., Clarke, S. G., and Read, L. K. (2009) A type III protein arginine methyltransferase from the protozoan parasite Trypanosoma brucei. *J Biol Chem* **284**, 11590-11600

10. Fuhrmann, J., Clancy, K. W., and Thompson, P. R. (2015) Chemical biology of protein arginine modifications in epigenetic regulation. *Chem Rev* **115**, 5413-5461

11. Lin, W. J., Gary, J. D., Yang, M. C., Clarke, S., and Herschman, H. R. (1996) The mammalian immediate-early TIS21 protein and the leukemia-associated BTG1 protein interact with a protein-arginine N-methyltransferase. *J Biol Chem* **271**, 15034-15044

12. Tang, J., Frankel, A., Cook, R. J., Kim, S., Paik, W. K., Williams, K. R., Clarke, S., and Herschman, H. R. (2000) PRMT1 is the predominant type I protein arginine methyltransferase in mammalian cells. *J Biol Chem* **275**, 7723-7730

13. Bedford, M. T. (2007) Arginine methylation at a glance. *J Cell Sci* **120**, 4243-4246

14. Cooper, C. S., and Foster, C. S. (2009) Concepts of epigenetics in prostate cancer development. *Br J Cancer* **100**, 240-245

15. Liu, K., Chen, C., Guo, Y., Lam, R., Bian, C., Xu, C., Zhao, D. Y., Jin, J., MacKenzie, F., Pawson, T., and Min, J. (2010) Structural basis for recognition of arginine methylated Piwi proteins by the extended Tudor domain. Proc Natl Acad Sci U S A 107, 18398-18403

16. Boisvert, F. M., Rhie, A., Richard, S., and Doherty, A. J. (2005) The GAR motif of 53BP1 is arginine methylated by PRMT1 and is necessary for 53BP1 DNA binding activity. Cell Cycle 4, 1834-1841

17. Yu, Z., Chen, T., Hebert, J., Li, E., and Richard, S. (2009) A mouse PRMT1 null allele defines an essential role for arginine methylation in genome maintenance and cell proliferation. Mol Cell Biol 29, 2982-2996

18. Yanzhong Yang, M. T. B. (2013) Protein arginine methyltransferases and cancer. Nature Reviews Cancer 13, 37-50

19. Sakurada, M., Shichiri, M., Imamura, M., Azuma, H., and Hirata, Y. (2008) Nitric oxide upregulates dimethylarginine dimethylaminohydrolase-2 via cyclic GMP induction in endothelial cells. Hypertension 52, 903-909

20. Sim, J. Y. (2010) Nitric oxide and pulmonary hypertension. Korean J Anesthesiol 58, 4-14

21. Maas, R., Wenske, S., Zabel, M., Ventura, R., Schwedhelm, E., Steenpass, A., Klemm, H., Noldus, J., and Boger, R. H. (2005) Elevation of asymmetrical dimethylarginine (ADMA) and coronary artery disease in men with erectile dysfunction. Eur Urol 48, 1004-1011; discussion 1011-1002

22. Yamagishi, S., Ueda, S., Nakamura, K., Matsui, T., and Okuda, S. (2008) Role of asymmetric dimethylarginine (ADMA) in diabetic vascular complications. Curr Pharm Des 14, 2613-2618

23. Achan, V., Broadhead, M., Malaki, M., Whitley, G., Leiper, J., MacAllister, R., and Vallance, P. (2003) Asymmetric dimethylarginine causes hypertension and cardiac dysfunction in humans and is actively metabolized by dimethylarginine dimethylaminohydrolase. Arterioscler Thromb Vasc Biol 23, 1455-1459

24. Sibal, L., Agarwal, S. C., Home, P. D., and Boger, R. H. (2010) The Role of Asymmetric Dimethylarginine (ADMA) in Endothelial Dysfunction and Cardiovascular Disease. Curr Cardiol Rev 6, 82-90
25. Vhuiyan, M., Thomas, D., Hossen, F., and Frankel, A. (2013) Targeting protein arginine N-methyltransferases with peptide-based inhibitors: opportunities and challenges. Future Med Chem 5, 2199-2206
26. Wysocka, J., Allis, C. D., and Coonrod, S. (2006) Histone arginine methylation and its dynamic regulation. Front Biosci 11, 344-355
27. Kirmizis, A., Santos-Rosa, H., Penkett, C. J., Singer, M. A., Green, R. D., and Kouzarides, T. (2009) Distinct transcriptional outputs associated with mono- and dimethylated histone H3 arginine 2. Nat Struct Mol Biol 16, 449-451
28. S. Gui et al., Investigation of the molecular origins of protein-arginine methyltransferase I (PRMT1) product specificity reveals a role for two conserved methionine residues. J Biol Chem 286, 29118-29126 (2011)
29. X. Zhang, X. Cheng, Structure of the predominant protein arginine methyltransferase PRMT1 and analysis of its binding to substrate peptides. Structure 11, 509-520 (2003).
30. R. Zhou et al., Molecular Mechanism underlying PRMT1 Dimerization for SAM Binding and Methylase Activity. J Chem Inf Model 55, 2623-2632 (2015)

V. Relationship between faith, Biochemistry and this research project.

On the first day of Biochemistry, every fall I introduce the class to my students and we go through the syllabus. I explain why we are studying Biochemistry, why is it important to me that they learn the material and concepts, and what I would like for them to remember about the class in 10 years or more. I like to begin the class by reading Genesis 1:28 “And God blessed them. And God said to them, Be fruitful and multiply and fill the earth and subdue it, and have dominion over the fish of the sea and over the birds of the heavens and over every living thing that moves on the earth.” After reading this verse, I explain what Biochemistry is about. Biochemistry is the study of living organisms, and what are the specific molecular features that make us different than non-living matter. Therefore, I tell them, one of the reasons why we study Biochemistry is to fulfill this commandment God gave to humanity back in Genesis. Because in order for us to be able to rule over living organisms, first we need to understand how they were designed to work, and what happens when those molecular systems do not work properly.

As we go through our semester, it is not uncommon for Biochemistry students to feel overwhelmed by the amount of class content, especially when we get to the point of discussing metabolism and all of the different metabolic routes in the cell. The reason for this is because biological pathways are indeed complex and very sophisticated, and there is a lot to learn about them. However, one thing that the students should recognize and I emphasize from day one of our class is that in the midst of complexity and chemical diversity, there is a set of principles that dictates the architecture and operation of those metabolic routes. It is my hope and my task to help my students see that biological systems do, indeed, conform to a simple set of rules. After understanding this I believe it becomes reasonable to think that an intelligent Creator played a role in the origin, history, and design of life.

In addition to that, when performing biochemical research, we spend most of our time performing laboratory procedures designed to measure the activities of biomolecules and biochemical systems. To get our experiments to work properly, we have to carefully design and optimize each test before executing it with exacting precision in the laboratory. Optimizing these assays is not easy. In fact, it could take weeks or even months of painstaking effort to get the experiments to work just right.
As I teach my research students in my lab how to design and optimize biochemical procedures, I would like for them to experience the fact that optimized systems don't just happen by chance, whether they are laboratory experiments, or well-designed cars or computers. Instead, optimization will always result from the mind and efforts of intelligent agents like themselves and therefore serves as a sure indicator that we were created in the image of a great designer.

In this specific project, we are aiming to modify the composition of a carefully designed 353 amino acid enzyme. And I would like for my research students to see how each of those amino acids was put together in its specific place with a purpose. And how modifying just one or two amino acids can completely alter the function that the protein was designed to have.

I believe that both in class and in the lab, Biochemistry provides a unique opportunity to observe the splendor and elegance of life's Chemistry, which should convince anyone that a good and perfect Creator is behind the origin, purpose and meaning of life.

VI. A time frame for the completion and a plan for the dissemination of the project.

Some preliminary work is currently being done and I believe we are well-positioned to test this hypothesis and perform the experiments by the end of 2022. Then the research results can be shared at the ACS national conference during the spring of 2023.

VII. Budget

4,500.00 USD for the purchase of an incubator shaker