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Comprehending dynamic protein methylation with mass spectrometry

Leila Afjehi-Sadat[1,2] and Benjamin A Garcia[1,2]

Protein methylation is a post-translational modification (PTM) which modulates cellular and biological processes including transcription, RNA processing, protein interactions and protein dynamics. Methylation, catalyzed by highly specific methyltransferase enzymes, occurs on several amino acids including arginine, lysine, histidine and dicarboxylic amino acids like glutamate. Mass spectrometry (MS)-based techniques continue to be the methods of choice for the study of protein PTMs. These approaches are powerful and sensitive tools that have been used to identify, quantify and characterize protein methylation. In addition, metabolic labeling strategies can be coupled to MS detection in order to measure dynamic and differential *in vivo* protein methylation rates. In this review, different applications of mass spectrometry technologies and methods to study protein methylation are discussed.

Addresses
[1] Epigenetics Program, Perelman School of Medicine, University of Pennsylvania, 1009C Stellar-Chance Laboratories, 422 Curie Boulevard, Philadelphia, PA 19104, USA
[2] Department of Biochemistry and Biophysics, Perelman School of Medicine, University of Pennsylvania, 1009C Stellar-Chance Laboratories, 422 Curie Boulevard, Philadelphia, PA 19104, USA

Corresponding author: Garcia, Benjamin A (bgarci@mail.med.upenn.edu)

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Introduction

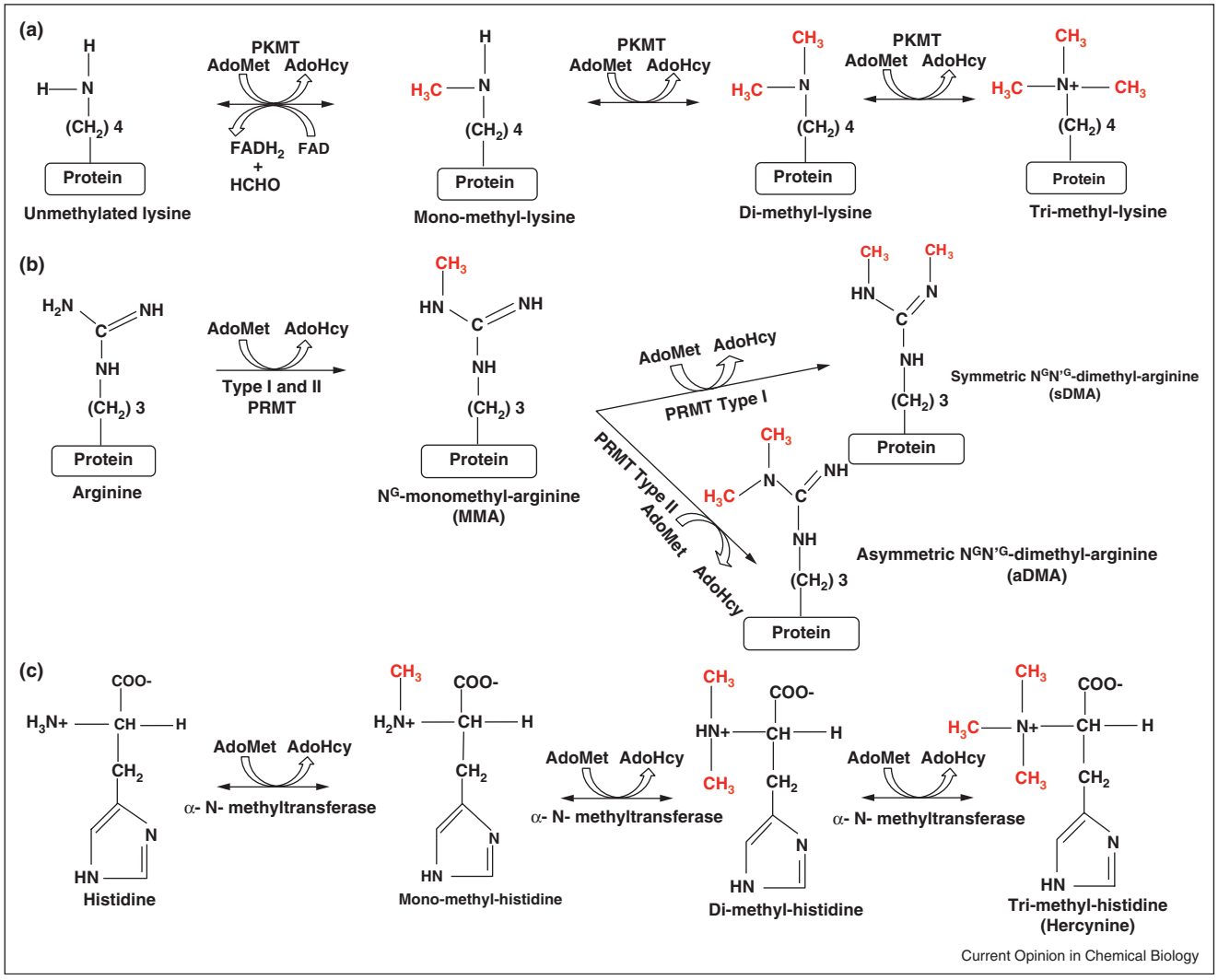
Post-translational modifications (PTMs) play crucial roles in modulating protein activity, turnover, and protein–protein interactions. Protein methylation is a fairly common type of protein PTM and has been implicated in several biological processes such as transcriptional regulation, RNA processing, metabolism and signal transduction [1]. Although methylation has been most commonly observed on lysine and arginine residues, methylation of other amino acids including histidine (H), cysteine (C), aspartic acid (D), glutamic acid (E), serine (S) and threonine (T) has been reported [2••,3]. Lysine methylation occurs by transferring one to three methyl groups from *S*-adenosyl-methionine (SAM) to the lysine ε-amine side chain, which leads to mono-methylated (me1), di-methylated (me2) or tri-methylated (me3) lysines (Figure 1). In the case of arginine, one or two methyl groups are added to its guanidine group which leads to mono-methylation or di-methylation [2••] (Figure 1). Methylation-specific enzymes (methyltransferases) can read specific protein sequence/motifs and further propagate existing methylation marks [2••]. For example, arginine methyltransferase enzymes often target proteins sequence including an RGG-RNA binding motif [4]. Furthermore, methylation has been shown to depend on a protein's existing methylation state and to be a dynamic modification. For instance, methylated lysines within histones have been shown to have measurable differential turnover rates [5].

PTMs, including protein methylation, have been traditionally identified by Edman degradation, amino acid analysis, radio isotope labeling or antibody-based methods including peptide and protein arrays. These methods suffer from being unspecific, low throughput, and having a low dynamic range for quantitative measurements. In addition, they fail to identify specific modification sites, cannot distinguish methylation state, and they often rely on prior knowledge of the modification. For instance, protein methylation has been detected by radioactive methods (review in [6•]) including using tritiated methyltransferase cofactor *S*-adenosyl methionine (SAM) as a methyl-donor. The weakness of this radioactive method is that radioisotopes of carbon and hydrogen are weak radio emitters and it is difficult to detect modified peptides efficiently. Another issue with current technologies is the small size of the methyl group which makes it challenging to develop high quality methylation specific antibodies. Protein methylation substrates can be identified by protein and peptide arrays, however, any hit needs to be validated with purified endogenous proteins by mass spectrometry. In recent years, MS-based methodology has proven to be superior for the analysis of PTMs including methylation due to improvements in the accuracy and sensitivity of MS instrumentation. MS methods have been developed to identify proteins carrying PTMs, to map (novel) PTM sites, to quantify the changes in PTM abundance at individual sites, and to characterize the cooperativity between interrelated PTMs at several sites on proteins [6•,7,8,9••] (Figure 2).

Mass spectrometry-based technology for PTM analysis: application to protein methylation

Post-translational modifications are functional groups including chemical species (phosphate, carbohydrate or

Figure 1



Biochemical mechanism of lysine, arginine and histidine methylation. **(a)** Lysine methylation: formation of mono-methylated, di-methylated and tri-methylated lysine by adding methyl group to ε amine of lysine residue. Conversion of *S*-adenosyl-L-methionine (AdoMet) to *S*-adenosyl-L-homocysteine (AdoHcy) leads to methyl group transfer to a protein. The methylation reaction is catalyzed by protein lysine-methyl-transferase (PKMT). The reversibility of the methylation reaction in presence of Fe(II) and α-ketoglutarate has been proved by the discovery of a demethylase. **(b)** Arginine methylation: addition of methyl groups to guanidine nitrogens of arginine forms NG -monomethyl arginine, NGN′G -dimethyl arginine — symmetric (sDMA) and asymmetric (aDMA). Type-I and type-II protein arginine methyltransferase (PRMT) are the catalytic enzyme for arginine methylation. **(c)** Histidine methylation: a methyl group will be added to the α-amino nitrogen atom of histidine and result in mono-methyl-histidine, di-methyl-histidine and tri-methyl-histidine (Hercynine). Histidine methylation is catalyzed by a single enzyme, histidine-α-*N*-methyltransferase [3].

methyl group) and functional polypeptides (ubiquitin and SUMO). PTMs can be added or removed from an amino acid side chain or protein termini or created by the cleavage of signal peptides from proteins or by covalent cross-linking between separate protein domains [10•]. These chemical changes on modified amino acids form a mass shift that can be measured by mass spectrometry (MS). Tandem mass spectrometry (MS/MS) provides valuable information about modified peptides. However, during MS/MS sequencing, it can be challenging to assign the mass shifts because the identified mass shifts may represent isobaric modifications or the sum of a few modifications. For example, the mass difference between tri-methylation (+42.05 Da) and acetylation (+42.01 Da) is very small (0.0364 Da) and can only be discriminated within <30 ppm mass accuracy on sensitive instruments such as the Fourier-Transform Ion Cyclotron MS (FT-ICR-MS) or Orbitrap systems. An additional way to discriminate between tri-methylation and acetylation is by the presence of diagnostic marker ions and neutral loss in MS/MS spectra [11].