Inhibition of NF-κB Activation by Arsenite through Reaction with a Critical Cysteine in the Activation Loop of IκB Kinase*

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Arsenite is a potent environmental toxin that causes various pathologies including cancers and skin disorders. Arsenite is believed to exert its biological effects through reaction with exposed sulfhydryl groups, especially pairs of adjacent thiols. Here, we describe the mechanism by which arsenite affects the NF-κB signaling pathway. Activation of transcription factor NF-κB depends on the integrity of the IκB kinase (IKK) complex. We found that arsenite potently inhibits NF-κB and IKK activation by binding to Cys-179 in the activation loop of the IKK catalytic subunits, IKKα/β. The affinity of IKKβ for trivalent arsenic was verified *in vitro* by the ability of IKKβ to enhance the fluorescence of an arsenic-substituted fluorescein dye. The addition of 1,2-dithiol antidotes or replacement of Cys-179 with an alanine residue abolished dye binding to and arsenite inhibition of IKKβ. Overexpression of IKKβ (C179A) protects NF-κB from inhibition by arsenite, indicating that despite the involvement of a large number of distinct gene products in this activation pathway, the critical target for inhibition by arsenite is on the IKK catalytic subunits.

The aqueous form of inorganic trivalent arsenic, the arsenite anion (AsO_3^{3-}), is a potent environmental toxin, exposure to which results in a variety of pathologies, most commonly skin disease and cancer, as well as internal cancers, peripheral neuropathy, and cardiovascular disease (1). Despite its toxicity, potassium arsenite (Fowler's solution) has been used in the treatment of psoriasis, a chronic inflammatory disease of the skin (2). However, treatment with Fowler's solution frequently results in malignant skin lesions and hyperkeratosis, as well as liver fibrosis and other pathologies (3–5). Similar problems were found to be associated with chronic exposure to high levels of arsenite through ground water contamination, as most re-

cently documented in West Bengal, where chronic arsenite intoxication has become a common occurrence (6). Chemically, arsenite reacts with sulfhydryl groups and exhibits very high affinity to vicinal thiols. Many of the adverse effects of arsenite on biological systems may therefore be caused by its reaction with closely spaced cysteine residues on critical cellular proteins (7).

The exact biological targets that account for most of the toxic effects of chronic arsenite exposure are not known. Assuming that some of the effects of arsenite exposure are due to aberrant functions of the cell cycle and signal transduction machineries, we examined how arsenite affects mitogen-activated protein kinases (MAPKs).[1] Incubation of cultured cells with sub-lethal doses of arsenite resulted in activation of all three major MAPK pathways, ERK, JNK, and p38 (8).[2] The activation of MAPKs by arsenite may explain its ability to act as a tumor promoter (1, 3, 7). Indeed, other tumor promoters, such as phorbol esters, also lead to MAPK activation.

Some of the effects that chronic arsenite exposure exerts on skin biology, such as disregulated keratinocyte proliferation and hyperkeratosis, closely resemble those associated with selective inhibition of NF-κB activity in keratinocytes (9, 10). We therefore investigated the effect of arsenite exposure on the NF-κB signaling pathway. NF-κB is a transcription factor that plays an important role in development of chronic inflammatory disease (11). NF-κB is kept in the cytoplasm of nonstimulated cells through interaction with specific inhibitors, the IκBs (12). In response to pro-inflammatory stimuli, the IκBs are rapidly phosphorylated and degraded through ubiquitin-dependent proteolysis, resulting in the release of free NF-κB dimers, which translocate to the nucleus to induce transcription of target genes (13–15). The protein kinase responsible for IκB phosphorylation and degradation in response to proinflammatory stimuli, is the IκB kinase (IKK). IKK is a complex of two catalytic subunits, IKKα and IKKβ, and a regulatory and structural subunit, IKKγ/NEMO (12). The IKK complex serves as a convergence point both for positive inputs that lead to NF-κB activation (12) as well as negative regulators that inhibit NF-κB activation (14). Ample evidence suggests that interference with IKK activity may lead to resolution of inflammatory disorders (14, 15). However, inhibition of IKK also sensitizes cells to induction of apoptosis (16, 17).

Here, we show that arsenite is a potent inhibitor of NF-κB

[1] The abbreviations used are: MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; JNK, Jun N-terminal kinase; MEKK, mitogen-activated protein kinase (MAPK)/ERK kinase kinase; NIK, NF-κB-inducing kinase; IKK, IκB kinase; TNFα, tumor necrosis factor α; BAL, British anti-Lewisite; DMPS, 2,3-dimercaptopropane-1-sulfonic acid; EDT, ethanoedithiol; FLAsH, fluorescein arsenical helix binder; WT, wild type.

[2] W. Li and M. Karin, unpublished results.

activation and that its interaction site includes a specific cysteine residue in the activation loop of the IKK catalytic subunits. Furthermore, we demonstrated that specific binding of an organoarsenical dye to IKKβ requires this cysteine residue (Cys-179) and that a substitution of Cys-179 with alanine generates an arsenite-insensitive form of IKKβ, the overexpression of which protects NF-κB activation from inhibition by arsenite.

MATERIALS AND METHODS

Cell Culture, Transfection, and Treatments—Cells were cultured in either Dulbecco's modified minimum essential medium (HeLa or HEK293) or RPMI 1640 (Jurkat) supplemented with 10% fetal calf serum and antibiotics. Cells were stimulated with either 20 ng/ml recombinant human tumor necrosis factor α (TNFα), 10 ng/ml interleukin-1β (Sigma), or 25 ng/ml 12-*O*-tetradecanoylphorbol-13-acetate. Sodium arsenite (Sigma), 2,3,-dimercaptopropanol, or British anti-Lewisite (BAL), and 2,3-dimercaptopropane-1-sulfonic acid (DMPS) (all from Sigma) were dissolved in Dulbecco's modified minimum essential medium and used at the indicated concentrations. 1,2-Ethanedithiol (EDT) was from Aldrich, and stock solutions were made in Me₂SO daily. Transfections were performed as described (14) using LipofectAMINE Plus (Life Technologies, Inc.) for HeLa and HEK293 cells. All of the expression vectors have been previously described (14, 18, 19). Cells were lysed in buffer B (50 mM Tris-HCl, pH 7.5, 400 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton, 0.5% Nonidet P-40, 10% glycerol, supplemented with phosphatase and protease inhibitors), and extracts were used for electrophoretic mobility shift and IKK assays as described previously (20–22).

FlAsH Labeling—The fluorescence emission spectrum of 250 nM (FlAsH-EDT₂) (23) was measured before and after incubation with 100 ng/ml (about 1 nM) IKKβ or other proteins. IKKβ, expressed in Sf9 cells using baculovirus expression vectors, was affinity-purified (14). The proteins were incubated with FlAsH-EDT₂ for 20 min in the absence of additional EDT before measuring the fluorescence emission spectrum as described (23).

RESULTS

Arsenite Inhibits NF-κB and IKK Activation—We examined the effect of arsenite on NF-κB activation and IκBα degradation in TNFα-stimulated HeLa cells. Both NF-κB activation and IκBα degradation were efficiently inhibited upon incubation with at least 12.5 μM arsenite or more (Fig. 1, *a* and *b*). No effect on the DNA binding activities of transcription factors Oct1 and Sp1 were observed in cells incubated with up to 100 μM arsenite (Fig. 1*a, lower panel*). Arsenite also inhibited the IκB kinase activity of the IKK complex (Fig. 1*c*). In HeLa cells, the half-maximal inhibitory (IC₅₀) concentration for arsenite toward NF-κB DNA binding activity was 8.7 μM, and the IC₅₀ toward IKK was 9.1 μM (Fig. 1*d*). Arsenite also inhibited IKK and NF-κB activation in response to either TNFα or interleukin-1 in HEK293 cells (results not shown). Similarly, arsenite inhibited IKK activity in phorbol ester-stimulated Jurkat cells (results not shown). Thus, inhibition of IKK catalytic activity, induction of NF-κB DNA binding activity, and IκBα degradation seem to occur independently of cell type and stimulus. As described previously (8), arsenite stimulated the kinase activities of JNK and p38 (data not shown) and did not prevent their activation by TNFα (Fig. 1*e*). Cell death was assessed in HeLa cells after arsenite treatment. No significant increase in cell death was found after exposure to 200 μM arsenite for 2 h (data not shown).

Direct Inhibition of IKKβ by Arsenite in Vivo and in Vitro— IKK can be activated by overexpression of two members of the MAPK kinase kinase (MAP3K) family: MEKK1 and the NF-κB-inducing kinase (NIK) (24). To determine whether arsenite inhibits NIK-induced IKK activation, HeLa cells were transiently co-transfected with Xpress-tagged NIK and HA-tagged IKKα or HA-tagged IKKβ (Fig. 2*a*). The cells were incubated with different concentrations of arsenite for 1 h, and endogenous IKK activity was measured 24 h after transfection. Under the conditions used, transiently expressed HA-IKKα or HA-
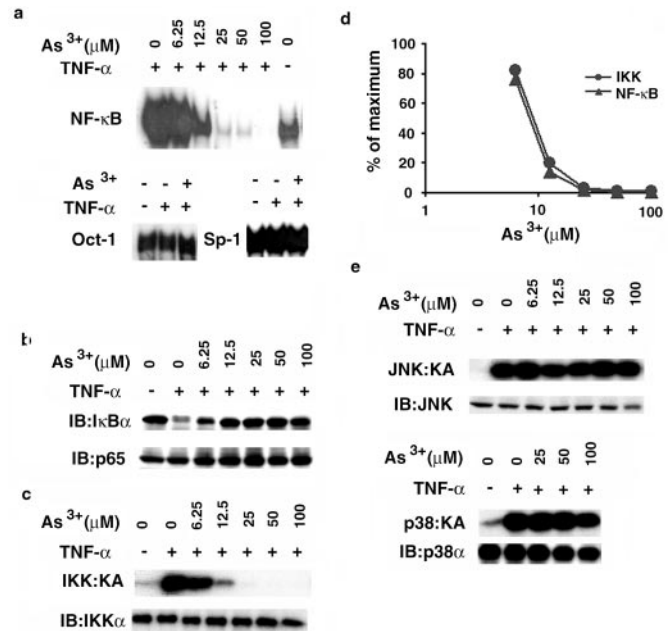

FIG. 1. **Arsenite inhibits induction of NF-κB DNA binding, IκBα degradation, and IKK activation.** *A*, HeLa cells treated with arsenite (0–100 μM) for 2 h were stimulated with TNFα for 15 min. After lysis, extracts were assayed for NF-κB DNA binding activity by electrophoretic mobility shift assay (*upper panel*). No effects of arsenite (100 μM) on constitutive Oct1 and Sp1 DNA binding activities were observed (*lower panel*) *b*, HeLa cells treated as above were analyzed for IκBα degradation by immunoblotting (*IB*) with a polyclonal anti-IκBα antibody. Equal loading was verified by reprobing the blot with anti-p65 (Rel A) antibody. *C*, the same extracts as above were analyzed for IKK kinase activity (KA) by an immunecomplex kinase assay. IKK recovery was verified by immunoblotting with an anti-IKKα antibody. *d*, dose response curves of the inhibition of NF-κB and IKK activities by arsenite. Both activities were quantified by phospho-imaging and are expressed as percentage of the maximal activity achieved in the absence of inhibitor. *e*, extracts from arsenite treated and untreated cells stimulated with TNFα for 15 min were analyzed for JNK1 (KA:JNK) and p38α (KA:p38) activities. Immunoblots of immunoprecipitated JNK1(IB:JNK) and p38α (IB:p38) are shown.

IKKβ molecules are incorporated into functional cytokine-responsive 900-kDa IKK complexes, which can be isolated with antibodies to the HA epitope (22). NIK-induced IKK activity was inhibited by arsenite regardless of whether HA-IKKα or HA-IKKβ was used (Fig. 2*a*). Arsenite was also effective in inhibiting MEKK1- and NIK-induced IKK activity in HEK293 cells (data not shown).

Of the two catalytic subunits, IKKβ plays the major role in responding to proinflammatory stimuli and mediating their effect on NF-κB (12). Previously, the IKKβ subunit was also shown to be inhibited by a high concentration of the anti-inflammatory drug aspirin (25) and by the cyclopentenone prostaglandin, 15-deoxy Δ¹²⁻¹⁴ PGJ₂ (14). To determine the effect of arsenite on IKKβ activity, we used a constitutively active IKKβ variant, IKKβ(EE), in which the two phospho-acceptor serine residues in the activation loop were substituted by glutamic acid (19). HeLa cells were transfected with HA-tagged IKKβ(EE); 24 h after transfection, cells were treated with arsenite for 1 h, and IKK activity was measured (Fig. 2*b*). Arsenite did not affect IKKβ expression as determined by Western blot analysis, whereas it strongly inhibited IKKβ catalytic activity.

We next investigated whether arsenite could inhibit IKK activity *in vitro*. HeLa cells were stimulated with TNFα for 15 min, endogenous IKK was immunoprecipitated, and its kinase activity was determined in the presence of increasing arsenite concentrations (Fig. 2*c*). Dose-dependent inhibition of IKK ac-
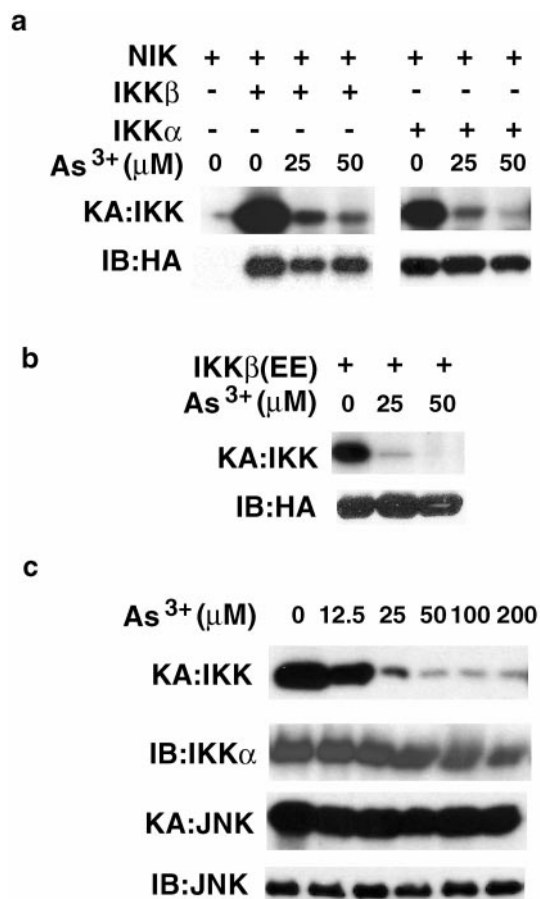
FIG. 2. **Arsenite directly inhibits IKK activity.** *a*, HeLa cells were co-transfected with Xpress-tagged NIK-and HA-tagged IKKα or IKKβ expression vectors, and then treated with different doses of arsenite for 1 h. Cells were lysed, immunoprecipitated with anti-HA antibody, and assayed for IKK activity and recovery 24 h after transfection. *b*, HeLa cells were transfected with HA-tagged constitutively active IKKβ(EE), and treated with various doses of arsenite for 1 h. Cells were lysed and assayed for HA-tagged IKKβ(EE) kinase activity and recovery 24 h after transfection. *c*, HeLa cells were stimulated with TNFα for 15 min. IKK was immunoprecipitated and incubated *in vitro* with the indicated concentrations of arsenite for 1 h followed by kinase assay (KA:IKK) and immunoblot analysis (IB:IKKα). JNK1 was also immunoprecipitated from HeLa cells stimulated with TNFα for 15 min. and its activity measured after pre-incubation *in vitro* for 1 h with the indicated concentrations of arsenite.

tivity was observed with an $IC_{50} = 14.2$ μM. No significant inhibition of immunoprecipitated JNK1 activity was observed upon incubation with up to 200 μM arsenite (Fig. 2*c*). Because many protein kinase inhibitors compete with ATP binding (26), we examined the effect of ATP on inhibition of IKK activity. However, preincubation in the presence of increasing concentrations of ATP had no effect on the inhibition of recombinant IKKβ activity by arsenite (data not shown).

Binding of a Fluorescent Arsenical Reagent to IKKβ—To verify whether trivalent arsenite binds IKKβ, we exploited a recently developed organoarsenical derivative of fluorescein, FlAsH-EDT$_2$, in which fluorescence is quenched until its arsenic substituents bind vicinal thiols in a polypeptide target (23). Normally FlAsH-EDT$_2$ is used in live cells in the presence of a modest excess of EDT or an analogous small 1,2-dithiol to prevent the FlAsH molecule from binding to sites with less than four preorganized cysteines. However, to make an *in vitro* binding assay comparable with treatment with arsenite in the absence of antidotes such as BAL, we measured the effect of purified recombinant IKKβ on the fluorescence of FlAsH-EDT$_2$ in the absence of excess 1,2-dithiols such as EDT or BAL. The
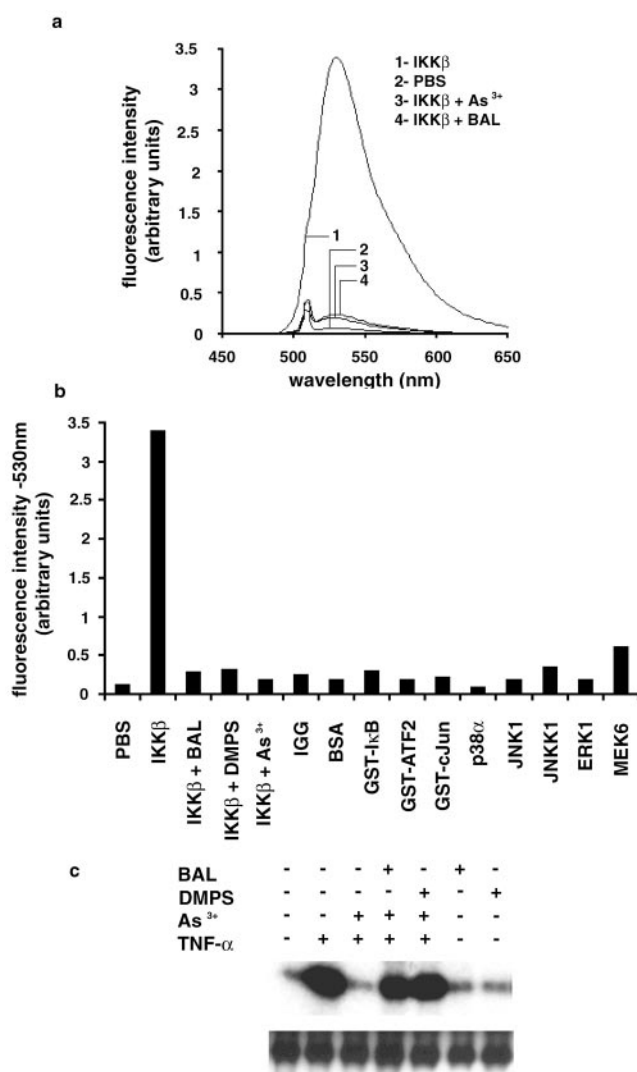
FIG. 3. **Direct binding of a fluorescent organoarsenical to IKKβ.** *a*, binding of FlAsH to IKKβ. FlAsH-EDT$_2$ (200 nM) was incubated for 20 min. in the presence of 100 ng/ml purified IKKβ (1), PBS (2), 100 ng/ml IKKβ pre-incubated for 1 h with 50 μM arsenite (3), or 100 ng/ml IKKβ with subsequent addition of 50 μM BAL for 5 min. (4). Fluorescence intensity at 530 nM was measured with excitation at 508 nM. *b*, relative fluorescence intensity of 200 nM FlAsH-EDT$_2$ in the presence of 100 ng/ml solutions of the indicated proteins in PBS. When indicated, the IKKβ sample was pre-incubated with BAL, DMPS or arsenite. *c*, HeLa cells were pre-incubated with 200 μM BAL or DMPS for 15 min. The cells were extensively washed to remove these compounds from the medium and cell surface and were incubated in the absence or presence of 100 μM arsenite followed by incubation in the absence or presence of TNFα for 15 min. After lysis, extracts were analyzed for IKK kinase activity (*KA*) by immunocomplex kinase assay. IKK recovery was verified by immunoblotting (*IB*) with an anti-IKKα antibody.

addition of IKKβ caused a large increase in fluorescence emission, whereas adding just buffer or IKKβ presaturated with 50 μM arsenite had a negligible effect (Fig. 3*a*). IKKβ alone was not fluorescent, and its ability to increase FlAsH fluorescence was essentially reversed by the addition of 10 μM EDT, the conditions under which FlAsH is normally used specifically to label tetracysteine motifs in living cells (Fig. 3*a*). This reversal by EDT confirms that the binding of IKKβ is mainly via the arsenic groups of FlAsH, and that under the normal antidote concentrations used in labeling of live cells, FlAsH should not be significantly affecting IKKβ activity. The convenience of assaying FlAsH fluorescence in the absence of excess EDT facilitated quick screening of a variety of other purified pro-

teins, none of which increased FlAsH fluorescence to anything like the extent of IKKβ (Fig. 3b). Thus, FlAsH as a model organoarsenical interacts much more strongly with IKKβ than with many other proteins in this or other signaling pathways. A fluorescein derivative comparable with FlAsH but containing only one arsenic[3] also increased fluorescence upon incubation with IKKβ (data not shown), indicating that only two cysteines are required for binding. However, the fluorogenic enhancement was by a smaller factor than with FlAsH-EDT$_2$, probably because the monoarsenic derivative was not as fully quenched before protein addition, and a large excess of the fluorophore over protein was present under the conditions used. To confirm the parallelism between FlAsH binding and arsenite inhibition, we tested whether 1,2-dithiols like BAL and DMPS, which can reverse the binding of FlAsH to IKKβ (Fig. 3, a and b), can also protect IKK activity from arsenite. Pre-incubation of cells with BAL or DMPS indeed prevented subsequent inhibition of IKK activity by arsenite (Fig. 3c). This result confirmed the effectiveness of these antidotes and suggested that they may be well enough retained in cells during a wash to combat a later arsenite challenge.

Because trivalent arsenic strongly prefers binding to vicinal thiols, we examined the IKKβ kinase domain for critically positioned cysteines. Although no consecutive cysteines were present, we noticed that both IKKα and IKKβ, but not JNK1 or p38α, contain a cysteine (Cys-179) within their activation loops (Fig. 4a). As IKKα and IKKβ form homo- and heterodimers (21), it is possible that two Cys-179 residues may be adjacent within the dimer. Alternatively, they may be located within close proximity to another cysteine within the folded protein. To examine whether Cys-179 is critical for sensitivity to arsenite, we replaced it with alanine using site-directed mutagenesis. Both WT IKKβ or IKKβ (C179A) were co-expressed with NIK in HeLa cells, and their sensitivity to arsenite was examined. Although both constructs were equally responsive to NIK, resulting in similar levels of kinase activity, the IKKβ (C179A) mutant was fully resistant to inhibition by 50 μM arsenite, a dose sufficient for complete inhibition of the WT enzyme (Fig. 4b). We also co-transfected an NF-κB-dependent transcriptional reporter (2XNFkB-LUC) with an expression vector encoding either WT IKKβ or IKKβ (C179A). A NIK expression vector was included to ensure maximal IKK activation. Although activation of NF-κB transcriptional activity in response to WT IKKβ was highly sensitive to arsenite, NF-κB activation in response to IKKβ (C179A) was insensitive to the inhibitor (Fig. 4c). Recombinant IKKβ (C179A) expressed in Sf9 cells using a baculovirus vector was also completely resistant to concentrations of arsenite that were inhibitory to WT IKKβ (Fig. 4d). Furthermore, IKKβ (C179A) exhibited decreased binding to FlAsH as judged by its much reduced ability to enhance fluorescence emission of FlAsH-EDT$_2$ (Fig. 4e). Collectively, these results strongly suggest that arsenite inhibits IKK by direct modification of its catalytic subunits at a site that includes Cys-179. When the IKKβ subunit is rendered resistant to modification, very little inhibition of NF-κB transcriptional activity occurs in arsenite-treated cells.

DISCUSSION

The pathological effects of arsenite are likely to be caused by modification of critical cysteine groups in cellular proteins or by its reaction with enzymes containing lipoic acid as a co-factor (7). Despite such a potentially indiscriminate mechanism of toxicity, we found that arsenite exerts rather specific effects on cellular signal transduction pathways. Previously, we demonstrated that exposure to sublethal doses of arsenite results in

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[3] S. R. Adams and R. Y. Tsien, unpublished results.
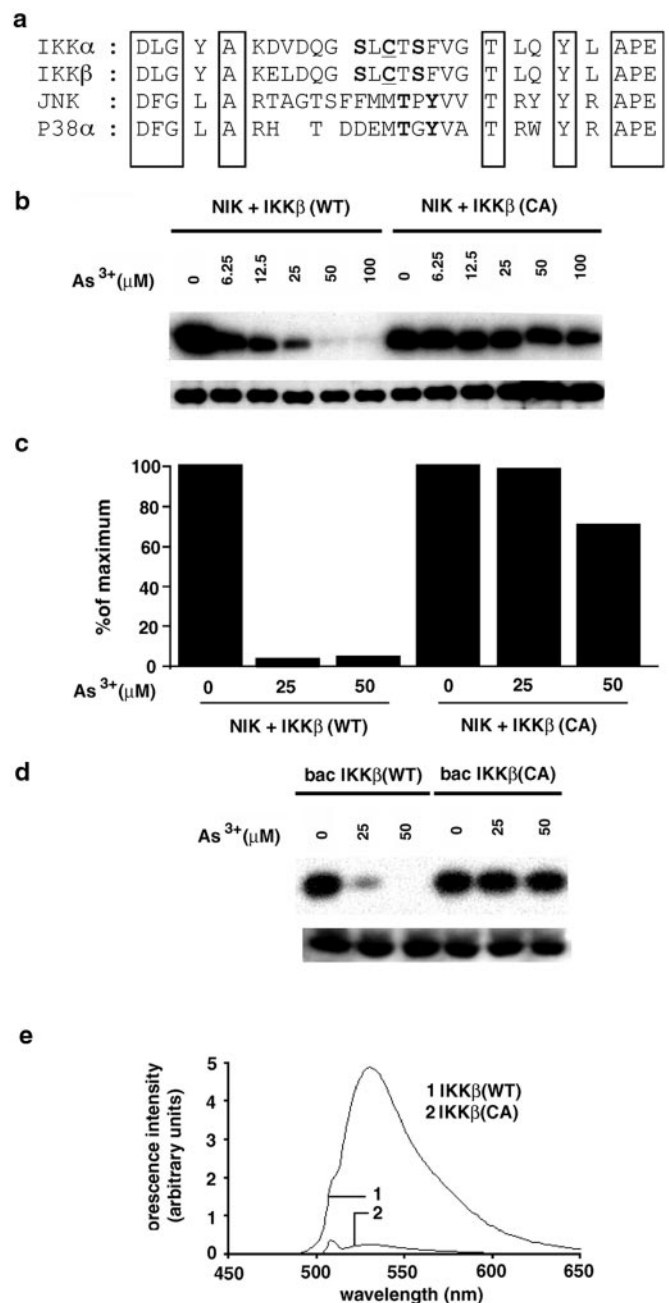
FIG. 4. **Arsenite directly inhibits IKKβ through modification of a specific cysteine residue in its activation loop.** *a*, alignment of the activation loops of IKKα, IKKβ, JNK1 and p38α. Invariant residues are boxed. Phosphoacceptor sites critical for kinase activation are in *boldface*. The cysteine residues of IKKα and IKKβ are bolded and underlined. *b*, HeLa cells were transiently transfected with for WT IKKβ or IKKβ (C179A) expression vectors together with a NIK expression vector. After 20 h, the cells were treated for 1 h with 0–100 μM arsenite. Kinase activity and expression were determined by immunocomplex kinase assay (*upper panel*) and immunoblotting (*IB*) with anti-HA antibodies (*lower panel*). *c*, HeLa cells were transiently transfected with a 2xNF-kB-LUC reporter and WT IKKβ or IKKβ (C179A) expression vectors. A NIK expression vector was included to ensure maximal IKK activation. At 3 h post-transfection the cells were treated with the indicated doses of arsenite. After 24 h the cells were lysed and luciferase activity was determined. The results represent averages of duplicate transfections, and in each case, maximal activity achieved in the absence of inhibitor was given an arbitrary value of 100% and the other values were calculated relative to that. *d*, Sf9 cells infected with WT IKKβ and IKKβ (C179A) baculoviruses were incubated with various concentrations of arsenite for 2 h at 28 °C. Kinase activity (*upper panel*) and expression (*lower panel*) were determined. *e*, fluorescence emission spectrum of 200 nm FlAsH-EDT$_2$ incubated with 100 ng/ml of WT IKKβ or IKKβ (C179A).

potent activation of various MAPK signaling pathways, most notably JNK and p38 (8). These effects were attributed to inhibition of MAPK phosphatases, enzymes that contain a critical cysteine in their catalytic pocket (8).[2] The present work demonstrates that in contrast to its positive effect on MAPK cascades, arsenite is a potent inhibitor of NF-κB activation because of its ability to directly inhibit IKK activity. Interestingly, the inhibition of IKK activity involves modification of Cys-179, which is critically positioned within the activation loop of the IKK catalytic subunits. Substitution of this cysteine with alanine prevents the inhibition of IKK activity and overexpression of the arsenite-resistant IKKβ (C179A) mutant protects NF-κB from arsenite-mediated inhibition. These results indicate that despite the involvement of at least 50 different proteins in NF-κB activation (12), each containing multiple cysteines, arsenite inhibits this system through modification of one critical site that includes Cys-179 in the IKKβ activation loop. Thus, even a highly reactive toxin such as arsenite can exert its predominant toxic effects through a small number of relevant targets.

Although arsenite exhibits much higher affinity to vicinal thiols than for single or widely spaced thiols (7), Cys-179 is not located nearby another cysteine within the IKKβ primary structure. However, it is likely that upon folding of the IKKβ polypeptide chain, Cys-179 is situated within a critical distance of another, yet to be identified cysteine. As the catalytic subunits of IKK form stable dimers (18, 21), it is possible that the second cysteine is present within the dimeric partner of IKKβ. Interestingly, Cys-179 of IKKα/β is highly reactive and was recently found to be a target for cyclopentenone prostaglandins, which, like arsenite, are potent IKK inhibitors (14). In that case, the inhibition is likely to be mediated by formation of a Michael adduct between the cytopentenone moiety and Cys-179 of IKKβ.

The ability to demonstrate direct binding of trivalent arsenite to IKKβ relied on the use of the fluorogenic organoarsenic FlAsH-EDT$_2$. This molecule was previously developed as a ligand that allows the specific fluorescent tagging of recombinant proteins that contain four appropriately spaced cysteines (23). Specificity for four cysteines relies on the presence of a small excess, typically 10 μM, of a 1,2-dithiol such as EDT to competitively suppress binding to isolated pairs of cysteines. In the absence of excess 1,2-dithiol, FlAsH forms relatively weak ($K_d \sim 0.1$. μM) nonfluorescent complexes with small peptides containing a single pair of consecutive cysteines.[3] On a full-size protein such as IKKβ, hydrophobic immobilization of FlAsH's second arsenic-EDT group on the protein surface may prevent that arsenic from quenching fluorescence, as described recently for the interaction of FlAsH and albumin (27). FlAsH or its mono-arsenic analog may be useful for detecting other vicinal thiols on full-size proteins *in vitro*.

Regulation of the NF-κB pathway is critical for the expression of genes involved in inflammation, immunity, and carcinogenesis. The IKK complex is believed to be an important site for integrating signals that regulate the NF-κB pathway. Recently, a number of drugs and naturally occurring compounds, including aspirin, sulindac, cyclopentenone prostaglandins, and cytochrome P450 epoxygenase-derived eicosanoids, have been shown to inhibit the NF-κB pathway through IKK (14, 28, 29). However, the mechanisms of this inhibition remain elusive. Our results on the mechanisms of inhibition of IKK by

arsenite reveal the critical role of a cysteine residue in the activation loop of IKKβ. The same cysteine is targeted by cyclopentenone prostaglandins and is essential for the sensitivity of IKK and the entire NF-κB pathway to these naturally occurring anti-inflammatory molecules (14).

Our results link some of the biological effects of arsenite on tissue pathology to inhibition of IKK and NF-κB. As a matter of fact, some of the consequences of chronic arsenite exposure on human skin, such as hyperkeratosis and hyperproliferative skin disease, are similar to the effect of NF-κB inhibition on skin (9, 10). Furthermore, we have observed dermatopathies involving blistering and hyperkeratotic lesions in mice that are mosaics for a null allele of the X-linked *Ikk*γ gene, coding for the essential regulatory subunit of the IKK complex (10). Thus, IKK is likely to be a highly relevant target for the chronic cutaneous toxicity of arsenite. In addition, through inhibition of IKK, and consequently NF-κB, arsenite can promote apoptosis. Previous work has shown that IKKβ-deficient mice die at mid-gestation because of sever liver apoptosis (10, 16, 17, 30). Thus, inhibition of IKK may certainly contribute to arsenite induced cytotoxicity.

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