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Rapid and direct measurement of methyltransferase activity in about 30 min

Joan M. Hevel*, Owen M. Price

Utah State University, Department of Chemistry & Biochemistry, Logan, UT 84321, United States

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ABSTRACT

Protein arginine methylation is a widespread eukaryotic posttranslational modification that occurs to both histone and non-histone proteins. The S-adenosyl-*L*-methionine (AdoMet or SAM)-dependent modification is catalyzed by the protein arginine methyltransferase (PRMT) family of enzymes. In the last several years a series of both direct and indirect assay formats have been described that allow the rate of methylation to be measured. Here we provide a detailed protocol to directly measure PRMT activity using radiolabeled AdoMet, reversed-phase resin-filled pipette tips (ZipTips®) and a liquid scintillation counter. Because the ZipTips® based quantitation relies only on the straightforward separation of unreacted AdoMet from a methylated substrate, this protocol should be readily adaptable to other methyltransferases. The method is fast, simple to employ with both peptide and protein substrates, and produces very little radioactive waste.

1. Introduction

Protein arginine methylation was originally identified more than 50 years ago in calf thymus extracts labeled with radioactive S-adenosylmethionine (AdoMet or SAM[a]) [1,2]. Initially, the role of this modification was not well understood and arginine methylation was overshadowed by the emerging understanding of other post-translational modifications. However over the last two decades, the role of protein arginine methylation as a major regulator of protein function in eukaryotic cells has been solidified (reviewed in [3–8]). This widespread modification is catalyzed by the family of enzymes called protein arginine methyltransferases (PRMTs), which transfer one or two methyl groups from AdoMet to an acceptor protein; the other product of the reaction is S-adenosyl-*L*-homocysteine (AdoHcy or SAH) (Fig. 1). The methylated product protein displays altered function in the cell, allowing for a variety of signals to be transduced or propagated. Not surprisingly, dysregulation of PRMT activity is correlated with a variety of pathologies (reviewed in [9–14]). Parsing out the PRMT-dependent aspects of a signaling cascade, evaluating endogenous regulation of PRMT activity and investigating pharmaceutical inhibition of the PRMTs all require one to quantitatively measure PRMT activity. In the last several years several different methods for quantifying protein methylation have been described [15–25]. In this review we will summarize the general approaches and considerations in measuring PRMT activity and discuss in detail a PRMT assay that is direct, easy to employ, fast, and quantitative.

2. Approaches to measuring methyl transfer

Monitoring the rate of AdoMet-dependent methyl transfer by PRMTs represents a significant challenge. Because AdoMet and AdoHcy (as well as the unmethylated and methylated peptide/protein) have indistinguishable visible spectra, the widely-used technique of UV–VIS spectroscopy cannot be employed directly. Given this obstacle, two main approaches exist to measure methylation rate [17]: 1) direct detection of reaction products using radioactivity as a tracer, or 2) a coupled assay that makes use of enzymes that will metabolize AdoHcy. Table 1 summarizes the characteristics of some of the currently used techniques. A more thorough description of methods to detect protein methylation (not necessarily *rate*) was previously reviewed [20]. Note that none of the techniques in Table 1 distinguish what type of arginine methylation has occurred (three different methylated products are possible depending on what PRMT isoform is used, what type of substrate is used, and how long the reaction proceeds). If the goal is to identify which methylated product is formed, we direct the reader to a review of such techniques [19]. While assays to identify specific PRMT substrates are in their infancy, bump-and-hole assays using a clickable AdoMet analogue with an engineered PRMT have been described

* Corresponding author.
E-mail address: joanie.hevel@usu.edu (J.M. Hevel).

[a] Abbreviations used: AdoMet (or SAM), S-adenosyl-*L*-methionine; AdoHcy (or SAH), S-adenosylhomocysteine; ATP, adenosine triphosphate; BSA, bovine serum albumin; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; MTAN, AdoHcy nucleosidase; PRMT, protein arginine methyltransferase; TFA, trifluoroacetic acid; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis.

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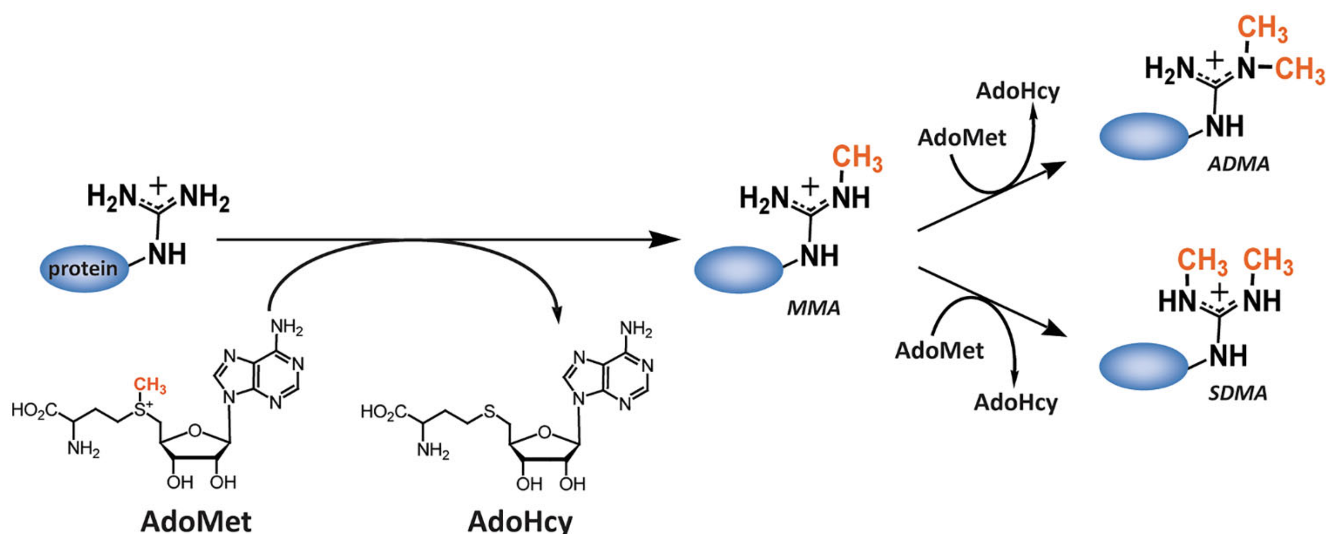

Fig. 1. Reactions catalyzed by the PRMTs. Nine human PRMT isoforms are capable of forming monomethylarginine (MMA), asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA) on targeted proteins using S-adenosyl-*L*-methionine (AdoMet or SAM) as the methyl source.

Table 1
Methods used to quantify the rate of protein arginine methylation.

	Key Element	Separation Technique	Product detected	Continuous or Discontinuous	Useful for protein substrates?
		P81 phosphocellulose paper			no
Direct	[^{3}H]-AdoMet	Streptavidin capture of biotinylated peptides	Radiolabeled product	Discontinuous	no*
		SDS-PAGE	peptide/protein		yes
		Zip-Tips			yes
Indirect	2–4 coupling enzymes	none	Luminescence, UV, fluorescence	Continuous and discontinuous	some

*biotinylation of proteins is possible but the technique has not been used yet to follow protein arginine methylation.

[26,27]. Additionally, a general approach using an AdoMet analogue as an activity-based probe to create a stable Enzyme-substrate-probe complex was recently demonstrated for Thiopurine methyltransferase (TPMT EC 2.1.1.67) [28]. Clickable AdoMet analogues [29] may soon be adapted for general use with all the PRMTs

All of the direct radiolabel-based techniques work by measuring the radioactive signal present in the peptide/protein product by either liquid scintillation counting or phosphorimaging. The key difference in the assays rests in how the unused radiolabeled AdoMet is separated from the newly radiolabeled product. Because a physical separation of the two is generally necessary, all of these assays operate in a discontinuous fashion; that is, aliquots at several timepoints are collected, each one undergoes the separation process, and each one is analyzed for the radiolabel content to establish a rate of methylation. The advantages of these assays are: 1) the certainty that what is being observed is indeed methylation (what is measured is the product of interest), 2) minimal steps in which altering assay conditions can affect the measured activity of the enzyme (the separation step), and 3) the use of reagents and equipment that are easily obtained or already present in most biochemical laboratories. The obvious disadvantage is that they all require the use of radioactivity. Two scintillation proximity assays have also been described using biotinylated histone peptide substrate that is captured by streptavidin-coated SPA beads or plates [18,30]. The scintillation proximity approach discriminates between radiolabeled peptide (bound to the SPA bead) and unused radiolabeled AdoMet by proximity, and thus does not require a dedicated separation step. Accordingly, these assays can be used in a high-throughput fashion but require radiometric instrumentation that can count in a 96-well format.[b]

Most of the indirect techniques require the presence of 2–4 coupling enzymes to metabolize AdoHcy and report a signal in the form of light in various forms, as reviewed elsewhere [20]. In order to accurately report on the rate of protein methylation, each method requires that the amount and rate of the coupling enzymes is not limiting in the reaction. Some of these techniques are available in commercial kits (G-biosciences) and have the major advantage of being continuous; that is, a single reaction is set up and followed over time, and a full rate of methylation is collected [16]. Many of the coupled assays are well-suited for high-throughput screens [20]. The disadvantages of these assays are: 1) the reliance on an alternative signal in lieu of an actual product of the reaction, 2) the increased number of steps in which altering assay conditions can affect the measured activity of the enzyme (each coupling enzyme), and 3) the need to purchase or obtain each of the coupling enzymes. Additionally, an aptamer-based assay that quantifies the byproduct AdoHcy (AptaFluor by BellBrook labs), and a luciferase based assay that quantifies ATP derived from AdoHcy (MTase-Glo by Promega) are both available. These assays have a fluorescence (AptaFluor) or luminescence (MTase-Glo) read-out and appear to have very good sensitivity, but operate on a discontinuous format.

Although all of the assays discussed above can be used to quantify the *rate* of methylation, some are more easily employed than others. For example, SDS-PAGE is a simple, straightforward approach that requires

[b] It is also possible to monitor protein arginine methylation by mass spectrometry (either by measuring the methylated product or the accumulation of AdoHcy [31] over time), however, this direct technique requires the use of a mass spectrometer. While used extensively to confirm and identify arginine methylation sites on peptides and proteins, the technique is not often used to measure *rates* of methylation.

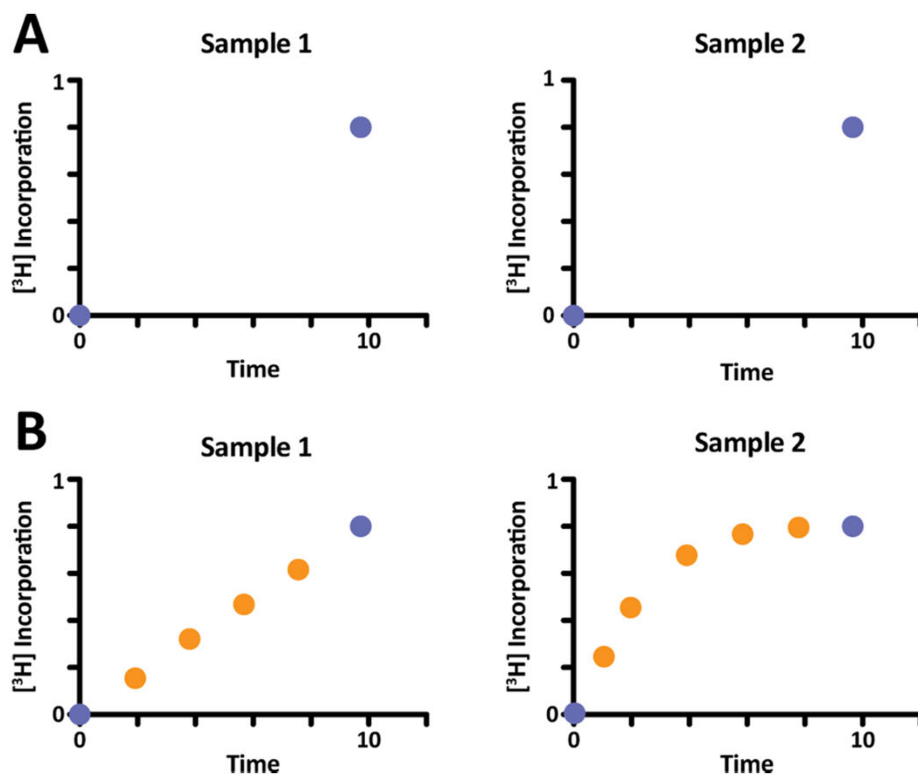

Fig. 2. Benefits to collecting methylation rates instead of single timepoints. In **(A)**, the collection of a single time point might suggest that PRMT activity is equal in Sample #1 and Sample #2. However, collection of an entire rate (additional points are shown in yellow) **(B)** indicates that the PRMT activity in Sample #1 is less than in Sample #2. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

little additional investment other than the radiolabeled AdoMet. However, the time incurred to run the gels, the number of gels needed, and the amount of radioactive waste that is created make this technique cumbersome when methylation *rates* are needed. So when are rates needed? In general, collecting *rates* minimizes analysis errors. To illustrate this point, take the following hypothetical example where two different samples were monitored for PRMT activity using a single 10-minute reaction time (Fig. 2A). Because the same amount of tritium was incorporated in 10 min, one might conclude that the protein arginine methyltransferase activity is the same in both samples. However, this conclusion would only be sound if the rate of methylation was linear during the entirety of the 10 min. The collection of additional timepoints to determine a rate yields a more complete, and sometimes very different story (Fig. 2B). Furthermore, if the goal is to collect kinetic parameters (such as K_m and V_{max} or k_{cat}), one needs to collect an *initial rate*, which typically represents just the first ~10–15% of product formed. These conditions necessitate an assay system with sufficient sensitivity to work below conditions of K_m and to be relatively fast. In the following sections, we review a simple to use methyltransferase assay that meets these criteria.

2.1. PRMT activity assay using [methyl-^{3}H]-AdoMet and ZipTips®

As discussed above, radiolabeled AdoMet can be used to directly detect a methylated peptide/protein product of a PRMT reaction. However, in order for this approach to work, the unused radiolabeled AdoMet must be removed from the radiolabeled methylated peptide/protein. SDS-PAGE separates the two radiolabeled species easily, but is slow, results in a great deal of waste, and suffers from poor sensitivity, especially with substrates that contain only a single arginine that becomes methylated [15]. In an attempt to design a faster assay strategy, we found that we could replace SDS-PAGE with a simple ZipTip® as a means for separating the two radiolabeled species (Fig. 3). We have successfully used this assay to determine methylation rates with both peptide and protein substrates [32–35]. Other than a scintillation counter, no additional equipment is necessary [15].

2.1.1. Materials

In general, each enzyme reaction will contain buffer, a peptide or protein substrate, a PRMT, [*methyl*-^{3}H]-AdoMet and unlabeled AdoMet. We also include AdoHcy nucleosidase (MTAN, EC 3.2.2.9) to degrade the AdoHcy that is produced during the reaction, bovine serum albumin (BSA) as a non-specific protein stabilizer, and EDTA/dithiothreitol (DTT) to prevent/repair oxidation that may occur to the enzyme. The enzyme reaction is incubated at 37 °C (or the optimal temperature of your enzyme), and aliquots are removed at various times over a 6–10 min-period and terminated using a guanidinium/trifluoroacetic acid solution. Each quenched time point is processed using a ZipTip®. Each component is discussed below.

Buffer: Commonly used buffers (20–100 mM, pH 7.5–8.0) that support PRMT catalysis are sodium phosphate, Hepes, and Tris. Buffer choice should be optimized to the particular PRMT isoform used.

Peptide and Protein Substrates: Peptide and protein substrates are used in PRMT reactions at a variety of concentrations. As a reference, we commonly use the acetylated R3 peptide (Ac-GGRGGFGGRGGFGGRGGFG) at a saturating concentration of 200 μM when measuring PRMT1 (EC 2.1.1.319) activity. The R3 peptide originates from the naturally occurring PRMT1 protein substrate fibrillarin [36]. The R3 peptide has three arginines that can be methylated by PRMT1 [37]. Changing the sequence, removing arginines, or decreasing the length of the peptide substrate can affect both K_m and k_{cat} [37–40]. Therefore, if a different peptide is to be used and saturating conditions are desired, assays below, at, and higher than 200 μM should be done to insure that 200 μM is indeed saturating. Protein substrates are typically used at lower concentrations, especially those that contain multiple arginines that can be methylated. For example, the PRMT1 protein substrate hnRNPK contains 7 arginines that can be methylated and showed saturation at < 10 μM [15].

PRMT: PRMTs can be recombinantly expressed or purchased from a variety of sources. Purification of the recombinant proteins is often simplified by the addition of a fusion tag such as glutathione-S-transferase (GST) or 6-HIS. In most cases it appears that either tag appended to the N-terminus results in active protein. However, in the case of

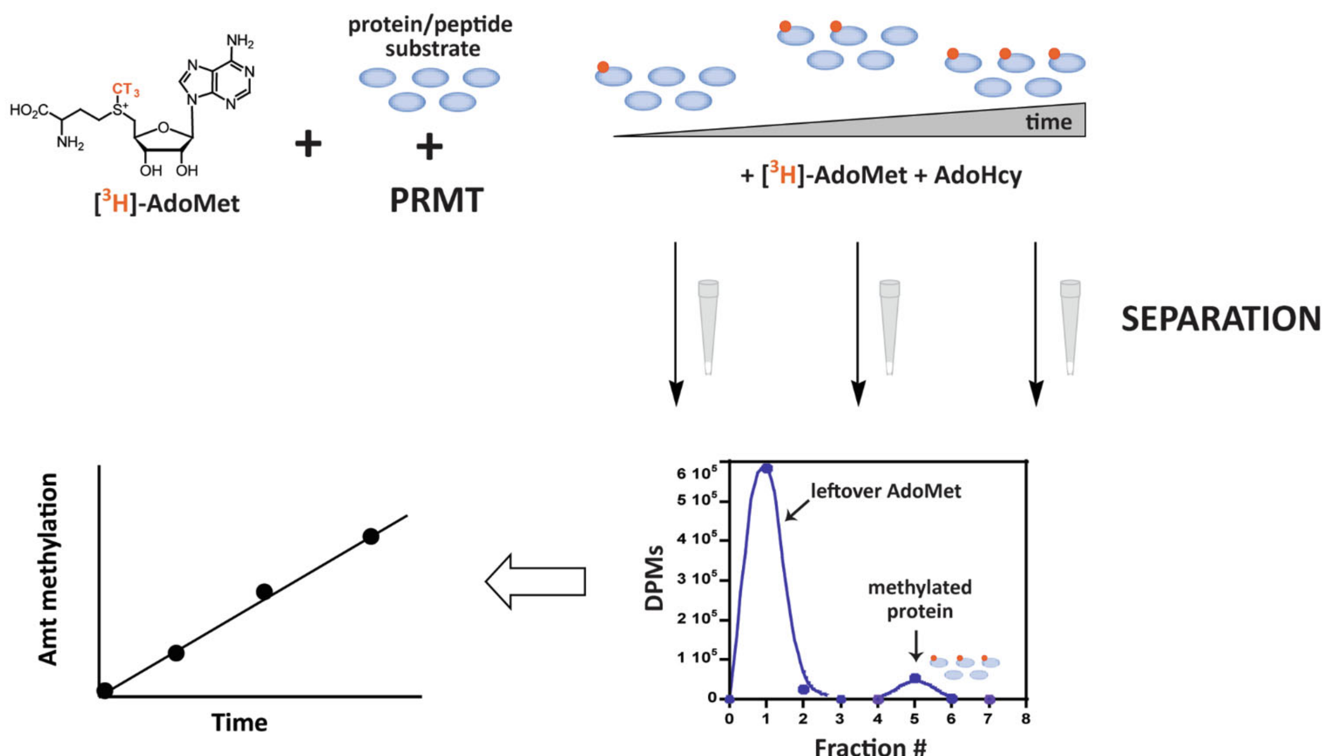

Fig. 3. Schematic showing the theory for using ZipTips® to measure methyltransferase activity [15].

PRMT1, we found that the GST-tagged enzyme exhibited lower activity than the HIS-tagged enzyme [15]. Additionally, fusion tags placed at the C-terminus of PRMT1 significantly impaired activity (unpublished data). Several PRMTs are regulated by redox chemistry with reduced enzyme displaying the highest activity [32]. The amount of oxidation to PRMT1 (and likely other isoforms) during purification is variable when no external reductant is used. Therefore, we recommend purifying and storing recombinant PRMT1, 6, 7 and 8 in 1 mM dithiothreitol and 1 mM EDTA.

AdoMet: [*methyl*-^{3}H]-AdoMet (specific activity ~ 70 Ci/mmol, 0.55 µCi/ µl) can be purchased from PerkinElmer. AdoMet is more stable in acidic conditions (hydrolysis in water occurs [41]) and is usually provided in dilute acid (e.g., 10 mM sulfuric acid/ethanol from Perkin Elmer) or made in dilute HCl and stored at ≤ −20 °C in small aliquots to avoid multiple freeze–thaw cycles. Care should be taken to store AdoMet under acidic conditions and at low temperatures to minimize hydrolysis and decomposition. The concentration of AdoMet solutions can be determined spectrophotometrically using the extinction coefficient at 256 nm of 15200 M^{-1} cm^{-1} at pH 1 [41]. Note that AdoMet harbors two chiral centers, and biological activity has only been reported for (S,S)-AdoMet. Spontaneous racemization of the AdoMet sulfonium has been reported [42,43]. Methods to specifically quantify [44] and purify [43,45] (S,S)-AdoMet have been described. The purity of commercial preparations of AdoMet range from 75 to 95%. In our assays, 1 µM [*methyl*-^{3}H]-AdoMet and 1 µM unlabeled AdoMet (for a total of 2 µM) is typically sufficient to observe activity. However, the K_m for AdoMet for a variety of PRMTs range from 2 to 20 µM for most PRMTs [40,46–49], with PRMT4/CARM1 showing a much lower K_m near 20 nM [50]. If saturating conditions for AdoMet are desired, the concentration of AdoMet can be increased (we increase the concentration with unlabeled AdoMet to keep costs down). Keep in mind that the enzyme reaction must employ sufficient buffering capacity to counter the acid from the additional AdoMet. To check this one can prepare a mock reaction containing only the final buffer concentration and the amount of acid that will be used in the final enzyme reaction and the pH can be measured.

Guanidinium/trifluoroacetic acid quench solution: Samples of the enzyme reaction mixture are quenched with an 8 M guanidine–HCL in 2.5% trifluoroacetic acid [TFA] solution. Cautions: TFA is both corrosive and an irritant to skin, eyes and mucous membranes.

ZipTips®: ZipTip$_{C4}$ or ZipTip$_{C18}$ pipette tips are available from Millipore. The ZipTip is a 10 µL pipette tip with 0.6 µL of reverse-phase (RP) chromatography resin (C4, silica, 15 µm, 300 Å pore size or C18, silica, 15 µm, 200 Å pore size) fixed at the tip. The tips have a binding capacity of approximately 3.3 µg protein per C4 tip and 5 µg per C18 tip so care should be taken to not overload the tip. The C18 tips are optimal when using peptides or proteins < 50 kDa as substrates; the C4 tips are optimal when using peptides or proteins 3–100 kDa as substrates. In general we use the C18 tips for most applications with peptide substrates, as most model peptide PRMT substrates used in our lab are ~1 kDa. For Histones and larger proteins C4 tips have worked well, with the exception of the PRMT substrate eIF4A1, which is not amendable to the ZipTip analysis for reasons that remain unclear. We note also that Pierce and Agilent sell a similar tip with slightly different amounts of RP resin and binding capacities.

MTAN, BSA, and EDTA/DTT: In order to remove any potential inhibition by the product AdoHcy, we typically add a final concentration of 10 nM AdoHcy nucleosidase (MTAN) to each enzyme reaction. The K_i for AdoHcy has been determined for several PRMT isoforms and ranges from 0.1 to 8 µM [40,46–48,50–52], and can vary depending on the substrate used. While we do not typically observe a difference in PRMT1 methylation rate in the presence or absence of MTAN (Fig. 4), it is included as a general precaution. Early on in the optimizing of PRMT1 assay conditions we found that the addition of BSA enhanced the measured activity of the enzyme, we rationalized by non-specifically stabilizing the enzyme. A BSA concentration of 0.4 µM was the lowest concentration that supported the highest PRMT1 activity. As noted above, the activity of several PRMT isoforms is regulated by redox chemistry. In order to repair and prevent further oxidation we add 1 mM DTT and 1 mM EDTA to each enzyme reaction.

Time: In order to ensure initial rate conditions we typically follow enzyme activity for a total of no more than 10 min using 100 nM

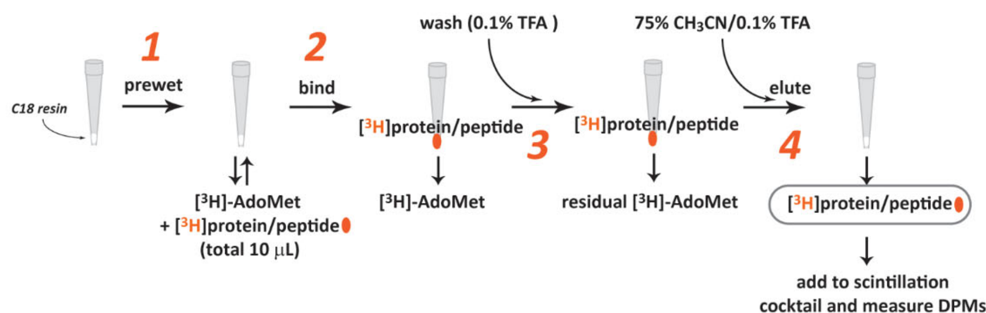

Fig. 4. Schematic showing methyltransferase assay workflow. The individual steps noted in the narrative are indicated by the orange numbers in the schematic. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

PRMT1. Different conditions, different substrates or PRMT isoforms may be more or less active. A total of 5–6 timepoints are taken from the enzyme reaction and processed with the ZipTips®.

Other solutions needed: Prewetting solution (75% acetonitrile in water), equilibration and wash solution (0.1% TFA in water) and elution solution (75% acetonitrile in 0.1% TFA) are required to process the samples using the ZipTips®.

2.1.2. Detailed protocol for PRMT1

Each PRMT1 activity assay is set up in the following manner (final concentrations):

Added to a tube on ice

1.0 µM unlabeled AdoMet
1.0 µM [*methyl*-^{3}H]-AdoMet (specific activity ~ 70 Ci/mmol, 0.55 µCi/µl)
50 mM Na_2HPO_4, pH 7.6
0.4 µM bovine serum albumin (BSA)
10 nM AdoHcy nucleosidase (MTAN)
1 mM DTT
1 mM EDTA
Added right before equilibration at 37 °C
100 nM PRMT1
Added to initiate the reaction
1 µM protein substrate or 200 µM R3 peptide substrate

Each reaction is equilibrated at 37 °C for 3 min and then initiated with peptide or protein substrate. The total volume of a typical reaction is 35 µL. Samples (5 µL) are removed at specified times (e.g., t = 0, 2, 4 and 6 min) and quenched in previously prepared tubes containing 6 µL of the quench buffer (8 M guanidine-HCL in 2.5% TFA).

Each time point is processed using the following protocol (also see Fig. 4): [Note that for clarity in this protocol the term "pipette up and down" means to place the pipette tip under the surface of the solution and leave it submerged as the solution is pipetted up and down across the resin. The term "Wash" means to pipette up the indicated solution and dispense it into a waste tube.]

1. A ZipTip$_{C18}$, one for each time point, is prewetted with 75% acetonitrile in deionized water by pipetting 10 µL up and down twice in a tube containing at least 500 µL of the wetting solution. The same wetting solution can be used to prewet multiple tips. After the tip has been prewetted, care should be taken not to move air through the resin. The resin is then equilibrated by washing twice with 10 µL of equilibration buffer (0.1% TFA in deionized water) for each wash.
2. Using the pre-equilibrated ZipTip $_{C18}$, each quenched reaction is pipetted up and down 20 times to bind the protein/peptide in the reaction to the C18 resin.
3. The ZipTip $_{C18}$ is washed 17 times with 10 µL of wash buffer (0.1% TFA in deionized water) to remove unreacted [*methyl*-^{3}H]-AdoMet.
4. Bound protein/peptide is eluted by pipetting up and down 10 times in 10 µL of elution buffer (75% acetonitrile in 0.1% TFA). Vapor from the elution buffer will build back-pressure in the pipette tip that tends to reduce the volume of solution that can be drawn into the pipette tip, however at this point the vapor can be pushed past the resin without affecting the data. Eluate is dispensed into an empty scintillation tube to which 5 mL of scintillation cocktail is added, and the sample is counted in a liquid scintillation counter. The total time to process each timepoint (not including scintillation counting) is $<$ 3 min.

Controls: Assays that lack the addition of PRMT1 provide the amount of background DPMs that bind and elute from the resin. Assays that lack the addition of a protein or peptide substrate evaluate possible automethylation to the PRMT enzyme itself. In our hands over the short time scale of 8 min, the two control assays usually give equivalent near-zero rates (Fig. 5).

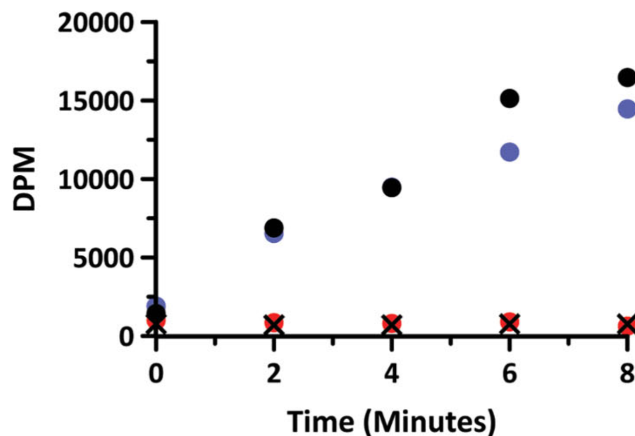

Fig. 5. Samples PRMT1 rates achieved with [*methyl*-^{3}H]-AdoMet and ZipTips®. Control reactions that did not contain substrate (red circles) or enzyme (black Xs) are shown. Full enzyme reactions in the presence (black circles) or absence (blue circles) of MTAN. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

TIPS:

1. Because AdoMet will hydrolyze in buffer solutions at pH 7.5–8, care should be taken to prepare only the reactions that will be run in one sitting, at one time.
2. For greatest efficiency when processing many samples, tubes containing sufficient volumes of the wetting, equilibration, and wash buffer should be measured out from larger stocks just prior to sample processing. For the previously mentioned buffers, one tube can be used to process many samples. However, for the elution buffer, one tube containing 10 µL of buffer should be prepared for

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each sample.
3. Use of a multichannel pipette to process many samples simultaneously is not recommended.
4. Using this protocol it is feasible for a single person to run multiple reactions simultaneously, however the start time for each reaction should be staggered by at least 30 s to allow adequate time to mix and sample each reaction before moving on to the next.
5. In order to quickly initiate the reaction and sample the t = 0 timepoint, two pipettes should be used. One pipette should be used to initiate and mix the reaction. The second pipette should be used to take the t = 0 timepoint and it should be set to the appropriate volume and loaded with a pipette tip prior to initiating the reaction.
6. Never allow the ZipTip® column to dry out during any steps preceding the elution step (step 4). Introducing air bubbles and channels in the resin can decrease the retention of peptide/proteins and introduce great variability into the assay.
7. We have observed that some small molecules will interfere with ZipTip processing. When testing PRMT effectors, a control reaction should be run using twice the normal volume (70 µL assay, 10 µL timepoints quenched in 12 µL of quench buffer). Each quenched timepoint should then be split into two equal volumes. This will yield two sets of samples in which the corresponding timepoints should give an identical signal. The effector should be added to one of these sets (after termination) in a volume of < 1 µL, and a blank should be added to the other set. The samples should then be processed with ZipTips as described above. If the effector does not interfere with ZipTip processing the signal from corresponding samples will be identical.

2.1.3. Variation, limitations and troubleshooting

In addition to [*methyl*-^{3}H]-AdoMet, [*methyl*-^{14}C]-AdoMet can also be used for greater sensitivity, but with more cost. Obviously, the key principle guiding success of this method is the ability to bind and elute the methylated product with near 100% efficiency. This can be tested by 1) radiolabeling a peptide/protein substrate with [*methyl*-^{3}H]-AdoMet and a PRMT, 2) splitting the sample into two equal parts and 3) counting one part as is and counting the other part after processing using the ZipTips. Counts from both samples should be the same. When tested with methylated hnRNP K, we found that binding and elution efficiencies were 100% [15]. Overloading the ZipTip columns would result in an underestimation of activity. Two of the greatest sources or error/variation in the assay come from pipetting. As noted above, once the tips become wet, they must remain wet until the final elution. Additionally, using a consistent, moderate pipetting speed is important. This is why we do not recommend a multi-channel pipette because the back pressure in each tip can vary, resulting in varied rates of adsorption and expulsion from the resin.

3. Concluding remarks

Monitoring the methylation rate of peptides and proteins can easily be accomplished using [*methyl*-^{3}H]-AdoMet, ZipTips®, pipettes, a water bath, and a liquid scintillation counter. The method is fast, produces very little waste, and directly reports on the desired methylated product.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ymeth.2019.10.002.

References

[1] W.K. Piak, S. Kim, Enzymatic methylation of protein fractions from calf thymus nuclei, Biochem. Biophys. Res. Commun. 29 (1967) 14–20.
[2] P.K. Woon, S. Kim, Protein methylase I, J. Biol. Chem. 243 (1968) 2108–2114.
[3] A.E. Raposo, S.C. Piller, Protein arginine methylation: an emerging regulator of the cell cycle, Cell Div. 13 (2018), https://doi.org/10.1186/s13008-018-0036-2.
[4] M.T. Bedford, S.G. Clarke, Protein arginine methylation in mammals: who, what, and why, Mol. Cell. 33 (2009) 1–13, https://doi.org/10.1016/j.molcel.2008.12.013.
[5] R.S. Blanc, S. Richard, Arginine methylation: the coming of age, Mol. Cell. 65 (2017) 8–24, https://doi.org/10.1016/j.molcel.2016.11.003.
[6] Y. Morales, T. Cáceres, K. May, J.M. Hevel, Biochemistry and regulation of the protein arginine methyltransferases (PRMTs), Arch. Biochem. Biophys. 590 (2016) 138–152, https://doi.org/10.1016/j.abb.2015.11.030.
[7] J. Sanzida, J. Davie, Protein arginine methyltransferases (PRMTs): role in chromatin organization, Adv. Biol. Regul. 57 (2015) 173–184.
[8] J. Fuhrmann, K.W. Clancy, P.R. Thompson, Chemical biology of protein arginine modifications in epigenetic regulation, Chem. Rev. 115 (2015) 5413–5461, https://doi.org/10.1021/acs.chemrev.5b00003.
[9] T. Teerlink, Z. Luo, F. Palm, C.S. Wilcox, Cellular ADMA: regulation and action, Pharmacol. Res. Off. J. Ital. Pharmacol. Soc. 60 (2009) 448–460, https://doi.org/10.1016/j.phrs.2009.08.002.
[10] T.B. Nicholson, T. Chen, S. Richard, The physiological and pathophysiological role of PRMT1-mediated protein arginine methylation, Pharmacol. Res. 60 (2009) 466–474, https://doi.org/10.1016/j.phrs.2009.07.006.
[11] Y. Yang, M.T. Bedford, Protein arginine methyltransferases and cancer, Nat. Rev. Cancer. 13 (2013) 37–50, https://doi.org/10.1038/nrc3409.
[12] J. Jarrold, C.C. Davies, PRMTs and arginine methylation: cancer's best-kept secret? Trends Mol. Med. (2019), https://doi.org/10.1016/j.molmed.2019.05.007.
[13] S.-C.M. Wang, D.H. Dowhan, G.E.O. Muscat, Epigenetic arginine methylation in breast cancer: emerging therapeutic strategies, J. Mol. Endocrinol. 62 (2019) R223–R237, https://doi.org/10.1530/JME-18-0224.
[14] J.H. Kim, B.C. Yoo, W.S. Yang, E. Kim, S. Hong, J.Y. Cho, The role of protein arginine methyltransferases in inflammatory responses, Mediators Inflamm. (2016), https://doi.org/10.1155/2016/4028353.
[15] B.B. Suh-Lailam, J.M. Hevel, A fast and efficient method for quantitative measurement of S-adenosyl-l-methionine-dependent methyltransferase activity with protein substrates, Anal. Biochem. 398 (2010) 218–224, https://doi.org/10.1016/j.ab.2009.09.005.
[16] K.M. Dorgan, W.L. Wooderchak, D.P. Wynn, E.L. Karschner, J.F. Alfaro, Y. Cui, Z.S. Zhou, J.M. Hevel, An enzyme-coupled continuous spectrophotometric assay for S-adenosylmethionine-dependent methyltransferases, Anal. Biochem. 350 (2006) 249–255, https://doi.org/10.1016/j.ab.2006.01.004.
[17] W.L. Wooderchak, Z.S. Zhou, J. Hevel, Assays for S-Adenosylmethionine (AdoMet/SAM)-Dependent Methyltransferases, Curr. Protoc. Toxicol. 38 (2008), https://doi.org/10.1002/0471140856.tx0426s38 4.26.1-4.26.12.
[18] J. Wu, N. Xie, Y. Feng, Y.G. Zheng, Scintillation proximity assay of arginine methylation, J. Biomol. Screen. 17 (2012) 237–244, https://doi.org/10.1177/1087057111414903.
[19] T.M. Lakowski, C. Zurita-Lopez, S.G. Clarke, A. Frankel, Approaches to measuring the activities of protein arginine N-methyltransferases, Anal. Biochem. 397 (2010) 1–11, https://doi.org/10.1016/j.ab.2009.09.021.
[20] M. Luo, Current chemical biology approaches to interrogate protein methyltransferases, ACS Chem. Biol. 7 (2012) 443–463, https://doi.org/10.1021/cb200519y.
[21] T.A. Klink, M. Staeben, K. Twesten, A.L. Kopp, M. Kumar, R.S. Dunn, C.A. Pinchard, K.M. Kleman-Leyer, M. Klumpp, R.G. Lowery, Development and validation of a generic fluorescent methyltransferase activity assay based on the transcreener AMP/GMP assay, J. Biomol. Screen. 17 (2012) 59–70, https://doi.org/10.1177/1087057111421624.
[22] G. Ibáñez, J.L. McBean, Y.M. Astudillo, M. Luo, An enzyme-coupled ultrasensitive luminescence assay for protein methyltransferases, Anal. Biochem. 401 (2010) 203–210, https://doi.org/10.1016/j.ab.2010.03.010.
[23] H. Gowher, X. Zhang, X. Cheng, A. Jeltsch, Avidin plate assay system for enzymatic characterization of a histone lysine methyltransferase, Anal. Biochem. 342 (2005) 287–291, https://doi.org/10.1016/j.ab.2005.04.028.
[24] E. Collazo, J.-F. Couture, S. Bulfer, R.C. Trievel, A coupled fluorescent assay for histone methyltransferases, Anal. Biochem. 342 (2005) 86–92, https://doi.org/10.1016/j.ab.2005.04.007.
[25] P. Rathert, X. Cheng, A. Jeltsch, Continuous enzymatic assay for histone lysine methyltransferases, BioTechniques. 43 (2007) 602–608, https://doi.org/10.2144/000112623.
[26] H. Guo, R. Wang, W. Zheng, Y. Chen, G. Blum, H. Deng, M. Luo, Profiling substrates of protein arginine n-methyltransferase 3 with s-adenosyl-L-methionine analogues, ACS Chem. Biol. 9 (2014) 476–484, https://doi.org/10.1021/cb4008259.
[27] Q. Lin, F. Jiang, P.G. Schultz, N.S. Gray, Design of allele-specific protein methyltransferase inhibitors, J. Am. Chem. Soc. 123 (2001) 11608–11613, https://doi.org/10.1021/ja011423j.

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[28] K.C. Catcott, J. Yan, W. Qu, V.H. Wysocki, Z.S. Zhou, Identifying unknown enzyme-substrate pairs from the cellular milieu with native mass spectrometry, ChemBioChem. 18 (2017) 613–617, https://doi.org/10.1002/cbic.201600634.

[29] J. Deen, C. Vranken, V. Leen, R.K. Neely, K.P.F. Janssen, J. Hofkens, Methyltransferase-directed labeling of biomolecules and its applications, Angew. Chem. Int. Ed. 56 (2017) 5182–5200, https://doi.org/10.1002/anie.201608625.

[30] M.S. Eram, S.P. Bustos, E. Lima-Fernandes, A. Siarheyeva, G. Senisterra, T. Hajian, I. Chau, S. Duan, H. Wu, L. Dombrovski, M. Schapira, C.H. Arrowsmith, M. Vedadi, Trimethylation of histone H3 lysine 36 by human methyltransferase PRDM9 protein, J. Biol. Chem. 289 (2014) 12177–12188, https://doi.org/10.1074/jbc.M113.523183.

[31] T.M. Lakowski, A. Frankel, Sources of S-adenosyl-l-homocysteine background in measuring protein arginine N-methyltransferase activity using tandem mass spectrometry, Anal. Biochem. 396 (2010) 158–160, https://doi.org/10.1016/j.ab.2009.08.043.

[32] Y. Morales, D.V. Nitzel, O.M. Price, S. Gui, J. Li, J. Qu, J.M. Hevel, Redox control of protein arginine methyltransferase 1 (PRMT1) activity, J. Biol. Chem. (2015), https://doi.org/10.1074/jbc.M115.651380.

[33] T.B. Cáceres, A. Thakur, O.M. Price, N. Ippolito, J. Li, J. Qu, O. Acevedo, J.M. Hevel, Phe71 in Type III trypanosomal protein arginine methyltransferase 7 (TbPRMT7) restricts the enzyme to monomethylation, Biochemistry 57 (2018) 1349–1359, https://doi.org/10.1021/acs.biochem.7b01265.

[34] S. Gui, W.L. Wooderchak, M.P. Daly, P.J. Porter, S.J. Johnson, J.M. Hevel, 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 (2011) 29118–29126, https://doi.org/10.1074/jbc.M111.224097.

[35] C. Wang, Y. Zhu, T.B. Caceres, L. Liu, J. Peng, J. Wang, J. Chen, X. Chen, Z. Zhang, X. Zuo, Q. Gong, M. Teng, J.M. Hevel, J. Wu, Y. Shi, Structural determinants for the strict monomethylation activity by trypanosoma brucei protein arginine methyltransferase 7, Structure 22 (2014) 756–768, https://doi.org/10.1016/j.str.2014.03.003.

[36] J. Najbauer, B.A. Johnson, A.L. Young, D.W. Aswad, Peptides with sequences similar to glycine, arginine-rich motifs in proteins interacting with RNA are efficiently recognized by methyltransferase(s) modifying arginine in numerous proteins, J. Biol. Chem. 268 (1993) 10501–10509.

[37] S. Gui, W.L. Wooderchak-Donahue, T. Zang, D. Chen, M.P. Daly, Z.S. Zhou, J.M. Hevel, Substrate-induced control of product formation by protein arginine methyltransferase 1, Biochemistry 52 (2013) 199–209, https://doi.org/10.1021/bi301283t.

[38] T.C. Osborne, O. Obianyo, X. Zhang, X. Cheng, P.R. Thompson, Protein arginine methyltransferase 1: positively charged residues in substrate peptides distal to the site of methylation are important for substrate binding and catalysis, Biochemistry 46 (2007) 13370–13381, https://doi.org/10.1021/bi701558t.

[39] W.L. Wooderchak, T. Zang, Z.S. Zhou, M. Acuña, S.M. Tahara, J.M. Hevel, Substrate profiling of PRMT1 reveals amino acid sequences that extend beyond the “RGG” paradigm †, Biochemistry 47 (2008) 9456–9466, https://doi.org/10.1021/bi800984s.

[40] M. Wang, R.-M. Xu, P.R. Thompson, Substrate specificity, processivity, and kinetic mechanism of protein arginine methyltransferase 5, Biochemistry 52 (2013) 5430–5440, https://doi.org/10.1021/bi4005123.

[41] R.M.C. Dawson, D.C. Elliott, W.H. Elliott, K.M. Jones, Data for Biochemical Research, Third, Oxford University Press, New York, 1989.

[42] S.E. Wu, W.P. Huskey, R.T. Borchardt, R.L. Schowen, Chiral instability at sulfur of S-adenosylmethionine, Biochemistry 22 (1983) 2828–2832, https://doi.org/10.1021/bi00281a009.

[43] J.L. Hoffman, Chromatographic analysis of the chiral and covalent instability of S-adenosyl-L-methionine, Biochemistry 25 (1986) 4444–4449, https://doi.org/10.1021/bi00363a041.

[44] L.M. Cannon, F.N. Butler, W. Wan, Z.S. Zhou, A stereospecific colorimetric assay for (S, S)-adenosylmethionine quantification based on thiopurine methyltransferase-catalyzed thiol methylation, Anal. Biochem. 308 (2002) 358–363, https://doi.org/10.1016/s0003-2697(02)00267-1.

[45] J. Zhang, J.P. Klinman, HPLC separation of the (S, S)- and (R, S)- forms of S-adenosyl-L-methionine, Anal. Biochem. 476 (2015) 81–83, https://doi.org/10.1016/j.ab.2015.02.004.

[46] O. Obianyo, T.C. Osborne, P.R. Thompson, Kinetic mechanism of protein arginine methyltransferase 1, Biochemistry 47 (2008) 10420–10427, https://doi.org/10.1021/bi800904m.

[47] T.M. Lakowski, A. Frankel, A kinetic study of human protein arginine N-methyltransferase 6 reveals a distributive mechanism, J. Biol. Chem. 283 (2008) 10015–10025, https://doi.org/10.1074/jbc.M710176200.

[48] O. Obianyo, P.R. Thompson, Kinetic mechanism of protein arginine methyltransferase 6 (PRMT6), J. Biol. Chem. 287 (2012) 6062–6071, https://doi.org/10.1074/jbc.M111.333609.

[49] M.B.C. Dillon, H.L. Rust, P.R. Thompson, K.A. Mowen, Automethylation of protein arginine methyltransferase 8 (prmt8) regulates activity by impeding S-adenosylmethionine sensitivity, J. Biol. Chem. 288 (2013) 27872–27880, https://doi.org/10.1074/jbc.M113.491092.

[50] S.L. Jacques, K.P. Aquino, J. Gureasko, P.A. Boriack-Sjodin, M. Porter Scott, R.A. Copeland, T.V. Riera, CARM1 preferentially methylates H3R17 over H3R26 through a random kinetic mechanism, Biochemistry. 55 (2016) 1635–1644, https://doi.org/10.1021/acs.biochem.5b01071.

[51] J.I. Brown, T. Koopmans, J. van Strien, N.I. Martin, A. Frankel, Kinetic analysis of PRMT1 reveals multifactorial processivity and a sequential ordered mechanism, ChemBioChem. 19 (2018) 85–99, https://doi.org/10.1002/cbic.201700521.

[52] C.L. Hendricks, J.R. Ross, E. Pichersky, J.P. Noel, Z.S. Zhou, An enzyme-coupled colorimetric assay for S-adenosylmethionine-dependent methyltransferases, Anal. Biochem. 326 (2004) 100–105, https://doi.org/10.1016/j.ab.2003.11.014.