Histone H1\textsuperscript{0} is known to consist of two subfractions named H1\textsuperscript{a} and H1\textsuperscript{b}. The present work was performed with the aim of elucidating the nature of these two subfractions. By using reversed-phase high performance liquid chromatography in combination with hydrophilic interaction liquid chromatography, we fractionated human histone H1\textsuperscript{0} into even four subfractions. Hydrophilic interaction liquid chromatographic analysis of the peptide fragments obtained after cleavage with cyanogen bromide and digestion with chymotrypsin suggested that the four H1\textsuperscript{0} subfractions differ only in their small N-terminal end of the H1\textsuperscript{0} molecule (30 residues). Edman degradation of the N-terminal H1\textsuperscript{0} peptide fragments and mass spectra analysis have indicated that human histone H1\textsuperscript{0} consists of intact histones H1\textsuperscript{a} (named H1\textsuperscript{a} Asn-3) and deamidated H1\textsuperscript{a} forms (H1\textsuperscript{a} Asp-3) having an aspartic acid residue at position 3 instead of asparagine. Moreover, both H1\textsuperscript{a} Asn-3 and H1\textsuperscript{a} Asp-3 are blocked (H1\textsuperscript{a}b Asn-3, H1\textsuperscript{a}b Asp-3) and unblocked (H1\textsuperscript{a}b Asn-3, H1\textsuperscript{a}b Asp-3) on their N terminus. Acid-urea gel electrophoretic analysis has shown that the histone subfraction, in the literature originally named H1\textsuperscript{a}, actually consists of a mixture of H1\textsuperscript{a} Asn-3 and H1\textsuperscript{a} Asp-3, whereas H1\textsuperscript{b} consists of H1\textsuperscript{b} Asn-3 and H1\textsuperscript{b} Asp-3. Furthermore, we found that hydrophilic interaction liquid chromatography separates rat and mouse histone H1\textsuperscript{0} just like human H1\textsuperscript{0} into four subfractions. Hydrophilic interaction liquid chromatographic analysis of brain and liver histone H1\textsuperscript{0} from rats of different ages revealed an age-dependent increase of both the N-terminally acetylated and the deamidated forms of H1\textsuperscript{b}. In addition, we found that the relative proportions of the four forms of H1\textsuperscript{b} histones differ from tissue to tissue.

DNA in eukaryotes is organized and compacted in chromatin. The fundamental subunit of chromatin is the nucleosomal core, which consists of 146 base pairs of DNA wrapped 1.75 times around an octamer of core histones (reviewed in Ref. 1). The linker histones H1, H5, and H1\textsuperscript{0} are associated with the core histone-DNA complex and with the linker DNA between adjacent nucleosomes and are thought to modulate the condensation/decondensation of the chromatin fiber, thus influencing many nuclear activities such as transcription, replication, recombination, and DNA repair (2). H1\textsuperscript{0} was first described in 1969 by Panyim and Chalkley (3, 4) as an H1-like protein present in mammalian tissues with little or no cellular proliferation and was later shown to increase at a terminal stage of differentiation (5–10). Some cells, however, accumulate significant amounts of the protein while still actively proliferating (11, 12) or accumulate it upon proliferation arrest without concomitant differentiation (12). In addition, H1\textsuperscript{0} seems to be the only histone undergoing changes during malignant transformation (13). Recently, it was found that transformation of NIH 3T3 fibroblasts by c-Ha-rasVal\textsuperscript{12} oncogene causes chromatin decondensation accompanied by alterations in the content of histone H1\textsuperscript{0} (14). All these findings suggest a role for H1\textsuperscript{0} in the regulation of either cell proliferation or cellular differentiation.

In every tissue in which H1\textsuperscript{0} has been detected, two subfractions were present (15–18). It appears that these two H1\textsuperscript{0} proteins, up to now named H1\textsuperscript{a} and H1\textsuperscript{b}, have specific individual functions in chromatin (15). The relative proportions of the two H1\textsuperscript{0} forms seem to differ from tissue to tissue (15) and exhibit age-dependent changes in rat brain cortical neurons (17). The two H1\textsuperscript{0}s are resolvable by ion-exchange chromatography on Bio-Rex 70 (16, 19) or acetic acid-urea gel electrophoresis (15–18). Most recently, Lindner et al. (20) developed a high performance capillary electrophoresis method allowing separation of H1\textsuperscript{0} and its subfractions from other histone H1 subtypes. The two H1\textsuperscript{0} proteins run coincidentally on sodium dodecyl sulfate-polyacrylamide gels, suggesting that the difference between them is one of charge and not of size (15). Since neither treatment with alkaline phosphatase nor exposure to alkaline conditions changed the separation of the H1\textsuperscript{0} peak into two subfractions, phosphorylation and ADP-ribosylation have been ruled out as possible post-translational modifications responsible for the different forms (16, 20, 21). Although some investigators speculated that the two forms of H1\textsuperscript{0} might be coded by different genes (15, 17), Doenecke et al. (22) found that the mammalian genomes contain only one H1\textsuperscript{0} gene.

To gain insight into the nature of the two H1\textsuperscript{0} subfractions, we took advantage of a combined reversed phase high performance liquid chromatography (RP-HPLC)\textsuperscript{3}/hydrophilic interaction liquid chromatography (HILIC) technique recently developed in our laboratory for separating acetylated core and phosphorylated H1 histones (23, 24). By applying this two-step HPLC method human placenta histone H1\textsuperscript{0} was resolved into four components, which were treated with cyanogen bromide and chymotrypsin. HILIC analysis of the peptide fragments

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\textsuperscript{3} The abbreviations used are: RP-HPLC, reversed-phase high performance liquid chromatography; HILIC, hydrophilic interaction liquid chromatography; AU-PAGE, acid-urea polyacrylamide gel electrophoresis; CE, capillary electrophoresis; TEA, triethylamine; MALDI-TOF-MS, matrix-assisted laser desorption ionization coupled with time-of-flight mass-spectrometry.
obtained indicated that the four H10 protein subfractions differ in their N-terminal end consisting of 30 residues. The subsequent Edman degradation of the N-terminal peptides and mass spectra analysis of the four untreated H10 subfractions demonstrated that human placenta histone H10 consists of a mixture of intact (H10 Asn-3) and deamidated forms (H10 Asp-3) both blocked (H10a Asn-3, H10a Asp-3) and unblocked (H10b Asn-3, H10b Asp-3) on their N terminus. Deamidation occurs at Asn-3 in the sequence Thr-Glu-Asn-Ser. Applying the procedure described for human placenta histone H10, we also analyzed histone H10 from rat and mouse liver and brain. We found four H10 subfractions consisting of all the possible combinations involving either acetylated or unacetylated N-terminal residues and/or Asn or Asp at position 3. We thus show for the first time the occurrence of N-terminally unblocked H10 histones and of in vivo deamidated forms of linker histones. Furthermore, we found an accumulation of both the N-terminally acetylated and the deamidated forms of H10 with aging and that the relative proportions of all four forms of H10 histones differ from tissue to tissue.

**EXPERIMENTAL PROCEDURES**

**Materials**—Sodium perchlorate (NaClO4) and triethylamine (TEA) were purchased from Fluka (Buchs, Switzerland), and hydroxypropylmethylcellulose (4000 centipoises) and trifluoroacetic acid were obtained from Sigma (Munich, Germany). All other chemicals were purchased from Merck (Darmstadt, Germany).

**Preparation of H1 Histones**—H1 histones were extracted from human placenta and from rat and liver with brain with perchloric acid (5%, v/v) according to the procedure of Lindner et al. (25) with slight modifications. The organs were homogenized in 2 volumes of buffer A (250 mM sucrose, 50 mM Tris-HCl, pH 7.5, 25 mM KCl, 5 mM MgCl2, 0.2 mM phenylmethylsulfonyl fluoride, 50 mM NaH2PO4, 45 mM sodium butyrate, and 10 mM 2-mercaptoethanol) in a Potter homogenizer. After centrifugation at 10,000 rpm for 3.500 rpm in the SS-34 rotor of a Sorvall centrifuge, the pellet was washed twice with buffer A and then homogenized in buffer A containing 0.2% Triton X-100 in a Dounce homogenizer. Nuclei thus obtained were centrifuged (10 min at 3,500 rpm), and the nuclear pellet was washed once with buffer A without Triton X-100. The pellet was centrifuged and then extracted with 1 volume of 0.5M stock solution prepared by adding TEA to phosphoric acid until used for HPLC.

**Hydrophilic Interaction Liquid Chromatography**—The histone fraction H10 (150 µg) isolated by RP-HPLC was analyzed on a PolyCAT A column (250 × 4.6 mm inner diameter; 5-µm particle size; 20 °C). The histone fractions obtained in this way were collected and, after adding 20 µl of 2-mercaptoethanol (0.2 µl, lyophilized, and stored at −20 °C).

**Amino Acid Sequence Analysis**—Peptide sequencing was performed on an Applied Biosystems Inc. (ABI) model 492 Procise protein sequencer. Sequence grade solvents were purchased from ABI. To partially deblock the acetylated N terminus, the histones were incubated in the presence of 1% formic acid overnight and then lyophilized and stored at −20 °C.

**Mass Spectrometric Analysis**—Determination of the molecular masses of the four histone H10 subfractions obtained by the HILIC run (Fig. 1B) was carried out by electrospray ion-mass spectrometry technique using a MAT 900 instrument (Finnigan/MAT GmbH, Bremen, Germany). Samples (5–10 ng) were dissolved in 50 µl of 70% formic acid containing 10 mg/ml cyanogen bromide (21, 27). The digest was then analyzed on a mass spectrometer equipped with a QGQ mass spectrometer (MALDI-TOF-MS) was used for determination of the molecular masses of the seven peptide fractions obtained by RP-HPLC separation (Fig. 3) of chymotrypsin-digested human placenta H10. MALDI-TOF-MS was performed on a KOMPACT MALDI III (Kratos Analytical, Manchester, UK) linear type mass spectrometer operating in the positive ion mode of detection. The matrix solution was prepared
by making a saturated solution of 4-hydroxy-α-cyanocinnamic acid with water/acetonitrile (1:2). Sample preparations were performed as in the following: typically 2 μl of the matrix stock solution was placed in an Eppendorf tube, and 1 μl of each sample peptide solution (10 μM/μl) and 1 μl of ubiquitin solution (10 μM/μl) were added as internal mass standard. The solution was briefly mixed using vortex stirring. 0.8 μl of the matrix/peptide/ubiquitin mixture was applied onto the sample slide. Acid-Urea Gel Electrophoresis—Polyacrylamide (15%) gel electrophoresis (16 cm × 18 cm × 0.75 mm) was carried out in acetic acid/urea (28). The gels were stained for 1 h with 0.1% Coomassie Blue in 40% (v/v) ethanol, 5% (v/v) acetic acid and destained in 20% (v/v) ethanol, 5% (v/v) acetic acid.

HILIC Separation of Human Placenta Histone H10 into Four Subfractions—Perchloric acid-extracted linker histones from human placenta were fractionated using RP-HPLC with a semi-preparative column filled with Nucleosil 300-5 C4, and a two-step water/acetonitrile gradient. The three fractions obtained (Fig. 1A) were characterized by SDS- and AU-PAGE (data not shown). The histone H10 fraction eluted at about 19 min as a single peak. By applying a new high performance capillary electrophoresis method (20, 29), this fraction was further separated into two major peaks (data not shown). This result was not further surprising since it is known (15–18) that long acid-urea-polyacrylamide gels also resolve histone H10 into two subfractions (designated H10a and H10b).

Excellent separations of modified core and H1 histones, recently achieved in our laboratory using the HILIC method (23, 24), prompted us to test this HPLC technique for fractionating histone H10 from human placenta. Therefore, the histone H10 fraction obtained in the RP-HPLC run (Fig. 1A) was subjected to HILIC using a PolyCAT A column with a triethylammonium phosphate buffer system, pH 3.0, in the presence of 70% acetonitrile. As shown in Fig. 1B, five major peaks were found. Since the peak eluting at about 15 min consists of protamine sulfate, which was generally added to the linker histone fractions isolated by RP-HPLC in order to stabilize the proteins (24), peaks 1–4 are due to H10 subfractions. The results obtained with CE and HILIC clearly indicate that the individual H10 subcomponents differ in both their charge and hydrophilicity. Furthermore, it is evident that the proteins of peaks 3 and 4 exhibit a more hydrophilic nature than do the proteins of peaks 1 and 2.

Characterization of the Four H10 Subfractions Obtained by HILIC—To characterize the proteins in Fig. 1B, each peak was first subjected to AU gel electrophoresis. It was found that peaks 1 and 2 in Fig. 1B correspond to the subfraction called H10a and peaks 3 and 4 to that of H10b (shown in Fig. 2). In order to localize the region responsible for the diversity of the H10 proteins, whole H10 histone obtained from the RP-HPLC run shown in Fig. 1A was treated with cyanogen bromide. Histone H10 contains only 1 methionine residue at position 30 and, therefore, cleavage with cyanogen bromide should produce two peptide fragments, a larger peptide from residue 31 to the C terminus and a smaller one originating from the N-terminal H10 domain (residues 1–30; shown in Table I). In our experiments, however, we were unable to detect the small peptide when using RP-HPLC and CE. This finding that the small CNBr peptide is not detectable agrees well with observations made by other investigators using gel electrophoresis (16, 30).

Both CE and HILIC analysis of the large C-terminal peptide produced by treatment with cyanogen bromide and characterized by Edman degradation revealed that this fragment was homogeneous (data not shown). Based on this result we assumed that the heterogeneity of H10 is due to differences within the first 30 residues of the N-terminal H10 region. In
order to obtain peptides containing this N-terminal region we digested whole H10 histone from human placenta with chymotrypsin and separated the peptides by means of RP-HPLC. The fragmentation yielded seven main peptide peaks, as shown in Fig. 3. The purity and homogeneity of the fractions were assessed by CE (data not shown). Fraction I alone was non-uniform and resolved into two components. To identify the seven peptide fractions, amino acid sequencing of the first three amino acids and MALDI-TOF analysis were performed. The result is shown in Table I. Thus, it was established that fraction I consists of a peptide containing the N-terminal 52 residues of histone H10. To unambiguously confirm our assumption that the N-terminal domain is responsible for the microheterogeneity of histone H10, we analyzed fraction I under HILIC conditions. As expected, four subfractions designated 1<sup>9</sup> to 4<sup>9</sup> were obtained (Fig. 4). The chromatogram closely resembled that obtained by HILIC analysis of undigested H10 (shown in Fig. 1B).

Sequence analysis performed with the intact H10 proteins was problematic because of chemical side reactions in the course of Edman degradation, resulting in non-reliable sequence analyses data. We consequently attempted to determine the amino acid sequence of the individual HILIC peaks of Fig. 4. Previous reports indicated that the N terminus of the H10 proteins is blocked, restricting the determination of primary structure in that region (15, 31). However, as shown in Table I, we did sequence all RP-HPLC fractions including the N-terminal fraction I of Fig. 3. We therefore concluded that the N-terminal residue is blocked in some, however not in all four, histone H10 fractions obtained by HILIC. This assumption proved to be true; although sequence determination of the H10 subfractions of HILIC peaks 3<sup>9</sup> and 4<sup>9</sup> was unproblematic, it was not possible to sequence the fractions of peaks 1<sup>9</sup> and 2<sup>9</sup>. Thus, the proteins designated H10<sub>a</sub> and H10<sub>b</sub> in Fig. 2 are H10 histones with N-terminal blocked and N-terminal unblocked residue, respectively.

Since HILIC resolves H10<sub>a</sub> and H10<sub>b</sub> into two further sub-

### Table I

| Chymotryptic peptides of human placenta histone H10 and human H10 primary structure |
|-------------------------------------------------------------------------------------------------|
| The peptide fractions of chymotrypsin-digested human placenta H10 were separated by RP-HPLC (Fig. 3) and analyzed by both amino acid sequencing of the first three amino acids and by MALDI-TOF-MS. Peptide I, amino-terminal sequence (residues 1–52); peptide II, IKS (53–69); peptide III, SIK (70–80); peptide IV, KQT (81–92); peptide V, RLA (93–106); and peptide VI, KKT (107–193). Histone H10 sequence data were taken from human H10 cDNA (32). |

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**Fig. 3.** RP-HPLC of peptide fractions of chymotrypsin-digested human placenta H10. Whole histone H10 from human placenta was digested with chymotrypsin as described under “Experimental Procedures.” The digest (containing ~100 μg of peptides) was injected onto a Nucleosil 300–5 C<sub>18</sub> column (250 x 3 mm). Analysis was performed at a constant flow of 0.35 ml/min using a two-step acetonitrile gradient starting at 80% A, 10% B (solvent A, water containing 0.1% trifluoroacetic acid; solvent B, 85% acetonitrile and 0.1% trifluoroacetic acid). The concentration of solvent B was increased linearly from 10 to 45% (60 min) and from 45 to 100% (10 min). The effluent was monitored at 210 nm. Peptide fractions I–VI were analyzed by CE, amino acid sequencing of the first three amino acids, and by MALDI-TOF-MS (data not shown). Fraction I was used for HILIC analysis (Fig. 4).
idue was found to be aspartic acid. We named the corresponding deamidated histone H1\(^0\) protein H1\(^{0b}\) Asp-3. As for the HILIC subfractions 1' and 2' containing the blocked N-terminal residue, we assumed that they could also differ by deamidation at position 3. To confirm this, we partially deblocked the HILIC fractions as described under “Experimental Procedures” and determined the amino acid sequences. In fact, subfractions 1' and 2' differed at position 3, subfraction 1' containing asparagine and subfraction 2' aspartic acid. The corresponding proteins were designated H1\(^{0a}\) Asn-3 and H1\(^{0a}\) Asp-3, respectively.

To examine the nature of the blocking residue of the human placenta H1\(^{0}\) histones, we subjected the four H1\(^{0}\) subfractions obtained by HILIC to ion-spray mass spectrometric analysis (data not shown). Significant mass differences were not observed between the H1\(^{0a}\) Asn-3 and H1\(^{0a}\) Asp-3 nor between the H1\(^{0b}\) Asn-3 and H1\(^{0b}\) Asp-3 histones. This result agrees well with our finding that the Asn-3 forms consist of intact H1\(^{0}\) and the Asp-3 forms of deamidated H1\(^{0}\) histones (mass difference is 1 Da). However, a mass difference of 43 Da was found between the H1\(^{0a}\) Asn-3 and H1\(^{0b}\) Asn-3 histones as well as between the H1\(^{0a}\) Asp-3 and H1\(^{0b}\) Asp-3 histones. This mass difference corresponds to the presence (H1\(^{0a}\) Asn-3 and H1\(^{0a}\) Asp-3) and absence (H1\(^{0b}\) Asn-3 and H1\(^{0b}\) Asp-3) of an acetyl group. We assume that the acetyl group is bound to the N-terminal nitrogen of the H1\(^{0}\) histone, thus blocking the Edman degradation of the H1\(^{0a}\) Asn-3 and H1\(^{0a}\) Asp-3 histones. In this context it should be noted that N-terminal acetylation is a characteristic of H1 histones (33). However, the occurrence of both the blocked and the unblocked protein forms appears unique to mammalian histones.

From the results obtained it was possible to compare the gel electrophoretic behavior of the four H1\(^{0}\) subcomponents with that of H1\(^{1a}\) and H1\(^{1b}\). AU gel electrophoresis (shown in Fig. 2) revealed that H1\(^{1a}\) Asn-3 and H1\(^{1a}\) Asp-3 (the less positively charged H1\(^{1}\) histones; lanes 5 and 4, respectively) migrate slower than H1\(^{1b}\) Asn-3 and H1\(^{1b}\) Asp-3 (the more positively charged histone H1\(^{1}\) proteins; lanes 3 and 2, respectively). It seems likely to us, therefore, that the H1\(^{0}\) subfractions, in the literature originally designated H1\(^{1a}\) and H1\(^{1b}\) (15–17), actually consist of a mixture of H1\(^{0a}\) Asn-3 and H1\(^{0a}\) Asp-3 and of

![Fig. 4. HILIC separation of peptide fraction I obtained by RP-HPLC of chymotrypsin-digested human H1\(^{0}\) histone.](image)

**Fig. 4.** HILIC separation of peptide fraction I obtained by RP-HPLC of chymotrypsin-digested human H1\(^{0}\) histone. The sample (~120 \(\mu\)g) was analyzed on a PolyCAT A column (250 × 4.6 mm) at 23 °C at a constant flow of 1.0 ml/min using a two-step gradient starting at 100% solvent A, 0% solvent B (solvent A, 70% acetonitrile, 0.015 M TEA/H\(_3\)PO\(_4\), pH 3.0; solvent B, 70% acetonitrile, 0.015 M TEA/H\(_3\)PO\(_4\), pH 3.0, and 0.68 M NaClO\(_4\)). The concentration of solvent B was increased from 0 to 65% B(5 min) and from 65 to 100% B (45 min). The HILIC fractions (designated 1'–4') were desalted using RP-HPLC. The peptide fractions obtained in this way were collected and applied on an ABI protein sequencer.

**Fig. 5.** HILIC analysis of histone H1\(^{0}\) from liver and brain of rats of various ages. H1\(^{0}\) was from the livers of rats aged 10 days (A) and 15 months (B) and the brains of rats aged 10 days (C) and 15 months (D). The H1\(^{0}\) fractions (~150 \(\mu\)g) isolated by RP-HPLC were analyzed on a PolyCAT A column (250 × 4.6 mm). Conditions were the same as for Fig. 1B. 1, H1\(^{0a}\) Asn-3; 2, H1\(^{0a}\) Asp-3; 3, H1\(^{0b}\) Asn-3; 4, H1\(^{0b}\) Asp-3.
Age-dependent Deamidation of H10 Histones

An obvious question raised by the present results was whether N-terminal acetylation and/or deamidation of histone H10 is a physiologically important process. In this context it should be noted that previous investigations have shown an age-related change in the proportion of H10 “subtypes” a and b in rat brain cortical neurons (17) and, moreover, differing ratios of histones H10a and H10b according to the tissue examined (15). We were interested, therefore, in examining the occurrence of the individual H10 forms prepared from various tissues (liver and brain) of rats aged 10 and 450 days using our HILIC technique. As can be seen from Fig. 5, the H10 pattern differs not only between rat liver and brain (Fig. 5, A and B compared with C and D) but also between young and old rat liver (Fig. 5A compared with B) and between young and old rat brain (Fig. 5C compared with D). In order to obtain more precise and, in addition, quantitative data on the age-dependent changes of N-terminally acetylated and deamidated H10 forms, we analyzed liver and brain H10 histones from rats aged 10 days, 30 days, 6 months, and 15 months. Fig. 6A reveals that the proportion of N-terminally acetylated H10 (sum of H10a Asn-3 and H10a Asp-3) is about 30% higher in senescent (15 months old) rat livers and brains than in young ones (10 days old). It is worth mentioning, however, that an increase of about 37% in acetylated histone H10 was already observed in rat brains of 30-day-old animals. The dramatic age-dependent increase of the deamidated histone H10 forms was about 7.5-fold higher in brain and about 3-fold higher in liver of rats 15 months of age than that in 10-day-old animals.

N-terminal acetylation of proteins is a common modification among eukaryotic proteins (34, 35). Although the structural or functional significance of N-terminal acetylation is unknown, it seems likely that the N terminus of a protein has a major effect on the regulation of protein metabolism (36) or on the rate of degradation (34–36). The N-terminal acetylation of a protein is usually complete (34). However, we found the N-terminally acetylated and unacetylated forms to occur simultaneously for the H10 histones. Regarding the linker histones, this kind of uncompleted acetylation of N-terminal residues seems to be limited to histone H10. For this reason, it is conceivable that this modification serves as a histone H10-specific molecular timer of histone turnover. It would be of interest, therefore, to explore further the biological consequences of a blocked and an unblocked N terminus of H10 histones.

Protein deamidation is a well-documented nonenzymatic process (37, 38). Some studies have shown an age-dependent increase of deamidation of proteins that turn over little or not at all during the lifetime of the organism (39, 40). This accumulation of deamidated forms of proteins possibly results from a decrease in the rate at which deamidated proteins degrade with age (41). The fate of deamidated asparaginyl residues in proteins is unknown. Robinson et al. (42) suggested that deamidation of proteins could serve as a molecular timer determining the lifetime of proteins. In a few cases the deamidation of specific asparagine residues has been connected with changes in protein function (43). Although deamidation caused by a deamidase was also described most recently (44, 45), in this study we did not find any evidence of such an enzymatic deamidation.

It should be noted that nonenzymatic deamidation is known to occur also in vitro during the isolation procedure and the handling and storage of peptides and proteins (46). It was important, therefore, to make sure that the described alter-
In histone H1\(^0\) forms are indeed the result of in vivo aging in the tissues. Despite the fact that all procedures for isolation and handling of proteins were carefully performed under identical conditions, the level of deamidated histone H1\(^0\) forms shows an age-dependent increase in the tissues investigated. Furthermore, neither the gentle extraction of linker histones using 0.5 M NaCl instead of 5% perchloric acid nor prolonged standing of the histones precipitated with 20% trichloroacetic acid (overnight instead of only 60 min) resulted in detectable changes in the amounts of deamidated forms (data not shown). It can thus be concluded that the deamidation events observed are actually in vivo processes in aging organs. This study, therefore, provides the first evidence of in vivo deamidation in linker histone proteins.

Whether the age-dependent increase of deamidated and/or N-terminally acetylated histone H1\(^0\) forms is responsible for the age-related decrease in transcriptional activity found in various mammalian tissues including the brain (47–49) is unknown at present. It is reported that this decline of transcriptional activity is primarily due to changes in the chromatin structures (49, 50). Both the N-terminal acetylation and the deamidation of Asn-3 reduce the positive charge of the N-terminal domain of histone H1\(^0\). It is conceivable, therefore, that the age-related changes in N-terminal acetylation and deamidation may directly affect the interaction of H1\(^0\) proteins with DNA. This effect might be one of the reasons for the structural changes in chromatin observed in aging tissues.

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