Development of a Plate-Based Screening Assay to Investigate the Substrate Specificity of the PRMT Family of Enzymes

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S *Supporting Information*

ABSTRACT: There are nine protein arginine methyltransferases (PRMTs 1−9) expressed in humans that vary in both subcellular localization and substrate specificity. The variation in substrate specificity between isozymes leads to competing effects that result in either activation or repression of tumor suppressor genes. Current methods used to study substrate specificity for these enzymes utilize radioisotopic labeling of substrates, mass spectrometry analysis of complex samples, or coupled assays that monitor cofactor degradation. Herein, we report the development of a rapid, nonradioactive, and sensitive method for screening multiple peptides in parallel to gain insight into the substrate specificity of PRMT enzymes. Our assay provides a major advantage over other high-throughput screening assays (e.g., ELISA, AlphaScreen chemiluminescence) by eliminating the need for purification of individual peptides and provides a timesaving, cost-effective alternative to the traditional PRMT assays. A one-bead one-compound (OBOC) peptide library was synthesized and subsequently screened against PRMT1 in a 96-well plate. This screen resulted in identification of a novel PRMT1 substrate with kinetic parameters similar to histone H4−21 (e.g., the best-known PRMT1 peptide substrate).

KEYWORDS: *protein arginine methyltransferase, histone, arginine, one-bead one-compound, substrate screening, high-throughput*
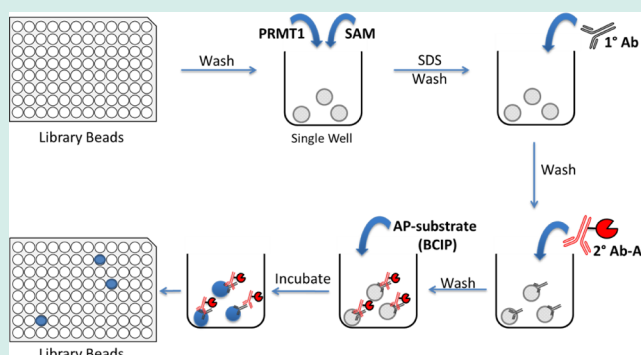
INTRODUCTION

The expression of eukaryotic genes is controlled by the accessibility of chromatin DNA to transcription factors that bind specific DNA sequences to promote gene transcription.[1] This accessibility can be controlled by post-translational modifications (PTMs) to the histone tails of chromatin.[1] These PTMs are generated by a variety of histone modifying enzymes, including histone acetylases, deacetylases, methyltransferases, demethylases, deiminases, and many others.[2,3] These enzymes can act as a switch that modulates the transcription of specific genes. For example, a post-translational modification can cause a conformational change of the histone that alters a DNA binding site for the transcription factor.[3] Alternatively, the post-translational modification may promote the recruitment of a chromatin modifying enzyme that alters the chromatin structure in an ATP-dependent manner.[4]

Arginine methylation, which is catalyzed by Protein Arginine Methyltransferases (PRMTs), is an example of a post-translational modification that alters transcription of various genes.[5−7] The PRMT enzymes transfer a methyl group from *S*-adenosylmethionine (SAM) to the guanidino group of arginine, with concomitant release of *S*-adenosylhomocysteine (SAH). Three types of PRMTs (I, II, and III) have been identified and characterized. Type I isozymes (PRMTs 1, 2, 3, 4, 6, and 8)

catalyze the formation of a monomethylarginine (MMA) followed by a second methyl transfer to produce asymmetric dimethylarginine (ADMA), whereby one of the nitrogens of the guanidino group has two methyl groups. Alternatively, Type II isozymes (PRMTs 5 and 9) catalyze the addition of one methyl group to each of the nitrogens of the guanidino group, initially forming MMA, and culminating with the formation of symmetric dimethylarginine (SDMA). PRMT7, a Type III PRMT, results only in the formation of MMA (Figure 1).[6,8] Arginine methylation has been shown to play a critical role in the onset and progression of a number of cancers (e.g., prostate, breast, lung), which may result from its ability to alter the accessibility, and thereby expression, of cancer related genes.[9] For example, PRMT1 methylates the N-terminal tail of Histone H4 at Arg3 to form ADMA, and this asymmetric dimethylation is associated with transcriptional activation of genes under control of ERα, p53, and others.[10,11] By contrast, conversion of the same arginine to SDMA, as catalyzed by PRMT5, has a repressive effect.[7,11,12] To date, very little is known about the factors that drive substrate specificity for one
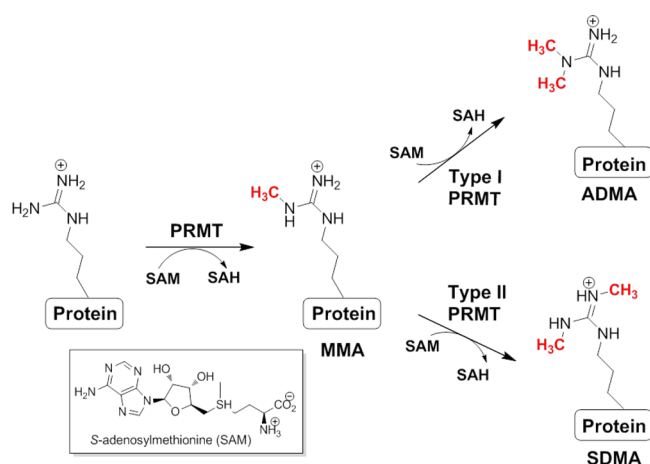
Figure 1. Enzymatic reaction catalyzed by the protein arginine methyltransferase family. PRMTs transfer a methyl group from *S*-adenosylmethionine (SAM) to the side chain of arginine to form monomethylarginine (MMA). Type I PRMTs result in the formation of asymmetric dimethylarginine (ADMA), and Type II PRMTs result in the formation of symmetric dimethylarginine (SDMA). A Type III PRMT can only catalyze the formation of MMA.

PRMT isozyme to add a repressive mark and another to add an activation mark on the same arginine in the histone tail.

Traditional methods for measuring the PRMT substrate specificity include radioisotope labeling of substrates, mass spectrometry to identify modified substrates in complex samples, or a coupled colorimetric assay for degradation of the SAM cofactor.[13,14] Evaluating the substrate specificity of peptides using these methods requires synthesis of individual peptides that incorporate a limited number of amino acids and/or post-translational modifications. There are a number of high-throughput (HT) amenable assays (e.g., AlphaScreen chemiluminescence, SAHH coupled assay, or the 384-well radioactive methyltransferase assay) designed for studying inhibition of the PRMT family, but most of these lack a HT method of screening peptides as PRMT substrates.[15] While these HT methods are quantitative, they require biotinylated peptides, expensive instrumentation (e.g., plate reader), and are only effective by using purified peptide substrates.[15] Given these methods are time-consuming and laborious, the number of potential peptide substrates that can be analyzed is limited; thus there has been a growing interest to develop novel methods for rapidly identifying recognition elements that help elucidate the substrate specificity for individual PRMT isozymes. Herein, we describe a rapid, nonradioactive, 96-well plate-based assay for identifying peptide substrates of PRMTs from a one-bead one compound (OBOC) peptide library. Our method provides a cost-effective and rapid assay that does not require any special instrumentation, synthesis of biotinylated peptides, and eliminates the labor-intensive purification of peptides before subsequent screening.

■ EXPERIMENTAL METHODS

Materials. Reagents for peptide synthesis were purchased from Anaspec (Fremont, CA), Advanced ChemTech (Louisville, KY), and Sigma-Aldrich (St. Louis, MO). Full length Protein Arginine Methyltransferase 1 (PRMT1) was expressed as a His-tagged fusion protein and purified to >95% using previously reported methods.[14] The methylated arginine antibody (Ab413) and the goat antimouse alkaline phosphatase antibody (Ab98690) were purchased from Abcam (Cambridge, MA). The 96-well filter plates (#8047 and

#5020) were purchased from Pall Life Sciences (Port Washington, NY). ^{14}C-labeled *S*-adenosylmethionine (SAM) and ^{14}C-labeled bovine serum albumin (BSA) were purchased from PerkinElmer Life Sciences (Waltham, MA).

Library Design and Synthesis. The peptide library was synthesized on 90 μm Tentagel S NH₂ resin (0.27 mmol/g; 130 mg). The library was synthesized from C-terminus to N-terminus using standard solid phase peptide synthesis procedures. A C-terminal methionine was directly attached to the resin followed by a linker sequence of Lys-*β*Ala-*β*Ala-*β*Ala. Each residue was added using 5 equiv of Fmoc-amino acid (Fmoc-Ala-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Gly-OH, Fmoc-His(Trt)-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, and Fmoc-Ser(tBu)-OH), 5 equiv of (1-cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylamino-morpholino-carbenium hexafluorophosphate (COMU), and 10 equiv of diisopropylethylamine (DIEA) as the coupling reagents (2 mL in DMF). Each coupling reaction was monitored by the Ninhydrin test. After synthesis of the linker, the beads were split evenly into the wells of a 96-well filter plate (Pall #8047). The library contained 96 different pentamer peptides composed of 7 amino acids (Ala, Arg, Gly, His, Leu, Lys, Ser). The amino acid sequence of each peptide was randomly determined using a random sequence algorithm and each peptide contained only a single arginine residue (Table S1). Synthesis of the peptide library was completed in individual wells of the filter plate by adding 5 equiv of the appropriate Fmoc-amino acid and coupling reagents described above (200 μL total volume per well). After the last residue was added, the beads were treated twice with 20% piperidine in DMF (10 min each). The side-chain protecting groups were deprotected with trifluoroacetic acid (TFA), triisopropylsilane (TIS), and water (95:2.5:2.5) for 2 h, washed with dichloromethane, dried, and stored at −20 °C.

Library Screening. Twenty beads from each well of the library were transferred to a new filter plate (Pall #5020) for screening. Beads were washed with water (10 times; 200 μL), TBST (10 times; 200 μL), and equilibrated with TBST overnight at room temperature. The library was equilibrated with assay buffer #1 (50 mM HEPES pH 8.0, 50 mM NaCl, 1 mM EDTA, and 0.5 mM DTT) with gentle shaking at 37 °C. After 30 min, assay buffer #1 was removed and the beads were equilibrated with assay buffer #2 (50 mM HEPES pH 8.0, 50 mM NaCl, 1 mM EDTA, 0.5 mM DTT, and 400 μM SAM) for 30 min with gentle shaking at 37 °C before adding 500 nM PRMT1. After 60 min, the reaction mixture was removed and the beads were washed with TBST (10 times; 200 μL) and equilibrated with 0.1% SDS for 10 min. The library beads were washed extensively with water (10 times; 200 μL) and TBST (10 times, 10 min each). The beads were blocked using 2% BSA in TBST for 1 h at room temperature with gentle agitation, then washed again with TBST (5 times; 200 μL). The library beads were equilibrated with the 1° antibody, which recognizes asymmetric dimethyl arginine (Ab413; 1:333 dilution), suspended in 2% BSA in TBST overnight at 4 °C with gentle rocking. After 14 h, the beads were equilibrated with TBS for 10 min and TBST for 10 min before adding the secondary antibody (Ab98690; 1:4000) for 60 min at room temperature with gentle rocking. The library was washed again for 10 min with TBS and TBST before adding 2.5 mg/mL alkaline phosphatase substrate, 5-bromo-4-chloro-3′-indolyphosphate *p*-toluidine salt (BCIP) in BCIP Buffer (100 mM Tris pH 9.0, 150 mM NaCl, and 1 mM MgCl₂, 200 μL total volume) for 30 min at room temperature. The enzymatic reaction was quenched by washing the beads with 0.1 M HCl. Finally, the beads were washed with TBS and TBST for 10 min each. Wells that contained a majority of beads that turned blue by inspecting and counting the beads under a stereo microscope were identified as "hit" sequences. The sequences of hit beads were entered into the WebLogo generator (http://weblogo.berkeley.edu/logo.cgi) to identify the consensus sequence. Note that three controls were included to ensure optimal screening conditions: an ADMA containing peptide (GG**ADMA**GG positive control), a substrate control plus PRMT1 (GG**R**GG; enzyme positive control), and a substrate control without PRMT1 (GG**R**GG; enzyme negative control).

Peptide Synthesis. Peptides (CH1, CH1R1K, CH1NL, AcCH1, AcFL-CH1, AcFL-CH1REV, FL-CH1REV, CH1REV, CH1NLREV, H4−5, H4−16, and H4−21) were synthesized on Wang resin using Fmoc chemistry. For each coupling, the resin was swelled in DMF before adding 5 equiv of the Fmoc-amino acid (Fmoc-Ala-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Gly-OH, Fmoc-His(Trt)-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, and Fmoc-Ser(tBu)-OH), HOBT, HBTU, and 10 equiv of NMM for 1 h at room temperature. The coupling reaction was monitored using the Ninhydrin test. Upon completion, the N-terminal Fmoc was removed with 20% piperidine (3 mL for 10 min, twice). Acetylated peptides were washed with DMF and DCM after the last amino acid was coupled to the resin, then treated with acetylating reagents (2 mL of a 1:1 acetic anhydride:triethylamine in 10 mL of a 1:1 DMF:DCM) for 1 h. After synthesis was completed, the side chain protecting groups and resulting peptides were cleaved from the resin with trifluoroacetic acid (TFA), triisopropylsilane (TIS), and water (95:2.5:2.5) for 2 h, precipitated with ether, and purified by reverse phase high-performance liquid chromatography. The peptide sequences were confirmed with electrospray ionization (ESI) mass spectrometry (Table S2).

Kinetic Evaluation of Peptides. PRMT1 activity was determined using a previously reported discontinuous gel-based assay that measures incorporation of ^{14}C into the peptide from ^{14}C-methyl-SAM cofactor.[14] Briefly, peptide and ^{14}C-methyl-SAM (15 μM) were preincubated in assay buffer (50 mM HEPES pH 8.0, 50 mM NaCl, 1 mM EDTA, and 0.5 mM dithiothreitol) for 10 min at 37 °C. The reaction was initiated by the addition of 400 nM PRMT1. Reactions were run in duplicate and quenched by the addition of 6× tris-tricine loading dye. The reaction mixtures were run on a 16.5% Tris-Tricine polyacrylamide gel and incorporation of the ^{14}C-methyl was determined using phosphorimage analysis. Note that ^{14}C-labeled BSA was used as an internal reference standard for quantification. Activity of duplicates agreed within 20% standard deviation and the initial rates were fit to eq 1, using GraFit, version 5.0.11.

$$v = V_{max}[S]/(K_m + [S]) \tag{1}$$

■ RESULTS AND DISCUSSION

Design and Synthesis of the Peptide Library. The N-terminal tail of Histone H4 was chosen as a template for the peptide library since a number of H4 peptide substrates have already been evaluated;[14,16,17] we hoped that our results would shed light on the factors governing substrate specificity and yield peptide sequences previously unknown to be PRMT1 substrates. A one-bead one compound (OBOC) peptide library was synthesized on Tentagel resin containing a Met-Lys-βAla-βAla-βAla linker. Inclusion of methionine facilitates in cleavage of the peptide from the bead in order to validate the peptide sequences, a feature that will be crucial when screening larger libraries. The remaining linker residues extend the library peptide from the bead. As a first generation library, we randomly incorporated the 7 amino acids found on the N-terminal tail of Histone H4 (Ala, Arg, Gly, His, Leu, Lys, and Ser) at 5 variable positions (Figure 2). The amino acid sequence of each peptide was randomly generated. Additionally, each peptide contained only a single arginine residue to make analysis more straightforward. The library included 93 random peptides that were synthesized in a 96-well filter plate using standard Fmoc procedures (Table S1). The remaining three wells contained a set of control peptides: a positive control (Linker-GG**ADMA**GG), a PRMT1 positive substrate control (Linker-GGRGG), and a PRMT1 negative substrate control (Linker-GGRGG; no enzyme). Each well contained approximately 20 beads that displayed multiple copies of the same peptide sequence (i.e., 20 identical beads/well). The sequences of these peptides were confirmed by cleavage of a
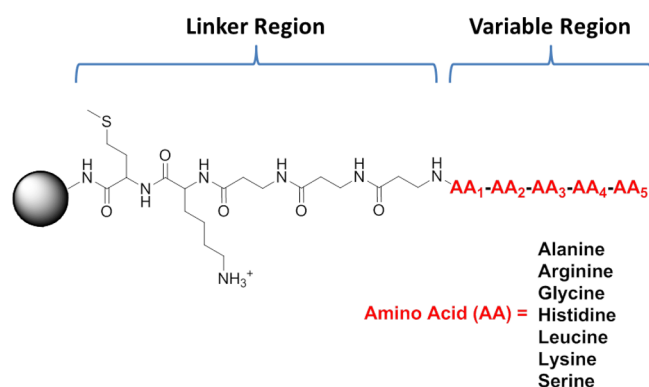
Figure 2. Design of the one-bead one compound peptide library. General structure of the peptide library and the amino acid building blocks used in the synthesis. The linker consisted of MKBBB, where B is β-alanine.

single bead from randomly chosen wells, and sequenced by ESI-MS/MS; the masses obtained were compared to those expected for that sequence (Table S3).

Screening the Peptide Library. PRMT1 is the most well characterized isozyme, and a number of studies have identified specific recognition elements that govern its substrate specificity.[14,17,18] However, these studies have only started to scratch the surface of categorizing the substrate specificity of PRMT1. Thus, we utilized our assay to identify previously unknown sequences from a peptide library that could be recognized and methylated by PRMT1. For this screen, the library was incubated with PRMT1 for 60 min. This time was determined using a series of control peptides and conditions; more than 95% of the beads containing the GGRGG peptide turned blue after incubation with PRMT1 for 60 min. Following incubation, the reaction was quenched with SDS and washed extensively. Beads containing peptides that were methylated by PRMT1 were identified using a 1° antibody that binds with ADMA only (Figure S1). We chose this antibody under the assumption that arginine residues completely converted to ADMA, that is, not MMA, are the most proficient substrates, thus only the best PRMT1 substrates would be identified in our screen. The 2° antibody was conjugated to alkaline phosphatase, and upon addition of the alkaline phosphatase substrate (BCIP), a blue precipitate was deposited on the beads. Therefore, only beads displaying ADMA became blue, thus providing a sensitive and easy method for detection (Figure 3).

As an additional method to improve reliability of our screen and to ensure that only the best PRMT1 substrates were chosen, each well contained 20 beads with the same peptide sequence, and wells resulting in dark blue color on the majority of beads were chosen as "hit" sequences. Only 11 of 93 wells met this criteria and were selected for further analysis (Figure 4). The sequences of these peptides were entered in the WebLogo generator (http://weblogo.berkeley.edu/logo.cgi) to identify sequence homology and determine a consensus sequence. The 11 hit sequences resulted in a consensus sequence of NH2-**RAHKH**-COO⁻. Interestingly, one of the hit wells contained the same sequence (Well G6; Figure 4).

Determining k_{cat} and K_m. To validate our screening methodology, we measured the kinetic values for a subset of the peptides using our traditional PRMT methyltransferase radioactive assay. The peptides synthesized and evaluated included the pentamer consensus sequence, the reverse pentamer
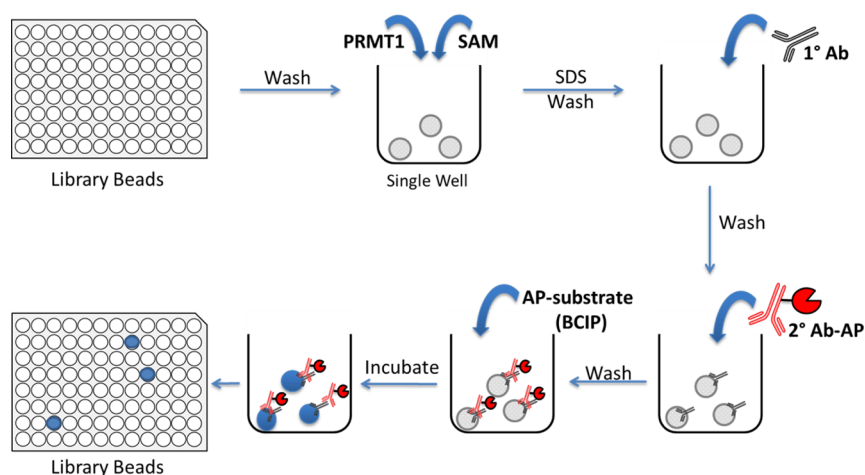
Figure 3. Substrate specificity screen of PRMT1 in a 96-well filter plate. The library was screened in the 96-well filter plate, where each well contained multiple beads composed of the same peptide.
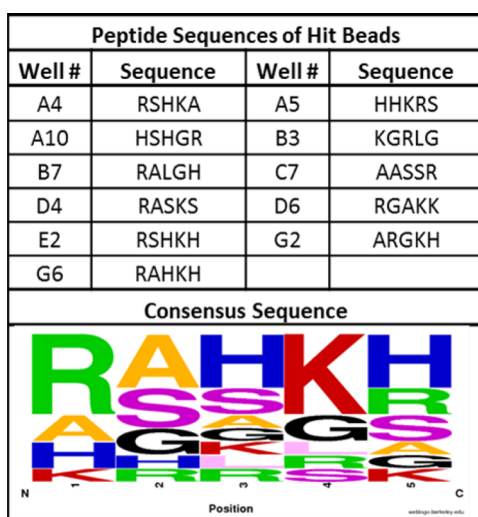
Figure 4. PRMT1 substrates (hits) identified from the 96-well peptide library. The peptide sequences were analyzed and the consensus sequence was determined using WebLogo generator.

consensus sequence, and the consensus sequence incorporated into the histone H4 N-terminal tail. Derivatives of these

peptides were also characterized to determine the effects of the linker peptide and acetylation of the N-terminus (Table 1).

The consensus sequence identified was first synthesized with and without the linker and kinetic values were determined. For the peptide containing the linker (CH1), the k_{cat} was 0.05 min^{-1} and the K_m was 563 μM (k_{cat}/K_m = 88.8 M^{-1} min^{-1}). The peptide without the linker (CH1NL) did not exhibit saturation with 1 mM of substrate, thus kinetic parameters were not determined. Furthermore, we replaced the arginine with a lysine in the CH1 peptide (CH1R1K) to demonstrate specificity of PRMT1 for the arginine residue, but methylation activity was not detected in this assay. We also evaluated the effect of acetylation on the N-terminus, given that the Arg is located at the N-terminus, by synthesizing and measuring the kinetic values of an acetylated version of the HKHAR peptide (AcCH1). However, the AcCH1 peptide had a k_{cat} and K_m of 0.03 min^{-1} and 851 μM, respectively (k_{cat}/K_m = 88.3 M^{-1} min^{-1}). These results were not surprising given that Osborne et al. previously reported that long distance interactions are important for peptide substrate binding.[14] We next hypothesized that the short peptide sequence alone was too small to have these favorable binding interactions. However, the presence of multiple copies of the peptide on the bead likely increases the effective concentration of the substrate presented to the enzyme, and hence explains why methylation occurs on

Table 1. Kinetic Characterization of Selected Peptides[a]

substrate	sequence	k_{cat} (min^{-1})	K_m (μM)	k_{cat}/K_m (M^{-1} min^{-1})
CH1	RAHKHBBBKM	0.050 ± 0.005	563 ± 106	88.8
CH1R1K	KAHKHBBBKM	nd[b]	nd[b]	nd[b]
CH1NL	RAHKH	nd[b]	nd[b]	nd[b]
AcCH1	Ac-RAHKHBBBKM	0.030 ± 0.001	851 ± 83	88.3
AcFL-CH1	Ac-RAHKHGGKGLGKGGAK	0.026 ± 0.001	218 ± 22	118
AcFL-CH1REV	Ac-HKHARGGKGLGKGGAK	0.47 ± 0.01	13.1 ± 1.3	3.58×10^4
FL-CH1REV	HKHARGGKGLGKGGAK	0.55 ± 0.01	6.8 ± 0.6	8.1×10^4
CH1REV	Ac-HKHARBBBKM	0.10 ± 0.01	1453 ± 175	67
CH1NLREV	HKHAR	nd[b]	nd[b]	< 25
AcH4−21	Ac-SGRGKGGKGLGKGGAKRHRKV	0.44 ± 0.01	1.61 ± 0.11	2.80×10^5
AcH4−16	Ac-SGRGKGGKGLGKGGAK	0.32 ± 0.01	170 ± 25	1.90×10^3
AcH4−5	Ac-SGRGK	nd[b]	nd[b]	nd[b]

[a]The concentration of SAM for determining peptide kinetics parameters was 15 μM. [b]The kinetic parameters of this peptide substrate were not determined because of low amount of product formation.

the bead. Furthermore, long distance interactions may also be mimicked through an avidity effect where one peptide on the bead provided the long distance interactions for substrate binding, and an adjacent peptide on the bead provided PRMT1 with the arginine to be catalyzed.

Knowing that interactions distal to the site of modification are important for catalysis of the substrate arginine in the histone H4 peptide (Arg3 of Histone H4), we incorporated our consensus peptide sequence into a histone H4 N-terminal tail to determine if this could enhance the kinetic rates for the short peptide. Previous studies concluded the complete histone H4 N-terminal tail (residues 1−21) was kinetically similar to the histone H4 protein and the best peptide substrate for PRMT1 ($k_{cat}/K_m = 2.80 \times 10^5$ M^{-1} min^{-1}).[14] Deleting residues from the C-terminus of the H4−21 peptide (residues 17−21; H4−16 peptide) decreased the k_{cat}/K_m by more than 100-fold ($k_{cat}/K_m = 1.90 \times 10^3$ M^{-1} min^{-1}), but still included some of the necessary long-range interactions for binding and catalysis at Arg3 of histone H4. For this reason and the fact the H4−16 peptide contained a single arginine, we chose this peptide as a template to determine if we had identified a better substrate in our screen. Specifically, we theorized that an enhancement in catalysis over the H4−16 peptide would confirm that our screen identified a novel peptide substrate. Note the H4−5 peptide based on the first five residues of the N-terminus of the H4−21 peptide showed no activity, thus supporting the need for including long-range interactions.

To test this hypothesis, the AcFL-CH1 peptide was synthesized. The sequence of this peptide replaces the first five residues of H4−16 (SGRGK) with our consensus sequence (RAHKH). The kinetics of this peptide were measured and provided a k_{cat} value of 0.026 min^{-1} and a K_m value of 218 μM ($k_{cat}/K_m = 118$ M^{-1} min^{-1}). Thus, the incorporation of the histone H4 N-terminal tail provided minimal improvements over the CH1 peptide.

Given the AcFL-CH1 did not provide a significant increase in catalytic efficiency, we hypothesized that PRMT1 may recognize the sequence on the bead in reverse. Note that the peptides are displayed on bead and the PRMT1 enzyme may recognize the reverse sequence, thus it is important to check both the forward and reverse sequence identified in the screen. Thus, we synthesized a reverse version of AcFL-CH1 by replacing the first five residues (RAHKH) with the reverse sequence of HKHAR (AcFL-CH1REV). The kinetic parameters were measured and resulted in values for the k_{cat} of 0.47 min^{-1} and a K_m of 13.1 μM. This resulted in a greater than 300-fold increase over AcFL-CH1 with a k_{cat}/K_m of 3.58×10^4 M^{-1} min^{-1}. More importantly, this initial screen of 93 peptides identified a peptide substrate with a k_{cat}/Km that is 20-fold greater than the H4−16 peptide.

In light of these data, we sought to determine the reasons for this large increase in catalytic efficiency by synthesizing a series of AcFL-CH1REV peptides. First, we evaluated the effect of acetylating the N-terminus by synthesizing an unacetylated FL-CH1REV peptide. Measuring the kinetic parameters of FL-CH1REV resulted in a k_{cat} value of 0.55 min^{-1} and a K_m of 6.8 μM (k_{cat}/K_m of 8.1×10^4 M^{-1} min^{-1}). This suggests the acetylated N-terminus does not provide any additional binding interactions for PRMT1 with this substrate. Given this information, we sought to determine if the long-range interactions provided by the H4−16 template was still necessary for this peptide. The reverse consensus sequence, identified from the screen, was synthesized with and without

the linker. Kinetic values were determined for both the CH1REV and CH1NLREV peptides. For the CH1REV, the k_{cat} was 0.1 min^{-1} and the K_m was 1453 μM (k_{cat}/K_m of 67 M^{-1} min^{-1}). The peptide without the linker (CH1NLREV) did not exhibit saturation with 1 mM of substrate, thus kinetic parameters were not determined. These data are consistent with previous studies showing that long-range interactions are necessary for binding to PRMT1.[14] However, amino acid substitutions around the substrate arginine may provide additional binding interactions around the PRMT1 active site that result in better PRMT1 substrates. This was evident from the greater than 42-fold increase of our FL-CH1REV peptide over the H4−16 peptide.

CONCLUSIONS

We have developed a sensitive and timesaving method for rapidly screening a series of potential PRMT peptide substrates. An initial screen of 93 distinct peptide sequences led to identification of one of the best-known peptide substrates for the enzyme with a k_{cat}/K_m of 8.1×10^4 M^{-1} min^{-1} (42-fold greater than the H4−16 peptide). This can be readily adapted to screen larger peptide libraries to determine the variations in the substrate specificity of the PRMT family. In addition, this methodology described herein provides alternative to other screening methodologies by eliminating the need for purifying individual peptides.

ASSOCIATED CONTENT

Ⓢ Supporting Information
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Further supplementary tables and figures (PDF)

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Notes
The authors declare no competing financial interest.

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