This article has been withdrawn by the authors. Lanes 2-4 of Fig. 4A were reused in Fig. 5C. Lanes 1 and 2 of Fig. 4B were reused as lanes 2 and 3 of Fig. 7B. Lanes 5 and 6 of Fig. 4C were reused in Fig. 5D. Lanes 1 and 2 of Fig. 5A were reused in Fig. 5B. The authors state that several gels were run for each experiment and that the images were cropped to create panels. They further state that the wrong panels were selected while assembling the figures as they appeared very similar. The authors assert that these errors do not change the results of this article.

Nucleoside-diphosphate kinase (NdK) (EC 2.7.4.6) is required for the maintenance of cellular ratios of nucleoside triphosphates and diphosphates. NdK catalyzes phosphoryl transfer from a nucleoside triphosphate to a nucleoside diphosphate and is ubiquitously present in different organisms. There is a wide body of literature available on the diverse array of cellular events and complex regulatory processes brought about by this enzyme in different organisms (1–7). In this regard, we reported earlier the biochemical characterization and extracellular secretory nature of NdK of Mycobacterium tuberculosis (mNdK) (Rv2445c) (7). Secretory NdKs from other organisms are secreted in situ and are involved in cell-to-cell communication and dissemination of the pathogen. Nucleoside diphosphate kinases from E. coli; GFP, green fluorescent protein; GST, glutathione S-transferase; NHE, nucleoside hyposensitive element; AP, apurinic/apyrimidinic; RU, response unit(s).

EXPERIMENTAL PROCEDURES

Materials—All chemicals and reagents were purchased from Sigma. Bacterial culture media were purchased from Difco Laboratories. mNdK and its mutant (H117Q) were expressed as GST fusion proteins in E. coli using the pGEX-2T expression vector (Amersham Biosciences). After thrombin cleavage, proteins were purified by chromatography on a Superdex 200 column as described earlier (18). DNA duplexes were prepared by heating an equimolar mixture of 5′-AGTC-
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TCTCCCTACCTCCACCCACCTCC ACACCTCTCCACAACCTGC-3' and 5'-GCTATGAGGTTGGGATGGAATGGAAGGGGAGGA-GACT-3' (complementary sequence) in 10 ml Tris buffer (pH 7.0) containing 200 mM NaCl at 95 °C followed by slow cooling. Subsequently, they were labeled with γ-32P-ATP in the presence of T4 polynucleotide kinase as described earlier (14). Similarly, a 45-bp ATGC repeat and its complementary oligonucleotides were annealed and labeled.

Construction of Expression Plasmid, Cell Culture, and Transfection—Genomic DNA isolated from M. tuberculosis H37Rv was used for PCR amplification of ndk. The nucleotide sequence of primers used was: 5'-ACCTAGTGATATCCTGACCGAAGCGC-3' with an EcoRI site at the 5'-end (forward primer) and 5'-GGCCACCCCGATCCTCCGCGGCGG-3' with a BamHI site at the 3'-end (reverse primer). The amplicons were digested with EcoRI-BamHI and ligated into pEGFP-N1 (Clontech Laboratories, Inc.) to obtain pmNdK-EGFP plasmid.

HeLa and COS-1 cells were maintained in 5% CO2 at 37 °C as a monolayer culture in Dulbecco’s modified Eagle’s medium supplemented with 10% (v/v) calf serum and 50 μg/ml gentamicyn. HeLa and COS-1 cells (2 × 106) were transfected with 1 μg each of pmNdK-EGFP or pEGFP-N1 using Lipofectin (Invitrogen). Transfection efficiencies of ~65% for HeLa cells and 40% for COS-1 cells were achieved under optimized conditions.

Fluorescence Microscopy and Immunoblot Analysis—At 24 h post-transfection, the cells were harvested by 0.05% trypsin, and the nuclei were isolated using lysis buffer (0.5% Nonidet P-40, 5 mM MgCl2, 25 mM KCl, and 10 mM Tris HCl (pH 7.5)) as described earlier (15). The nuclei were counterstained with the DNA-binding dye Hoechst 33258, and the images were acquired using a fluorescence microscope (Nikon Corp.). Localization of mNdK-EGFP in the isolated nuclei was also analyzed by immunoblotting using polyclonal antisera against mNdK as described earlier (7).

DNA Nicking Assay—HeLa and COS-1 cells were treated with and centrifuged at 2580 × g to isolate nuclei. The nuclei were incubated with 250 ng of mNdK or 1 unit of DNase I (10 ng) (Amersham Biosciences) for 4 h at 37 °C. At the end of the incubation, the nuclei were pelleted, washed with lysis buffer, and incubated with a Klenow fragment of DNA polymerase in a reaction containing 5 mM MgCl2, 25 mM KCl, and 10 mM Tris HCl (pH 7.5), followed by a 7 min incubation at 70 °C. Thereafter, GST-tagged mNdK (250 ng/ml) was passed over the chip for 10 min at 2 μl/min. Various concentrations of pUC19myc plasmid DNA (1.85–99.60 μg/ml) dissolved in filtered and degassed reaction buffer I containing 0.005% surfactant P20 (Biacore AB) were injected (10 μl/min for 3 min) to study its interaction with chip-bound mNdK. After each binding cycle, glycine (10 mM, pH 2.0) was injected (10 μl/min for 30 s) to remove both GST-mNdK and plasmid, allowing the base line to return back to that of the captured antibody. A blank-flow cell (without attached mNdK) was used to account for any bulk or solvent effect not specific to the interaction. Data analysis was done by plotting the signal (in response units) obtained against various concentrations of pUC19myc plasmid. The data were fitted into the equation: RUeq = RUmax (ligand/[Kd] + [ligand]), where RUmax is the measured response, RUeq is the maximum response, and Kd is the equilibrium dissociation constant.

Free Radical Detection Assays—Plasmid cleavage was studied under both aerobic and anaerobic (nitrogen atmosphere) conditions as described by Crumbliss and Basolo (23). The effect of superoxide dismutase on plasmid cleavage was investigated as described earlier by Hertzberg and Dervan (24).

The electron paramagnetic resonance (EPR) spectra of plasmid cleavage assays were recorded on X band (~9 GHz) using a Varian E-109 spectrometer with 100 kHz field modulation. A quartz flat sample cuvette was used to contain a 0.2 ml sample). Data analysis was done by plotting the signal (in response units) obtained against various concentrations of pUC19myc plasmid. The data were fitted into the equation: RUeq = RUmax (ligand/[Kd] + [ligand]), where RUmax is the measured response, RUeq is the maximum response, and Kd is the equilibrium dissociation constant.

Bioinformatics Analysis—DNA sequence alignment of mNdK with the critical residues (lysine 12, proline 13, and histidine 117, involved in autophosphorylation and DNA nicking) is phylogenetically conserved (Fig. 1). mNdK and its amino acid sequence was compared with the “non-redundant databases” and to find conserved motifs by Clustal W (version 1.82). The gap extension and gap distance penalties were kept as 0.05 and 10, respectively, during the alignment (20).

Sequence alignment of mNdK (NM23-H2 and NM23-H1) (26), which suggests that mNdK DNA (14), and histidine117, involved in autophosphorylation and DNA nicking (Fig. 1). mNdK and its amino acid sequence was compared with the “non-redundant database” using BLAST (19). Similar sequences were retrieved from the program were used for the search tool to find conserved motifs by Clustal W (version 1.82). The gap extensions and gap distance penalties were kept as 0.05 and 10, respectively, during the alignment (20).

Plasmid Cleavage Assay—Supercoiled pUC19myc plasmid (having a nuclease hypersensitive element (NHE) of the c-myc promoter) was incubated with mNdK or mNdK-H117Q in the presence of varying concentrations of ATP (0.25–0.5 μM), ADP (2 μM), or AMP (2 μM) in reaction buffer I (50 μM Tris-HCl (pH 7.9) containing 100 mM KCl, 1.5 mM MgCl2, 50 μg/ml bovine serum albumin, and 2% glycerol) for 30 min at 37 °C. The reaction was terminated by incubating the reaction mixture with stop solution (1% SDS, 10 mM EDTA, and proteinase K (200 μg/ml)) for 30 min at 55 °C. Samples were resolved on a 0.5% agarose gel. Moreover, the pUC19myc plasmid used for the study was examined for the presence of apurinic/apyrimidinic (AP) (version 1.82) or abasic sites using 1,2-ethylenediamine (21).

DNA Binding Assays—An electrophoretic mobility shift assay was used to determine the binding of mNdK with duplex DNA. Briefly, 5'-32P-labeled 45-bp duplex DNA containing an NHE sequence (5 ng) was incubated with mNdK or mNdK-H117Q in the presence of EDTA and ATP in reaction buffer I for 30 min at 37 °C. After incubation, samples were resolved on a 5% native PAGE, vacuum-dried, and autoradiographed.

Surface Plasmon Resonance Analysis—The interaction of mNdK with plasmid DNA was studied using a BIACore 2000 instrument (Biochip AB, Uppsala, Sweden) as described earlier (22). Anti-GST antibody (2 μl/min) was chemically coupled (density ~3000 RU) on the CM5 sensor chip activated by treatment with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride and N-hydroxysuccinimide, and then the unoccupied chip surface was deactivated using ethanolamine. Thereafter, GST-tagged mNdK (250 ng/ml) was passed over the chip for 10 min at 2 μl/min. Various concentrations of pUC19myc plasmid DNA (1.85–99.60 μg/ml) dissolved in filtered and degassed reaction buffer I containing 0.005% surfactant P20 (Biacore AB) were injected (10 μl/min for 3 min) to study its interaction with chip-bound mNdK. After each binding cycle, glycine (10 mM, pH 2.0) was injected (10 μl/min for 30 s) to remove both GST-mNdK and plasmid, allowing the base line to return back to that of the captured antibody. A blank-flow cell (without attached mNdK) was used to account for any bulk or solvent effect not specific to the interaction. Data analysis was done by plotting the signal (in response units) obtained against various concentrations of pUC19myc plasmid. The data were fitted into the equation: RUeq = RUmax (ligand/[Kd] + [ligand]), where RUmax is the measured response, RUeq is the maximum response, and Kd is the equilibrium dissociation constant.

Nuclear Localization of mNdK—Because M. tuberculosis is an intracellular pathogen, the extracellular secretion of mNdK (7), like the Ndks of other species (8–11), encouraged us to study its possible role in the host pathogen interaction. To ascertain the subcellular localization of mNdK in host cells, we cloned it in a mammalian expression vector (pEGFP-N1) and transfected it into HeLa and COS-1 cells. Fluorescence microscopy of nuclei isolated from these cells transfected with pmNdK-EGFP plasmid revealed that the mNdK protein localizes in the nucleus, whereas nuclei isolated from cells transfected with pEGFP-N1 did not exhibit green fluorescent protein (GFP)-associated fluorescence (Fig. 2A). Nuclear localization of mNdK was further confirmed by probing the nuclear lysate of HeLa cells transfected with pEGFP-N1 and pmNdK-EGFP, using mNdK-specific polyclonal antibodies. A 40-kDa protein (corresponding to the predicted molecular mass of NdK-GFP fusion protein) reacted with anti-NdK antibody in the nuclear lysate of HeLa cells transfected with pmNdK-EGFP, whereas this protein was absent in the nuclear lysate of untransfected and pEGFP-N1-transfected cells (Fig. 2B). Similar results were obtained with the COS-1 cell line (data not shown).

DNA Nicking by mNdK—NM23-H1, the human homolog of mNdK, has been shown to localize in the nucleus and to have DNase I-like activity (15). Considering the homology of mNdK with human NdK, we investigated its effect on chromosomal DNA nicking and plasmid cleavage.
DNA using isolated nuclei of HeLa and COS-1 cells. mNdK incubated with isolated nuclei showed no sign of oligonucleosomal DNA fragmentation when analyzed by 1% agarose gel electrophoresis (data not shown). To assess more subtle forms of DNA damage, single-stranded breaks were identified by radiolabeling the nicked ends with Klenow and resolving them by denaturing agarose gel electrophoresis. Alkaline agarose gel electrophoresis and scintillation counting showed high incorporation of [32P]dCTP in DNA isolated from mNdK and DNase I-treated nuclei (1220 cpm/ng in mNdK-treated nuclei and 38273 cpm/ng in DNase I-treated nuclei) (Fig. 3). On comparison, mNdK showed 3% of the DNA cleavage activity obtained with bovine pancreas DNase I.

**mNdK Cleaves Supercoiled pUC19myc Plasmid—** NM23-H1 and NM23-H2 were shown earlier to bind and nick the NHE region of the c-myc promoter (14). Incubation of mNdK with pUC19myc plasmid showed magnesium-dependent nicking and cleavage of the plasmid (Fig. 4A, lane 3). The requirement of metal ions for mNdK activity was further confirmed by the observation that cleavage was inhibited in the presence of EDTA (Fig. 4B), whereas some other divalent ions used had varied effect on the activity of mNdK. It was found that Mn2+ and Ni2+ could replace Mg2+ (Fig 4A, lanes 4 and 7), whereas Zn2+ and Ca2+ failed to complement Mg2+ (Fig. 4A, lanes 5 and 6). mNdK did not cleave pUC19 plasmid, suggesting that the cleavage was sequence-specific (Fig. 4C, lanes 2 and 4). Similarly, mNdK did not cleave pGEX-5X-3 (Amersham Biosciences) lacking the NHE region of the c-myc promoter (data not shown), which revealed that DNA cleavage by mNdK is sequence-specific. The presence of abasic sites in the plasmid used to assess the DNA-associated properties of mNdK was ruled out by treating the plasmid with 1,2-ethylenediamine (21). 1,2-Ethylenediamine was unable to cleave the pUC19myc plasmid, which indicates that plasmid used for the cleavage assays lack AP sites (Fig. 4C, lane 6). The DNA cleavage activity of mNdK was catalytic, as increasing the amount of DNA resulted in increased cleavage when plasmid was incubated with equal amount of mNdK (data not shown).

**ATP Inhibits DNA Cleavage Activity of mNdK—** The relationship between kinase activity and the DNA cleavage property of mNdK was examined by ascertaining the effect of ATP on DNA cleavage and using the kinase mutant of mNdK (H117Q). The presence of ATP in the reaction mixture abrogated the cleavage of plasmid by mNdK (Fig. 5A). ATP-mediated inhibition was not abrogated by the increased concentration of MgCl2 (20 mM), suggesting that inhibition of plasmid cleavage by ATP was not

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**Fig. 1.** Sequence comparison of mNdK with NdK of different organisms. Gaps are indicated by dashes and COOH-terminal residues by a degree sign. Absolute conservation is indicated by an asterisk, and a relatively conserved position is indicated by a colon. A hyphen indicates the gaps introduced to optimize alignment. The primary protein sequences given are: MYCTU, M. tuberculosis; MYCLE, M. leprae; MYCSM, M. smegmatis; ECHCO, E. coli; PSEA, Pseudomonas aeruginosa; MYXXA, Myxococcus xanthus; NM23-H2, human NdK; DROME, Drosophila melanogaster; DICDI, Dictostelium discoideum.
because of scavenging of magnesium ions (Fig. 5A, lane 4). However, ADP and AMP had no effect on the plasmid cleavage activity of mNdK (Fig. 5B). To further examine ATP-induced inhibition, we used a mNdK mutant, H117Q, which is deficient in ATP binding, hydrolysis, and autophosphorylation activities (7). This mutant of mNdK also showed plasmid cleavage activity (Fig. 5C). It was observed that ATP also inhibited the

FIG. 2. Nuclear localization of mNdK. A, nuclei isolated from HeLa cells transfected with pmNdK-EGFP (panels 1 and 2) or pEGFP-N1 (panels 3 and 4) were counterstained with Hoechst 33258 and visualized by fluorescence microscopy. The blue and green colors show the fluorescence of Hoechst 33258 (panels 1 and 3) and GFP (panels 2 and 4), respectively. B, immunoblot of nuclei isolated from HeLa cells that are untransfected (lane 1) and cells transfected with either pEGFP-N1 (lane 2) or pmNdK-EGFP (lane 3).

FIG. 3. mNdK-mediated in situ DNA nicking. Isolated nuclei were incubated with mNdK for 4 h, and then DNA nicks were radiolabeled with dCTP using Klenow fragment of DNA polymerase. Genomic DNA isolated from nuclei was subjected to alkaline agarose gel electrophoresis followed by autoradiography. lane 1, control; lane 2, DNAse I; lane 3, mNdK. SC, supercoiled; LIN, linear; OC, open circle/nicked circular form of plasmid.

because of scavenging of magnesium ions (Fig. 5A, lane 4). However, ADP and AMP had no effect on the plasmid cleavage activity of mNdK (Fig. 5B). To further examine ATP-induced inhibition, we used a mNdK mutant, H117Q, which is deficient in ATP binding, hydrolysis, and autophosphorylation activities (7). This mutant of mNdK also showed plasmid cleavage activity (Fig. 5C). It was observed that ATP also inhibited the

FIG. 4. Plasmid cleavage by mNdK. mNdK (250 ng) was incubated with pUC19myc (500 ng), and plasmid was analyzed on a 1% agarose gel. A, effect of divalent metal ions. All reactions were carried out in 50 mM Tris-HCl (pH 8.0), 100 mM KCl, 50 mM bovine serum albumin, and 2% glycerol. Lane 1, pUC19myc alone; lane 2, pUC19myc and mNdK; lanes 3–7, pUC19myc and mNdK supplemented with 1.5 mM MgCl2, MnCl2, ZnCl2, CaCl2, and NiCl2, respectively. B, effect of EDTA. Reactions were carried out in reaction buffer I supplemented with EDTA. Lanes 1–3 show 0, 1.5, and 3 mM EDTA, respectively. C, specificity of cleavage and lack of AP lyase activity in mNdK. All of the reactions were carried out in reaction buffer I. Lane 1, pUC19myc alone; lane 2, pUC19 alone; lane 3, pUC19myc and mNdK; lane 4, pUC19 and mNdK digested with BamHI; lane 5, pUC19myc and 1,2-ethylenediamine (25 mM); lane 6, pUC19myc and 1,2-ethylenediamine (25 mM) plus mNdK; lane 7, pUC19myc, mNdK and 1,2-ethylenediamine (25 mM).
Intracellular pathogens secrete several proteins to gain advantage for their survival and pathogenesis. Recently, it has been shown that M. tuberculosis secretes protein kinase G and tyrosine phosphatases that modulate host defense mechanisms and help in the survival of the organism (29, 30). Our earlier studies established that mycobacterial NdK, like the NdKs of other pathogenic organisms (7, 9, 11), is a secretory protein and helps in the survival of the organism (29, 30). Our earlier studies showed that mNdK localizes like reaction. To validate this hypothesis, EPR experiments were carried out, and indeed superoxide formation was detected during the course of the reaction (Fig. 7C), which was analyzed on a 1% agarose gel.

**Fig. 5. Inhibition of plasmid cleavage activity of mNdK by ATP.** Cleavage activity of pUC19myc (500 ng) by mNdK or H117Q in the presence of 0, 0.25, and 0.5 mM ATP was analyzed on a 1% agarose gel. A, effect of ATP. Lanes 1–3, mNdK; lane 4, H117Q. B, effect of ADP and AMP. Lane 1, ADP (2.0 mM); lane 2, AMP (2.0 mM). C, cleavage of pUC19myc by H117Q. Lane 1, control; lane 2, mNdK; lane 3, H117Q. D, plasmid alone; supercoiled; LIN, linear; OC, open circular; SC, single-stranded DNA.

DISCUSSION

Intracellular pathogens secrete several proteins to gain advantage for their survival and pathogenesis. Recently, it has been shown that M. tuberculosis secretes protein kinase G and tyrosine phosphatases that modulate host defense mechanisms and help in the survival of the organism (29, 30). Our earlier studies established that mycobacterial NdK, like the NdKs of other pathogenic organisms (7, 9, 11), is a secretory protein and is cytotoxic to macrophage cells in the presence of ATP (7). The human homologs of mNdK, NM23-H1 and NM23-H2, have been shown to bind and nick the NHE region of the c-myc promoter (14). These observations prompted us to investigate the significance of mNdK secretion and its DNA-associated properties. In the present study, we show that mNdK localizes into the nucleus and causes DNA damage. Nuclear localization of mNdK was shown by expression of mNdK as a GFP fusion protein in HeLa and COS-1 cells using fluorescence microscopy and immunoblotting (Fig. 2). mNdK induces a type of DNA damage (single-stranded nicks) that leads to larger DNA fragments not detected by conventional nondenaturing agarose gels. The in situ DNA damage by mNdK was observed by alkaline agarose gel electrophoresis (Fig. 3). Incorporation of radiolabeled dCTP at the site of nicks with Klenow fragment of DNA polymerase confirmed that mNdK causes in situ nicking of chromosomal DNA.

DNA nicking was further studied by using pUC19 plasmid containing the NHE sequence of the c-myc promoter (pUC19myc). It was observed that cleavage of pUC19myc plasmid mediated by mNdK resulted in relaxation and subsequent linearization of the plasmid due to DNA nicking (Fig. 4). It has been reported by Freifelder and Trumbo (31) that single strand breaks can be produced in double-stranded DNA in a variety of ways and that some of these breaks in opposite strands are in close proximity lead to double strand breaks. Proteinase K treatment after completion of plasmid cleavage reaction results in a band corresponding to the linearized form of the plasmid (Fig. 4), which was further corroborated by the observation that the product obtained from plasmid digestion by proteinase K migrated at the same position as mNdK-cleaved plasmid. These observations collectively suggest that DNA cleavage activity by mNdK is not related to AP lyase activity. Earlier, Bennet et al. (17) have given compelling evidence regarding eNdK lacking detectable uracil-DNA glycosylase activity, which was in contradiction to the earlier report by Postel et al. (3). Therefore, we also investigated the magnesium ion-dependent AP lyase activity of mNdK. The sequence comparison of mNdK with uracil-DNA glycosylase of M. tuberculosis (Swiss Protein accession number P95119) and E. coli (Swiss Protein accession number BAA10923.1) showed that five highly conserved structural motifs (QDPYH, AIPPS, LLLN, GS, and HPSPLSAHHR), which serve as a signature for the uracil-DNA glycosylases, are absent in mNdK. 1,2-Ethenediamine has been shown to cleave the abasic/AP sites in double-stranded DNA (21). It was found that 1,2-ethenediamine did not cleave the pUC19myc (Fig. 4C, lane 6), thereby proving that this plasmid does not have AP sites. Finally, mNdK cleaved pUC19myc plasmid specifically and had no effect on other plasmids such as pUC19 and pGEX-5X-3. These observations collectively suggest that DNA cleavage activity by mNdK is not related to AP lyase activity.

Earlier, it was hypothesized that ATP-mediated inhibition of DNA cleavage activity of NM23-H2 could be because of (a) the kinase activity of NdK, (b) competition by Mg^{2+} with ATP for the active site, (c) quenching of Mg^{2+} by ATP directly, or (d) the inhibitory effect of phosphorylation of His-118 (14). We also elucidated the effect of ATP on the DNA cleavage and binding properties of mNdK. Abrogation of the DNA binding and cleavage properties of mNdK by ATP was not restored even after increasing the magnesium concentration to 15-fold (Fig. 5A, lane 4), suggesting that inhibition of DNA cleavage by ATP is
not due to magnesium ion scavenging. Histidine 117 of mNdK, a highly conserved residue among other NdKs, was shown to be essential for its autophosphorylation (7, 32–34), and H117Q mutant failed to complement an ndk-disrupted strain of Pseudomonas aeruginosa (35). Interestingly, H117Q mutant of mNdK was found to have both DNA binding and cleavage activities (Fig. 5C, lane 3, and Fig. 6A, lane 4). The DNA cleavage property of H117Q mutant was also found to be inhibited by ATP, indicating that plasmid cleavage occurs by a mechanism that is independent of the ATP binding/hydrolysis property of NdK. Earlier reports indicated that tightening of the erythrocytic NdK conformation was observed in the presence of ATP, which led to the changes in its activity toward p-chloromercuribenzoate (36). Accordingly, it can be hypothe-
able metal ion forms a coordination complex with mNdK, and subsequently mNdK delivers this metal ion to the site of DNA helix where activation of molecular oxygen occurs through electron transfer mechanism resulting in DNA cleavage. The proposed hypothesis was strengthened by the observation that mNdK-mediated cleavage of DNA was completely inhibited under N\textsubscript{2} atmosphere, suggesting the necessity of molecular oxygen for the reaction (Fig. 7A, lane 3). The involvement of oxygen and metal ions in the DNA cleavage prompted us to investigate the nature of species involved in this electron transfer reaction. The presence of superoxide species in the mNdK-mediated plasmid cleavage reaction was detected by EPR studies (Fig. 7C), and the calculated g value of 2.0039 was similar to the g value of the chemical system containing potassium superoxide in dimethyl sulfoxide (40). An EPR signal was not observed in the absence of Mg\textsuperscript{2+}, molecular oxygen, DNA, or mNdK (Fig. 7C), implying that all these components are part of a redox system responsible for the free radical generation.

The nuclear localization and DNase-like activity of mNdK, similar to NM23-H1 (15), suggest that mNdK can be a potent virulence factor. Secreted mNdK by \textit{M. tuberculosis} might help in the dissemination of this intracellular pathogen, and subsequent establishment of the disease, by killing host cells via its DNase activity. Our experiments mimic \textit{in vivo} infection, and it is likely that the same phenomenon may occur during mycobacterial infection.

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