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Acetylation of the human DNA glycosylase NEIL2 and inhibition of its activity

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ABSTRACT

Post-translational modifications of proteins, including acetylation, modulate their cellular functions. Several human DNA replication and repair enzymes have recently been shown to be acetylated, leading to their inactivation in some cases. Here we show that the transcriptional coactivator p300 stably interacts with, and acetylates, the recently discovered human DNA glycosylase NEIL2, involved in the repair of oxidized bases both *in vivo* and *in vitro*. Lys49 and Lys153 were identified as the major acetylation sites in NEIL2. Acetylation of Lys49, conserved among Nei orthologs, or its mutation to Arg inactivates both base excision and AP lyase activities, while acetylation of Lys153 has no effect. Reversible acetylation of Lys49 could thus regulate the repair activity of NEIL2 *in vivo*.

INTRODUCTION

Oxidative damage in DNA is induced by reactive oxygen species (ROS), including H_2O_2, the $O_2^{-}\cdot$ radical and the OH· radical, and is widely believed to play a critical role in the etiology of aging, many degenerative diseases and cancer (1–3). Reaction of ROS with DNA produces a wide variety of DNA base lesions as well as strand breaks (4). Most of these DNA lesions, except double-strand breaks, are repaired via the base excision repair (BER) pathway, which is initiated with excision of the damaged base by a DNA glycosylase (5,6). The glycosylases involved in repair of oxidatively damaged bases often have a broad substrate range for damaged bases, but with preference for specific substrate lesions (7). All oxidized base-specific DNA glycosylases possess intrinsic AP lyase activity as a result of which the DNA strand is cleaved at the abasic (AP) site resulting from base excision (7).

The marked evolutionary conservation of BER enzymes from bacteria to mammals suggests the importance of the BER pathway in maintaining genomic integrity. Two DNA glycosylases, 8-oxoguanine-DNA glycosylase 1 (OGG1) and NTH1, involved in the repair of oxidized bases, were previously characterized in mammals (8,9). 7,8-Dihydro-8-oxoguanine (8-OxoG), an abundant and arguably the predominant mutagenic lesion induced by ROS, is excised by OGG1 and Fpg (MutM) in *Escherichia coli* (9,10). Similarly, a number of pyrimidine lesions, including 5,6-dihydrouracil (DHU) and 5-hydroxyuracil (5-OHU), generated from cytosine by ionizing radiation and ROS, are excised by *E.coli* endonuclease III (Nth) and its mammalian ortholog NTH1, and also by *E.coli* endonuclease VIII (Nei) (8,11,12). The mammalian glycosylases OGG1 and NTH1 belong to the *E.coli* Nth family, whose members use an internal Lys (lysine) residue as the active site nucleophile and carry out β lyase reaction to cleave the AP site in order to produce 3′-phospho α,β-unsaturated deoxyribose and 5′-phosphate termini (13). *Escherichia coli* Fpg and Nei constitute a distinct family of DNA glycosylases that utilize N-terminal Pro as the active site, carry out a βδ elimination reaction, and generate 3′-phosphate and 5′-phosphate termini after removal of both the base lesion and deoxyribose (11,14).

We and others have recently identified and characterized two human orthologs of *E.coli* Fpg/Nei and named them NEIL (Nei-like)-1 and NEIL2 (originally named NEH1 and NEH2), respectively (15–19). Both enzymes use N-terminal Pro as the active site, excise multiple oxidized derivatives of purines and pyrimidines and carry out βδ elimination like Fpg and Nei. The 37 kDa NEIL2 is primarily involved in excising oxidative products of cytosine, with the highest activity for 5-OHU, which is also a substrate of NEIL1. However, unlike NEIL1 whose expression level increases in the S phase, NEIL2 expression is not cell cycle dependent (15,16).

CREB binding protein (CBP) and its homolog p300 have intrinsic histone acetyltransferase (HAT) activity and are transcriptional co-activators for a number of sequence-specific transcription factors (TFs) that integrate diverse signaling pathways (20–23). These proteins are responsible for the bulk of HAT activity *in vivo* and play a critical role in chromatin remodeling (22,24). CBP/p300 and their associated factor (P/CAF) acetylate not only histones, but also many TFs, and thus are also called factor acetyltransferases (FATs) (25). Recent evidence suggested the involvement of p300 in DNA replication and repair because of its physical interaction with proliferating cell nuclear antigen (PCNA), which has a central role in these processes (26). PCNA has recently been shown to be acetylated in an S-phase-specific manner (27). Furthermore, many DNA-metabolizing proteins are acetylated by p300/CBP; these include flap endonuclease 1 (FEN1), DNA polymerase β (Polβ) and G·T-specific thymine-DNA glycosylase (TDG) (28–30). Both FEN1 and Polβ interact stably with p300 *in vivo* (28,29). Multiple p300-mediated acetylation sites in FEN1 have been identified whose

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acetylation levels are significantly enhanced after UV irradiation *in vivo* (28). Interestingly, acetylation decreases the nuclease activity of FEN1, presumably by reducing its DNA-binding affinity (28). On the other hand, acetylation of TDG by p300 did not affect its DNA glycosylase activity (30). Thus the role of acetylation in BER appears to be complex.

In this study, we show that NEIL2 is acetylated both *in vivo* and *in vitro* by p300, with which it also forms a stable complex. *In vitro* acetylation of NEIL2 significantly decreases its 5-OHU excision activity, thus suggesting a regulatory effect of acetylation on its enzymatic activity and further supporting involvement of p300 in the DNA base excision repair process.

MATERIALS AND METHODS

Purification of proteins

Wild-type (WT) NEIL2 was cloned into the *E.coli* expression plasmid pRSETB as described earlier (16). The K49R, K153R and K49R/K153R mutants of NEIL2 were generated by PCR and similarly cloned into pRSETB. The identity of all recombinant DNAs generated by PCR was confirmed by sequencing. The WT and mutant NEIL2s were purified as before (16). FLAG-tagged p300 (HAT domain) expressed from recombinant baculovirus was purified by affinity chromatography from virus-infected Sf9 cells using FLAG antibody affinity matrix (Sigma) according to the manufacturer's instructions (31).

Cell culture and plasmids

Human colon carcinoma HCT 116 cells (a gift from B. Vogelstein), were grown at 37°C in McCoy 5A (Gibco Life Technologies) medium supplemented with 10% fetal bovine serum, penicillin (100 U/ml) and streptomycin (100 μg/ml). The C-terminal FLAG-tagged-NEIL2 mammalian expression plasmid was constructed as described earlier (16).

In vitro acetylation of NEIL2

WT or mutant NEIL2 (5 μg) was incubated with 0.2 μg recombinant p300 (HAT domain), together with 1 μCi [^{3}H]acetyl CoA (AcCoA, 200 mCi/mM, NEN) in 50 μl HAT buffer containing 50 mM Tris–HCl pH 8.0, 0.1 mM EDTA, 10% (v/v) glycerol, 1 mM dithiothreitol and 10 mM Na-butyrate at 30°C for 45 min (31). ^{3}H-labeling of the proteins due to acetylation was analyzed by SDS–PAGE and fluorography with enhancing solution (Amplify, Amersham).

In vivo acetylation of NEIL2

HCT 116 cells [(2–4) × 10^6 cells/dish] were transfected with 1 μg FLAG-tagged NEIL2 expression plasmid using LipofectAMINE 2000 reagent (3 μl) (Gibco Life Technologies); 40 h later, the cells were incubated in McCoy 5A medium (Gibco Life Technologies) containing 1 mCi/ml [^{3}H]Na-acetate (5 Ci/mmol, NEN) for 1 h. The cells were then washed twice with cold phosphate-buffered saline (PBS) and lysed on the plates with a cold lysis buffer containing 50 mM Tris–HCl pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 1 mM NaF, 1 mM Na-orthovanadate, 10 mM Na-butyrate and a protease inhibitor cocktail. The cell extract was immunoprecipitated for 3 h at 4°C with anti-FLAG M2 antibody (Sigma) cross-linked to agarose beads. The beads were washed with cold TBS (50 mM Tris–HCl pH 7.5, 150 mM NaCl), and FLAG-NEIL2 was eluted from the immunocomplex by gentle shaking for 30 min with TBS containing 300 ng/μl FLAG peptide (Sigma), followed by SDS–PAGE (12% polyacrylamide) and fluorography.

Identification of acetyllysine (AcLys) residues

Recombinant NEIL2 (50 μg) was acetylated with p300 HAT domain (1 μg) and 0.5 mM acetylCoA (AcCoA) together with 4 μCi of [^{3}H]AcCoA (200 mCi/mM) in 50 μl at 30°C for 1 h. Acetylated NEIL2 (AcNEIL2) was subsequently digested with 0.5 μg of endoproteinase AspN (Roche) in 50 mM sodium phosphate pH 8.0 (100 μl) for 6 h. The digested peptides were separated by reverse phase HPLC and the radioactivity in the fractions measured in a liquid scintillation spectrometer. N-terminal sequencing of radiolabeled peptides was performed via Edman degradation at the UTMB Protein Chemistry Core facility. The acetyllysine (AcLys) residue was unambiguously identified as phenylthiohydantoin-AcLys (PTH-AcLys) based on elution time. Two peptides corresponding to amino acid residues 39–56 and 141–160 of NEIL2 were chemically synthesized and HPLC purified at the UTMB Protein Chemistry Core facility. After acetylation with p300 HAT (0.2 μg) and 2 mM acetylCoA at 30°C for 1 h, the peptides (5 μg) were analyzed by MALDI-TOF (Applied Biosystems) or Edman degradation.

Assay of NEIL2 with duplex oligo substrate

We assayed the DNA glycosylase/AP lyase activity of NEIL2 with a 5′ ^{32}P-labeled duplex oligo substrate, as described earlier (16,32). After labeling with ^{32}P at the 5′ terminus with polynucleotide kinase and [γ^{32}-P]ATP, a 51mer oligo 5′-GCT TAG CTT GGA ATC GTA TCA TGT AXA CTC GTG TGC CGT GTA GAC CGT GCC-3′ (Midland Certified Reagent Co., Midland, TX), with X indicating 5-OHU, was annealed with the complementary strand (with G opposite 5-OHU). The labeled oligo duplex was incubated in 15 μl with WT or mutant NEIL2 at 37°C for 20 min in 50 mM HEPES pH 8.0, 50 mM KCl, 100 μg/ml bovine serum albumin and 5% glycerol. The reaction was stopped with 80% formamide in 10 mM NaOH, and the cleaved DNA was separated by denaturing gel electrophoresis in 18% polyacrylamide containing 8 M urea in 90 mM Tris–borate pH 8.3 and 2 mM EDTA. The radioactivity in the DNA bands was quantitated by PhosphorImager (Molecular Dynamics) analysis. For acetylation, WT or mutant NEIL2 (5 μg) was incubated with either active or heat-inactivated p300 (0.2 μg), together with 1 mM AcCoA in HAT assay buffer at 30°C for 45 min. The enzymes were diluted in 50% glycerol in PBS, and DNA strand cleavage was assayed as before (16).

Co-immunoprecipitation analysis

HCT 116 cells were lysed in cold lysis buffer 40 h after transfection with 1 μg NEIL2-FLAG or p300 expression plasmid, and the extracts (2 mg/ml) were immunoprecipitated with either anti-FLAG M2 antibody (Sigma) or p300 antibody (N-15, Santa Cruz) as described earlier (33). The immunoprecipitates were separated by SDS–PAGE (6% polyacrylamide for p300 and 12% for FLAG-NEIL2) after resuspension in 2× Laemmli buffer. Immunoblot analysis was carried out

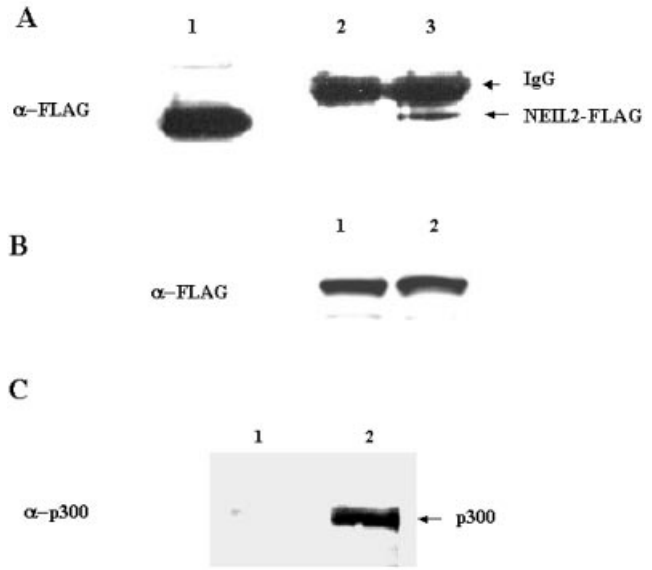

Figure 1. *In vivo* interaction of NEIL2 and p300. (**A**) Extracts of HCT 116 cells cotransfected with expression plasmids for FLAG-tagged NEIL2 and p300 were immunoprecipitated with anti-p300 antibody (lane 3) or pre-immune sera (lane 2) and then blotted with anti-FLAG-antibody. Lane 1, FLAG-tagged NEIL2 in cell extract as marker. (**B**) Lanes 1 and 2, western analysis of extracts with anti-FLAG antibody. (**C**) Extracts of HCT 116 cells transfected with FLAG-tagged NEIL2 (lane 2) or empty vector (lane 1) were immunoprecipitated with anti-FLAG antibody and the immunoprecipitates analyzed for p300 by western blotting.

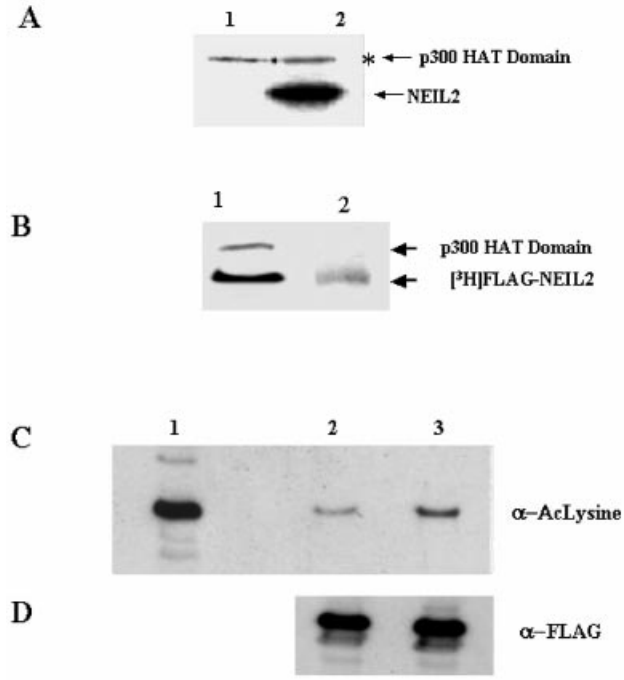

Figure 2. *In vitro* and *in vivo* acetylation of NEIL2. (**A**) *In vitro* acetylation of NEIL2 (lane 2) with purified p300 HAT domain and [^{3}H]acetylCoA followed by SDS–PAGE and fluorography. Lane 1, no NEIL2. (**B**) Extracts of HCT 116 cells transfected with FLAG-tagged NEIL2 (lane 2) and labeled with [^{3}H]Na-acetate were immunoprecipitated with anti-FLAG antibody and analyzed by SDS–PAGE and fluorography. Lane 1, *in vitro* acetylated [^{3}H] NEIL2 marker; upper band, autoacetylated p300 HAT. (**C**) Western analysis with anti-AcLys antibody of anti-FLAG immunoprecipates. HCT 116 transfected with FLAG-NEIL2 (lane 2), or FLAG-NEIL2 plus p300 expression plasmids (lane 3). Lane 1, AcNEIL2 marker. (**D**) Western analysis with anti-FLAG antibody to compare NEIL2 levels in (C).

with anti-p300 or FLAG antibodies (33). Acetylated FLAG-NEIL2 was identified using an anti-AcLys antibody (New England Biolab).

RESULTS

Interaction of NEIL2 with p300 *in vivo*

Earlier studies showing acetylation of DNA replication and repair proteins by p300 suggested that p300 might interact with NEIL2 during repair of oxidatively damaged DNA bases (26,28,29). We tested this possibility by co-immunoprecipitation analysis of p300 in HCT 116 cells transfected with FLAG-tagged NEIL2. NEIL2 was detected in the immunoprecipitate of p300 (Fig. 1A, lane 3), but not in the control (lane 2). The western blot (with FLAG-specific antibody) showed similar levels of ectopic expression of FLAG-tagged NEIL2 in both cases (Fig. 1B). To confirm stable interaction between p300 and NEIL2, we carried out reciprocal studies by immunoprecipitating ectopically expressed FLAG-NEIL2 from HCT 116 extract, and showed the presence of p300 in the immunoprecipitate (Fig. 1C, lane 2). The control (immunoprecipitate of empty vector transfectant extract) did not contain p300 (Fig. 1C, lane 1). Together, these results indicate that NEIL2 forms a stable complex with p300 *in vivo*.

In vitro and *in vivo* acetylation of NEIL2 by p300

We next tested whether p300 could acetylate NEIL2 *in vitro*. We incubated NEIL2 and recombinant p300 HAT domain in the presence of [^{3}H]AcCoA. Incorporation of a significant amount of radioactivity into NEIL2 indicated that this enzyme was acetylated by the p300 *in vitro* (Fig. 2A, lane 2).

We then confirmed the *in vivo* presence of acetylated NEIL2 (AcNEIL2). HCT 116 cells were transfected with FLAG-tagged NEIL2 and pulse-labeled (1 h) with [^{3}H]sodium acetate. Immunoprecipitation of the total extract with an anti-FLAG antibody, followed by SDS–PAGE and fluorography, showed the presence of radioactivity in the FLAG-NEIL2 band (Fig. 2B, lane 2). To verify the presence of AcNEIL2 in the cell extract, we tested for AcLys residues by western analysis of the FLAG immunoprecipitate with anti-AcLys antibody, because Lys is the only acetyl acceptor in proteins (Fig. 2C). Only one band (corresponding to AcNEIL2) was observed in the immunoblot. The AcNEIL2 used as a marker in this experiment (Fig. 2C, lane 1) was produced by *in vitro* acetylation of NEIL2 with p300 and AcCoA. Figure 2C shows the presence of AcNEIL2 in the FLAG immunoprecipitates of HCT 116 transfected with FLAG-NEIL2 expression vector alone (lane 2) or cotransfected with p300 expression vector (lane 3). It is evident that the level of AcNEIL2 in HCT 116 cells was higher when p300 was simultaneously over-expressed. However, the level of the NEIL2 polypeptide remained unchanged, as indicated by western analysis of the same samples with anti-FLAG antibody (Fig. 2D). These results strongly suggest that p300 is the major enzyme for acetylation of NEIL2 *in vivo*.

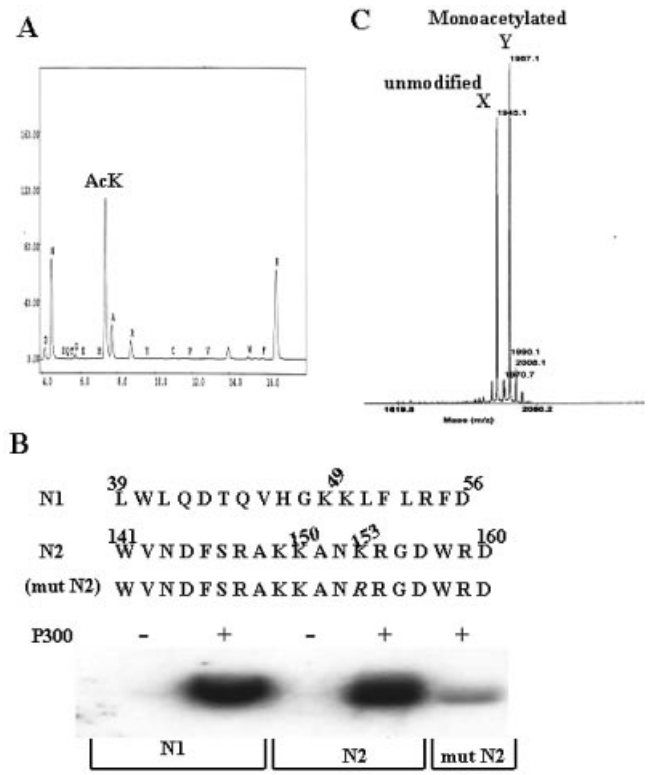

Figure 3. Identification of AcLys residues in NEIL2. Recombinant NEIL2 (50 μg) was acetylated with [^{3}H]AcCoA, as described in Materials and Methods. (**A**) Radiolabeled peptides were separated after protease digestion; N-terminal sequencing identified AcLys (AcK) as PTH-AcLys. (**B**) Acetylation of N1, N2 and mutant (mut) N2 peptides with p300 and [^{3}H]AcCoA, followed by SDS–PAGE and fluorography. (**C**) Mass spectroscopic analysis of *in vitro* acetylated N1 peptides. The smaller peak (X) corresponds to the unmodified N1 peptide (*m*/*z* 1945.1) and the larger peak (Y) corresponds to the monoacetylated form (*m*/*z* 1987).

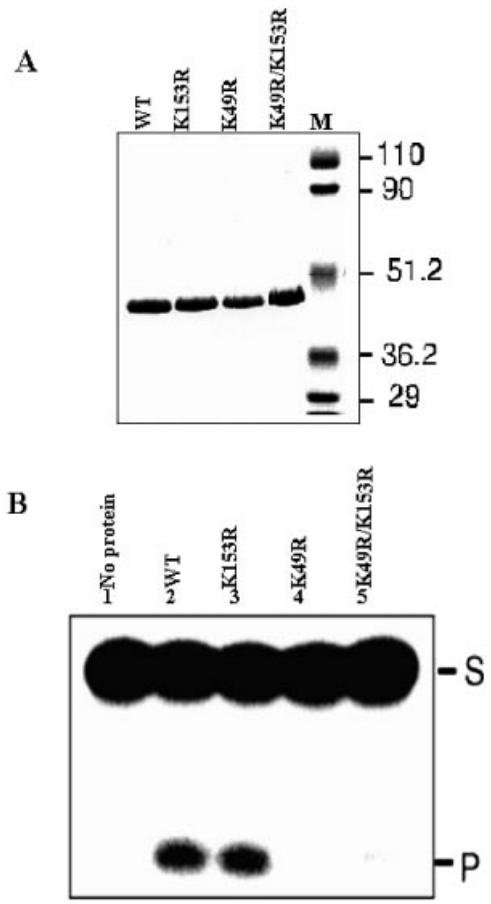

Figure 4. (**A**) Coomassie Blue staining of purified WT and mutant NEIL2 after SDS–PAGE. (**B**) Strand incision activity of WT and mutant NEIL2: 50 nM WT NEIL2 (lane 2), K49R, (lane 4), K153R or K49/K153R (lanes 3 and 5) was incubated with 500 nM 5′ ^{32}P-labeled 5-OHU-containing oligo duplex at 37°C for 20 min, and the reaction products were separated on a denaturing polyacrylamide (18%) gel. Lane 1, no protein; S, substrate; P, product.

Identification of acetyl acceptor Lys residues in NEIL2

After acetylation of NEIL2 with p300 and [^{3}H]AcCoA as above, followed by digestion with endoprotease N (AspN), the NEIL2 peptides were separated by reverse phase HPLC (8). Three fractions containing major radioactivity were identified during HPLC. N-terminal sequencing of one of the fractions containing the highest ^{3}H-labeling showed the presence of a single peptide sequence and indicated that Lys49 of NEIL2 was preferentially acetylated. Figure 3A shows a peak corresponding to acetylated lysine at the 49 position of NEIL2 in one round of Edman degradation. The AcLys residues were identified during Edman degradation as PTH-AcLys which eluted just before PTH-Ala. N-terminal sequencing of the second fraction showed the presence of another peptide sequence and indicated that Lys153 of NEIL2 was preferentially acetylated, although very low levels of acetylation were also observed at Lys149 and Lys150 (data not shown). The third fraction with a low level of ^{3}H showed the presence of two peptides sequence corresponding to the N-terminal part of NEIL2. However, no acetylated lysine residues were observed in this fraction by N-terminal sequencing. To confirm that Lys49 and Lys153 are indeed the primary acetylation sites in NEIL2, two peptides, corresponding to residues 39–56 (N1) and 141–160 (N2) of NEIL2, were chemically synthesized and used as substrates for *in vitro* acetylation by p300. Figure 3B shows that both peptides were acetylated, while mutation of K153 to arginine (R) in the N2 peptide (mut N2) significantly decreased the amount of acetylation. The low level of acetylation in the mutant N2 peptide may be due to acetylation of K149 or K150. Mass spectrometric analysis of *in vitro* acetylated N1 peptide confirmed the presence of one monoacetylated species whose mass was 42 mass units higher than that of the untreated peptide (Fig. 3C). We finally confirmed Lys49 and Lys153 as acetylation sites in these peptides by Edman degradation (data not shown). Thus Lys49 and Lys153 are the major targets in NEIL2 for acetylation by p300.

Activity analysis of WT NEIL2 and that mutated at acetyl acceptor Lys residues

Once we identified Lys49 and Lys153 as the primary acetylation sites on NEIL2, it was important to test the effect of acetylation on its enzymatic activity. We assayed the DNA glycosylase activities of WT, K49R, K153R and K49R/K153R mutants of NEIL2, each purified to homogeneity, as described in Materials and Methods (Fig. 4A). While WT

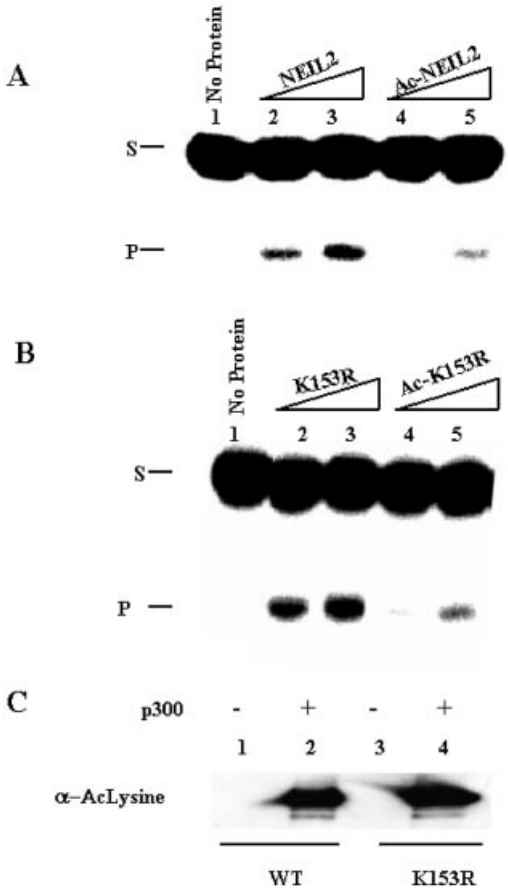

Figure 5. Incision activity of NEIL2 and AcNEIL2. (**A**) NEIL2 (5 μg) was incubated with either native p300 HAT domain (0.5 μg) (lanes 4 and 5) or heat-inactivated HAT domain (lanes 2 and 3) and 1 mM AcCoA for 45 min at 30°C; 50 and 100 nM of WT NEIL2 (lanes 2 and 3) or AcNEIL2 (lanes 4 and 5) were incubated with 500 nM of 5′ ^{32}P-labeled 5-OHU·G oligo at 37°C for 20 min, and the cleaved products were analyzed as in Figure 4. Lane 1, no protein. (**B**) Incision activity of the AcK153R mutant. The K153R mutant (5 μg) was acetylated *in vitro* and the incision activity was measured as in (A). (**C**) Western analysis (using anti-AcLys antibody) of WT (lanes 1 and 2) and mutant NEIL2 (lanes 3 and 4) after acetylation as in (A) and (B).

NEIL2 was highly active as expected (Fig. 4B, lane 2), the K49R and K49R/K153R mutants showed no activity for incising the DNA strand containing 5-OHU (lanes 4 and 5). In contrast, the K153R mutation had no significant effect on the strand incision (base excision) activity (lane 3). These results indicate that K153 does not have an essential role in NEIL2 glycosylase/AP lyase activity, whereas K49 is essential, at least for the DNA glycosylase activity.

Inhibition of NEIL2 activity by acetylation

In view of our observation that Lys49 is critical for the DNA glycosylase activity of NEIL2, we investigated whether acetylation affects the overall activity of NEIL2. Purified WT NEIL2 was incubated with AcCoA in the presence of active p300 HAT domain (Fig. 5A, lanes 4 and 5) or heat-inactivated protein (lanes 2 and 3) and then assayed for activity. As shown in Figure 5A, incubation with active p300 decreased NEIL2 activity in a dose-dependent manner (lanes 4 and 5). Our p300 HAT preparation showed no contaminating nuclease or 5-OHU incision activity when incubation was carried out with the 5-OHU oligo in the absence of NEIL2 (data not shown). Furthermore, p300 HAT alone, without AcCoA, had no effect on the activity of NEIL2 (data not shown). Western analysis of acetylated WT NEIL2 with anti-AcLys antibody confirmed acetylation of NEIL2 (Fig. 5C, lane 2). Because Lys49 is essential for NEIL2 activity and is also a major target for acetylation, we investigated whether the decreased activity of AcNEIL2 was due to acetylation of Lys49 of NEIL2, or to acetylation of both Lys49 and Lys153. The K153R mutant of NEIL2 (in which only Lys49 could be acetylated) was acetylated with p300. Acetylation significantly decreased the activity of both WT NEIL2 and its K153R mutant (Fig. 5A and B). Western analysis of acetylated WT and K153R NEIL2 using anti-AcLys antibody confirmed acetylation of NEIL2 in both cases (Fig. 5C, lanes 2 and 4). These results show that acetylation of Lys49 alone, but not acetylation of Lys153, significantly decreased the activity of NEIL2.

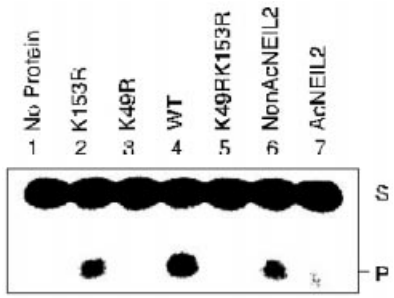

Figure 6. AP-lyase activity of control, acetylated and mutant NEIL2. The indicated enzymes (20 nM) were incubated with 500 nM duplex oligo containing an AP site, and the products separated in a urea–polyacrylamide (15%) gel at 37°C for 20 min. Lane 1, no protein. All reactions were terminated with SDS (0.5%) and glycerol (5%) without heating, as described earlier (42).

To further explore whether acetylation of NEIL2 affects its AP-lyase activity, we analyzed the activity of WT, mutant (K49R, K153R and K49R/K153R) and acetylated NEIL2 using a ^{32}P-labeled AP-site-containing oligo substrate (15,34). Figure 6 shows that the K49R and K49R/K153R mutants and AcNEIL2 were unable to cleave AP sites by βδ elimination, unlike the K153R mutant and WT NEIL2. We therefore conclude that Lys49 plays an essential role not only in the DNA glycosylase activity step, but also in the subsequent AP lyase reaction (Fig. 6, lanes 6 and 7) which is severely depressed by acetylation of this residue.

DISCUSSION

The role of post-translational modifications of proteins involved in cell signaling and transcription has been extensively investigated. However, the physiological significance of modifications to DNA repair proteins is not well understood. In this study we show for the first time that the activity of a DNA glycosylase involved in repair of oxidized bases is modulated by acetylation. We also show that NEIL2 stably interacts with p300 *in vivo*, which also suggests involvement of this transcription coactivator in BER.

We identified Lys49 and Lys153 in NEIL2 as the major targets for *in vitro* acetylation by p300, and showed that acetylation strongly inhibits both DNA glycosylase and AP lyase activities. Comparable inhibition of the activities of the WT and K153R mutant by acetylation indicates that acetylation of Lys49 is primarily responsible for the reduced activity of AcNEIL2. It is important to note that Lys49 of NEIL2 is a conserved residue in the Fpg/Nei family (17). It has been shown that mutation of K52 in *E.coli* Nei or K56 in Fpg (homologous to K49 in NEIL2) significantly reduces the DHU or 8-oxoG-excision activity, respectively. Surprisingly, both mutants retained the AP lyase activity (35,36). In contrast, the K49R NEIL2 mutant lacks both DNA glycosylase and AP lyase activities, reflecting some structural difference between the bacterial and mammalian enzymes. The X-ray crystallographic structures of Nei and Fpg covalently bound to an AP site indicate that K52 of Nei or K56 of Fpg makes contact with phosphate residues near the base lesion (35,36). Thus removing such contacts with a DNA-phosphate backbone, which may be necessary for base excision, may disrupt the architecture of the active site (35). It is possible that the loss of positive charge in Lys prevents its ionic interaction with phosphate residues (35,36). However, the fact that the K49R mutant is inactive suggests a more subtle role of the Lys residue. Elucidation of the substrate-bound structures of NEIL2 and acetylated NEIL2 will be necessary to explain the precise effect of acetylation on its AP lyase activity.

Although Lys153 in NEIL2 is an additional target for acetylation, its acetylation does not affect the enzymatic activity. It is possible that this acetylation has a distinct *in vivo* function, for example in modulating interaction of NEIL2 with other BER proteins. Such interactions have been invoked in the model of coordination in the BER process (37). We have observed DNA ligase IIIα and Polβ, the downstream proteins in BER stably interact with NEIL2, which could be affected by its acetylation (T. K. Hazra, L. Wiederhold, I. Boldogh, J. B. Leppard, P. Kedar, F. Karimi-Busher, A. Das, M. Wienfeld, A. E. Tomkinson, R. Prasad, S. H. Wilson and S. Mitra, unpublished data).

p300 has been shown to acetylate many nonhistone proteins such as p53, GATA1, E2F etc. (38–40). The consequences of this acetylation vary for different target proteins. Functional interaction of FEN 1, Polβ, TDG and PCNA with p300, and modulation of their activity upon acetylation, suggest that acetylation of repair proteins contributes to regulation of the BER process (28–30). Acetylation of FEN1 by p300 decreased its endonuclease activity *in vitro*, which appears to result from lower substrate binding due to the loss of an essential positive charge at the C-terminus (28). Inactivation of NEIL2 upon acetylation is similar to the loss of the dRP-lyase activity of Polβ upon acetylation of Lys72 (29), which was shown to be essential for the β-elimination reaction (41). Thus Lys72 in Polβ and Lys49 in NEIL2 are critical residues for enzymatic activity, and are also targets for acetylation. In contrast, acetylation of a G/T(U) mismatch-specific glycosylase, TDG, does not affect its binding or cleavage of G/T-U mispaired DNA but weakens its interaction with APE1 (30).

Acetylation-dependent inactivation of NEIL2 is unexpected because this enzyme should remain active *in vivo* in repairing oxidatively damaged bases generated continuously. However, the physiological conditions under which NEIL2 is acetylated in cells are not clear at present. It is possible that NEIL2 acetylation takes place after the enzyme has completed reaction at a lesion site. In this scenario, acetylation of K49 inactivates the enzyme, while acetylation of K153 enhances interaction with downstream BER proteins, such as Polβ and DNA ligase III, and recruits them to the damage repair sites. Another possibility is that under normal physiological conditions NEIL2 remains in an inactive form due to acetylation, and deacetylation is needed to reactivate the enzyme after a repair cycle. Which histone (factor) deacetylase (HDAC) is required for deacetylation of NEIL2 is currently unknown. It is thus tempting to speculate that under oxidative stress this deacetylase removes the acetyl group to activate NEIL2. We showed earlier that acetylated APE1 is deacetylated by group I HDACs and involved in transcriptional regulation of the parathyroid hormone gene (33). In any event, the present study supports the possibility that oxidative damage base repair is regulated *in vivo* partly via reversible modification of component proteins in a fashion analogous to that seen in transcriptional regulation.

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