Deacetylase Activity Associates with Topoisomerase II and Is Necessary for Etoposide-induced Apoptosis*

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DNA topoisomerase II (topo II) is a ubiquitous nuclear enzyme that is involved in DNA replication, transcription, chromosome segregation, and apoptosis. Here we show by immunoprecipitation, pull down with glutathione S-transferase fusion proteins, and yeast two-hybrid analysis that both topo IIα and -β physically interact with the histone deacetylase HDAC1. The in vitro DNA decatenation activity of recombinant topo IIα and -β is inhibited by association with catalytically inactive, recombinant HDAC1. We provide evidence for the in vivo significance of the topo II-HDAC1 association, showing that inhibition of HDAC activity with trichostatin A suppresses apoptosis induced by the topo II poison etoposide, but not by the topo I inhibitor camptothecin. We suggest that chromatin remodeling by an HDAC-containing complex facilitates both topo II-catalyzed DNA rearrangement and etoposide-induced DNA damage in vivo.

For completion of cell division, the DNA of replicated chromosomes must be disentangled to allow the segregation of sister chromatids. In humans, this is achieved by the unique decatenation activity of DNA topoisomerase II (topo II). Topo II is essential for normal and neoplastic cellular proliferation, and several common anti-cancer drugs exert their cytotoxic effects through this enzyme (1, 2).

Topoisomerase II activity in mammalian cells has been attributed to at least two isoforms. Topo IIα (p170) associates with chromosomes during prophase and throughout mitosis and is thought to be a major component of the nuclear scaffold (3, 4). It has a peak of expression during G2/M of the cell cycle (5). In contrast, the closely related topo IIβ (p180) isoform is thought to have a more general role in DNA metabolism, with expression levels that remain relatively constant during cell and growth cycles (5).

Both isoforms interact with the C-terminal region of the tumor suppressor protein, p53 (6). p53 is a component of a multiprotein complex that contains the histone deacetylase HDAC1 and the corepressor Sin3a (7–11).

HDAC1, and the closely related HDAC2, are both components of two separate multiprotein complexes. The NuRD/Mi-2 repression complex contains both nucleosome remodeling and histone deacetylase activities (12), whereas the Sin3 complex contains only the latter (9). Both complexes contain the Rb-associated proteins RbAp46 and RbAp48 and associate with various, sometimes DNA-binding, transcriptional repressor and corepressor proteins (11). The Xenopus NuRD complex (which contains homologues of mammalian HDAC1, RbAp48, and the methyl-CpG-binding protein MDB3) copurifies with DNA topoisomerase II (13), raising the possibility that mammalian topo II isoforms and HDAC1 may interact in a multiprotein complex.

Here we show that HDAC1 and DNA topoisomerase II isoforms physically interact both in vivo and in vitro. We also show that the HDAC inhibitor, TSA, suppresses apoptosis induced by the topo II poison etoposide, but not by the topo I inhibitor camptothecin. Our results raise the interesting possibility that chromatin remodeling by a topo II-HDAC-containing complex is involved in topo II-catalyzed DNA rearrangements and/or generation of etoposide-induced DNA strand breaks in vivo.

MATERIALS AND METHODS

Cells, Reagents, and Materials—The human cell lines HL-60 (promyelocytic leukemia; p53 null) and HeLa were grown in RPMI 1640 medium containing 8% fetal calf serum. Regions of HDAC1 cDNA were subcloned into the pGEX3T-4 family of vectors (Amersham Pharmacia Biotech) and verified by sequencing. GST fusion proteins were purified essentially as described previously (6). Recombinant human DNA topoisomerase IIα and -β were made in a yeast system and purified as described previously (14). Characterization and use of rabbit polyclonal antibodies against topo IIα (18511a) and topo IIβ (18513b) are described elsewhere (15). A polyclonal rabbit antibody against mammalian HDAC1 was raised against a synthetic peptide corresponding to amino acid residues 467–482 and affinity-purified as described previously (16). Antibody against topo I was obtained commercially (Topogen, Gen, number 2012).

Immunoprecipitations, in Vitro Binding Assays, and Western Blot Analysis—HeLa whole cell extract was prepared by lysing cells in incubation buffer (50 mM Tris, pH 7.5, 150 mM NaCl, 5 mM EDTA, 5 mM EGTA, 10% (v/v) glycerol) containing 1.0% (v/v) Nonidet P-40, 0.5% (w/v) sodium deoxycholate, 0.1% (w/v) SDS, protease inhibitors (0.1 mM phenylmethylsulfonyl fluoride and “Complete Mini™” tablets, Roche Molecular Biochemicals) and 50 units of DNase I (Amersham Pharmacia Biotech) per 107 cells. The lysate was incubated in ice for 10 min, and the clarified supernatant was used in standard immunoprecipitations as described previously (6, 7). To confirm specificity, cognate blocking peptide (10 μg) was incubated with the antibody for 30 min before the addition of extract. Preimmune serum and irrelevant antibodies were used as controls. GST pull down experiments used equivalent amounts of GST fusion proteins prebound to glutathione-Sepharose beads (Amersham Pharmacia Biotech) as described previously (6).

Interactions with recombinant topo IIα were performed in incubation buffer containing 0.1% (v/v) Nonidet P-40.

 Yeast Two-hybrid Assays—Yeast strains CG-1945 from a Matchmaker Two-Hybrid System II kit (CLONTECH) were transformed with appropriate binary combinations of constructs containing the GAL4 DNA-binding domain and the GAL4 activation domain, as recommended by the manufacturers. HIS3 reporter gene expression was
were also labeled with either FITC-annexin V conjugate (PharMingen).

1. Anti-HDAC1 antibody did not immunoprecipitate de-

amounts of topo II

A

B

FIG. 1. Evidence for the physical association of topo IIα and HDAC1 proteins. A, Immunoprecipitation of endogenous topo IIα (molecular mass, 170 kDa), with the indicated antibodies (10 μg of total IgG), from 100 μg of HeLa whole cell extract (WCE). B, immunoprecipita-
tion of deacetylase activity by antiserum against topo IIα and topo IIβ (18511α and 18513β) from HeLa WCE. Deacetylase activity is inhibited by TSA (10 ng/ml). Activity immunoprecipitated is expressed as a percentage of total input deacetylase activity, less a background of nonenzymatic release of [3H]acetate of 1.94%. Percentages are averages by 5 μM of immobilized (bound) GST-HDAC1, but not by immobilized GST. Topo IIα is also depleted from the supernatant (unbound) by GST-HDAC1. B, pull down of endogenous HDAC1 (molecular mass, 82 kDa) by immobilized GST-
topo IIα and topo IIβ, containing the CTD of each isoform. C, pull down of deacetylase activity with immobilized GST-topo IIα CTD and topo IIβ CTD. Bars represent ranges of two experiments.

and topo IIβ immunoprecipitate 6–9% of total deacetylase activity from HeLa whole cell extract (Fig. 1B). The activity is fully inhibited by TSA. Negative control immunoprecipitations with preimmune serum, an irrelevant antibody (anti-CDK7), or an antibody against DNA topoisomerase I (topo I) did not bring down activity above that of the no-antibody control.

We performed in vitro pull down experiments of endogenous protein with GST fusion proteins. Full-length mammalian HDAC1, tagged with a GST moiety, but not GST itself, bound endogenous topo IIα in whole cell extract (Fig. 2A). In the converse experiment, a GST fusion protein containing the C-terminal domain (CTD) of topo IIα was able to pull down endogenous HDAC1 (Fig. 2B). The fusion protein of the CTD of topo IIβ was also able to pull down small amounts of HDAC1 (Fig. 2B). Fusion proteins of the CTD of topo IIα and topo IIβ were able to pull down between 9 and 11% of deacetylase activity (Fig. 2C), comparable with the amounts brought down by immunoprecipitation (Fig. 1B). Whereas GST-topo IIβ pulls down less HDAC1, as detected on Western blots, than comparable amounts of GST-topo IIα (Fig. 2B), the two different fusion proteins bring down similar amounts of deacetylase activity (Fig. 2C). A possible explanation for this quantitative discrepancy is that other deacetylases, in addition to HDAC1, are preferentially associated with topo IIβ.

GST fusion proteins containing the C-terminal domain of HDAC1 interact with recombinant topo IIα (Fig. 3). This domain has previously been shown to contain the LXCXE motif (residues 414–418), that appears to mediate interactions with the retinoblastoma protein pRb (19). In contrast, an N-terminal HDAC1 fusion protein, containing the catalytic site, showed minimal interaction with recombinant topo IIα (Fig. 3).

A yeast two-hybrid system (18) was used to test for direct in vivo interaction between topo II and HDAC1. Inserts were constructed to express the topo IIα and topo IIβ C-terminal domains (6) and the HDAC1 region 220–482 (Fig. 3). Expression of the integrated, GAL4-dependent HIS3 reporter gene was used to detect interactions between “bait” and “prey” proteins in vivo. Topo IIα CTD or topo IIβ CTD as bait, together with HDAC1 as prey, allowed growth of large colonies (over 2 mm diameter) on His-selective medium. All three proteins were ineffective when expressed individually (Fig. 4).

To explore the biological significance of the topo II-HDAC1 interaction, we tested the ability of full-length recombinant
HDAC1 to modulate the functional properties of recombinant topo IIα and IIβ. Both of these enzymes can decatenate kinetoplast DNA (kDNA) to minicircle monomers, a process that requires a double-stranded break in the kDNA to allow strand passage. The addition of increasing amounts of HDAC1 to the reaction decreases the decatenation of kDNA by topo IIα and IIβ (Fig. 5). Addition of GST alone did not affect decatenation by either topo IIα and IIβ.

Suppression of Etoposide-mediated Apoptosis by the HDAC Inhibitor Trichostatin A—We tested the effect of the HDAC inhibitor trichostatin A (20) on apoptosis induced by the chemotherapeutic agent etoposide (VP-16). Etoposide causes topoisomerase II-mediated DNA damage by increasing the steady-state concentration of covalent DNA cleavage complexes (1, 2, 4). Cells treated with etoposide acquire an apoptotic morphology, notably the condensation of chromatin at the nuclear periphery and blebbing of the plasma membrane (2, 21). HL-60 cells displayed apoptotic chromatin condensation after only 1.5-h treatment with either 100 μM etoposide or 5.8 μM camptothecin, an inhibitor of topo I (Fig. 6A). Plasma membrane changes during early apoptosis include the exposure of phosphatidylserine to the external cellular environment (22). This change was measured by binding of FITC-conjugated annexin V and counting of labeled cells by fluorescence microscopy (Fig. 6B). Activation of cysteine aspartyl proteases (caspases) (21) during the apoptosis of HL-60 cells was assayed with a fluorescent substrate and FACS analysis of viable cells (Fig. 6C). Chromatin condensation, membrane changes, and caspase activation all demonstrated that prior treatment with 100 nM TSA suppresses the apoptotic effect of etoposide (Fig. 6, A–C). In contrast, TSA did not affect apoptosis induced by the topo I inhibitor camptothecin (Fig. 6, A–C). Note that topo I does not associate with detectable amounts of deacetylase activity (Fig. 1B). An identical anti-apoptotic effect of TSA treatment was also observed for the human lung adenocarcinoma cell line H1299 and HeLa cells (data not shown).

DISCUSSION

The results presented show that the histone deacetylase HDAC1 is physically associated with each of the two isoforms of human topoisomerase II, topo IIα and topo IIβ. The association occurs in vivo, being detectable by coimmunoprecipitation from human cell extracts and by yeast two-hybrid assay. It also

FIG. 3. In vitro interaction of recombinant topo IIα and HDAC1. Regions of HDAC1 cDNA were subcloned and expressed as GST fusion proteins. Immobilized fusion proteins (5 μg) were tested for interaction with 1 μg of recombinant topo IIα.

FIG. 4. Yeast two-hybrid assay showing that the CTDs of topo IIα and topo IIβ can interact with HDAC1 CTD (amino acids 220–482) in vivo. Expression of the reporter gene HIS3 in yeast strain CG-1945 (CLONTECH) was determined by two parallel series of spot assays on selective medium plates lacking tryptophan, leucine, and histidine (−Trp, −Leu, −His), but in the presence of 25 mM 3-amino-1,2,4-triazole to suppress background growth (6, 18). Colony size was compared with that on plates lacking tryptophan and leucine (−Trp −Leu) as control. Strong growth in −His medium occurs only in cells in which the bait and prey proteins physically interact.

FIG. 5. Functional association of topo II and histone deacetylase. Decatenation of 1.4 μg of kinetoplast DNA concatemer (conc.) to monomers of minicircle DNA by recombinant topo IIα (0.60 pmol) and topo IIβ (0.22 pmol) is inhibited by increasing amounts of GST-HDAC1 (1.4–13.9 pmol), but not by GST alone (4.2–13.9 pmol).

FIG. 6. TSA suppresses apoptosis induced by topo II-mediated DNA damage, but not by topo I inhibition, in HL-60 cells. A, cells were treated as indicated (see “Materials and Methods” for details). Nuclear changes were visualized by indirect fluorescence microscopy after staining with Hoechst 33342 with the addition of propidium iodide (PI) to visualize necrotic cells. The nuclear morphology of cells was scored for apoptotic cells (PI−; clear bars) and necrotic cells (PI+; gray bars) and expressed as percentages of total cells in the field of view. Values are averages of two separate experiments. **B**, plasma membrane changes were detected by the binding of annexin V conjugated to FITC, after treatment with TSA, etoposide, or camptothecin as indicated (see “Materials and Methods”). Staining was visualized by indirect fluorescence microscopy and expressed as percentages of total cells. Bars represent S.D. values for three separate experiments. **C**, broad-spectrum caspase activity was detected by labeling live cells with the FAM-VAD-FMK fluorescein-conjugated caspase substrate (see “Materials and Methods”) after treatment with inhibitors, as indicated. Labeled cells were detected by FACS analysis, and activity is expressed as the percentage of the viable cell subpopulation that stains positive for the substrate. Values are averages of two separate experiments.
occurs in vitro. GST-coupled recombinant topo IIα and topo IIβ pull down significant amounts of HDAC activity from cell extracts, while recombinant HDAC1 inhibits the in vitro deacetylation activity of recombinant topo IIα. Since completion of the work reported here, Tsai et al. (23) have reported essentially the same findings for the two very similar deacetylases HDAC1 and HDAC2. Interestingly, whereas Tsai et al. (23) find evidence for an interaction between topo IIα and various regions of HDAC2, including N-terminal residues 1–57, we find that only the C-terminal region of HDAC1 (residues 220–482) interacts with topo II in vitro. These two deacetylases seem to differ in their mode of interaction with topo II.

In experiments to assess the biological significance of the topo II-HDAC interaction, we analyzed the effect of the deacetylase inhibitor TSA on processes known to require topo II activity. The most striking effect so far has been on the ability of the topo II poison etoposide to drive cells into apoptosis. We show that treatment with TSA prior to the addition of etoposide suppresses apoptosis in a variety of cell lines. The effect is seen even with HL60 cells, in which apoptosis is detectable within less than 1 h, a finding that minimizes the probability that inhibition of apoptosis is due to pleiotropic effects of TSA, such as its ability to alter cell cycle progression. The inhibitory effect of TSA was detected in several p53-null cell lines, so the interaction between HDAC1 and p53 (7) cannot be responsible. Microscopically detectable chromatin remodeling is a diagnostic characteristic of cells in the later stages of apoptosis, and recent reports indicate that both topo II and histone acetylation play a role in this process (24). However, our results indicate that this is not the stage at which TSA exerts the inhibitory effect reported here. We have shown that TSA inhibition is detectable even when using an assay that measures one of the earliest changes of apoptosis, namely the alteration in membrane phospholipids detected by binding of annexin V (22). These findings argue that TSA is acting at a relatively early stage in apoptosis, prior to the onset of major changes in nuclear ultrastructure. It remains possible that TSA also affects more subtle chromatin changes, possibly those determining expression of genes required for progression through apoptosis (25). These effects are not mutually exclusive. Indeed, recent results indicate that both topo II and changes in acetylation act at various stages in the pathways by which cells progress through apoptosis (24, 26–28).

In attempting to explain the effect of TSA on etoposide-induced apoptosis, it is important to note that etoposide is a topo II poison that blocks the enzyme after DNA cleavage but induced apoptosis, it is important to note that catalytically active HDAC1, in the context of a multiprotein complex that includes both HDAC and chromatin remodeling activities (23), is also inhibitory. It might even be the case that, in vivo, topo II activity is inhibited only by association with HDAC rendered catalytically inactive by inhibitors such as TSA. Such an effect would complement the suppression of topo II activity brought about by inhibition of chromatin remodeling.

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