Synthesis and Validation of Substrates for PRMT1 using Plate-Based Screening Assay

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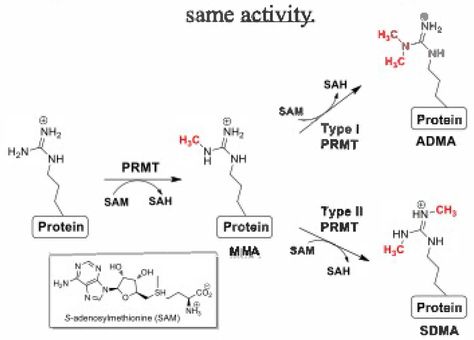
PRMT (Protein Arginine Methyltransferase) is a mammalian enzyme that catalyzes methylation of arginine residues in a polypeptide chain. PRMT is categorized as 3 different types. The methylation can occur as asymmetric dimethylation (ADMA, PRMT 1, 2, 3, 4, 6, and 8), symmetric dimethylation (SDMA, PRMT 7, 5 and 9) or monomethylation (MMA, PRMT 7), Type I, II and III respectively. PRMT1 generates ADMA on arginine residues of the Histone H4 N-terminal tail, which can lead to transcription of cancer-related genes. Alternatively, PRMT5 can modify the same arginine residue to produce SDMA, which represses the development of those same cancer-related genes. A better understanding of the substrate specificity of these enzymes can assist in the development of novel isozyme-specific pharmaceuticals.[1] To identify these differences, we synthesized a 96-well plate of peptides based on the Histone H4 N-terminal tail, screened them against PRMT1 using a screening method previously developed in the Knuckley lab.[2] This medium-throughput screen identified 7 "hit" peptide sequences and consensus sequences based on the "hit" peptides were synthesized by solid-phase peptide synthesis. Each of these consensus sequences varied at the N-terminus, while retaining the more distal positive charges of H4-16 peptide. The peptides were validated using a MTase-Glo™ Methyltransferase Assay to determine if they were indeed substrates for PRMT1. The kinetic values indicate their efficiency as PRMT1 substrates and further investigations are being conducted to identify the differences in the substrate specificity regarding PRMT4 and PRMT5. These continued efforts will help us gain a better understanding of the role PRMT isozymes play in the onset of cancer, while assisting in the design of novel pharmaceuticals to battle this disease.

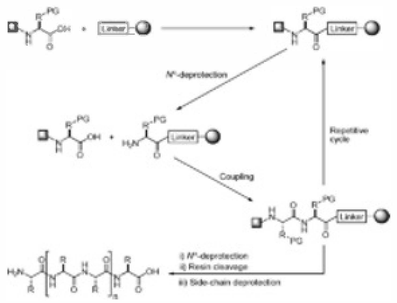
Background

❖ PRMT1 and PRMT5 target arginine residues on histone tails and methylate the guanidino nitrogen on arginine asymmetrically or symmetrically, respectively. Overexpression of PRMT1 is associated with the development of certain cancerous genes in the body while PRMT5 is repressive of that same activity.

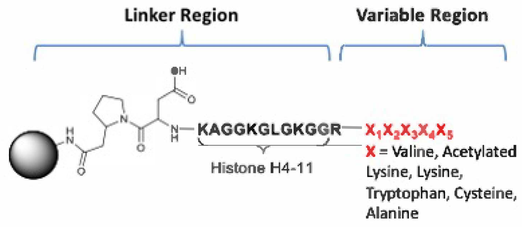


Library Synthesis and Screening

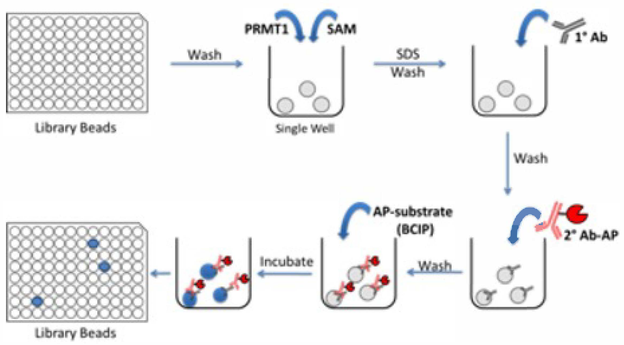
❖ Solid Phase Peptide Synthesis (SPPS) "couples" individual amino acid residues together with the use of coupling agents and the removal of protecting groups.

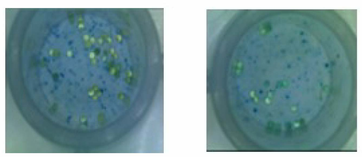


❖ The H4-11-R Linker (KAGGKGLGKGGR) was synthesized via SPPS in a syringe and then the solid phase beads were divided evenly into a 96 well plate for synthesis of a variable region of five residues, creating 96 different peptides.



❖ The 96 well plate was then screened with PRMT1 and a series of antibodies. When a peptide from the well was a PRMT1 substrate, the antibody wash would leave a blue residue so that potential substrate "hits" could be identified.





(a) Non-Hit (b) Hit (Blue)
Images of wells beneath a microscope to identify "hits"

Results

❖ After screening against PRMT1, there were 7 "hit" compounds.

Hits	
Well Location	**Sequence N to C**
B7	AMKVV
D5	VKVCM
C6	KAMKK
F8	MKCKV
G2	MCCVA
G7	MWWAA
C1	MKVAA

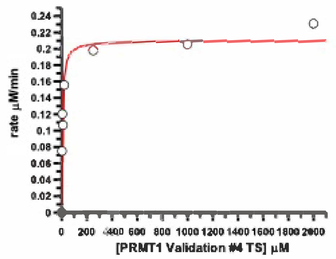
V=Valine **A**=Alanine **K**=Lysine **M**=Acetylated Lysine **C**=Cysteine **W**=Tryptophan

❖ Consensus sequence libraries were generated for the PRMT1 "hit" sequences and are pictured below. The consensus sequence was typed C to N terminus, as it was coupled.

❖ 4 peptides were synthesized based on the consensus sequence to validate these are true substrates. The kinetic values were measured using a standard methyltransferase assay.

Sequence Name	Sequence N to C
ValPep1	**AKCKM**-RGGKGLGKGGAK
ValPep2	**AKKKM**-RGGKGLGKGGAK
ValPep3	**VVCKM**-RGGKGLGKGGAK
ValPep4	**VVKKM**-RGGKGLGKGGAK

Kinetic Assay Results			
Sequence Name	**K_M (M)**	**K_{cat} (min^{-1})**	**K_{cat}/K_M (M^{-1} min^{-1})**
ValPep1	13.5×10^{-6}	1.321	9.75×10^{4}
ValPep2	16.5×10^{-6}	0.694	4.20×10^{4}
ValPep3	6.49×10^{-5}	0.690	1.10×10^{5}
ValPep4	5.69×10^{-6}	1.051	1.85×10^{5}



Parameter	Value	Std. Error
Vmax	0.2102	0.0104
Km	5.6931	1.3184

Future Directions

❖ Screening the same peptide library against other PRMT isozymes, such as PRMT4 and PRMT5, to determine differences in the substrate specificity amongst PRMT family members.

❖ Develop more specific PRMT inhibitors using this information.

References

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III. Maude, Tai S.; Davies, L.R.; Liu, R.P.; Harris, B.; Kocieński, S.A.; Aggeli, A. P.J.; Peptide synthesis and self-assembly. *Topics in current chemistry*. **2011** *310*, 27-69 .

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