International Journal of Mass Spectrometry 278 (2008) 89–94

Contents lists available at ScienceDirect

International Journal of Mass Spectrometry

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

Characterization of acetylation of *Saccharomyces cerevisiae* H2B by mass spectrometry

Kangling Zhang*

Mass Spectrometry Facility, School of Medicine, Loma Linda University, 11021 Campus Street, Loma Linda, CA 92350, United States

ARTICLE INFO

Article history:
Received 4 August 2008
Received in revised form 21 August 2008
Accepted 22 August 2008
Available online 2 September 2008

Keywords:
Histone
Histone H2B
Post-translational modification (PTM)
Acetylation
Mass spectrometry

ABSTRACT

Following the identification of histone H3 modifications in *Saccharomyces cerevisiae* [K. Zhang, Int. J. Mass Spectrom. 269 (2008) 101–111], here, we report a detailed characterization of post-translational modifications by LC/MS/MS analysis of tryptic and Glu-C digests of H2B proteins isolated from *S. cerevisiae*. We show that both H2B.1 and H2B.2 are acetylated at K6, K11, K16, K21 and K22 while H2B.2 has an additional acetylation site at K3. All the acetylation sites of yeast H2B except K3 of H2B.2 are located at the same positions on aligned protein sequences of Arabidopsis H2B variants that were reported previously to be acetylated at K6, K11, K27, K32, K38 and K39. A unique acetylation motif AEK is observed in the H2B variants of these two species, indicating a plant/yeast H2B specific acetyltransferase may exist.

1. Introduction

Histone modification is one of the epigenetic factors in regulating gene expression [1]. The modifications include lysine acetylation, methylation and ubiquitylation, arginine and N-terminal alanine methylation, and serine and threonine phosphorylation. These modifications are believed to be regulated intra- and intro-molecularly to play a vital role in genomic development. To this context, several hypotheses such as the "histone code", "methyl/phos" switch, "acetyl/phos" switch and "ubi/methyl" interaction have been proposed [2–5].

In the past decade, extensive effort has been directed to the study of the mechanism of histone modification in the regulation of gene expression. During this period, scientists in mass spectrometry have also been very active in the identification of post-translational modification sites of proteins including histones. The progress is largely attributed to the innovation of two ionization technologies, MALDI as well as the electrospray. Consequently, many new modification sites in histones and histone variants have been identified by mass spectrometry [6–10]. Using immunoblotting or immunofluorescence imaging with antibodies raised based upon the information of modification sites provided by mass spectrometry, functional studies on histone modifications have broadened our knowledge of biochemistry in chromosomal activity [11–13]. It becomes apparent that a combination of mass spectrometric and biological techniques is a successful approach to investigate the role of histone modifications in gene expression, especially for the new modification sites [14].

Previously, our laboratory and Gruissem's laboratory identified six acetylation sites, K6, K11, K27, K32, K38 and K39, in Arabidopsis H2B [15,16]. *Saccharomyces cerevisiae* (budding yeast) genome has two H2B genes, which encodes proteins that only differ by four amino acids on the N-terminus. Yeast H2B protein sequences are highly homologous to those of Arabidopsis H2B variants. Complete identification of yeast H2B post-translational modification sites has not been reported. Following our previous report of the identification of post-translational modification sites in yeast H3 [17], here, we report the identification of modifications in yeast H2B by LC/MS/MS analysis of the digests of H2B proteins with proteases trypsin and Glu-C. We found that both H2B.1 and H2B.2 are acetylated at K6, K11, K16, K21 and K22; and H2B.2 has an additional acetylation site at K3. With the exception of acetylation at K3 of H2B.2, yeast H2Bs are acetylated on the same positions as Arabidopsis H2B on their aligned protein sequences.

2. Experimental

2.1. Isolation of histone H2B from yeast S. cerevisiae

Core histones were isolated from budding yeast as described previously [17]. Histone H2B was purified by reversed phase HPLC. A capillary HPLC (Agilent 1100, Agilent Technologies) was used to

* Tel.: +1 909 558 7691; fax: +1 909 558 4887.
E-mail address: kzhang@llu.edu.

1387-3806/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijms.2008.08.018

perform the purification. A phenomenex 2 mm × 150 mm Jupiter C4 column (5 μm particle diameter, 100 Å pore size) with mobile phase of solution A (0.1% trifluoroacetic acid in water) and solution B (0.065% trifluoroacetic acid in acetonitrile) was used with a two-step gradient from 38 to 55 of mobile phase B for 50 min, 55% of mobile phase B for 5 min, and then from 65% to 85% of mobile phase B for 13 min at a flow rate of 50 μl/min. 10 μl of sample with a concentration of about 30 μg/μl core histones in water was injected for each run. H2B fraction together with fractions containing other histone subgroups were manually collected, dried and stored under −20 °C.

2.2. Chemical modification of lysines

The H2B fraction was totally dried and then reacted with deuterated aceticanhydride in deuterated acidic acid solution over night. The reaction solutions were completely dried to remove deuterated aceticanhydride and acidic acid that remained and then dissolved in 25 mM ammonium bicarbonate buffer.

2.3. Enzymatic digestion of histone H2B

The HPLC fraction containing about 30 μg of H2B was dried and re-dissolved in 40 μl of 25 mM ammonium bicarbonate buffer solution and digested at 37 °C with 0.25 μg trypsin for 2 h or with 1.0 μg Glu-C overnight.

2.4. Sequence analysis by QTOF mass spectrometry

Sequence analyses of peptides were done by LC-ESI/MS/MS on the QTOF Global and Ultima instruments which were coupled with Agilent 1100 capillary HPLC and Waters CapLC, respectively. Agilent 1100 HPLC ran at a flow rate of 6 μl/min for a gradient of 60 min starting with mobile phase 2% B (0.1% formic acid in acetonitrile) and ramping to 85% B on the Agilent Zorbax SB-C18 column (5 μm, 150 mm × 0.5 mm). Mobile phase A is 0.1% formic acid in water. Waters CapLC ran at a flow rate of approximately 0.5 μl/min for a gradient of 120 min on the Waters Atlantic C18 column (3 μm, 150 mm × 75 μm). The same mobile phases as Agilent HPLC were used. A collision energy varying from 20 to 40 eV, which was decided by the precursor ion masses, was applied in the hexapole collision cell with Ar (12 psi) as the collision gas. The QTOF Global ran at a capillary voltage of 3.0 kV and a cone voltage of 75 V, and the QTOF Ultima ran at a capillary voltage of 1.9 kV and a cone voltage of 45 V. The source block temperature was 80 °C and desolvation temperature was 120 °C.

2.5. Determination of post-translational modification sites

The raw data from the QTOF instruments were converted into the peak list files (*.pkl) by Proteinlinx 2.0 software. The PKL files were submitted to MASCOT for the search of proteins and modifications under the setting of variable modifications of acetylation, phosphorylation, etc. Moreover, Prospector (prospector.ucsf.edu) software was run for *de nova* sequencing of peptides and determining modification sites.

3. Results

3.1. Acetylation sites identified in tryptic digests of H2B

In order to characterize H2B modifications, H2B proteins were purified by reversed-phase HPLC from the core histones that were isolated from the budding yeast. The H2B proteins were then digested by trypsin and analyzed by LC/MS/MS to identify peptides containing post-translational modifications. Modifications were identified first by MASCOT search with an input of variable modifications including acetylation and phosphorylation. All the MS/MS spectra were also manually analyzed by Prospector to confirm the modification sites indicated by MASCOT and to determine modification sites missed by MASCOT search by taking into account of lysine/arginine methylation; lysine acetylation; and serine, threonine, and tyrosine phosphorylation.

The upper half of Table 1 shows a list of peptides from tryptic H2B digests which were detected and sequence confirmed by tandem mass spectrometry. Peptides at *m*/*z* 482.8 (2+) and 483.8 (2+), which show similar fragmentation patterns in the MS/MS spectra, were observed with a mass increment of 42 Da and assigned to peptides, $^{22}K_{ac}$TSTSVDGK and $^{22}K_{ac}$TSTSTDGK, which differ by 2 Da for their masses, representing the difference between V27 and T27. The two peptides, from H2B.1 and H2B.2, respectively, have been determined to be acetylated. In both spectra (Fig. 1A and B), peaks at *m*/*z* 126.1 and 143.1 were detected, indicating acetylation and not tri-methylation [6–8], because tri-methylation normally produces a neutral loss ion of tri-methylamine (−59 Da) [8]. Acetylation at K22 was determined by the b2 and b3 ions both of which have a mass increment of 42 Da compared with their unmodified counterparts. The C-terminal fragmentation ions, the y ions, were observed without an increase of mass, indicating no acetylation at the C-terminal lysine (K28). Moreover, modified lysines are immune to tryptic digestion, further excluding the possibility of acetylation at this site.

MS/MS spectrum of the triply charged precursor ion at *m*/*z* 351.6 (Fig. 1C) showed the fragmentation pattern matching the peptide sequence, APAE$^{16}K_{ac}$KPAAK, where K16 was determined to be acetylated by the observation that y6–y8 have a mass increment of 42 Da and y1–y5 have not. In other words, the mass difference of 170.1 between y5 and y6 represented an acetylated (or tri-methylated) lysine at position 6, not 5, counted from the C-terminus. Again, the acetylation specific ion at *m*/*z* 126.1 was detected in the spectrum, confirming acetylation and none tri-methylation of the peptide. According to our experience, proline in the peptide that could produce a weak immonium ion at *m*/*z* 126.06 would not interfere with the assignment of lysine acetylation which produced an immonium ion at *m*/*z* 126.10 because a high resolution and well-calibrated mass spectrometer, such as the QTOF, was to resolve these two ions. In the middle of K16 and P18, acetylation at K17 was not detected by mass spectrometry, indicating proline may bend the peptide making the access of acetyltransferases to its adjacent lysine unfavorable in one specific direction.

As shown in Fig. 1D, MS/MS spectrum of the precursor ion at *m*/*z* 681.8 (3+) showed the fragmentation pattern matching the triple acetylated peptide, APAE$^{16}K_{ac}$KPAA$^{21}K_{ac}$$^{22}K_{ac}$TSTSTDGK. The clear fragmentation information, major y series of ions and minor b series ions as well as the internal fragmentation ions gave an unambiguous assignment of the peptide sequence and acetylation at three lysines, K16, K21 and K22. Detection of acetylation specific immonium ion at *m*/*z* 126.1 and non-detection of tri-methylation specific ions confirm specified acetylation rather than tri-methylation. Meanwhile, K6 was determined to be the acetylation site in the H2B.1 peptide acSSAAE$^{6}K_{ac}$KPASK and K3 was acetylated in the H2B.2 peptide acSA$^{3}K_{ac}$AEK. The MS/MS spectra of these two peptides are shown in Fig. 1E and F. The acetylation specific immonium ion at *m*/*z* 126.1 was detected in both spectra. Fig. 1F showed an overlap of the MS/MS spectrum of the peptide, IATEAS^{82}K with a precursor ion at *m*/*z* 360.2, indicating the two peptides were co-eluted from the HPLC column. The mixed fragmentation ions did not prevent us from the elucidation of acetylation at K3. Moreover, from the MS/MS spectra corresponding to the N-terminal peptides of H2B.1 and H2B.2, the N-terminal

Table 1
LC/MS/MS detected peptides from tryptic and Glu-C digests of H2B

Exp. mass (Da)	Peptide sequence	In H2B variants
Trpsin digest of H2B (sequence coverage: 90%)		
594.4 (2+)	$Ac^{1}SSAAE^{6}K_{ac}KPASK$	H2B.1
359.3 (2+)	$Ac^{1}SA^{3}K_{ac}AEK$	H2B.2
351.6 (3+)	$^{12}APAE^{16}K_{ac}KPAAK$	H2B.1 and/or H2B.2
681.8 (3+)	$^{12}APAE^{16}K_{ac}KPAA^{21}K_{ac}{}^{22}K_{ac}TSTSTDGK$	H2B.2
482.8 (2+)	$^{22}K_{ac}TSTSVDGK$	H2B.1
483.8 (2+)	$^{22}K_{ac}TSTSTDGK$	H2B.2
641.3 (2+)	$^{37}KETYSSYIYK$	H2B.1 and/or H2B.2
577.3 (2+)	$^{38}ETYSSYIYK$	H2B.1 and/or H2B.2
404.6 (3+); 606.4 (2+)	$^{50}QTHPDTGISQK$	H2B.1 and/or H2B.2
591.3 (3+); 886.5 (2+)	$^{61}SMSILNSFVNDIFER$	H2B.1 and/or H2B.2
824.8 (3+)	$^{61}SMSILNSFVNDIFERIATEAAK$	H2B.1 and/or H2B.2
360.2 (2+)	$^{76}IATEASK$	H2B.1 and/or H2B.2
340.2 (2+)	$^{81}LAAYNK$	H2B.1 and/or H2B.2
404.3 (2+)	$^{81}LAAYNKK$	H2B.1 and/or H2B.2
408.7 (2+)	$^{96}EIQTAVR$	H2B.1 and/or H2B.2
584.4 (3+)	$^{96}EIQTAVRLILPGELAK$	H2B.1 and/or H2B.2
428.8 (2+)	$^{112}HAVSEGTR$	H2B.1 and/or H2B.2
571.8 (2+)	$^{120}AVTKYSSSTQA$	H2B.1 and/or H2B.2
Glu-C digest of H2B (sequence coverage: 84%)		
356.9 (3+)	$^{6}K_{ac}KPASKAPAE$; $KKPAS^{11}K_{ac}APAE$	H2B.1 and/or H2B.2
555.8 (2+)	$^{6}K_{ac}KPASK_{ac}APAE$	H2B.1 and/or H2B.2
454.3 (3+)	$^{16}KKPAAKKTSTSVD$	H2B.1
454.9 (3+)	$^{16}KKPAAKKTSTSTD$	H2B.2
468.9 (3+)	$^{16}KKPAAKK_{ac}TSTSTD$	H2B.2
483.0 (3+)	$^{16}K_{ac}KPAAKK_{ac}TSTSTD$	H2B.2
406.0 (3+)	$^{29}GKKRSKVRKE$	H2B.1 and/or H2B.2
486.5 (4+); 648.4 (3+)	$^{39}TYSSYIYKVLKQTHPD$	H2B.1 and/or H2B.2
614.3 (3+)	$^{55}TGISQKSMSILNSFVND$	H2B.1 and/or H2B.2
460.3 (4+); 613.4 (3+)	$^{80}ASKLAAYNKKSTISARE$	H2B.1 and/or H2B.2
437.3 (3+)	$^{97}IQTAVRLILPGE$	H2B.1 and/or H2B.2
486. 2 (3+); 728.9 (2+)	$^{117}GTRAVTKYSSSTQA$	H2B.1 and/or H2B.2

serine residues were witnessed to be acetylated, as a common phenomenon was also observed in other histone subgroups.

Table 1 also listed the unmodified tryptic peptides of H2B. Included with the modified peptides (*Note*: Their unmodified counterparts were not shown on the list), the tryptic peptides detected and sequence confirmed by LC/MS/MS covered 90% of the H2B peptide sequence leaving only one peptide KRSKARK with multiple trypsin cut sites undetected.

3.2. Acetylation sites identified in Glu-C digests of H2B

Since histone H2B has multiple peptide regions crowded with basic amino acids R and K subject to trypsin cleavage, the enzymatically digested peptides of small size might not be well retained on a reversed-phase HPLC column and subsequently not detected by mass spectrometry. In order to identify any modification sites that might be missed for detection by LC/MS/MS analysis of the tryptic peptides, alternatively, we performed the LC/MS/MS analysis of the Glu-C digests of histone H2B. Glu-C cuts E and D in the buffer of ammonium bicarbonate (pH 7.8), leaving lysines or arginines with and without modification intact in peptides, and therefore providing complementary sequence coverage to the tryptic digest.

The lower half of Table 1 showed a list of peptides from Glu-C digests of H2B which were detected and sequence confirmed by tandem mass spectrometry. Peptides at m/z 468.9 (3+) and 483.0 (3+) were observed with a mass increment of 42 and 84 Da, and assigned to peptide KKPAAKKTSTSTD of histone H2B.2 with one and two acetylations, respectively. In Fig. 2A, MS/MS spectrum of the first peptide showed the b2 ion at m/z 257.2, indicating that the N-terminal lysines K16 and K17 were not modified. The observation that b8–b10 ions of the peptide have a mass increment of 42 Da compared with the same ions produced from the unmodified counterpart, indicated that either K21 or K22 or mixture of both was acetylated. We did not know whether K22 was preferentially acetylated to K21, or the vice versa, because the b7 ion that includes K21 and none of y series ions were detected to distinguish modifications between the two lysines. In Fig. 2B, MS/MS spectrum of the second peptide showed the b2 ion at m/z 299.2, indicating that the N-terminal lysine either K16 or K17 was acetylated. K16 was likely the modification site since K17 was hindered for acetylation by its right neighbor P18 as demonstrated in the information gained from the MS/MS spectra of the tryptic peptides. The observation that all of b9–b10 ions and a group of internal fragmentation ions of the peptide have a mass increment of 42 Da indicated again that either K21 or K22 or mixture of both was acetylated. Surprisingly, acetylation on the analogous peptides for H2B.1 was not detected albeit the unmodified counterpart was detected as shown with a peak at m/z 454.3 Da.

MS/MS spectrum of the precursor ion at m/z 356.9 (3+) as shown in Fig. 2C established the peptide KKPASKAPAE with a mass increment of 42 Da, indicating either one acetylation or tri-methylation in the peptide. A peak at m/z 126.1 was observed confirming that it is acetylation and not tri-methylation. The observation of b2 ion at m/z 299.2 indicated the acetylation is at K6. Meanwhile, the appearance of the internal fragmentation ions at m/z 426.3 corresponding to acetylated PASK, at m/z 594.3 corresponding to acetylated PASKAP, and at m/z 552.3 and 623.4 corresponding to unmodified PASKAP and PASKPA, respectively, revealed that K11 was partially acetylated. Therefore, the spectrum shown in Fig. 1C was from a mixture of two peptides $^{6}K_{ac}KPASKAPAE$ and $KKPAS^{11}K_{ac}APAE$, where K6 and K11 were acetylated, respectively. Acetylation at these two sites was further confirmed by the detection and sequence confirmation of the di-acetylated peptide $^{6}K_{ac}KPAS^{11}K_{ac}APAE$ with a peak at m/z 555.8 (2+) (Fig. 2D).

LC/MS/MS analysis of Glu-C digest of H2B proteins reconfirmed acetylation at K6, K16, K21 and K22. Acetylation at K11 was iden-

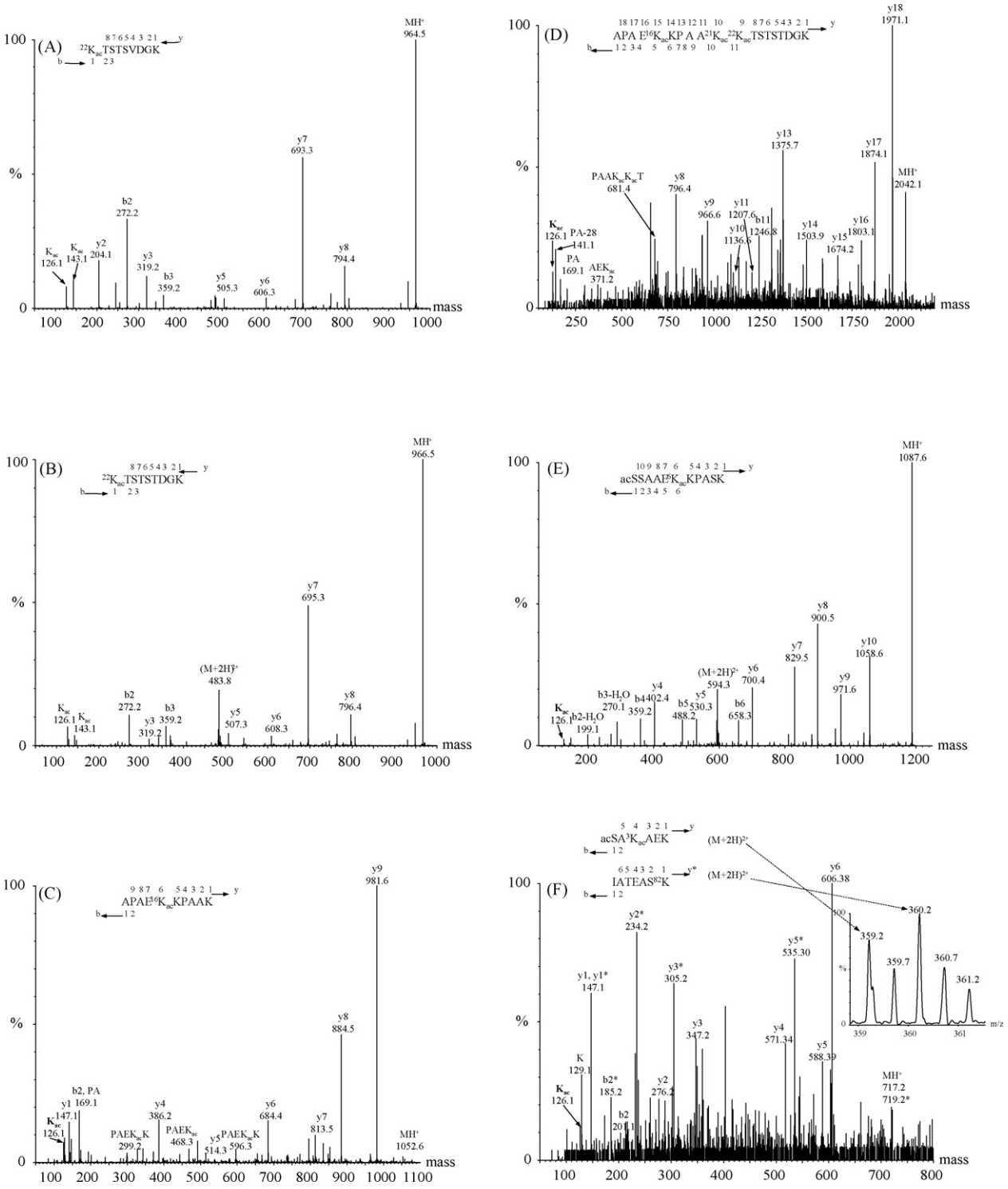

Fig. 1. H2B acetylation sites identified by LC/MS/MS analysis from trypsin digests. (A) MS/MS spectrum of the precursor ion at m/z 482.8 (2+). The spectrum was deconvoluted as singly charged ions spectrum as done for all the spectra throughout this paper. (B) MS/MS spectrum of precursor ion at m/z 483.8 (2+). (C) MS/MS spectrum of precursor ion at m/z 351.6 (3+). (D) MS/MS spectrum of precursor ion at m/z 681.8 (3+). (E) MS/MS spectrum of precursor ion at m/z 594.4 (2+). (F) MS/MS spectrum of precursor ion at m/z 359. 3 (2+) mixed with the MS/MS spectrum of precursor at m/z 360. 2.

tified from the Glu-C digest. Table 1 also listed the unmodified Glu-C digest peptides of H2B. Included with the modified peptides, the Glu-C peptides detected and sequence confirmed by LC/MS/MS took up 84% of the H2B peptide sequence. A combination of tryptic and Glu-C peptides represented full sequence coverage of H2B proteins.

4. Discussion

Because two yeast H2B variants share significant sequence identity (only four amino residues differ on the N-terminus) and they are co-eluted on the reversed-phase HPLC column, it is not possible to assign lysine acetylation sites to a specific H2B variant. K6, K11

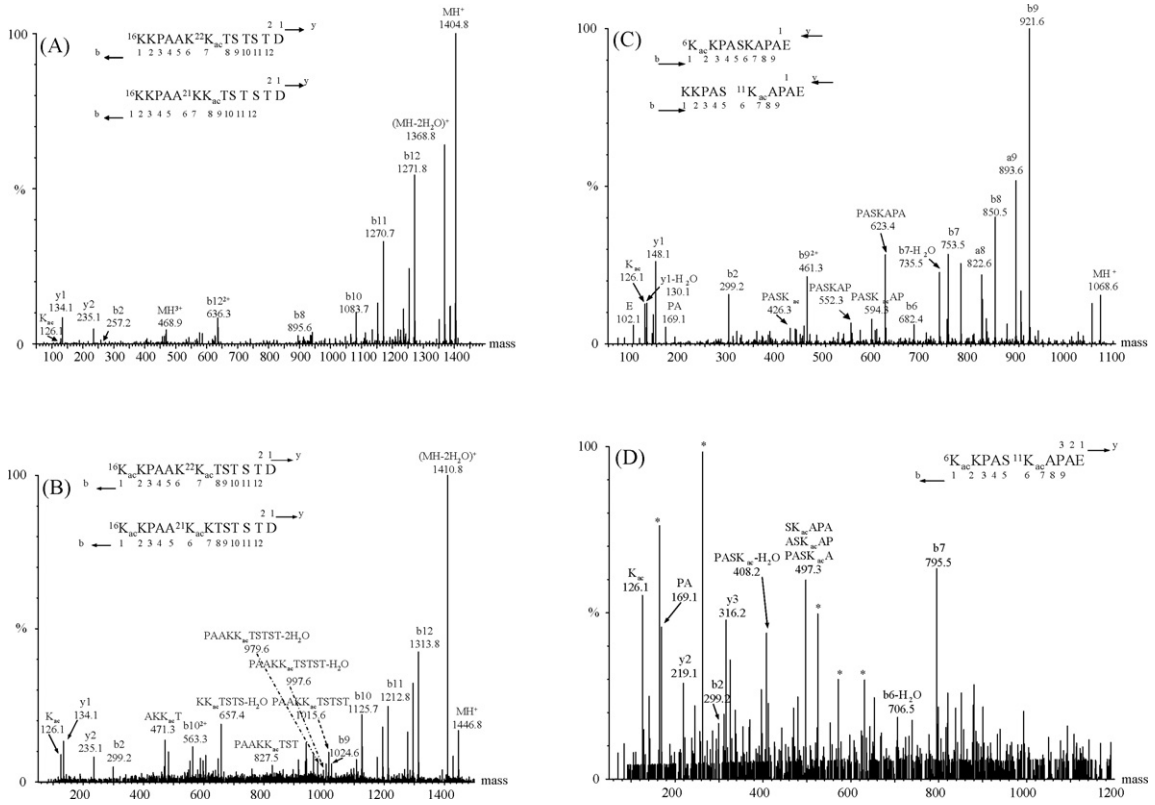

Fig. 2. H2B acetylation sites identified by LC/MS/MS analysis from Glu-C digests. (A) MS/MS spectrum of the precursor ion at *m*/*z* 468.9 (3+). (B) MS/MS spectrum of the precursor ion at *m*/*z* 483.0 (3+). (C) MS/MS spectrum of the precursor ion at *m*/*z* 356.9 (3+). (D) MS/MS spectrum of the precursor ion at *m*/*z* 555.8 (2+). *Unresolved impurity peaks.

and K16 acetylation was found on the tryptic or Glu-C peptides whose sequences are shared by both H2B.1 and H2B.2. Therefore, acetylation at these three sites was assigned to H2B.1 and/or H2B.2. Nevertheless, K22 is certainly acetylated in both H2B.1 and H2B.2 variants because acetylation at this site was determined from the two distinctive tryptic peptides belonging to the two variants. H2B.2 has an additional acetylation site at K3, whereas at this position of H2B.1, the lysine residue is replaced by the alanine residue that cannot be modified. We only detected K21 acetylation in H2B.2 and this site acetylation appeared in the double or triple-acetylated peptides in which K6 and/or K22 were also acetylated. It is predictable that K21 is also acetylated in H2B.1 because of the striking similarity of the peptide sequence at the neighborhood of this site between the two H2B variants (Fig. 3). Since hyperacetylation was only observed in H2B.2, it is obvious that either the cell line may have a preference for acetylation on H2B.2 over H2B.1 or the protein expression level of H2B.1 is lower than that of H2B.2 and thereby K21 acetylation in the hyperacetyated form in H2B.1 is below the instrumental detection limit.

Two Arabidopsis H2B variants (HTB1, At1g07790; HTB4, At5g59910) out of total of seven known variants were renamed in this paper and in previous paper published in PLoS ONE as H2B.1

```
                          10        20        30        40        50        60        70
                   ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Yeast H2B.1        MSSAAEKKPAS----------------KAPAEKKP-AAKKTSTSVD------GKKRSKVRK---ETYSSY
Yeast H2B.2        MSAKAEKKPAS----------------KAPAEKKP-AAKKTSTSTD------GKKRSKARK---ETYSSY
Arabidopsis H2B.1  MAPRAEKKPAEKKPAAEKPVEEKSKAEKAPAEKKPKAGKKLPKEAG----AGGDKKKKMKKKSVETYKIY
Arabidopsis H2B.2  MAPKAEKKPAEKKPASEKPVEEKSKAEKAPAEKKPKAGKKLPKEAG----AGGDKKKKMKKKSVETYKIY

                          80        90       100       110       120       130       140
                   ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Yeast H2B.1        IYKVLKQTHPDTGISQKSMSILNSFVNDIFERIATEASKLAAYNKKSTISAREIQTAVRLILPGELAKHA
Yeast H2B.2        IYKVLKQTHPDTGISQKSMSILNSFVNDIFERIATEASKLAAYNKKSTISAREIQTAVRLILPGELAKHA
Arabidopsis H2B.1  IFKVLKQVHPDIGISSKAMGIMNSFINDIFEKLASESSKLARYNKKPTITSREIQTAVRLVLPGELAKHA
Arabidopsis H2B.2  IFKVLKQVHPDIGISSKAMGIMNSFINDIFEKLAQEASKLARYNKKPTITSREIQTAVRLVLPGELAKHA

                          150
                   ....|....|....|..
Yeast H2B.1        VSEGTRAVTKYSSSTQA
Yeast H2B.2        VSEGTRAVTKYSSSTQA
Arabidopsis H2B.1  VSEGTKAVTKFTSS---
Arabidopsis H2B.2  VSEGTKAVTKFTSS---
```

Fig. 3. Sequence alignment of yeast H2B and Arabidopsis H2B. **K** in bold: acetylation.

and H2B.2 [16]. These two H2B variants show significantly higher ion abundance in mass spectrometry than those of other remaining H2B variants. Except for the difference at positions 3 and 15, the peptide sequences of these two H2B variants are almost identical (Fig. 3). They are both acetylated at sites of K6, K11, K27, K32, K38 and K39. Interestingly, the peptide sequences of the two yeast H2B variants are highly homologous to those of the two Arabidopsis H2B variants. And surprisingly, the acetylation sites identified from yeast H2B are located at the same positions as observed in Arabidopsis H2B after the protein sequences of these two species are aligned using the ClustalW Multiple Alignment tool (Fig. 3). Several acetylation sites are observed within the AEK motif, indicating there may be distinct acetyltransferases responsible for acetylation of H2B proteins in yeast and Arabidopsis involved in the regulation of gene expression by adopting one specific but not yet specified mechanism.

All the acetylation sites identified in yeast H2B are on the N-terminus. LC/MS/MS analysis of the tryptic and Glu-C digests of H2B covers the full protein sequence, revealing no modifications in the middle region and the C-terminus. Neither lysine nor arginine methylation was observed. Chemically acetylating lysines of proteins with regionally clustered lysine and arginine residues expands the size and hydrophobicity of tryptic peptides (acetylated lysines will have higher hydrophobicity and not be cleaved by trypsin), demonstrating stronger electrospray signals for acetylated peptides as well as providing quantative information of acetylation [18]. However, in our experiments, LC/MS/MS analysis of the digest of yeast H2B proteins that were treated with d_6-aceticanhydride and then digested by trypsin did not yield additional modifications (data not shown).

Yeast H2B has been reported to be phosphorylated at S10 [19]. The phosphorylation was not detected in our LC/MS/MS experiments, most likely due to its low abundance or dephosphorylated in the process of purification of histones since no phosphorylase inhibitors were added to the isolation solutions. Yeast histone H2B is also known to be ubiquitinated at K123 [5]. Ubiquitination at this site was not detected in our LC/MS/MS experiments, demonstrating lower level of H2B ubiquitination in yeast than Arabidopsis in which ubiquitination at K143 was readily detected by mass spectrometry [15,16]. Nevertheless, identification of the acetylation sites in yeast H2B and the observation of their modification similarities with Arabidopsis H2B sets the stage for further epigenetic study of H2B acetylation in a complex plant system by using a simple yeast system.

References

[1] P.A. Jones, S.B. Baylin, Cell 128 (2007) 683.
[2] B.D. Strahl, C.D. Allis, Nature 403 (2000) 41.
[3] Q. Fischle, Y. Wang, C.D. Allis, Nature 425 (2003) 475.
[4] P. Cheung, K. Tanner, W. Cheung, P. Sassone-Corsi, J. Denu, C. Allis, Mol. Cell 5 (2000) 905.
[5] H.H. Ng, R.M. Xu, Y. Zhang, K. Struhl, J. Biol. Chem. 277 (2002) 34655.
[6] K. Zhang, H. Tang, L. Huang, J.W. Blankenship, P.R. Jones, F. Xiang, P. Yau, A.L. Burliname, Anal. Biochem. 306 (2002) 259.
[7] J.Y. Kim, K.W. Kim, H.J. Kwon, D.W. Lee, J.S. Yoo, Anal. Chem. 74 (2002) 5443.
[8] K. Zhang, P.M. Yau, B. Chandrasekhar, R. New, R. Kondrat, B.S. Imai, E.M. Bradbury, Proteomics 4 (2004) 1.
[9] K.F. Medzihradszky, X. Zhang, R.J. Chalkley, S. Guan, M.A. McFarland, M.J. Chalmers, A.G. Marshall, R.L. Diaz, C.D. Allis, A.L. Burlingame, Mol. Cell. Proteomics 5 (2006) 194.
[10] D. Bonenfant, M. Coulot, H. Towbin, P. Schindler, J. Oostrum, Mol. Cell. Proteomics 5 (2006) 541.
[11] H.H. Ng, D.N. Ciccone, K.B. Morshead, M.A. Oettinger, K. Struhl, PNAS 100 (2003) 1820.
[12] F. Xu, K. Zhang, M. Grunstein, Cell 121 (2005) 375.
[13] C.B. Miller, F. Xu, K. Zhang, M. Grunstein, Genes Dev. 20 (2006) 711.
[14] W. Peng, C. Togawa, K. Zhang, S. Kurdistani, Genetics 179 (2008) 277.
[15] E. Bergmüller, P.M. Gehrig, W. Gruissem, J. Proteome Res. 6 (2007) 3655.
[16] K. Zhang, V.V. Sridhar, J. Zhu, A. Kapoor, J.-K. Zhu, PloS ONE 2 (2007) e1210.
[17] K. Zhang, Int. J. Mass Spectrom. 269 (2008) 101.
[18] C.M. Smith, P.R. Gafken, Z. Zhang, J.B. Gottschling, J.B. Smith, D.L. Smith, Anal. Biochem. 316 (2003) 23.
[19] S. Ahn, R.L. Diaz, M. Grunstein, C.D. Allis, Mol. Cell 24 (2006) 211.