Nickel Compounds Are Novel Inhibitors of Histone H4 Acetylation

Limor Broday, Wu Peng, Min-Hao Kuo, Konstantin Sahnikow, Maria Zoroddu, and Max Costa

Department of Environmental Medicine [L. B., W. P., K. S., M. C.] and Kaplan Comprehensive Cancer Center [K. S., M. C.] New York University School of Medicine, New York, New York 10016; Department of Biochemistry, Michigan State University, East Lansing, Michigan 48824-1319 [M.-H. K.]; and University of Sassari, 07100 Sassari, Italy [M. Z.]

Abstract

Environmental factors influence carcinogenesis by interfering with a variety of cellular targets. Carcinogenic nickel compounds, although generally inactive in most gene mutation assays, induce chromosomal damage in heterochromatic regions and cause silencing of reporter genes when they are located near telomere or heterochromatin in either yeast or mammalian cells. We studied the effects of nickel on the lysine acetylation status of the NH2-terminal region of histone H4. At nontoxic levels, nickel decreased the levels of histone H4 acetylation in vivo in both yeast and mammalian cells, affecting only lysine 12 in mammalian cells and all of the four lysine residues in yeast. In yeast, lysine 12 and 16 were more greatly affected than lysine 5 and 8. Interestingly, a histidine Ni+2 anchoring site is found at position 18 from the NH2-terminal tail of H4. Nickel was also found to inhibit the acetylation of H4 in vitro using purified recombinant histone acetyltransferase. To our knowledge, this is the first agent shown to decrease histone H4 acetylation at nontoxic levels.

Introduction

Certain nickel compounds including crystalline nickel sulfide (NiS) and subsulfide (Ni3S2) are potent carcinogens that induce a wide variety of tumors in experimental animals and are implicated in the etiology of human respiratory cancers after inhalation exposure (1). The potent carcinogenic activity exhibited by crystalline nickel subsulfide was found to be due to the ability of the NiS particles to enter cancer target cells by phagocytosis (2). After entry, the particles are dissolved intracellularly, yielding higher cytoplasmic and nuclear concentrations of nickel ions than could be achieved when cells are exposed to water-soluble nickel salts (2). Whereas higher cellular levels of nickel ions can be obtained by raising exposure concentrations to soluble nickel salts, the delivery of nickel to intracellular compartments is more uniform with the soluble salts causing greater toxicity but less nuclear nickel and thus less transformation.

The major damage that is produced by nickel particles occurs in the heterochromatic regions of chromosomes (3). In transgenic gpt+ Chinese hamster cell lines, nickel induced the inactivation of a reporter gene located specifically near a heterochromatic region (4). The same position effect variegation that caused epigenetic silencing of gene expression in mammalian cells was also demonstrated in the yeast Saccharomyces cerevisiae (5). This phenomenon, termed TPE (telomeric position effect; Ref. 6), was measured using a telomeric marker gene that was repressed as a result of the growth of yeast cells in the presence of nickel chloride. Soluble nickel was effective in this system because it was taken up actively by yeast cells through a magnesium transport system.

Silenced DNA is packaged into condensed heterochromatic regions. These regions contain poorly acetylated histones, whereas euchromatin regions contain transcriptionally active genes that are associated with NH2-terminal acetylated histones (7). The connection between acetylation and transcription was demonstrated when p55 from Tetrahymena was shown to have HAT3 activity and to be highly homologous to the yeast GCN5 that had already been known to be a transcriptional ‘adaptor’ in yeast (8).

To further elucidate the effect of nickel compounds on transcriptional repression, we measured the effects of these compounds on the acetylation status of histone H4. The acetylation pattern and protein interactions of the NH2 termini of H3 and H4 in yeast telomeres were extensively studied and were found to be crucial for the establishment of gene silencing (9). We show that nickel is a potent inhibitor of histone H4 acetylation in yeast and mammalian cells. In vitro inhibition was also detected using the liquid HAT assay. The possible mode of action and the specificity of nickel targeting is also considered.

Materials and Methods

Chemicals. Nickel chloride anhydrous was obtained from Morton Thiokol (Danvers, MA), nickel subsulfide was obtained from INCO (Toronto, Canada), cobalt chloride (CoCl2·6H2O) was obtained from Mallinckrodt Chemical Works (St. Louis, MO), cadmium chloride anhydrous was obtained from Fisher Scientific (Fairlawn, NJ), and cupric sulfate anhydrous was obtained from Fisher Scientific (Fairlawn, NJ). The antibodies for H4-acetyl-lysine were obtained from Serotec Inc. (Raleigh, NC) and from Chemicon International Inc. (Temecula, CA), and H3 and H4 histones (calf thymus) were purchased from Roche Molecular Biochemicals (Indianapolis, IN).

Yeast Strains and Mammalian Cell Culture. S. cerevisiae strains UCC506 and YJS-URA-Tel were gifts of Daniel E. Gottschling (Fred Hutchinson Cancer Research Center, Seattle, WA) and Virginia A. Zakian (Princeton University, Princeton, NJ), respectively (6). To assay metal-induced changes, a fresh colony was grown in standard synthetic complete medium (Difco) until 3–6 × 102; then the cells were diluted in a fresh synthetic complete medium (5 × 104 cells per ml), and the metal was added. The cells were grown for the indicated number of cell generations, the metal was washed out using dH2O, and histones were purified.

The lung carcinoma A549 cells were grown in monolayer in F-12K medium containing 10% fetal bovine serum and 100 units/ml of penicillin. The culture was maintained at 37°C in a humidified atmosphere of 95% air:5% CO2. The cells were plated onto 150-mm-diameter tissue culture dishes on day 0. On day 1, the indicated amount of Ni3S2 particles in F-12K medium was added to the cell dishes (cells were about 40% confluent). On day 3, when cells were 75–80% confluent, the treatment was stopped by washing away the Ni3S2 particles in cold PBS. Attached cells were collected from the dishes by scraping, and the histone were purified from these cells.

Histone Purification. Preparation of histones from yeast cells was done as described by Ekwall et al. (10). Preparations of histones from A549 cells was done according to Cousens et al. (11) with the following modifications: the
Washed cells were suspended in lysis buffer (11) containing TSA (100 ng/ml) and PMSF (1 mM). After pipetting up and down for about 20 times, the nuclei were washed three times in the lysis buffer and once in 10 mM Tris and 13 mM EDTA (pH 7.4). The histones were extracted from the pellet in 100 μl of 0.4 M H₃SO₄ on ice for 1 h. After centrifugation for 10 min at 12,000 rpm, the supernatant was removed and mixed with 10 volumes of cold acetone and kept at −20°C overnight. The histones were collected by centrifugation and air-dried and then were suspended in 4 M urea and stored at −20°C.

**In Vitro Acetylation.** Recombinant Gcn5p was expressed and purified from *Escherichia coli* as described by Kuo et al. (12). HAT assays were performed in 20-μl reactions containing: 13 μg of calf histone H4 (or 18 μg of calf histone H3), 10 mM HEPES (pH 7.8), 0-50 mM NaCl, 0-0.4 mM NiCl₂, 4% glycerol, 10 mM n-butyrate, Gcn5p (15 ng), and 0.1 μCi of [³H]acetyl-CoA (4-6 Ci/mmol). The reaction was incubated at 30°C for 30 min. Aliquots were spotted on P-81 filter paper and washed as described by Mizzen et al. (13), and the level of [³H]acetyl-CoA incorporation was determined by scintillation counting (two replicates for each reaction). The HAT activity remaining after exposure to nickel was determined in at least three separate experiments. All of the reported values were corrected by subtracting appropriate background levels determined for vector (only) controls for each reaction condition and histone substrate.

**Results and Discussion**

To identify whether nickel exerted its activity on gene silencing by a mechanism that involved changes in histone acetylation, we measured the effect of this metal on histone H4 acetylation in *S. cerevisiae* using antibodies specific for the particular acetylated isoforms (14). Histone H4 acetylation at lysine 5, 8, 12, and 16 increased during growth in the logarithmic phase (Ref. 15; Fig. 1a). The addition of 0.5 mM NiCl₂ suppressed the growth-related accumulation of lysine acetylation, causing a decrease in acetylation at all four of the lysine residues (Fig. 1a). This inhibition could be detected after 2-3 cell generations in the presence of nickel. Previously, we have shown that nickel caused the silencing of a marker gene positioned near the telomere (TPE) in yeast cells grown for 5–7 cell generations (5).

**Fig. 1.** Nickel inhibits histone H4 acetylation in *S. cerevisiae* cells. *a*, *S. cerevisiae* UCC506 cells (5 × 10⁵) were grown with or without NiCl₂ (0, 0.2, or 0.5 mM) for 1, 3, or 6 cell generations and early stationary phase (24 h, ST). Three μg of histones from bulk chromatin preparations were separated on 15% SDS-PAGE gels and subjected to Western blotting with antibodies specific for H4 acetyl-lysine 5, 8, 12, or 16 or stained with Coomassie Blue (5 μg; Lanes 1–8). *b*, the experiment was started at 5 × 10⁵ cells/ml, and NiCl₂ (0.5, 1, or 2 mM) was added after three cell generations, i.e., at 4 × 10⁶ cells/ml, for an additional two cell generations or until early stationary phase (24 h, ST; Lanes 9–14); the histones were then subjected to the same analysis as in *a*.

**Fig. 2.** The effect of NiCl₂, CuSO₄, CdCl₂, and CoCl₂ on the level of histone H4 acetylation in *S. cerevisiae*. Cells were grown (started at 5 × 10⁵ cells/ml) in the presence of NiCl₂ (*a*), CuSO₄ (*b*), CdCl₂ (*c*), and CoCl₂ (*d*) for four to five cell generations. Histones from bulk chromatin preparations were subjected to the same analysis as described above. Western analysis was done with antisera to acetylated H4, αH4Ac (acetylated at lysines 5, 8, 12, and 16), or with antisera to H4 acetylated at lysine 12 only, αH4Ac.12. The experiment was done with the strain UCC506 for all of the metals. In addition, CuSO₄ was also incubated with the strain YJS-URA-TEL, which was found in our previous study (5) to be more sensitive to this metal, probably because of different levels of copper ion uptake, sequestration, or defense against reactive oxygen intermediates.
temporal sequence suggested that the inhibition of histone H4 acetylation by nickel induced changes in gene expression that subsequently led to higher levels of gene silencing localized in areas near heterochromatin. The effects of Ni\textsuperscript{2+} on histone acetylation were observed at levels that were not toxic to cells (5).

In logarithmically growing yeast cells, the level of histone acetylation is high and the acetylation and deacetylation processes occur at a slow rate (15, 16). When nickel was added to a culture at this stage, a decrease in the acetylation levels required higher concentrations of nickel and longer incubation periods (Fig. 1b). In addition, the decrease in histone H4 acetylation differed in extent at positions 5, 8, 12, and 16. The lysine residue at position 12 was the most sensitive, and two cell generations with 1 mM NiCl\textsubscript{2} were sufficient to inhibit acetylation at this position (Fig. 1b). These results suggested that nickel has preference to specific residues on H4. Indeed, the unique structure of the NH\textsubscript{2}-terminal tail of histone H4 contains a histidine residue at position 18, which is close to the posttranslational modified lysine sites. This histidine residue was shown by us to be an anchoring site for nickel coordination.\textsuperscript{4} Binding of nickel to this residue may interfere differently with the process of acetylation of specific lysine residues that are located in its vicinity and, thus, explain the differential activity of nickel (i.e., inhibition by Ni\textsuperscript{2+} interaction with this substrate).

The effect of other metals on histone acetylation in S. cerevisiae in comparison with nickel is shown in Fig. 2. CdCl\textsubscript{2} up to 100 \( \mu \text{M} \) or CoCl\textsubscript{2} (0.2–2 mM) did not inhibit H4 acetylation; however, CuSO\textsubscript{4} inhibited H4 acetylation at 0.5 mM, which was in the nontoxic concentration range for the yeast strain examined (UCC506). A comparison with a strain that was more sensitive to Cu\textsuperscript{2+} (YJS-URA-TEL) showed the same effect, but this occurred at toxic levels (50 \( \mu \text{M} \) for this strain), which inhibited growth. Interestingly, histidine 18 in H4 was also shown to be an anchoring site for Cu\textsuperscript{2+} binding as well as for nickel binding.\textsuperscript{4}

The effect of nickel on the levels of histone H4 acetylation was also examined in lung carcinoma A549 cells treated with soluble and insoluble nickel compounds. In mammalian cells, the core histones of transcriptionally active chromatin regions were undergoing high rates of acetylation and deacetylation, whereas in regions of repressed chromatin, the turnover rate of acetylation was slow. The bulk of the chromatin has core histones in unacetylated and monoacetylated states (16). In A549 cells, soluble nickel compounds did not change the level of histone H4 acetylation (not shown), whereas nickel subsulfide particles decreased the levels of acetylation in a concentration-dependent manner (Fig. 3). The major effect was on Lys-12, which was also shown to be more sensitive to nickel in yeast cells, especially when nickel was added to a logarithmic culture (Fig. 1b). Thus, the effect of nickel was not equal with respect to the different lysine residues also in mammalian cell lines. In contrast to yeast cells, there was only a slight effect on Lys-16 (Fig. 3), probably because this is the first residue on H4 to be acetylated in mammalian cells (16) and, thus, was less influenced by nickel binding.

Under physiological ionic conditions, the core histone NH\textsubscript{2}-termini

---

are not stably bound to nucleosomal DNA in either native chromatin or nucleosomal arrays (17). Therefore, we decided to examine the effect of nickel on the in vitro acetylation reaction under different ionic concentrations, which may influence the coordination of nickel with its putative histone target. Nickel inhibited H4 acetylation in vitro when incubated with the histone substrate and the recombinant yeast HAT GCN5 in the presence of elevated concentrations of NaCl (Fig. 4A). The highest concentration examined was 50 mM NaCl because higher concentrations of NaCl by itself inhibited the enzyme activity (not shown). A comparison to Histone H3 under the same assay conditions (50 mM NaCl), showed no inhibition of the acetylation reaction by nickel (3 mM; Fig. 4B). These results strengthen the assumption that nickel binds to histidine 18 of H4 in vivo, thus preventing the addition of the acetyl group to the nearby lysine residues and establishing a repressed chromatin state. At physiological pH, the ε-amino group of the lysine substrate is protonated and, therefore, nonreactive. This group is deprotonated by an active-site amino acid residue of the HAT enzyme that acts as a general base catalyst (18). It has been suggested that the coordination of nickel with H4 at histidine 18 involves the lateral chain of lysines, and, thus, it prevented the deprotonation process and the subsequent acetylation.4

The process of histone acetylation conferred an important regulatory role in gene transcription, replication, repair, and recombination. The acetylation status of chromatin histones, which are the major proteins found in chromatin, was determined by the equilibrium between activities of HATs and histone deacetylases (8, 19). Our results suggested that nickel may have suppressed gene expression and, in particular, the expression of tumor suppressor genes through inhibition of histone H4 acetylation. To our knowledge, nickel is the first agent known to inhibit histone H4 acetylation. The effect occurs at nontoxic levels and probably results from nickel binding to the H4 substrate with histidine 18 being the anchoring site. This specific inhibition may be useful in studying the role of histone acetylation in the transcriptional activation of genes because a specific inhibitor of HAT activity has not been identified yet. Additionally, because nickel has been shown to increase DNA methylation in mammalian cells, the loss of histone H4 acetylation may be coupled with DNA methylation (20) in the programming of gene expression inactivation as a result of exposure to this metal.

References
Nickel Compounds Are Novel Inhibitors of Histone H4 Acetylation

Limor Broday, Wu Peng, Min-Hao Kuo, et al.


Updated version  Access the most recent version of this article at:  http://cancerres.aacrjournals.org/content/60/2/238

Cited articles  This article cites 19 articles, 7 of which you can access for free at:  http://cancerres.aacrjournals.org/content/60/2/238.full#ref-list-1

Citing articles  This article has been cited by 3 HighWire-hosted articles. Access the articles at:  http://cancerres.aacrjournals.org/content/60/2/238.full#related-urls

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.