Biochemical and Biophysical Research Communications 372 (2008) 811–815

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

# Hsl7 is a substrate-specific type II protein arginine methyltransferase in yeast

Joyce Sayegh, Steven G. Clarke *

*Department of Chemistry and Biochemistry and the Molecular Biology Institute, UCLA, 607 Charles E. Young Drive East, Los Angeles, CA 90095-1569, USA*

ARTICLE INFO

*Article history:*
Received 20 May 2008
Available online 2 June 2008

*Keywords:*
Cell cycle
Chromatin modification
Protein arginine methylation
Posttranslational modification of protein
Histones
Methyltransferases

ABSTRACT

The *Saccharomyces cerevisiae* protein Hsl7 is a regulator of the Swe1 protein kinase in cell cycle checkpoint control. Hsl7 has been previously described as a type III protein arginine methyltransferase, catalyzing the formation of ω-monomethylarginine residues on non-physiological substrates. However, we show here that Hsl7 can also display type II activity, generating symmetric dimethylarginine residues on calf thymus histone H2A. Symmetric dimethylation is only observed when enzyme and the methyl-accepting substrate were incubated for extended times. We confirmed the Hsl7-dependent formation of symmetric dimethylarginine by amino acid analysis and thin layer chromatography with wild-type and mutant recombinant enzymes expressed from both bacteria and yeast. This result is significant because no type II activity has been previously demonstrated in *S. cerevisiae*. We also show that Hsl7 has little or no activity on GST-GAR, a commonly used substrate for protein arginine methyltransferases, and only minimal activity on myelin basic protein. This enzyme thus may only recognize only a small subset of potential substrate proteins in yeast, in contrast to the situation with Rmt1, the major type I methyltransferase.

© 2008 Elsevier Inc. All rights reserved.

Methylation and demethylation of lysine and arginine residues of the N-terminal tails of histones play crucial roles in the regulation of gene expression [1]. In the budding yeast *Saccharomyces cerevisiae*, *HSL7* was identified as an essential gene in mutants where the histone H3 tail was deleted [2]. The amino acid sequence of Hsl7 is similar to those of known yeast and mammalian protein arginine methyltransferases [3,4]. It is most similar (27.5% identity over 680 residues) to PRMT5, the predominant type II protein arginine methyltransferase of mammalian cells that catalyzes symmetric dimethylarginine formation in a variety of methyl-accepting substrates including histone H3 [5,6]. However, such an enzymatic function has not been shown to date for Hsl7. Indeed, evidence has been presented that Hsl7 is not involved in histone arginine methylation in *S. cerevisiae,* although it has a robust type III activity forming ω-monomethylarginine with mammalian histone H2A [7]. Hsl7 is present in a complex at the yeast bud neck with the Hsl1 and Swe1 proteins that acts to monitor septin assembly as a cell cycle checkpoint [8–11]. Disruption of the *HSL7* gene in *S. cerevisiae* leads to an elongated bud phenotype and a delay in $G_2$ of the cell cycle [2]. How this function of Hsl7 may be linked to chromatin modification in the nucleus is unclear, although it has been suggested that histone tail methylation and acetylation may act to bypass the morphogenesis checkpoint [11].

It was initially reported that Hsl7, expressed as a FLAG-tagged fusion protein in wild-type yeast cells, had methyltransferase activity on substrates that included calf thymus histones H2A and H4 as well as myelin basic protein [12]. However, later studies were not able to detect methyltransferase activity in GST-Hsl7 or Hsl7-myc fusion proteins expressed in yeast or bacterial cells using a variety of methyl-accepting substrates [13]. A complication of these studies is that Hsl7 purified from wild-type yeast cells can be contaminated with the major endogenous protein arginine methyltransferase Rmt1 [7]. When a FLAG-tagged fusion of Hsl7 was purified from yeast cells lacking the Rmt1 methyltransferase, a preparation was obtained that was unable to methylate histone H4 or myelin basic protein, but that was able to readily methylate calf thymus histone H2A [7]. Two mutant constructs of Hsl7 with either a deletion of the GAGRG sequence of its methyltransferase motif I or a double mutation of the motif to GAVRV were unable to catalyze any methylation reaction, demonstrating that the activity observed with the wild-type enzyme on histone H2A was in fact due to Hsl7 methyltransferase activity [7]. Interestingly, Hsl7 is not essential for the major protein arginine methylation modification of endogenous *S. cerevisiae* histones [7]. Amino acid analysis indicated that the only product of the Hsl7 enzymatic reaction was ω-monomethylarginine, suggesting that this enzyme was not in fact the catalytic homolog of the mammalian PRMT5 enzyme, which is clearly a type II enzyme able to catalyze the addition of a second methyl group to the arginine residue to form symmetric dimethylarginine [5], but a potentially novel type III methyltransferase.

In this study we ask if Hsl7 has the ability to form dimethylated arginine residues if incubated for extensive times with methyl-

* Corresponding author. Fax: +1 310 825 1968.
*E-mail address:* clarke@mbi.ucla.edu (S.G. Clarke).

0006-291X/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2008.05.121

accepting substrates. We show here that Hsl7 can in fact act as a type II protein arginine methyltransferase, forming symmetric dimethylarginine like the mammalian PRMT5 enzyme. However, Hsl7 is clearly not the functional homolog of PRMT5; the endogenous substrates of Hsl7 still remain to be identified.

## Materials and methods

*Purification of GST-GAR, GST-Hsl7 wild-type and GST-Hsl7$^{G387A}$.* GST-GAR is a fusion protein containing the first 148 amino acids of human fibrillarin [19]. GST-Hsl7 and GST-Hsl7$^{G387A}$ are fusion proteins expressed from plasmids pDLB2211 and pDLB2212, respectively. These plasmids were obtained from Dr. Daniel Lew at the Duke University Medical Center and were constructed as described in Theesfeld et al. [14] to encode the wild-type enzyme and a form with a glycine to alanine substitution in the second conserved glycine residue of motif I (ILVAGAGRG to ILVAGAARG). Plasmids encoding these proteins were used to transform *Escherichia coli* strains DH5α (GST-GAR) and BL21 (GST-Hsl7 and GSTHsl7$^{G387A}$); in each case cells were grown in 1 L LB media in the presence of 100 μg/ml ampicillin at 37 °C until an optical density of $OD_{600}$ of 0.6 was obtained. The cells were induced with 0.4 mM isopropyl-β-D-thiogalactopyranoside (IPTG) at 25 °C for 16 h, and the cells were harvested by centrifugation at 5000*g* and washed twice with phosphate-buffered saline solution (PBS) containing 137 mM NaCl, 2.7 mM KCl, 1.4 mM $KH_2PO_4$, and 4.3 mM $NaHPO_4$, pH 7.4. The cells were sonicated on ice in the presence of protease inhibitor (100 μM of phenylmethylsulfonyl fluoride from a 1000-fold concentrated stock in DMSO). Lysed cells were centrifuged at 23,300*g* for 50 min at 4 °C and the supernatant was mixed with 0.5 ml of glutathione–sepharose 4B beads (GE Healthcare Biosciences AB, Uppsala, Sweden). After three washes with 10 ml of PBS, the GST-fusion proteins were eluted with 1 ml of 30 mM glutathione, 50 mM Tris–HCl, pH 7.5, and 120 mM NaCl.

*Expression and purification of wild-type and mutant FLAG-HSL7* in *S. cerevisiae*. A yeast-*E. coli* shuttle vector containing a gene engineered to express a FLAG-tagged fusion of Hsl7 (pTKB-FLAG-Hsl7; [12]) was a gift from Dr. Sidney Pestka at Rutgers University. A plasmid expressing a site-directed double mutant (FLAG-Hsl7 (GAGRG→GAVRV)) where valine residues replace the glycine residues at position 387and 389 of Hsl7 [7] was transformed into *S. cerevisiae* strain AFY5130 (*MAT**a**, ade2, rmt1::LEU2, hsl7::URA3*) [7]. Expressed FLAG-tagged proteins were purified as described [7].

*In vitro methylation of GST-GAR, H2A, and MBP.* Methylation reactions were performed in duplicate using 2 μg of bacterially expressed GST-Hsl7 and GST-Hsl7$^{G387A}$ in the presence and absence of 10 μg of the substrates GST-GAR, H2A (purified from calf thymus, Roche Molecular Chemical, Indianapolis, IN), or MBP (purified from bovine brain, lyophilized powder, product M1891, Sigma Chemical, St. Louis, MO), and 0.61 μM *S*-adenosyl-L-[*methyl*-$^3$H]methionine ([$^3$H] AdoMet, 81.0 Ci/mmol, dilute HCl: ethanol (9:1), pH 2.0–2.5, Amersham Pharmacia Biotech, Piscataway, NJ) in 100 mM sodium phosphate buffer (pH 7.5) in a final volume of 60 μl for 1, 5, or 20 h at 37 °C. Reactions were incubated at 37 °C for 1, 5, and 20 h, and frozen at −20 °C. Similar methylation reactions were performed with 2 μg of *S. cerevisiae* expressed wild-type FLAG-Hsl7 or FLAG-Hsl7$^{G387V,\ G389V}$ enzyme and 10 μg of calf thymus H2A. These reactions were incubated for 20 h at 30 °C and stored at −20 °C.

*Chemical analysis of* $^3$H-*methylated species by amino acid analysis.* Proteins from *in vitro* methylation reactions were precipitated in 60 μl of 25% (w/v) trichloroacetic acid with 20 μg of bovine serum albumin as a carrier, then hydrolyzed as previously described [15]. The hydrolyzed sample was added to 50 μl of water and 500 μl of citrate buffer (0.2 M $Na^+$, pH 2.2) along with 1.0 μmol each of unlabeled standards of ω-$N^G$-monomethylarginine (acetate salt, Sigma Chemical, St. Louis, MO)(ω-MMA) and ω-$N^G$, $N^G$-dimethylarginine (hydrochloride, Sigma Chemical, St. Louis, MO)(ADMA), and ω-$N^G$, $N'^G$-dimethylarginine (di(*p*-hydroxyazobenzene-*p*′-sulfonate) salt, Sigma Chemical, St. Louis, MO)(SDMA). Amino acids were separated by high resolution cation-exchange chromatography as described [15].

## Results and discussion

*Yeast Hsl7 is a type II protein arginine methyltransferase forming symmetric dimethylarginine residues*

Amino acid analysis of short term incubation mixtures of wild-type and mutant GST-Hsl7 with [$^3$H] AdoMet and calf thymus histone H2A demonstrated that the GST-Hsl7 fusion protein is an ac-

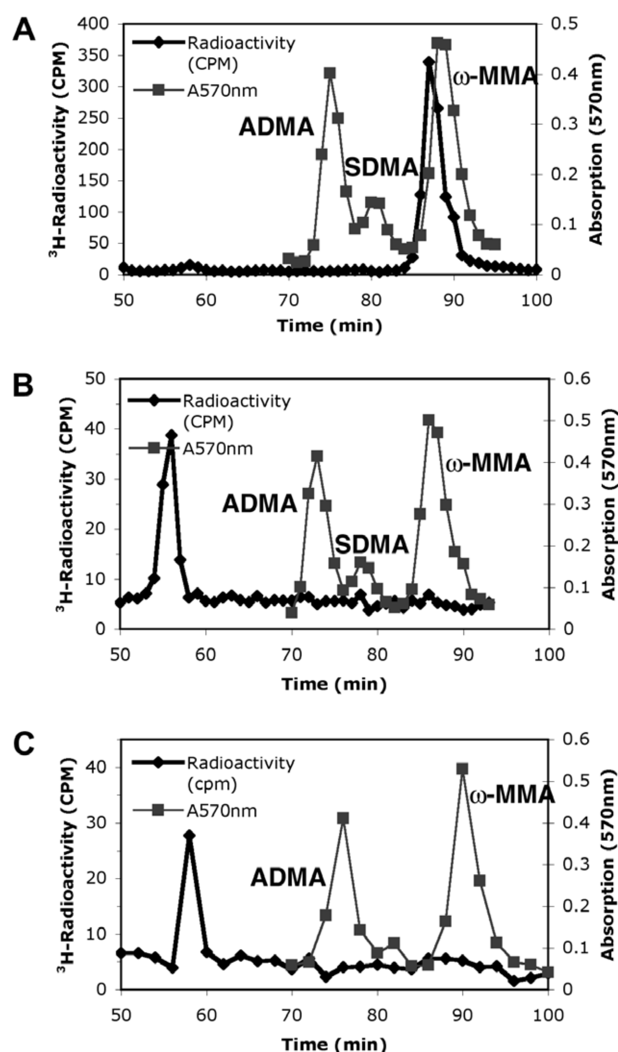


**Fig. 1.** Predominant type III protein arginine methyltransferase activity of Hsl7 with calf thymus histone H2A. Amino acid analysis of $^3$H-methylated arginine residues when histone H2A was incubated for 1 h at 37 °C with wild-type GST-Hsl7 (A) or mutant GST-Hsl7$^{G387A}$ (B) in the presence of $^3$H-AdoMet as described in the Materials and methods. A control is also shown when the wild-type enzyme is incubated in the absence of histone H2A (C). $^3$H-Radioactivity from mixing 200 μl of each fraction with 400 μl water and 5 mL scintillation fluor (Safety Solve, Research Products International, Mt. Prospect, IL) is seen in the black line with diamonds. Unlabeled standards of ω-MMA, ADMA, and SDMA were detected with a ninhydrin assay using 100 μl of each fraction [16] and are shown as absorption at 570 nm by gray lines with squares. As expected, the elution position of the tritiated methyl arginine derivatives is slightly ahead of the non-isotopically labeled standards [17].

Download English Version:

https://daneshyari.com/en/article/1935363

Download Persian Version:

https://daneshyari.com/article/1935363

Daneshyari.com