Eur J Nutr 41 : 78 – 84 (2002)

ORIGINAL CONTRIBUTION

Biotinidase catalyzes debiotinylation of histones

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Received: 27 September 2001
Accepted: 6 January 2002

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Summary *Background* Post-translational modifications of histones play important roles in processes such as regulation of gene expression and DNA repair. Recently, evidence has been provided that histones in human cells are modified by covalent attachment of biotin. *Aim of the study* To determine whether the reverse process (debiotinylation of histones) occurs in biological samples and whether debiotinylation is an enzyme-mediated process; and to characterize the enzyme that mediates debiotinylation of histones. *Methods* Plasma and lymphocytes from healthy adults and a biotinidase-deficient patient were used as sources of debiotinylating enzymes. Debiotinylation of histones by plasma and lymphocyte proteins was measured using a colorimetric 96-well plate assay. *Results* Histones were debiotinylated rapidly if incubated with human plasma or lysates of lymphocytes. The following observations are consistent with the hypothesis that debiotinylation is an enzyme-mediated process: (*i*) Hydrolysis was slower at 4 °C compared to 37 °C; (*ii*) debiotinylating activity was destroyed when biological samples were heated at 90 °C for 30 min preceding incubation with biotinylated histones; and (*iii*) rates of debiotinylation were pH dependent. Rates of histone debiotinylation were significantly decreased in biotinidase-deficient samples. *Conclusion* Debiotinylation of histones in human samples is an enzyme-mediated process that is at least partly catalyzed by biotinidase.

Key words Biotin – Biotinidase – Biotinylation – Debiotinylation – Histone

Introduction

Histones are the primary proteins that mediate the folding of DNA into chromatin [1]. The binding of DNA to histones is of electrostatic nature; binding is mediated by the association of negatively charged phosphate groups of DNA with positively charged ε-amino groups and guanidino groups of histones.

In vivo, histones are modified posttranslationally by covalent attachment of groups such as acetate [2–4], ubiquitin [5], and poly (ADP-ribose) [6–8]. These groups are attached to either ε-amino groups (lysine residues) or guanidino groups (arginine residues) of histones; each such modification takes away one positive charge from histones, leading to weakened association between DNA and histones. Some histone modifications (e. g., acetylation of histones) correlate with increased transcription of DNA and DNA repair mechanism [poly (ADP-ribosylation) of histones] [1].

Recently, evidence has been provided for another posttranslational modification of histones: Hymes et al. have proposed a reaction mechanism by which the enzyme biotinidase (E. C. 3.5.1.12) mediates covalent bind-

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ing of biotin to histones [9] in addition to the classical role of biotinidase in intermediary metabolism. The classical role of biotinidase in metabolism is to hydrolyze biocytin (biotinyl-ε-lysine), a degradation product of biotin-dependent carboxylases [10]. Hydrolysis of biocytin releases free biotin which is recycled in the synthesis of new holocarboxylases. Hymes et al. have extended this classical view by showing that cleavage of biocytin by biotinidase leads to the formation of a biotinyl-thioester intermediate (cysteine-bound biotin) at or near the active site of biotinidase [9, 11]. In a next step, the biotinyl moiety is transferred from the thioester to a free amino group in histones. Based on these pioneering studies, our laboratory has demonstrated that human cells biotinylate histones and that cells respond to proliferation with increased biotinylation of histones [12].

In the present study we hypothesized that cells synthesize an enzyme that catalyzes debiotinylation of histones. Theoretically, such an enzyme enables cells to reduce biotinylation of histones, e. g., to regulate gene expression in response to changes of the cellular environment or physiological requirements. By analogy, cells reduce transcriptional activity of genes by decreasing acetylation and ubiquitinylation of histones [1, 5]. In the present study, we specifically (*i*) developed a 96-well plate assay to determine rates of histone debiotinylation; (*ii*) determined whether debiotinylating activity is present in biological samples; (*iii*) determined whether debiotinylation of histones is an enzyme-mediated process; (*iv*) characterized the enzyme that mediates debiotinylation of histones.

Experimental methods

Isolation of plasma and lymphocytes

Heparinized blood was collected from apparently healthy donors and from a patient that had been diagnosed with the inborn error biotinidase deficiency. Plasma and lymphocytes were collected by gradient centrifugation as described previously [13]. Both plasma and lymphocytes were used to measure activities of enzymes that catalyze debiotinylation of histones as described below. This study was approved by the Institutional Review Boards at the University of Nebraska-Lincoln and the University of Wisconsin-Madison.

Histone debiotinylase assay

Histone H1 (Sigma, St. Louis, MO) was biotinylated enzymatically [9] to produce substrate for analysis of debiotinylating enzyme(s): 1 mg of histone H1 was dissolved in 0.1 mL of 20 mM sodium acetate, pH 4.5, and diluted with 18.9 mL of 50 mM Tris, pH 8.0. Enzymatic biotinylation is catalyzed by biotinidase and requires biocytin as substrate [9]. Thus, 0.4 mL of 750 µM biocytin, and 0.6 mL of human plasma (as source of biotinidase) were added to the histone solution. The sample was incubated at 37 °C for 45 min. Next, 30 mL of 50 mM sodium carbonate buffer, pH 9.6, was added and the solution was used immediately to coat plates, i. e., to adsorb biotinylated histone H1 to the surface of 96-well plates. For coating, 100 µL of biotinylated histone H1 solution was added per well and plates were incubated at 4 °C overnight. Coating solution was removed from wells and plates were incubated for at least 1 h with 200 µL/well of 0.1 % bovine serum albumin (wt./vol) and 0.05 % Tween–20 (vol./vol) in phosphate-buffered saline to block unoccupied adsorption sites. Plates containing blocking solution lost less than 10 % of biotinylated histones if stored at 4 °C for 10 days (data not shown). We noted substantial differences in the adsorption efficiency of histones to plates from different manufacturers during the coating process. For the studies reported here, we used polypropylene 96-well flat bottom plates from Becton Dickinson (Franklin Lakes, NJ; catalog number 353072).

Plates coated with biotinylated histone H1 were used to characterize enzymes that catalyze debiotinylation of histones in biological samples. In a typical experiment, debiotinylation of histones was measured as follows. After coating and blocking, plates were washed twice with phosphate-buffered saline. The following samples (150 µL/well) were added to release biotin from its covalent binding to histone: (*i*) human plasma (1 vol. of plasma diluted with 2 vol. of 50 mM Tris, pH 8.0); (*ii*) human lymphocytes; 8 x 10^6 lymphocytes were pelleted and lysed by freezing at –70 °C and thawing; the lysate was suspended in 2 mL of 50 mM Tris, pH 8.0. (*iii*) 50 mM Tris, pH 8.0 (control). Plates were incubated at 37 °C for 3 min. Samples were removed and plates were washed twice using phosphate-buffered saline. Next, 100 µL of horseradish peroxidase-conjugated avidin was added to the empty wells; this solution was prepared as follows: 1 mg of lyophilized horseradish peroxidase-conjugated avidin (Pierce; Rockford, IL; catalog number 21123) was reconstituted with 1 mL of water; 1 mL was diluted with 0.1 % bovine serum albumin (wt./vol) in phosphate-buffered saline. Blanks were prepared without horseradish-peroxidase conjugated avidin. Plates were incubated with horseradish peroxidase-conjugated avidin for 1 h at room temperature. Plates were washed twice with 0.05 % Tween–20 in phosphate-buffered saline (vol./vol). One hundred microliters of TMB 1-component microwell substrate (Kirkegaard & Perry Laboratories, Gaithersburg, MD; catalog number 50–76–04) were added to each well and plates were incubated for 0.5 h at room temperature. Finally, reactions were terminated by addition of 100 µL stop solution

(Kirkegaard & Perry Laboratories, catalog number 50–85–04) and the absorbance was read at 450 nm in an Emax microwell plate reader (Molecular Devices, Sunnyvale, CA). Note that high debiotinylating activity leads to a decrease of biotinylated histones and, thus, to a decrease of absorbance at 450 nm. The assay procedure described here was modified for individual experiments as described in Results, e. g., by addition of inhibitors or by incubation at 4 °C.

Chemical biotinylation of substrates

In some experiments, naturally occurring and synthetic polypeptides were biotinylated chemically for subsequent use as substrates in the debiotinylation assay. For chemical biotinylation, 1 mg of either bovine serum albumin, poly-L-lysine (mol. wt. 20,000–30,000), or histone H1 from calf thymus (all from Sigma) were dissolved in 0.5 mL of 50 mM bicarbonate buffer, pH 8.5. Immediately before use, 3.5 mg of sulfo-NHS-biotin (sulfosuccinimidobiotin; Pierce, Rockford, IL) was dissolved in 1 mL of water; 50 µL of this biotinylating agent was added to the protein solution and samples were incubated at room temperature for 1 h [14]. Finally, samples were dialyzed three times against 250 volumes of water to remove unreacted sulfo-NHS-biotin. Biotinylated proteins were used to coat 96-well plates as described above with the following modification. Protein concentration in the coating solution was adjusted to 2 mg/mL (compared to 20 mg/mL for enzymatically biotinylated histone) to adjust for the high stoichiometry of biotinylation achieved by sulfo-NHS-biotin. Activities of debiotinylating enzyme were determined using these plates as described above for enzymatically biotinylated histones.

Determination of biotinidase activity

Biotinidase activity in plasma was measured as the hydrolysis rate of N-*D*-biotinyl-*p*-aminobenzoic acid as described by Knappe [15] and Backman-Gullers [16] and modified by Nilsson [17]. Forty microliters of plasma were mixed with 740 µL of 54 mM sodium phosphate buffer, pH 6.0, containing 1.08 mM disodium EDTA and 4.3 mM cysteamine hydrochloride (prepared freshly); samples were warmed at 37 °C for 10 min. Then, 20 µL of 6 mM of N-*D*-biotinyl-*p*-aminobenzoic acid were added. After 30 min at 37 °C, 80 µL of 2 M trichloroacetic acid were added and protein was removed by centrifugation (2,500 *g* for 10 min). Six-hundred microliters of the supernatant were mixed with 200 µL water. At room temperature and at 3-min intervals, 80 µL of 14.5 mM sodium nitrite (prepared freshly), 80 µL of 43.8 mM ammonium sulfamate, and 80 µL of 3.86 mM *N*–1-naphtyl ethylenediamine hydrochloride were added in succession and allowed to incubate for 10 min before measuring the absorbance at 546 nm. One unit of biotinidase activity is defined as the amount of enzyme that releases 1 µmol of *p*-aminobenzoic acid per minute [18].

Statistics

Significance of differences among groups was tested by one-way ANOVA if more than two treatment groups were compared. Effects of incubation time and treatment were tested by two-way ANOVA (time by treatment). Fisher's Protected Least Significant Difference procedure was used for posthoc testing [19]. Paired comparisons were made using the paired, two-tailed t-test. StatView 5.0.1 (SAS Institute; Cary, NC) was used to perform all calculations. Differences were considered significant if $P < 0.05$. Data are expressed as mean ± 1 SD.

Results

Normal plasma contained a factor that mediated debiotinylation of histone H1. For example, 96-well plates were incubated either with native plasma, heat-denatured plasma (90 °C for 30 min), or Tris buffer (control) for up to 30 min. At timed intervals, biotinylation of histone H1 was determined as described above using horseradish peroxidase-conjugated avidin as a probe for biotin. Incubation with native plasma caused a rapid debiotinylation of histone, as judged by the decrease of absorbance at 450 nm (Fig. 1). Debiotinylating activity was destroyed if plasma was heated prior to incubation with biotinylated histones. Likewise, incubation with enzyme-free Tris buffer did not cause debiotinylation of histone H1. These data are consistent with the presence

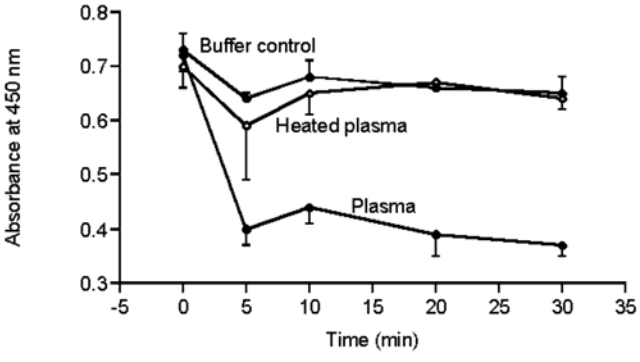

Fig. 1 Debiotinylation of histone H1 in the presence of native human plasma, heat-denatured plasma, or Tris buffer. Histone H1 was debiotinylated more rapidly in the presence of native plasma compared to heat-denatured plasma or Tris buffer ($P < 0.01$ by two-way ANOVA; n = 6 repeats using the same plasma sample).

of one or more enzymes in plasma that catalyze debiotinylation of histones.

Human lymphocytes also contain catalytic activity that mediates debiotinylation of histones. For example, incubation of biotinylated histone H1 with lysed lymphocytes at 37 °C for 5 min caused a decrease of absorbance from 0.71 ± 0.01 (buffer control) to 0.51 ± 0.01 (lymphocytes); $P < 0.01$ (n = 6 repeats, using the same lymphocyte sample). For the majority of the experiments described here, plasma rather than lymphocytes was used as a source of debiotinylating enzyme based on the following line of reasoning: (*i*) the enzyme biotinidase was identified as a candidate enzyme to catalyze debiotinylation of histones (see below); plasma contains high activity of biotinidase [18, 20]. (*ii*) Large quantities of plasma are available conveniently, including plasma from patients with the inborn error of metabolism, biotinidase deficiency.

The following observations are consistent with the hypothesis that debiotinylation of histones was caused by enzymes rather than by spontaneous degradation of biotinylated histones. (*i*) Debiotinylation of histones was significantly decreased at 4 °C compared to 37 °C. For example, if biotinylated histone H1 was incubated with plasma at 4 °C for 3 min, biotinylation status decreased by only about 7 % as judged by the decrease of absorbance at 450 nm from 1.20 ± 0.19 to 1.11 ± 0.10 ($P = 0.53$; n = 5–6 repeats, using the same plasma sample). In contrast, if 96-well plates were incubated at 37 °C for 3 min, biotinylation of histones decreased by about 54 % as judged by the decrease of absorbance from 1.15 ± 0.23 to 0.53 ± 0.08 ($P < 0.01$; n = 6 repeats, using the same plasma sample). Moreover, heating of plasma at 90 °C for 30 min prior to incubation with histone inactivated debiotinylating activity (see above).

(*ii*) Debiotinylation of histones is pH dependent. Biotinylated histone H1 was incubated with plasma at 37 °C for 3 min at pH values that spanned a range from 6.0 to 10.0. Debiotinylating activity reached a maximum (i. e., absorbance minimum) at about pH 8 (Fig. 2).

The enzyme biotinidase has broad substrate specificity with regard to cleaving biotinyl amides (see Discussion). Thus, we determined whether biotinidase might account for debiotinylation of histones. If biotinylated histone H1 was incubated with plasma from a biotinidase-deficient patient, no significant debiotinylation of histones was observed (Fig. 3 a; deficient plasma versus buffer control). Histone H1 was debiotinylated more rapidly if incubated with normal control plasma compared to biotinidase-deficient plasma.

Likewise, debiotinylation of histone H1 was reduced in biotinidase-deficient lymphocytes compared to normal lymphocytes. If biotinylated histone H1 was incubated with lysed biotinidase-deficient lymphocytes (0.4×10^6 cells) at 37 °C for 3 min, absorbance at 450 nm decreased by only 8 % compared to enzyme-free control

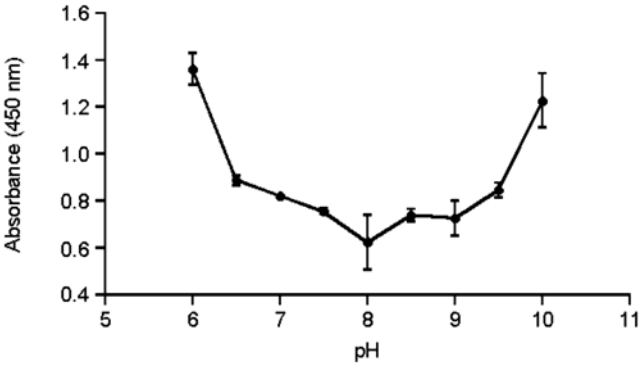

Fig. 2 Debiotinylation of histone H1 by plasma enzymes is pH dependent. Biotinylated histone H1 was incubated with plasma at pH values that spanned a range of 6.0 to 10.0. In this experiment, the standard protocol was modified by diluting 1 volume of plasma with 2 volumes of either one of the following buffers (all stock solutions at 50 mM): citrate-phosphate buffer (pH 6.0 and 6.5), phosphate buffer (pH 7.0 and 7.5), Tris buffer (pH 8.0 to 9.0), and carbonate-bicarbonate buffer (pH 9.5 and 10.0). Note that the lower the absorbance the greater the enzyme activity (n = 3 repeats using the same plasma sample).

(Tris buffer, Fig. 3 b). In contrast, if biotinylated histone H1 was incubated with lysed normal lymphocytes, absorbance at 450 nm decreased by 16 % compared to enzyme-free control.

The diagnosis of biotinidase deficiency in our patient was confirmed by measuring biotinidase activity in plasma. Biotinidase activity equaled 0.1 units/L. This is less than 2 % of the biotinidase activity in plasma from healthy individuals in this study (7 –12 units/L).

Next, we determined whether chemically biotinylated proteins (bovine serum albumin and histone H1) and a synthetic polypeptide (poly-L-lysine) are also debiotinylated if incubated with plasma. The broad substrate specificity of plasma biotinidase would be consistent with rapid debiotinylation of these compounds. Indeed, all three biotinylated compounds were debiotinylated rapidly when incubated with plasma at 37 °C for 3 min (Table 1). Debiotinylation rates were decreased when biotinidase-deficient plasma rather than normal plasma was used for incubations: 32 ± 9.7 % of biotin was removed from albumin (51 ± 16 % for normal plasma); 45 ± 5 % of biotin was removed from poly-L-lysine (63 ± 2.9 % for normal plasma); and 22 ± 3 % of biotin was removed from histone H1 (33 ± 2.8 % for normal plasma). The observation that synthetic compounds are debiotinylated at a slower rate when incubated with biotinidase-deficient plasma compared to normal plasma is consistent with our observations made for enzymatically produced biotinylated histone H1. In summary, these observations are consistent with the hypothesis that biotinidase might account for debiotinylation of histones. It remains uncertain why debiotinylation of histones by biotinidase has a maximal rate at pH 8 (see above), whereas hydrolysis of biocytin by biotinidase occurs maximally at pH 6–7.5 [17, 20].

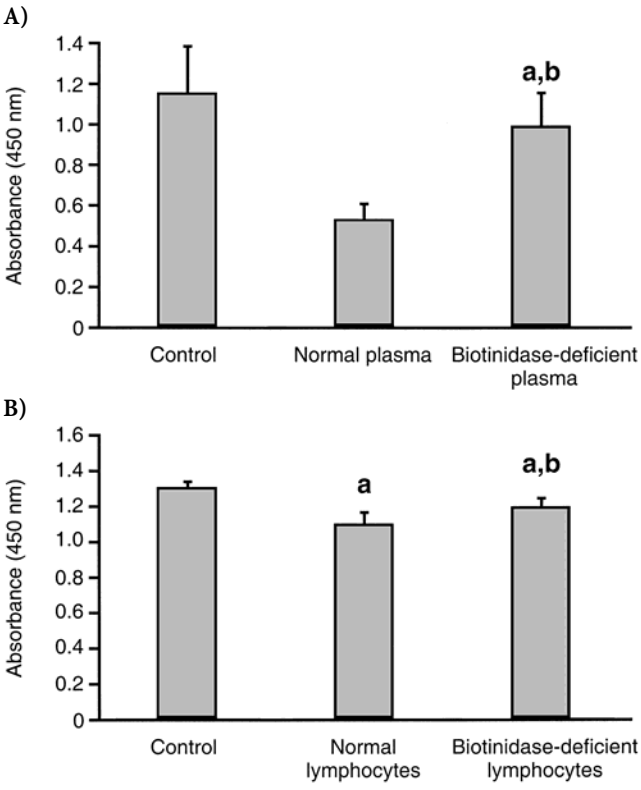

Fig. 3 Debiotinylation of histone H1 by biotinidase-deficient biological samples. (**A**) Histone H1 is not debiotinylated by biotinidase-deficient plasma. Biotinylated histone H1 was incubated with either buffer (enzyme-free control), normal plasma, or plasma from a biotinidase-deficient patient. a Not significantly different from buffer control ($P = 0.10$; $n = 6$; values for biotinidase-deficient plasma are the intraexperimental variation); b significantly different from normal plasma ($P < 0.01$; $n = 6$ repeats). (**B**) The rate of debiotinylation of histone H1 is reduced in biotinidase-deficient lymphocytes compared to normal lymphocytes. Biotinylated histone H1 was incubated with either buffer (enzyme-free control), normal lymphocytes, or lymphocytes from a biotinidase-deficient patient ($n = 5$–6 repeats; values for biotinidase-deficient plasma are the intraexperimental variation). [a] Significantly different from buffer control ($P < 0.01$); [b] significantly different from normal lymphocytes ($P < 0.05$).

Table 1 Rates of debiotinylation of bovine serum albumin, poly-L-lysine, and histone H1[a]

Sample	Control	Plasma
	Absorbance at 450 nm	
Albumin	0.48 ± 0.09	0.22 ± 0.03[b]
Poly-L-lysine	2.48 ± 0.08	0.93 ± 0.09[b]
Histone H1	3.28 ± 0.09	2.19 ± 0.10[b]

[a] Bovine serum albumin, poly-L-lysine, and histone H1 from calf thymus were biotinylated chemically using sulfosuccinimidobiotin. Ninety-six well plates were coated with these biotinylated compounds. Then, plates were incubated at 37 °C for 3 min with either plasma (containing debiotinylating enzyme) or Tris buffer (control). After incubation, biotinylation of proteins was determined using the avidin-based procedure described in the text.

[b] Significantly different from enzyme-free control ($P < 0.01$; $n = 6$ repeats, using the same plasma sample).

Next, we determined whether plasma proteases might account for some of the release of free biotin from biotinylated histones. In these experiments, 1 mL of protease inhibitor cocktail (Sigma catalog number P–8340) was added per 100 µL of plasma prior to incubation with biotinylated histones. The protease inhibitor cocktail contains 4-(2-aminoethyl)-benzenesulfonyl fluoride (an inhibitor of serine proteases), aprotinin (an inhibitor of serine proteases), leupeptin (an inhibitor of serine and thiol proteases), bestatin (an inhibitor of aminopeptidases), pepstatin (an inhibitor of acid proteases), and trans-epoxysuccinyl-L-leucylamido-(4-guanidino)butane (an inhibitor of cystein and thiol proteases). Addition of protease inhibitors caused a modest decrease of the rate of histone debiotinylation (Fig. 4). This finding suggests that proteases account for some hydrolysis of biotinylated histone H1 in addition to debiotinylation of histone H1 by biotinidase.

The decreased rate of histone debiotinylation in the presence of protease inhibitors was not caused by inhibition of biotinidase, based on the following line of evidence. Addition of protease inhibitor cocktail (1 µL) to plasma (100 µL) did not affect biotinidase activity: 24 ± 2.0 units/L (plasma with protease inhibitors) versus 20 ± 1.1 units/L (plasma without inhibitors; $P > 0.05$). Note that biotinidase activity was measured using N-*D*-biotinyl-*p*-aminobenzoic acid as substrate, i. e., the classical analysis procedure was used.

Finally, we quantified the activity of debiotinylating enzyme in biological samples from a group of healthy individuals in order to provide some information about interindividual variation. Plasma from six apparently healthy individuals (four women/two men; one African-American, one Asian, four Caucasians) was collected and debiotinylating activity was determined as described above. Incubation of biotinylated histones with plasma for 3 min at 37 °C caused a decrease of absorbance at 450 nm from 1.09 ± 0.12 (Tris control) to

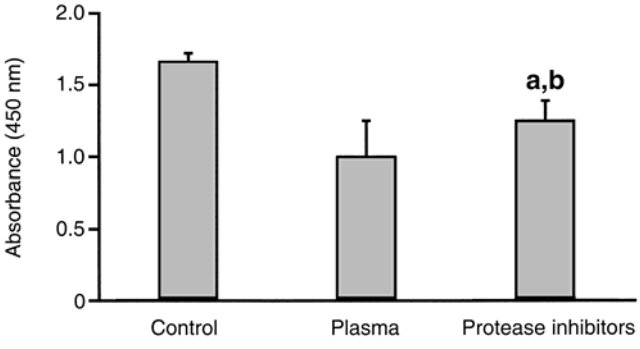

Fig. 4 Debiotinylation of histone H1 is decreased in the presence of protease inhibitors. Biotinylated histone H1 was incubated either with buffer (enzyme-free control), plasma, or plasma containing protease inhibitors. [a, b] Significantly different from control and inhibitor-free plasma ([a] $P < 0.01$ versus control; [b] $P < 0.05$ versus inhibitor-free plasma; $n = 6$ repeats using the same plasma sample).

0.64 ± 0.08 (plasma); mean (± SD) decrease of absorbance was 0.45 ± 0.13 (range: 0.29 to 0.62). In addition, debiotinylating activity was measured in lymphocytes from five apparently healthy individuals (four women/one man; four Caucasians, one Hispanic). Incubation of biotinylated histones with lysed lymphocytes for 3 min at 37 °C caused a decrease of absorbance at 450 nm from 0.98 ± 0.06 (Tris control) to 0.77 ± 0.04 (lymphocytes); mean (± SD) decrease of absorbance was 0.22 ± 0.08 (range: 0.16 to 0.35). These observations suggest that interindividual variation in healthy subjects is moderate.

Discussion

The study described here provides evidence that human plasma and cells contain an enzyme that catalyzes debiotinylation of histones. The following lines of evidence suggest that this enzyme is identical with biotinidase: (*i*) Debiotinylation of histone H1 is significantly reduced in biotinidase-deficient plasma; (*ii*) biotinidase has a broad substrate specificity with regard to its debiotinylating activity: biotinidase debiotinylates naturally occurring compounds as well as synthetic compounds such as biotinyl-ε-lysine, N-*D*-biotinyl-*p*-aminobenzoic acid, and biotinyl peptides [10, 20]. Moreover, biotinidase also hydrolyzes some substrates that do not contain biotin such as β-naphthylacetate and lipoyl-e-lysine [17, 20]. Notwithstanding the important role of biotinidase as a debiotinylating enzyme, we cannot rule out the possibility that proteases also account for some hydrolysis of biotinylated histones, based on our experiments with protease inhibitors.

Previous studies and the present study are consistent with the hypothesis that biotinidase has activity to catalyze both biotinylation of histones [9] and debiotinylation of histones. Theoretically, biotinylation of histones might be a side product from the hydrolysis of biocytin in cells. If this were the case, cells would probably seek rapid hydrolysis of biotinylated histones in order to restore the biotin-free status of histones. Alternatively, biotinylation of histones might be a mechanism by which cells regulate processes such as transcription of DNA. Previous studies in our laboratory provided evidence that human cells respond to proliferation with increased biotinylation of histones, suggesting a physiologic role for biotinylation of histones [12].

If biotinylation of histones were an important mechanism to regulate cellular processes, how would cells regulate biotinylation status of histones despite the fact that both biotinylation and debiotinylation of histones are catalyzed by the same enzyme? We offer the following explanations: (*i*) Enzymes other than biotinidase might also catalyze biotinylation or debiotinylation of histones. For example, holocarboxylase synthetase (E. C. 6.3.4.10) catalyzes covalent binding of biotin to ε-amino groups of lysine residues in four mammalian carboxylases [21]. The possibility that holocarboxylase synthetase catalyzes binding of biotin to histones is an untested hypothesis.

(*ii*) Covalent modification of biotinidase might be a mechanism to favor either biotinylation or debiotinylation of histones. Currently, glycosylation is the only posttranslational modification of biotinidase that has been identified [22].

(*iii*) The presence of cofactors might favor either biotinylation or debiotinylation of histones. For example, high concentrations of the substrate biocytin may increase the rate of histone biotinylation. Similarly, biotinylated peptides might inhibit debiotinylation of histones by competing for binding to biotinidase. In contrast, the optimum pH is similar (pH 8) for both the biotinylating activity [9] and the debiotinylating activity of biotinidase. Thus, changes of the pH in the microenvironment of histones should not affect biotinylation status.

In conclusion, this study provides strong evidence that human cells and body fluids contain an enzyme (probably biotinidase) that catalyzes debiotinylation of histones. There is still great uncertainty as to whether this enzyme is important for regulation of biotinylation status of histones. Nevertheless, the existence of the debiotinylating enzyme suggests that the biotinylation status of histones might be physiologically significant, e. g., to regulate transcriptional activity of DNA. Alternatively, the debiotinylating enzyme might be important to rapidly eliminate a side product derived from hydrolysis of biocytin (biotinylated histones) in order to avoid derangement of normal metabolism of histones.

Acknowledgments This work was supported by NIH grant DK 60447 and the United States Department of Agriculture/National Research Initiative Competitive Grants Program project award 2001–35200–10187. A contribution of the University of Nebraska Agricultural Research Division, Lincoln, NE 68583. Journal Series No. 13424.

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