# Substrate and sequential site specificity of cytoplasmic histone acetyltransferases of maize and rat liver

Doris Kölle[a], Bettina Sarg[b], Herbert Lindner[b], Peter Loidl[a],*

[a]*Department of Microbiology, University of Innsbruck Medical School, Fritz-Pregl-Str. 3, A-6020 Innsbruck, Austria*
[b]*Department of Medical Chemistry and Biochemistry, University of Innsbruck Medical School, Fritz-Pregl-Str. 3, A-6020 Innsbruck, Austria*

**Abstract** The cytoplasmic B-type histone acetyltransferase was purified to apparent homogeneity from maize embryos. We established a novel protocol for easy large-scale preparation of acetylated core histone species, using preparative acetic acid-urea-Triton PAGE. The pure maize histone acetyltransferase B was highly specific for histone H4 under various assay conditions, modifying H4 up to the di-acetylated isoform. Only non-acetylated H4 isoform was accepted as substrate, whereas mono-acetylated H4 could not be further acetylated. The enzyme selectively acetylated lysines 12 and 5 in a sequential manner. The same results were obtained with a partially purified cytoplasmic histone acetyltransferase of rat liver. Protein sequencing results were supported by immunological characterization of acetylated H4 subspecies with site-specific H4-acetyllysine antibodies.

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*Key words:* Chromatin; Histone acetylation; Histone acetyltransferase; Nucleosome assembly; Maize; Histone H4

## 1. Introduction

Lysine residues in the N-terminal extensions of core histones are posttranslationally acetylated by histone acetyltransferases; the reaction can be reverted by histone deacetylases [1]. Histone acetylation has long been uniquely correlated with transcriptionally active chromatin. The basic idea was that the highly charged N-terminal extensions interact with DNA when non-acetylated; after acetylation and the resulting charge reduction, this interaction would be weakened and therefore the nucleosome structure loosened, so that transcription factors could gain access to the DNA. Whilst this view was conceptually supported by the identification of histone acetyltransferases as transcriptional regulators [2], the very recent 2.8 Å resolution of the nucleosome core particle structure [3] argued against such a simplistic model, because regions of acetylation are not tightly bound to DNA and are disordered and fully accessible to other proteins. It is therefore possible that core histone acetylation rather acts in the destabilization of higher order structures of chromatin, but also as a specific flag for regulatory or enzymatic proteins that are involved in transcription, replication or DNA repair in a way similar to protein phosphorylation in signal transduction pathways [4,5]. One of the most important questions in this context is how specific and selective histone acetyltransferases and deacetylases are with respect to substrates, lysine sites and the sequential order of site usage.

We started to systematically investigate substrate and site specificity of the involved enzymes. In this first report we present data on purified histone acetyltransferase B (HAT-B) of maize embryos [6,7] and compare results with those obtained with partially purified HAT-B of rat liver. One of the experimental requirements for such investigations is the availability of large amounts of highly pure, acetylated core histone subspecies. Instead of multiple chromatographies we used preparative acetic acid-urea-Triton PAGE to isolate sufficient amounts of pure core histone species and acetylated H4 subspecies. We demonstrate that HAT-B of maize and rat liver is highly specific for the non-acetylated H4 isoform which is acetylated sequentially at lysine 12 and lysine 5 into a di-acetylated isoform. Characterization of acetylated H4 subspecies with site-specific H4-acetyllysine antibodies revealed that lysine 12 is modified before lysine 5.

## 2. Materials and methods

### 2.1. Preparation of HATs

Maize HAT-B was purified essentially as described [6]. For purification of the cytoplasmic HAT-B of rat liver, three livers of rats were minced with a razor blade and homogenized in buffer 1 (50 mM Tris-HCl, pH 7.5, 0.25 M sucrose, 25 mM KCl, 5 mM $MgCl_2 \cdot 6H_2O$, 0.5 mM phenylmethylsulfonyl fluoride, 1 mM dithiothreitol; 25 g of tissue per 100 ml of buffer) in a motor-driven Potter-Elvehjem homogenizer with cooling in an ice bath. The homogenate was centrifuged for 20 min at $800 \times g$ at 4°C. The supernatant was filtered through nylon membranes (mesh 100 μm) and then centrifuged for 30 min at $50\,000 \times g$ at 4°C. The clear supernatant was dialyzed against buffer B (15 mM Tris-HCl, pH 8.0, 10 mM $NH_4Cl$, 0.25 mM EDTA, 10 mM 2-mercaptoethanol, 10% (v/v) glycerol) and subjected to Q-Sepharose Big Beads chromatography (column volume 50 ml). After loading and washing with buffer B, proteins were eluted with a linear gradient (300 ml) of 10 mM–0.5 M NaCl (in buffer B). Fractions of 5 ml were collected and assayed for protein content ($A_{280}$) and histone acetyltransferase activity.

### 2.2. Large-scale purification of acetylated core histone subspecies

Chicken erythrocyte core histones were isolated as described [8]. Aliquots of 10 mg were dissolved in distilled water (50 μl) and mixed with 200 μl of sample buffer (6 M urea, 10 mM 2-mercaptoethanol, 0.9 M acetic acid, 0.37% Triton X-100) for electrophoretic separation in a preparative acetic acid-urea-polyacrylamide gel with Triton [9]. The cylindrical gel (28 mm diameter; 9.8 cm separating gel; 0.8 cm stacking gel) was run in a 491 Prep Cell (Bio-Rad, Hercules, CA, USA) with a central cooling unit. After pre-electrophoresis of the gel for 7 h at 15 mA (const.), the histones were electrophoresed for 85 h (70 h at 50 V (const.), 13 h at 100 V (const.)). Collection of histones (fraction volume 5 ml) was started 37 h after the start of electrophoresis, using 10 mM 2-mercaptoethanol in distilled water for elution, at a flow rate of 1 ml/min. Aliquots of 100 μl of every tenth fraction were freeze-dried, dissolved in 15 μl SDS sample buffer [10], and then subjected to SDS-PAGE (precast 14% gels, Novex, USA) with subsequent silver staining [11]. Core histones eluted in the following order: H4 (fractions 55–140), H2B (fractions 120–210), H3 (fractions 240–330), H2A (fractions 510–570). After SDS-PAGE screening, fractions were analyzed in acetic acid-urea-Triton (AUT)

*Corresponding author. Fax: (43) (512) 5072866.
E-mail: Peter.Loidl@uibk.ac.at

polyacrylamide gels (80×80×0.75 mm); for this purpose 300 µl of every third fraction of the H4 region was freeze-dried, dissolved in 15 µl of sample buffer (6 M urea, 10 mM 2-mercaptoethanol, 0.9 M acetic acid, 0.37% Triton X-100), and subjected to electrophoresis (0.5 h at 100 V (const.), 2.5 h at 150 V (const.)) after pre-electrophoresis of the gel for 16 h at 50 V (const.). Gels were stained with Coomassie blue. According to the electrophoretic pattern fractions were pooled, so that homogeneous samples with respect to acetylated histone subspecies were achieved. The pooled fractions were finally concentrated by ultrafiltration (Amicon membrane YM1, 1 kDa cut-off), freeze-dried and stored at −50°C.

To separate acetylated H4 subspecies in those few fractions where two isoforms were present, these fractions were pooled, concentrated, freeze-dried, and subjected to sulfopropyl-Sephadex C-25 chromatography (Pharmacia-Biotech, Uppsala, Sweden). A total of 800 µg of histone H4 was dissolved in 530 µl of buffer G (0.4 M guanidinium hydrochloride, 6 M urea, 0.1 M 2-mercaptoethanol, pH 3.0) and applied to an SP-Sephadex column (volume 6 ml). After washing the column with buffer G, acetylated isoforms were eluted with a linear gradient (80 ml) of 0.4–0.6 M guanidinium hydrochloride in 6 M urea/0.1 M 2-mercaptoethanol at a flow rate of 1 ml/h. Fractions of 1 ml were collected. For protein estimation in a turbidimetric assay, aliquots of two fractions (100 µl each) were precipitated with 900 µl of 22% (w/v) trichloroacetic acid for 30 min. These samples were then measured for absorbance at 400 nm. Fractions containing protein were then analyzed by AUT-PAGE. Fractions were pooled according to the electrophoretic pattern; pools were desalted in Sephadex-G25 Hi-Trap columns (Pharmacia-Biotech), freeze-dried and stored at −50°C.

### 2.3. Histone acetyltransferase assays

For determination of enzyme activity in chromatographic fractions an established standard assay was employed [6].

For determination of substrate specificity a modified assay was performed. Enzyme (250 µl of pure maize HAT-B [6] or of the Q-Sepharose HAT-B peak fraction of rat liver) was incubated with 10 µl chicken erythrocyte core histone solution (8 mg/ml), and 30 µl [14C]acetyl-CoA (25 µCi/ml) for 30 min at 37°C. To eliminate chemical, non-enzymatic acetylation, we increased the salt concentration of the assay mixture to 0.4 M. We routinely included 0.1 µM Trichostatin A in our assay mixture. 15 µl of both an insulin solution and an apoferritin solution (10 mg/ml each) were included to inhibit proteases, if present, by an excess of proteins. After incubation, the reaction was terminated by addition of 28 µl buffer N (2.5 M NaCl, 0.5 M Tris-HCl, pH 8.0) and 35 µl of novobiocin solution (10 mg/ml; Boehringer Mannheim, Germany). The whole mixture was incubated at 4°C for 1 h, and centrifuged for 10 min at $10\,000 \times g$. The pellet was washed twice with 0.9 ml ethanol. Finally, the pellet was dried under vacuum and subjected to electrophoretic analysis by SDS-PAGE or AUT-PAGE with subsequent exposure on phospho-storage image screens. Screens were scanned in a Molecular Dynamics STORM phosphorimager.

For assays using purified, acetylated H4 isoforms, non- or monoacetylated H4 was taken instead of total chicken core histones in the standard assay.

For determination of site specificity of HAT-B of maize embryos and rat liver, a modified assay was performed. 100 µl of pure maize HAT-B or partially purified rat liver HAT-B was incubated with 80 µg of non-acetylated H4 (H4Ac0), 500 mM NaCl, 200 nCi [14C]acetyl-CoA, and 0.1 µM Trichostatin A in a final volume of 250 µl for 1 h at 37°C. After incubation, histones were precipitated with novobiocin (see above).

### 2.4. Determination of site specificity of HAT-B by protein microsequencing

The whole histone pellet was applied to a precast SDS-14% polyacrylamide slab gel (Novex) and electrophoresed for 1.5 h at 150 V (const.). After electrophoresis the gel was equilibrated in blot buffer (50 mM boric acid/NaOH, pH 9.0, 10% (v/v) methanol) for 15 min and blotted onto Immobilon P^SQ membrane (Millipore, USA) for 1.5 h at 25 V (const.) at 4°C in a Novex electrophoresis chamber with a blot module. Protein bands on the membrane were stained with Coomassie blue for 3 min and destained by diffusion in 40% methanol/10% acetic acid.

The H4 band was excised from the dry blot and sequencing of H4 was performed on an Applied Biosystems Inc. Model 492 Procise

protein sequenator. Sequencer grade solvents were purchased from ABI. In order to partially deblock the acetylated N-terminus, the PVDF-blotted H4 was incubated within the cartridge with gaseous trifluoroacetic acid for 50 min at 48°C. After incubation, the PVDF membrane was washed with ethyl acetate and butylchloride. This procedure, incubation and consecutive washing steps, was repeated twice. Finally Edman degradation of 17–18 amino acids was performed using standard pulsed-liquid phase sequencing cycles. Fractions were collected after conversion of cleaved amino acids into PTH derivatives and radioactivity was determined in a liquid scintillation spectrophotometer.

### 2.5. Immunodetection of acetylated H4 sites

For immunodetection of acetylated lysine sites in the products after maize HAT-B reaction, a standard HAT assay was performed, except that purified non-acetylated H4 was taken instead of total core histones. After enzymatic reaction, the whole assay solution was mixed with sample buffer and subjected to AUT-PAGE. Part of the gel was blotted to nitrocellulose membrane (blot buffer: 5% acetic acid) and the remaining part was stained with Coomassie blue. The blot lane was analyzed with antibodies raised against H4 N-terminal peptides acetylated at lysine 5 or 12 (kindly supplied by Christian Seiser, Vienna). Immunodetection was done with the ECL detection kit (Amersham International plc). For quantitative evaluation of immunosignals, both the Coomassie blue-stained AUT gel lanes and the corresponding immunodetection were scanned in a laser densitometer. Optical densities of the bands after immunodetection (mono- and diacetylated H4; ECL detection) were related to the optical density of the corresponding bands of the Coomassie blue-stained gel. This immunosignal/protein ratio was expressed in arbitrary units.

## 3. Results and discussion

In all experimental systems investigated so far, two types of HATs are present: multiple nuclear A-type enzymes that are responsible for acetylation of core histones in nucleosomes, and a cytoplasmic B-type enzyme that acetylates newly synthesized core histones, predominantly H4, at the time of DNA replication and nucleosome assembly [1,12]. Whereas numerous models have been proposed to explain the functional significance of the action of nuclear A-type HATs, the biological role of the cytoplasmic HAT is less clear. Cloning of the encoding cDNAs of both enzyme types clearly showed that A-type enzymes are distinct from the cytoplasmic HAT-B [13–

Table 1
Substrate specificity of HAT-B of maize embryos and rat liver

| Histone | HAT-B activity (cpm) | |
|---|---|---|
| | maize | rat |
| H4[a] | 1900 | 857 |
| H3 | 75 | 96 |
| H2A | 80 | 94 |
| H2B | 76 | 83 |
| _[b] | 65 | 90 |
| H4ac0 | 1718 | 594 |
| H4ac1 | 96 | 95 |
| Total histones[c] | 124 | 110 |

Purified HAT-B from maize embryos or partially purified HAT-B from rat liver was incubated with pure, individual core histones or histone subspecies for 30 min as described in Section 2. 500 mM NaCl was included in the assay mixture to prevent chemical, non-enzymatic acetylation.
[a]Purified H4 consisted of approx. 50% non-acetylated (H4ac0), 30% mono-acetylated (H4ac1), 15% di-acetylated, and 5% tri- and tetra-acetylated (H4ac3, ac4) subspecies.
[b]HAT-B was incubated in the presence of [14C]acetyl-CoA without histones.
[c]Total core histones were incubated with [14C]acetyl-CoA without HAT-B in the presence of 500 mM NaCl.
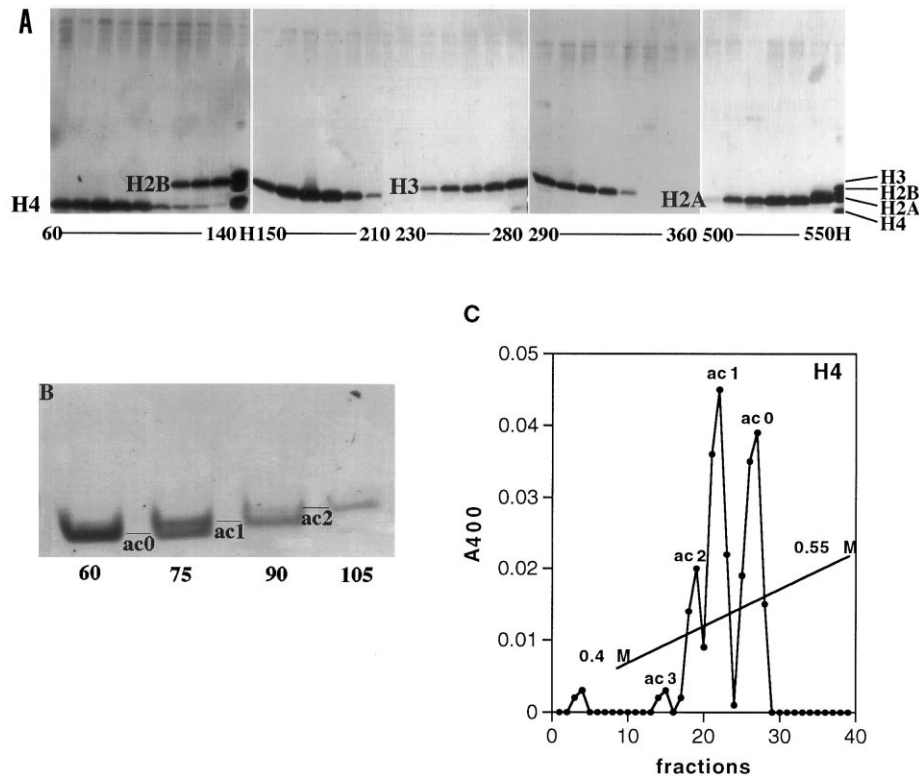
Fig. 1. Purification of individual core histone species and acetylated subspecies by preparative AUT-PAGE and SP-Sephadex chromatography. A: Chicken erythrocyte core histones were subjected to preparative AUT-PAGE. The resulting fractions were analyzed by SDS-14% PAGE. Fraction numbers of the AUT-PAGE are indicated; 100 µl of every tenth fraction was freeze-dried and applied to the SDS gel. H indicates lanes with total core histones as markers; individual core histones are indicated. Gels were stained with silver. B: Selected fractions of the preparative AUT-PAGE were analyzed on AUT slab gels. Aliquots of 500 µl of fractions 60, 75, 90 and 105 were freeze-dried and applied on AUT slab gels; positions of non- (ac0), mono- (ac1), and di- (ac2) acetylated H4 subspecies are indicated. C: Fractions of the preparative AUT-PAGE that contained two H4 subspecies were pooled, concentrated and applied onto an SP-Sephadex column. Acetylated H4 subspecies (ac0–ac3) were eluted by a linear guanidinium hydrochloride (0.4–0.6 M) gradient. Protein concentration was monitored by a turbidimetric measurement ($A_{400}$). Fractions were subsequently analyzed by AUT-PAGE.

17]. To learn more about possible functions of enzymes involved in histone acetylation, it is important to gain further insight into the substrate specificity and lysine site specificity. We set out to study this question in detail for both HATs and deacetylases. With respect to HAT-B, numerous reports have been published on specificity, but there are still considerable discrepancies among the different results. For that reason, we studied purified maize HAT-B and compared results to partially purified HAT-B of rat liver.

To address substrate specificity and site selectivity it is of essential importance to have sufficient amounts of highly pure core histone species and acetylated subspecies. Instead of a chromatographic approach to purify histones, we used preparative electrophoresis in AUT-polyacrylamide gels. Fig. 1A shows the electrophoretic pattern on SDS-polyacrylamide slab gels after separation of 8 mg of chicken erythrocyte core histones in AUT-PAGE. Using this technique, one can isolate large amounts of histone species in pure form by a simple one-step technique. The core histones eluted in the following order: H4, fractions 55–120; H2B, fractions 140–210; H3, fractions 240–330; H2A, fractions 510–570 (Fig. 1A). This method does not just allow the purification of sufficient amounts of individual histones for substrate specificity studies, it also makes it possible to obtain pure acetylated histone isoforms for studies of site specificity. Fig. 1B shows the electrophoretic separation in AUT-polyacrylamide slab gels of H4-containing

fractions after preparative AUT-PAGE. As can be seen, acetylated subspecies of histone H4 can be recovered in pure form. Typically, fractions 55–80 contained non-acetylated
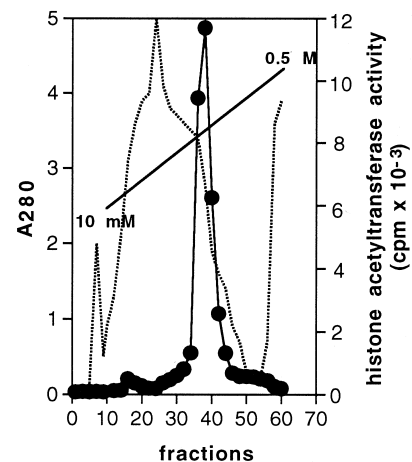


Fig. 2. Chromatographic separation of rat liver HAT-B by Q-Sepharose Big Beads chromatography. The high-speed supernatant of soluble cytoplasmic proteins was applied to a Q-Sepharose Big Beads column (50 ml). After washing the column, bound proteins were eluted with a linear salt gradient (10 mM–0.5 M). Every second fraction was assayed for histone acetyltransferase activity (cpm) and protein content ($A_{280}$).
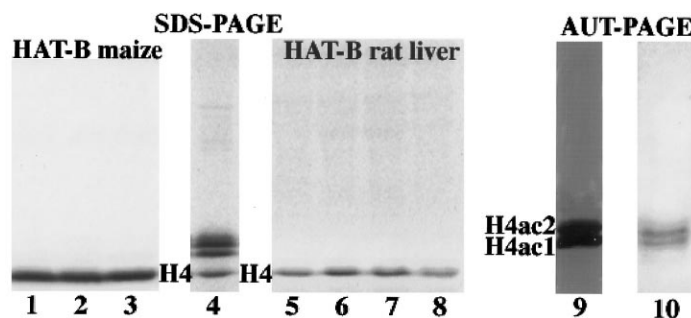
Fig. 3. Substrate specificity of maize and rat liver HAT-B. Purified maize HAT-B [6] (lanes 1–3; different preparations) and rat liver HAT-B (lanes 5–8 corresponding to fractions 37–40 of chromatography in Fig. 2) were incubated with total chicken erythrocyte core histones and [$^{14}$C]acetyl-CoA. Subsequently samples were subjected to SDS-PAGE. Dried gels were exposed to phospho-storage image screens. For comparison, lane 4 shows the reaction products of a crude preparation of rat nuclear A-type HATs; the position of H4 is indicated. Both enzymes (lane 9: maize HAT-B; lane 10: rat liver HAT-B) were also incubated with pure non-acetylated H4 subspecies in the presence of [$^{14}$C]acetyl-CoA; subsequently the reaction products were electrophoresed in AUT-polyacrylamide slab gels with exposure to phospho-storage image screens; the positions of mono- and di-acetylated H4 subspecies (H4ac1, H4ac2) are indicated.

H4, fractions 70–95 contained mono-acetylated H4, and fractions 90–110 contained di-acetylated H4, with some overlapping fractions that contained two subspecies (Fig. 1B). According to the electrophoretic pattern, fractions were pooled, so that homogeneous samples with respect to acetylated histone subspecies were created; these subspecies served as substrates for studies of the specificity and site selectivity of the cytoplasmic HAT-B, but can also be used for studies of deacetylase specificity. Those fractions of the preparative AUT gel that contained two acetylated isoforms were subjected to SP-Sephadex chromatography in a second step to resolve individual isoforms (Fig. 1C).

We have recently purified HAT-B of germinating maize embryos to homogeneity (as judged by silver-stained SDS-PAGE) by a sequence of eight chromatographic steps [6] and used this enzyme for our specificity studies. To be able to compare the obtained results with a mammalian enzyme we partially purified rat liver cytoplasmic HAT-B by Q-Sepharose chromatography (Fig. 2); gel filtration chromatography of the peak fraction revealed that the native enzyme has a molecular mass of ∼100 kDa (results not shown). This correlates well with HAT-B of HeLa cells which was estimated to be a 100 kDa protein [18].

To verify the substrate specificity, both HATs were incubated with total chicken erythrocyte core histones with subsequent electrophoretic separation of the histones and exposure to phospho-storage image screens. Fig. 3 shows that purified maize HAT-B as well as partially purified rat liver HAT-B were highly specific for histone H4. The enzymes did not accept any other core histone as substrate. Faint labelling that may sometimes be observed in H2A or H3 is due to chemical acetylation. This non-enzymatic acetylation occurs even at salt concentrations up to 0.3 M and is ignored in most reports on in vitro investigations of HATs. Therefore, we now routinely include 0.5 M salt in the assay mixture when we determine substrate specificities; this salt concentration does not inhibit the activity of HAT-B. For comparison we included the result obtained with a crude preparation of maize A-type HATs which acetylated all core histones (Fig. 3). The same substrate specificity of HAT-B was obtained when we incubated the enzymes with purified individual core histone species in the standard assay instead of the mixture of total core histones; again H4 was the only substrate for HAT-B from both organisms (Table 1). Incubation of the enzymes

with the purified, non-acetylated H4 isoform led to the generation of mono- and di-acetylated H4 (Fig. 3, lanes 9 and 10); no higher levels of acetylation could be introduced by HAT-B, as judged from the electrophoretic pattern on AUT gels and autoradiography.

We then analyzed whether HAT-B enzymes can accept purified, mono-acetylated H4 and convert this to the di-acetylated isoform. Strikingly, mono-acetylated H4 was not at all accepted as a substrate (Table 1), indicating that maize and rat liver HAT-B are essentially selective for non-acetylated H4. This probably reflects the in vivo situation since the enzyme is assumed to acetylate newly synthesized H4 molecules prior to assembly into nucleosomes. It should be pointed out that neither the substrate specificity of the enzymes nor the level of acetylation introduced by the enzymes changed when non-acetylated H4 was incubated together with purified H3 (results not shown). This was tested because chromatin assembly factor 1 (CAF-1) has been shown to be physically associated with newly synthesized H3 and H4 [19].

There are considerable discrepancies in the level of acetylation and the site usage of B-type HATs in the literature. HAT-B of pea was shown to acetylate non-acetylated H4 into up to tri-acetylated subspecies in vitro [20], with no distinct site selectivity; the pea enzyme modified lysines 5 and 12 with similar affinity, but also lysine 16. HAT-B of crude *Drosophila* extracts only acetylated lysine 12, when assayed with H4 or synthetic H4 N-terminal peptides [21]. In vivo studies in HeLa cells and *Drosophila* clearly demonstrated di-acetylation of newly synthesized H4 with a clear preference for lysines 5 and 12 [22]. Yeast Hat1p, which is the catalytically active subunit of the B enzyme, only introduces mono-acetylation in H4 by modification of lysine 12 [13,14]; surprisingly, recombinant Hat1p was able to di-acetylate H4 by modifying lysines 5 and 12 [14]. On the other hand, CAF-1 copurifies with a chromatin assembly complex (CAC) that is associated with newly synthesized H4 in up to the tri-acetylated H4 subspecies [23]; about 30% of each was present as non-, mono-, and di-acetylated H4 subspecies and a small, but significant percentage (1–2%) was tri-acetylated. In this case lysines 5, 8, and 12 were modified [23], which is again discrepant with Mingarro et al. [20] where lysines 5, 12, and 16 were acetylated. There is no trivial explanation for all these divergent results. An important point is that in vitro studies are not directly comparable with in vivo results because multiple
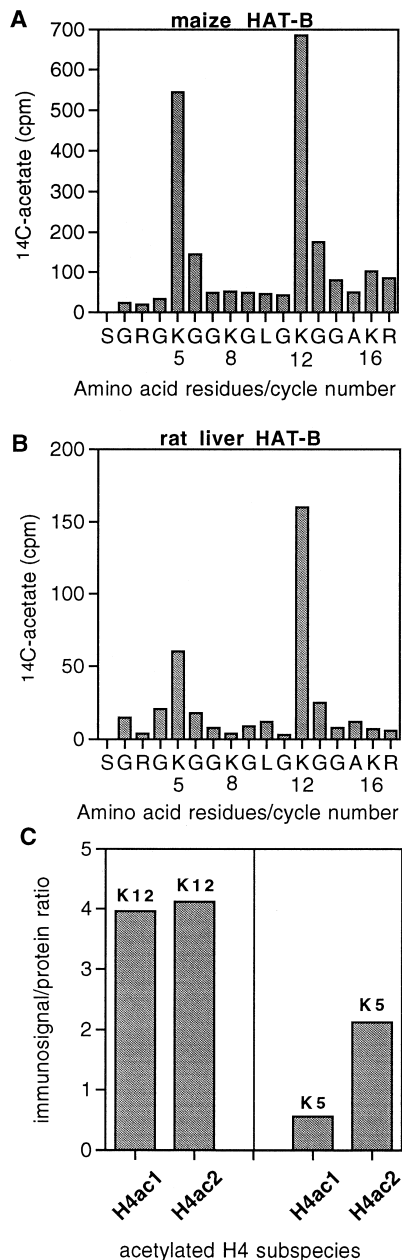
Fig. 4. Site specificity of HAT-B of maize embryos and rat liver. Highly purified HAT-B from maize (A) and partially purified HAT-B from rat liver (B) were incubated with pure non-acetylated H4 subspecies in the presence of [14C]acetyl-CoA. The reaction products were subjected to SDS-PAGE with subsequent blotting onto PVDF membrane. The H4 band was visualized by Coomassie blue staining. The band was excised and microsequenced after chemical deblocking. Fractions were collected after conversion of cleaved amino acids into PTH derivatives. The amino acid sequence was determined and radioactivity was measured in the liquid scintillation spectrophotometer. C: Aliquots of the reaction product of A were subjected to AUT-PAGE. Parallel lanes were either stained with Coomassie blue or blotted onto nitrocellulose membrane for subsequent immunodetection, using antibodies against acetylated lysine residues (K5, K12). Mono- and di-acetylated H4 lanes (stained gel) were quantitated by densitometry, as were immunosignals obtained with the different antibodies over mono- or di-acetylated H4. The ratio between the immunosignal over each band and the corresponding protein content is expressed in arbitrary units.

HATs are present and a certain pattern of acetylation can be changed by the action of histone deacetylases. Another possibility to explain the discrepant results may be that investigations have been performed with either crude or highly purified (recombinant) HATs; moreover, the histone substrates differed with respect to purity, especially purity of acetylated isoforms. For that reason we established an easy one-step method for large-scale purification of pure histones and pure acetylated subspecies. Using pure maize HAT-B and pure non-acetylated H4, we could unambiguously demonstrate by protein microsequencing that HAT-B exclusively modifies lysines 12 and 5 (Fig. 4A). No label was incorporated into lysines 8 or 16. The same result was obtained with partially purified rat liver HAT-B (Fig. 4B); it has to be emphasized that the chromatographic fraction of rat liver HAT-B that we used was essentially free of nuclear HAT-A activity. To investigate the sequential usage of lysines 12 and 5, we used site-specific acetyllysine antibodies raised against different H4 peptides that each carried one particular acetylated lysine residue. Immunodetection on blots after AUT-PAGE of in vitro reaction products of maize HAT-B with non-acetylated H4 clearly showed that the ratio between immunosignal and total protein was unchanged ($\sim$1) when anti-lysine 12 immunosignals were compared in the mono- and di-acetylated H4 subspecies (Fig. 4C). However, this ratio increased threefold ($\sim$0.6 to $\sim$2.1) when the immunosignal of anti-lysine 5 was compared in mono- and di-acetylated H4. No immunosignals were observed with antibodies against lysines 8 and 16. We conclude that HAT-B has a sequential order of site usage, with lysine 12 being acetylated before lysine 5.

Large-scale preparation of pure individual histone species and acetylated subspecies by preparative AUT-PAGE also enables us to study substrate and site specificity of histone deacetylases [24,25], a topic that has not been reported in detail until now. We are currently working on the identification of the maize histone deacetylase that specifically recognizes the lysine 12/5 acetylation pattern generated by B-type HATs.

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