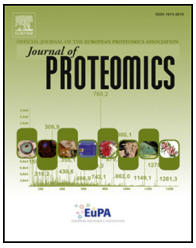
JOURNAL OF PROTEOMICS 114 (2015) 226–233

Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/jprot



Proteomic analysis of protein methylation in the yeast *Saccharomyces cerevisiae*

Keyun Wang[a,b,1], *Yongjin J. Zhou*[a,1], *Hongwei Liu*[a,1], *Kai Cheng*[a,b], *Jiawei Mao*[a,b], *Fangjun Wang*[a], *Wujun Liu*[a], *Mingliang Ye*[a], *Zongbao K. Zhao*[a,*], *Hanfa Zou*[a,*]

[a]*Division of Biotechnology, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, China*
[b]*University of Chinese Academy of Sciences, Beijing 100049, China*

ARTICLE INFO

Article history:
Received 24 January 2014
Accepted 20 July 2014

Keywords:
Saccharomyces cerevisiae
Methylation
Proteomics
LC/MS/MS

ABSTRACT

Protein methylation catalyzed by SAM-dependent methyltransferase represents a major PTM involved in many important biological processes. Because methylation can occur on nitrogen, oxygen and sulfur centers and multiple methylation states exist on the nitrogen centers, methylproteome remains poorly documented. Here we present the *m*ethylation by isotope labeled SAM (MILS) strategy for a highly-confident analysis of the methylproteome of the yeast *Saccharomyces cerevisiae* based on the online multidimensional μHPLC/MS/MS technology. We identified 43 methylated proteins, containing 68 methylation events associated with 64 methylation sites. More than 90% of these methylation events were previously unannotated in Uniprot database. Our results indicated, 1) over 2.6% of identified *S. cerevisiae* proteins are methylated, 2) the amino acid residue preference of protein methylation follows the order Lys ≫ Arg > Asp > Asn ≈ Gln ≈ His > Glu > Cys, and 3) the methylation state on nitrogen center is largely exclusive. As our dataset covers various types of methylation centers, it provides rich information about yeast methylproteome and should significantly contribute to the field of protein methylation.

Biological significance

In this paper, we presented the *m*ethylation by isotope labeled SAM (MILS) strategy for a highly-confident analysis of the methylproteome of the yeast *S. cerevisiae* and collected a comprehensive list of proteins methylated on a set of distinct residues (K, R, N, E, D, Q, H, C). Our study provided useful information about the amino acid residue preference and methylation state distributions on nitrogen centers of protein methylation in *S. cerevisiae*.

1. Introduction

Protein methylation catalyzed by SAM-dependent methyltransferase represents a major PTM [1] involved in many important biological processes [2–4]. Protein methylation can occur on nitrogen, oxygen and sulfur centers and multiple methylation states, namely, mono-, di- and trimethylation, can occur on

Abbreviations: SAM, S-adenosyl-L-methionine; PTM, post-translational modification; HPLC-MS/MS, high performance liquid chromatography tandem mass spectrometry; FDR, false discovery rate.

* *Corresponding authors at: Division of Biotechnology, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian 116023, China.* Tel.: +86 411 84379066, +86 411 84379610 ; fax: +86 411 84379211, +86 411 84379620.
E-mail addresses: zhaozb@dicp.ac.cn (Z.K. Zhao), hanfazou@dicp.ac.cn (H. Zou).

[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.jprot.2014.07.032
1874-3919/

nitrogen center [5]. The dynamic nature of protein methylation further promotes efforts to understand the importance and function of this PTM [3,6]. Irrespective of copious publications in this field, studies have been extensively focused on the N-methylation on Lys or Arg residue [2–4,7–13]. Because the introduction of methyl groups within a protein usually has little effect in terms of analytical properties [2], challenges are remarkable for a global analysis of the methylated protein. Therefore, a comprehensive survey of methylproteome of *Saccharomyces cerevisiae* remains elusive [14–17]. Several fundamental questions in the field remain unanswered. For example, to what extent a proteome is methylated? Are there any preferences among different methylation centers or amino acid residues? In the case of methylation on nitrogen center, what is the distribution profile for different methylation states? Here we present the methylproteome of the yeast *S. cerevisiae* and the *m*ethylation by *i*sotope *l*abeled SAM (MILS) strategy dedicated for the analysis of the SAM-auxotrophic yeast [18] proteome based on the online multidimensional μHPLC/MS/MS technology. Our dataset addressed most of those questions and provided rich information for further research on protein methylation.

Several strategies have been applied to identify protein methylation. Traditional [methyl-^{3}H] labeling in combination with fluorography is laborious and it is difficult to locate the methylation sites [19]. The data processing tool FindMod was used to analyze partial yeast proteome and 83 methylation events on Lys or Arg were found [17]. The approach combining antibody-enrichment of Arg-methylated proteins and MS analysis identified 200 putatively methylated proteins, yet exact methylation sites were unavailable [14]. In recent years, antibody enrichment was widely utilized to the global identification of methylation sites on Lys or Arg residue [8–11]. It should be noted that the use of pan-antibody significantly advanced the detection of protein methylome, however, the enrichment efficiency remains to be improved. The strategy based on stable isotope labeling by amino acids in cell culture was also employed to quest protein methylation, where the cell culture is supplemented with [$^{13}CD_3$]methionine, the biological precursor of labeled methyl donor, [$^{13}CD_3$]SAM [8–10,15]. In this method, the confidence of identified methylation sites was enhanced by the presence of MS ion pairs. However, a few issues are obvious. First, [$^{13}CD_3$]methionine per se can also be incorporated into the proteome, which would complicate the MS data analysis. Moreover, SAM-dependent transmethylation activities co-produce S-adenosylhomocysteine (SAH), which can be hydrolyzed to homocysteine. Methionine synthase, an essential housekeeping enzyme for sulfur and cofactor metabolism, can methylate homocysteine to produce unlabeled methionine depending on methyltetrahydrofolate [20]. Such an inevitable cellular process can dilute the labeled methyl donor, leading to complication in data analysis and high false negative results.

In this study, we designed the MILS strategy to analyze the yeast methylproteome by taking the advantage that yeast can take up sulfonium compounds including SAM from the culture medium. When [CD_3]SAM was used, the SAM-auxotrophic yeast cells generated [CD_3]-modifications by protein methyltransferases. Because the biosynthetic route of methionine to SAM was blocked, the formation of fresh, regular SAM from the methylation co-product SAH upon [CD_3]SAM consumption was not possible. This prevented regular methylation events. Moreover, we showed that the conversion of [CD_3]SAM into [CD_3]methionine was negligible, which prevented the incorporation of [CD_3]methionine into protein biosynthesis. Therefore, the MILS strategy had higher labeling specificity over the conventional use of [CD_3]methionine, which could reduce false negative results and increase the confidence of our dataset. A total of 43 methylated proteins were identified, containing 68 methylation events associated with 64 methylation sites. More than 90% of these methylation events were previously unannotated in the Uniprot database or in the Pang's study [17].

2. Materials and methods

2.1. SAM and [CD_3]SAM synthesis

The AdoMet synthetase gene *MetK* was PCR amplified from the *Escherichea coli* genome with the primer pair MetK-F/MetK-R. *MetK* and expression vector pET28a (Invitrogen) were digested with *Nde* I and *EcoR* I and ligated, resulting in pMetK, which was transformed into *E. coli* Bl21 (DE3). The procedure for preparation of SAM synthase was modified from a previous study [21]. Briefly, Bl21(DE3) (pMetK) was cultivated at 30 °C and 200 rpm in 500 mL Luria–Bertani (LB) rich medium with 50 μg/mL kanamycin sulfate up to an optical density at 600 nm (OD_{600}) of 0.4–0.6. Then, 0.2 mM isopropyl β-D-1-thiogalactopyranoside was added to induce the recombinant protein expression. After 12 h, the cells were collected by centrifugation (2000 *g*, 5 min), resuspended in 50 mL of 100 mM Tris–HCl buffer (pH 8.0) containing 1 mM EDTA, and treated with 50 μg/mL lysozyme at 30 °C for 30 min. After adding phenylmethylsulfonyl fluoride to a final concentration of 0.1 mM, the cells were lysed by ultrasonication in an ice bath and the lysate was centrifuged for 20 min at 12,000 *g* in a Hitachi 46 rotor to remove the cell debris. After dialysis against Tris–KCl buffer which contains 50 mM Tris–HCl, 50 mM KCl and 1 mM EDTA (pH 8.0), the crude AdoMet synthase was either used immediately or stored at –20 °C with 20% glycerol until needed.

Enzymatic preparation of SAM and [CD_3]SAM was performed in 90 mL of 100 mM Tris–HCl buffer (pH 8.0) in the presence of 50 mM KC1, 26 mM $MgC1_2$, 1 mM EDTA, 20% (v/v) acetonitrile, 13 mM ATP, 10 mM methionine or [CD_3]methionine, and 10 mL of crude AdoMet synthase. The reaction was incubated at 30 °C for 12 h, and the progress of the reaction was analyzed by thin layer chromatography. Acetonitrile was removed under reduced pressure. The aqueous phase was centrifuged, and the supernatant was collected, concentrated, re-dissolved in 40 mL of 1.0 M HCl, and centrifuged again to remove residual protein. The supernatant was concentrated and the residue was re-dissolved in 30 mL of H_2O, and passed through an IRC86 cation resin column (formate form, elute: H_2O, 0.02 N HCl to 0.04 N HCl). The absorbance of the elute was monitored at 254 nm, and SAM or [CD_3]SAM fractions were collected, and lyophilized to give a white solid (150 mg, 95%). ^{1}H-NMR spectra of synthetic SAM and [CD_3]SAM were shown in Fig. S1.