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Modulation of transcription factor NF-κB binding activity by oxidation–reduction *in vitro*

(diamide/*N*-ethylmaleimide/2-mercaptoethanol/AP-1/serum response factor)

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ABSTRACT **NF-κB is a widely used regulator of inducible and tissue-specific gene control. In the cytosol, when complexed to an inhibitory molecule, IκB, NF-κB is in an inactive form and cannot bind DNA. Activation of cells with appropriate stimuli results in the dissociation of NF-κB from IκB and its translocation to the nucleus as an active binding protein. We now demonstrate that NF-κB binding *in vitro* can be inhibited by agents that modify free sulfhydryls. Binding is eliminated after treatment with *N*-ethylmaleimide, an alkylating agent, and diamide, an oxidizing agent. The diamide effect can be reversed by 2-mercaptoethanol. Further, 2-mercaptoethanol acts synergistically with deoxycholate plus Nonidet P-40 in converting inactive cytosolic NF-κB to an active DNA-binding form. It is therefore possible that modulation of the redox state of NF-κB could represent a post-translational control mechanism for this factor.**

Regulatory proteins that influence gene expression can be controlled both transcriptionally and post-transcriptionally. For example, the T-cell-specific DNA-binding protein NFAT-1, which is critical for interleukin 2 gene expression, is regulated principally at the level of transcription (1), whereas the binding of CREB (2) is influenced by its phosphorylation state, and *in vitro* the iron response element RNA-binding protein (IRE-BP) is influenced by the redox state of the protein (3).

NF-κB is an inducible DNA-binding protein detected in multiple cell types and has been implicated in the transcriptional induction of many genes (4). NF-κB is at least in part controlled by its cellular localization (5, 6). It consists of a DNA-binding subunit of 50 kDa (7, 8) that associates with a second protein of 65 kDa (9). In the cytosol of uninduced non-B cells, NF-κB exists in an inactive form complexed to an inhibitory molecule, IκB (5, 6). Activation of cells by appropriate stimuli results in the dissociation of NF-κB from IκB and its translocation to the nucleus as a complex containing both p50 and p65 (5, 6). NF-κB therefore represents a paradigm for the study of cytoplasmic-to-nuclear signaling and induced gene expression (4). We now demonstrate that NF-κB binding *in vitro* can be modulated by variations in the redox state of the protein. We discuss the potential implications of this phenomenon.

MATERIALS AND METHODS

Oligodeoxynucleotides. Complementary oligonucleotides were prepared on an Applied Biosystems DNA synthesizer (model 381A), annealed, purified on native polyacrylamide gels, and used as unlabeled competitors or as probes after end-labeling with [α-^{32}P]dCTP and the Klenow fragment of DNA polymerase I. Radiolabeled probes were separated from free nucleotides by using push columns (Stratagene).

Cells and Preparation of Nuclear and Cytoplasmic Extracts. Jurkat (acute lymphocytic T-cell leukemia), MT-2 (human T-cell lymphotropic virus I-infected T cell line), and HeLa (cervical carcinoma-derived cell line) were grown in RPMI 1640 medium supplemented with 10% (vol/vol) fetal bovine serum, 2 mM glutamine, penicillin (100 units/ml), and streptomycin (100 μg/ml). Where indicated, cells were stimulated with phorbol 12-myristate 13-acetate (PMA, 50 ng/ml) 10–14 hr before harvesting. Nuclear extracts were prepared per Dignam *et al.* (10) with the omission of dithiothreitol (DTT) in each buffer. The post-nuclear supernatant was centrifuged at 50,000 × *g* and the supernatant was concentrated using a Centricon-10, dialyzed against buffer D (10) not containing DTT [buffer D(−)], and saved as cytosolic extracts. Protein concentrations were determined by the bicinchoninic acid (BCA) method. Diazinedicarboxylic acid bis(*N*,*N*-dimethylamide) (diamide, Sigma) was constituted as a 1 M solution in water. *N*-Ethylmaleimide (NEM, Calbiochem–Behring) was constituted as a 250 mM solution in water. Dialysis of nuclear extracts, after treatment with diamide or NEM, was performed using 1500 vol of buffer D(−) for ≥4 hr.

***In Vitro* Transcription and Translation.** pBluescript vector containing a truncated (*Rsa* I digestion at nucleotide 1372, amino acids 1–399) human NF-κB/KBF-1 (p50) cDNA insert (11) was linearized with *Xba* I and transcribed into mRNA by using T7 RNA polymerase with the cap analog to produce capped transcripts according to the manufacturer's recommendations (Pharmacia). After purification on a push column, 1 μg of mRNA was used in rabbit reticulocyte lysate and wheat germ extract cell-free translation systems, according to the manufacturer's directions (Promega).

Electrophoretic Mobility-Shift Assays (EMSAs). DNA-binding reaction mixtures (20 μl) contained 10 mM Tris·HCl (pH 7.6), 50 mM NaCl, 1 mM EDTA, 1 μg of poly(dI-dC), and 1.5–2.5 × 10^4 cpm of the probe. Depending on the experiment, 5 μg of nuclear extracts, 10 μg of cytosolic extracts, 2 μl of rabbit reticulocyte lysates, or 4 μl of wheat germ extracts were added prior to the probe. Diamide, NEM, 2-mercaptoethanol (2-ME), sodium deoxycholate (DOC), and Nonidet P-40 (NP40) were added to the binding reaction mixtures at 20°C, for approximately 10 min before addition of the probe. Binding reactions and subsequent analyses on native 4% polyacrylamide gels were performed as described (12). Autoradiograms were analyzed using an UltraScan XL densitometer (LKB).

RESULTS

Alkylation by NEM Inhibits NF-κB Binding *in Vitro*. As shown (12–15), NF-κB binding activity was present in nuclear

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Abbreviations: PMA, phorbol 12-myristate 13-acetate; DTT, dithiothreitol; NEM, *N*-ethylmaleimide; EMSA, electrophoretic mobility-shift assay; 2-ME, 2-mercaptoethanol; NP-40, Nonidet P-40; DOC, sodium deoxycholate; IL-2Rα, α chain of interleukin 2 receptor.

extracts prepared from PMA-induced Jurkat T-cells and from MT-2 T cells (Fig. 1*A*, lane 1). Treatment of extracts with NEM, a chemical agent that irreversibly alkylates free sulfhydryls, significantly inhibited subsequent DNA–protein complex formation at concentrations of 4 mM or greater (lanes 2–6). NEM inactivated NF-κB binding even if added after the probe (data not shown). These results suggested that free sulfhydryl(s) were functionally important for NF-κB binding activity *in vitro*. As expected, the NEM-induced loss of binding activity was not reversed by 2-ME (lane 7).

Oxidation Reversibly Inactivates NF-κB Binding *in Vitro*. To further investigate the role of sulfhydryl groups in NF-κB binding, we used diamide, which can catalyze the oxidation of free sulfhydryls (17). Treatment of nuclear extracts with 12 mM diamide abolished NF-κB binding activity (Fig. 1*B*, lane 2 vs. lane 1). This effect was also seen at concentrations as low as 1 mM diamide (data not shown). In contrast to the irreversible alkylation by NEM, the diamide effect was completely reversed by 2-ME (lanes 3–5) or DTT (data not shown). Treatment of nuclear extracts with reducing agents, however, did not significantly increase NF-κB binding activity (Fig. 1*C*, lane 1 vs. lanes 2 and 3), suggesting that the majority of NF-κB in nuclear extracts as isolated was already optimally reduced. Nuclear extracts prepared in the presence or absence of DTT behaved similarly (data not shown). Inactivation of NF-κB binding by diamide and NEM were not reversed by dialysis (Fig. 1*D*, lanes 2 and 5 vs. lane 1). As expected, after dialysis, the diamide but not the NEM effect was reversed by 2-ME (lanes 3 and 4 and lanes 6 and 7). Dialysis alone did not significantly affect NF-κB binding (lane 8). Diamide treatment can result in the formation of an unstable sulfenyl hydrazene intermediate (18); the fact that dialysis did not restore NF-κB binding suggested that diamide was instead inducing the formation of a stable oxidized compound (17, 18). This conclusion was supported by the apparent insensitivity of NF-κB to NEM treatment when nuclear extracts were treated with diamide (Fig. 1*E*). Although NEM alone irreversibly diminished NF-κB binding (lanes 2 and 3 vs. lane 1), after treatment with diamide and then NEM, binding activities could be reversed by 2-ME (lane 5 vs. lane 4), indicating that the oxidation of free sulfhydryls by diamide prevented alkylation by NEM.

What is the nature of the oxidized form? It could involve intrachain disulfide bond(s) within NF-κB, disulfide bond(s) between NF-κB and another molecule or potentially could depend on the oxidation of sulfhydryl(s) into sulfonic acid. The fact that dialyzed 2-ME-treated extracts remained sensitive to diamide (data not shown) made less likely the formal possibility of a mixed disulfide bond between NF-κB and a small dialyzable molecule such as glutathione.

Cytosolic NF-κB Binding Is Maximally Activated by a Combination of Both Dissociating and Reducing Agents. Does the *in vitro*-oxidized form of NF-κB generated by diamide have a physiologically important counterpart *in vivo*? Although we have not proven that it does, we have obtained data consistent with this possibility. Since NF-κB binding is inducible, we investigated whether an oxidized form was expressed in cytosolic extracts prepared from noninduced cells. As noted above, NF-κB can exist in the cytosol in an inactive form complexed to IκB. NF-κB binding was activated by agents such as DOC plus NP-40 (5, 6) (Fig. 2*A*, lane 2 vs. lane 1) that dissociate this complex. 2-ME alone also could induce NF-κB binding activity in uninduced T-cell cytosolic extracts (lanes 6 and 7), but the appearance of the shifted complex was somewhat different from that seen using DOC plus NP-40 (lane 2). 2-ME and DOC plus NP-40 acted synergistically in their induction of NF-κB binding activity (lanes 3 and 4). Competition with unlabeled IgκB oligonucleotide confirmed the specificity of this binding (lane 5). The synergistic effect of 2-ME and DOC plus NP-40 was dose dependent between 0.05 and 2% 2-ME (Fig. 2*B*). Presumably 2-ME did not increase cytosolic NF-κB binding solely by promoting its dissociation from IκB because, at optimal concentration of 2-ME, DOC plus NP-40 could still increase binding activity (Fig. 2*C*). It is also unlikely that the partial activation of cytosolic NF-κB by 2-ME represented the binding of the NF-κB–IκB complex, because of the comparatively similar mobilities of the complexes resulting from DOC vs. 2-ME treatment. It is therefore possible that an oxidized form of NF-κB (not necessarily associated with IκB) exists in the cytosolic fraction of uninduced cells.

Modulation of NF-κB Binding by Oxidation–Reduction Occurs Through KBF-1/NF-κB p50. As noted above, NF-κB consists of a DNA-binding subunit of 50 kDa denoted as p50 (7, 8) or KBF-1 (11) associated with a 65-kDa protein (9). KBF-1/p50 is synthesized as a precursor protein of 105 kDa, which is then processed to its functional size (11, 19). To verify that the effect of redox variations was occurring

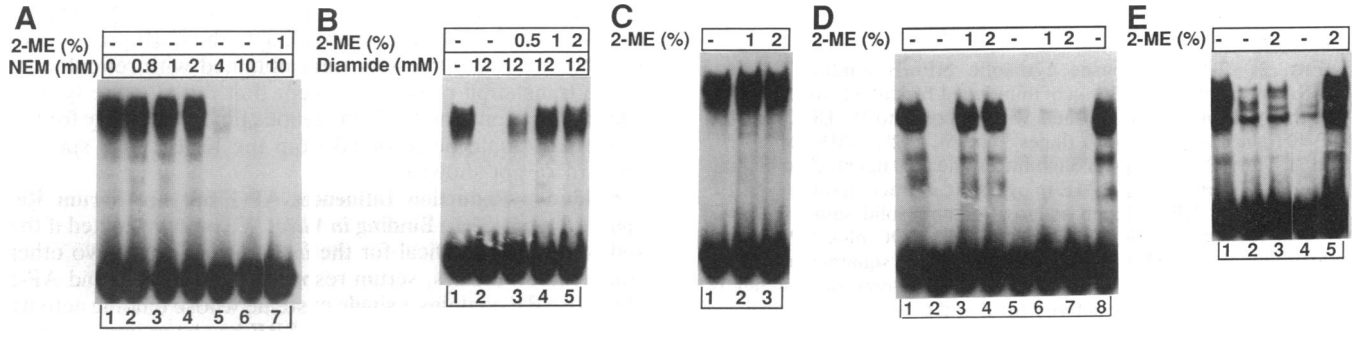

FIG. 1. Alkylation or oxidation of sulfhydryls eliminates NF-κB binding *in vitro*. (*A*) NEM inhibits NF-κB binding. EMSAs were performed as described (9) using an IgκB probe (ref. 16; TCGACAGAGGGACTCTCCCAGAGGC) and PMA-induced Jurkat nuclear extracts. NEM was not added (lane 1) or was added to the binding reaction mixture as indicated (lanes 2–7). 2-ME was also added after NEM (lane 7). Probe was always added last. (*B*) Oxidation reversibly inactivates NF-κB. EMSA using 5 μg of PMA-induced Jurkat nuclear extracts and the IgκB probe. Extracts were not treated (lane 1) or were treated with 12 mM diamide (lanes 2–5). 2-ME was subsequently added as indicated (lanes 3–5) (1% 2-ME is approximately 142 mM). (*C*) 2-ME does not significantly affect binding of nuclear NF-κB. EMSA using the IgκB probe and PMA-induced Jurkat nuclear extracts treated with 0, 1, or 2% 2-ME (lanes 1–3). (*D*) Dialysis does not reverse the effects of NEM and diamide. EMSA using the IgκB probe and 5 μg of MT-2 nuclear extracts untreated (lane 1), treated at room temperature for 5 min with 120 mM diamide (lanes 2–4), with 60 mM NEM (lanes 5–7), or mock-treated (lane 8) prior to dialysis vs. buffer D(−). 2-ME was added prior to probe (lanes 3, 4, 6, and 7). (*E*) Oxidation protects NF-κB from alkylation by NEM. EMSA using 5 μg of PMA-induced Jurkat nuclear extracts and an IgκB probe. Extracts were not treated (lanes 1–3) or were treated with 120 mM diamide (lanes 4 and 5) for 5 min at room temperature and were dialyzed against buffer D(−). The extracts were then untreated (lanes 1) or were treated with 60 mM NEM (lanes 2–5) and subjected to a second dialysis against buffer D(−) prior to binding to the DNA probe. 2-ME was added as indicated (lanes 3 and 5).

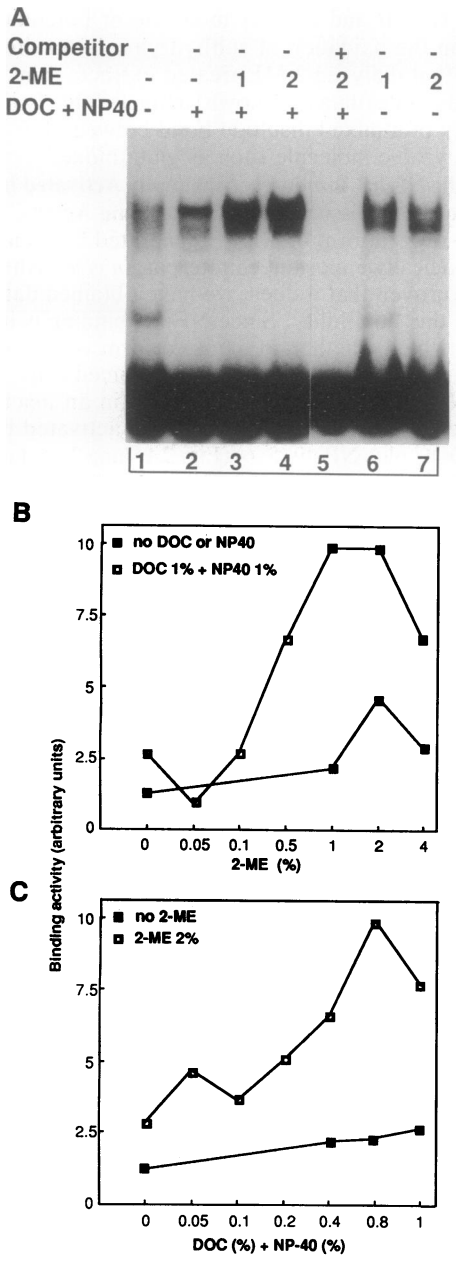

FIG. 2. 2-ME increases cytosolic NF-κB binding activity. EMSAs performed with 10 μg of uninduced Jurkat cytosolic extracts and the IgκB probe. (*A*) Extracts were exposed to 1% DOC plus 1% NP-40 (lanes 2–5), 1% 2-ME (lanes 3 and 6), or 2% 2-ME (lanes 4, 5, and 7). The unlabeled Ig-κB oligonucleotide (100 ng) blocked binding (lane 5). (*B*) Titration of 2-ME in cytosolic extracts treated with 1% DOC plus 1% NP-40 (open squares) or not (solid squares). 2-ME (0–4%) was added prior to the probe. (*C*) DOC plus NP-40 were added (equal amounts) as indicated with (open squares) or without (solid squares) 2-ME. *B* and *C* represent densitometric scanning of autoradiograms. Binding activity for *B* and *C* were each normalized to the binding of cytosolic extracts in the absence of 2-ME, DOC, and NP-40 and are expressed in arbitrary units.

through KBF-1/p50, we used *in vitro* translation of a construct corresponding to a truncation of the cloned KBF-1 cDNA. This truncation corresponds to amino acids 1–399 of KBF-1 and retains full NF-κB binding activity (11). In both wheat germ extracts (Fig. 3*A*, lane 1) and rabbit reticulocyte lysates (lane 2), the primary translation product had the expected size of 45–48 kDa. In an EMSA performed with the wheat germ translation product and the IgκB probe, a band of retarded mobility was observed (Fig. 3*B*, lane 1). The band was competed with an excess of unlabeled IgκB oligonucleotide (lane 5) but not with an excess of an unrelated oligonucleotide or a mutated IgκB probe (lanes 6 and 7), demonstrating the specificity of binding. No similar shifted band was observed in the absence of mRNA (data not shown). Although 5 mM DTT was present in the wheat germ extracts, a high concentration of 2-ME (1%) significantly increased binding to the IgκB probe (lane 2). Further, binding was abolished by pretreatment of the translation products with 12.5 mM diamide (lane 3) and this loss of binding was fully reversed when 2-ME (1%) was subsequently added in the binding reaction (lane 4). Treatment of the translation product with NEM also abolished binding to the IgκB probe (data not shown). Similar results were obtained with reticulocyte lysate translation products, except that reticulocyte lysates additionally contained an endogenous binding activity for the probe that could be resolved from the KBF-1/p50 specific band (data not shown).

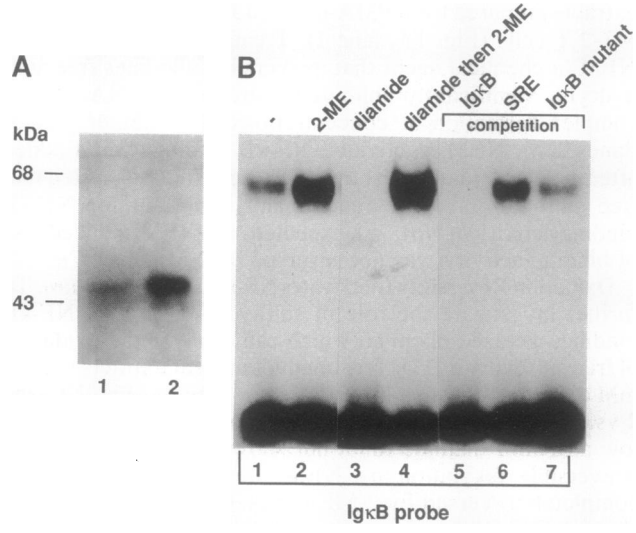

FIG. 3. Modulation of NF-κB binding by oxidation–reduction is mediated through the KBF-1/p50 subunit of NF-κB. (*A*) mRNA corresponding to the KBF-1/p50 cDNA was translated *in vitro* in wheat germ extract (lane 1) and reticulocyte lysate (lane 2) cell-free translation systems in the presence of [^{35}S]methionine and analyzed by SDS/PAGE in a 7.5% gel. After electrophoresis, the gel was fixed, treated with enhancing solution, dried, and exposed to autoradiography. (*B*) EMSA performed with 4 μl of the wheat germ extract translation product and the IgκB probe. 2-ME (1%; lane 2), diamide (12.5 mM; lane 3), or diamide (12.5 mM) followed by 2-ME (1%; lane 4) were added prior to the probe. Competition with 50 ng of the IgκB (lane 5), an oligonucleotide displaying the serum response element (SRE) binding site (11) (lane 6), or a mutated IgκB oligonucleotide TCGAGAGCTCACTCTCCCAGAGGC (lane 7) is shown.

Oxidation–Reduction Influences AP-1 but not Serum Response Factor (SRF) Binding *in Vitro*. We next evaluated if the redox state was critical for the *in vitro* binding of two other transcription factors, serum response factor (SRF) and AP-1 (Fig. 4). SRF contains a single cysteine whose binding activity is constitutive in most cell lines. SRF has been implicated in the transcription of the c-*fos* (21–23), actin (24, 25), and interleukin 2 receptor α (IL-2Rα) chain genes (40). AP-1 is a PMA-inducible nuclear factor (20) formed by the association of products of the *jun* gene family with the Fos protein (26, 27) and is responsible for the transcriptional activation of certain PMA-inducible genes (20). Fos and Jun contain eight and three cysteines, respectively, one of which is located in the DNA-binding domain of both proteins (28, 29).

For the SRF experiments, we first used MT-2 nuclear extracts and an oligonucleotide corresponding to positions −270 to −237 of the IL-2Rα enhancer, which contains adjacent κB-like and CC(A + T-rich)$_6$GG (CArG) box sites

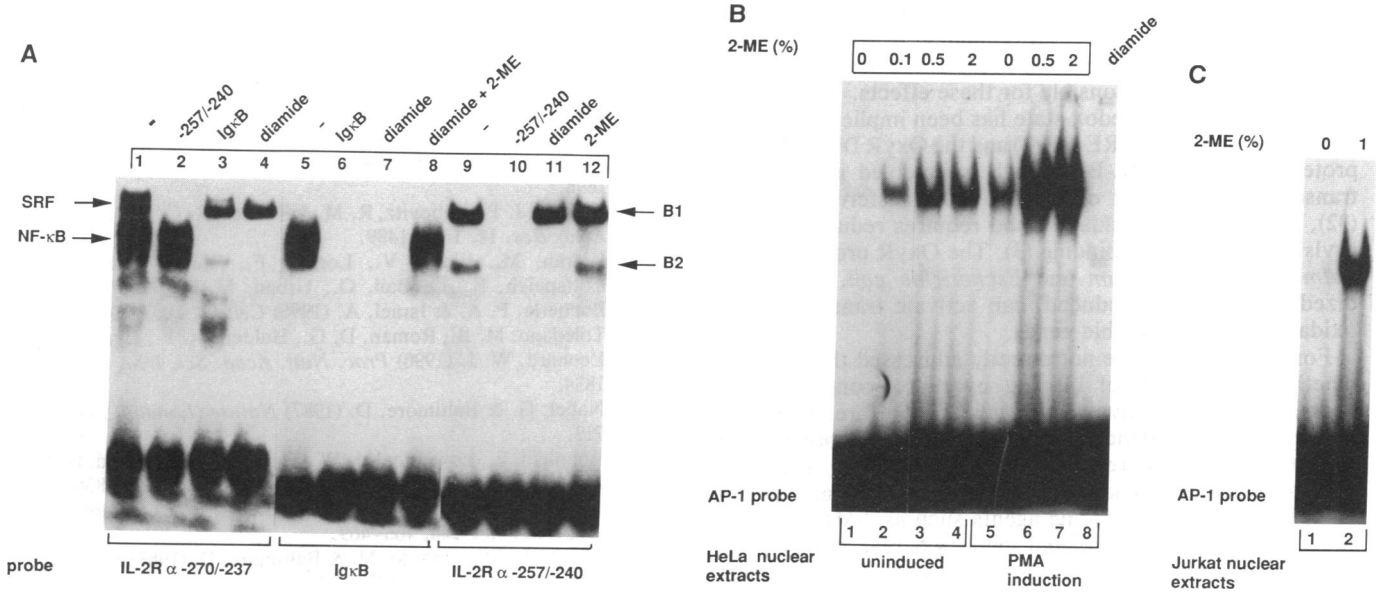

FIG. 4. Binding of AP-1 but not SRF is sensitive to redox variations. (*A*) EMSA performed with 5 μg of MT-2 nuclear extracts and labeled IL-2Rα −270/−237 oligonucleotide (lanes 1–4), IgκB probe (lanes 5–8), or labeled IL-2Rα −257/−240 oligonucleotide (lanes 9–12). Diamide (25 mM; lanes 4, 7, 8, and 11) and 2-ME (2%; lanes 8 and 12) were added prior to probe. Competition with IL-2Rα −257/−240 (lanes 2 and 10) or Ig-κB (lanes 3 and 6) oligonucleotides is shown. SRF and NF-κB complexes are indicated by arrows. (*B*) EMSA performed with 5 μg of uninduced (lanes 1–4) or PMA-induced (lanes 5–8) HeLa nuclear extracts and an AP-1 probe (20). 2-ME in the concentrations indicated (lanes 1–7) or 50 mM diamide (lane 8) were added prior to the probe. (*C*) EMSA was performed with 5 μg of uninduced Jurkat nuclear extracts and the AP-1 probe without (lane 1) or with 1% 2-ME (lane 2).

capable of binding NF-κB and SRF, respectively (12) (Fig. 3*A*, lane 1). These complexes are specifically competed, respectively, by the IgκB (lane 3) and the IL-2Rα (positions −257 to −240; −257/−240) oligonucleotide, which only contains the CArG box (lane 2). Diamide abolished only NF-κB binding (lane 4). When the IgκB probe was used, as expected, binding was competed by the IgκB oligonucleotide (lane 6 vs. lane 5). Diamide also eliminated binding (lane 7), but this effect was reversed by 2-ME (lane 8). By using the IL-2Rα −257/−240 oligonucleotide, we confirmed that SRF was insensitive to diamide and 2-ME (lanes 11 and 12 vs. lane 1, band B1). Band B1 corresponds to p67 SRF, as demonstrated by the ability of antisera raised against this protein to further retard mobility of this band (data not shown). A second more rapidly migrating band, B2, of unknown identity was sensitive to diamide (lane 11 vs. lane 9). The insensitivity of p67 SRF to oxidation–reduction indicates that diamide and 2-ME are not nonspecifically influencing all DNA–protein binding reactions *in vitro*.

We next evaluated AP-1 binding using a probe containing its consensus binding site (TGACTCA) (20) and HeLa or Jurkat nuclear extracts. In uninduced cell extracts, AP-1 binding activity was barely detectable (Fig. 3 *B*, lane 1, and *C*, lane 1) but treatment of extracts with 2-ME activated binding in a dose-dependent fashion (Fig. 3 *B*, lanes 2–4, and *C*, lane 2) consistent with the results of Abate *et al.* (30). AP-1 binding activity was induced in PMA-treated cells (Fig. 4*B*, lane 5) yet was dramatically increased after 2-ME treatment (lanes 6 and 7). Because 2-ME treatment of extracts from uninduced cells results in much less AP-1 binding (lanes 3 and 4) than 2-ME treatment of PMA-induced cells (lanes 6 and 7), 2-ME alone is not equivalent to PMA induction of AP-1. Similar to its effect on NF-κB binding, diamide abolished AP-1 binding (lane 8), and 2-ME could reverse the diamide effect (data not shown).

DISCUSSION

We have demonstrated that modification of free sulfhydryl(s) can eliminate NF-κB binding *in vitro*. Specifically, we have demonstrated the ability of both alkylating (NEM) and oxidizing (diamide) agents to eliminate binding. We have also shown that NF-κB from the cytosol of uninduced Jurkat cells was present in an inactive form and that 2-ME could increase binding *in vitro* and act synergistically with DOC plus NP-40. Further, we have demonstrated, by using *in vitro* translation products generated from the KBF-1/p50 cDNA that the redox sensitivity of NF-κB binding occurred through its KBF-1/p50 DNA-binding subunit. The presence of oxidized inactive form(s) of NF-κB in uninduced cell cytosolic extracts but not in activated nuclear extracts is consistent with the possibility that NF-κB binding could be physiologically regulated through the modulation of its redox state and that reduction represents a necessary step for full binding activity. Further, the observed synergy between reducing and dissociating agents (Fig. 2) argue that these agents involve distinct molecular mechanisms in the activation of cytosolic NF-κB. Although we cannot determine whether the *in vitro* forms of NF-κB we have isolated accurately reflect the redox state of the protein *in vivo*, it is noteworthy that cytosolic extracts contained minimal to absent NF-κB binding activities whether they were prepared in the absence or presence of DTT, whereas nuclear extracts contained potent binding activity in both cases (refs. 5, 6, and 12 and this report). It is also unclear what role the sulfhydryls might play. They presumably could be important as part of a metal binding domain, for protein–DNA interactions, or protein–protein interactions (either interchain or intrachain). It has been demonstrated that KBF-1/p50 is synthesized as an inactive 105-kDa precursor that is processed to generate the functional molecule (11, 19). It is interesting to speculate that the carboxyl-terminal part that must be removed to generate the active p50 molecule might interfere with critical sulfhydryl(s). Site-directed mutagenesis of the KBF-1/p50 cDNA with expression of wild-type and mutant proteins should allow clarification of this point.

We have also demonstrated that, whereas AP-1 behaves similarly to NF-κB in its sensitivity to redox variations *in vitro*, p67 SRF does not. This observation is particularly interesting in that NF-κB and AP-1 binding are inducible but SRF binding is not. These data for AP-1 are consistent with

those of Abate *et al.* (30) who have demonstrated that oxidation–reduction affects AP-1 binding *in vitro* and identified a critical cysteine residue in the DNA-binding domain that is apparently responsible for these effects.

The control of the redox state has been implicated for the RNA-binding protein IRE-BP (3) and the OxyR DNA-binding protein (31). The IRE-BP, which is involved in the post-transcriptional control of ferritin and transferrin receptor (32), is sensitive to oxidation and requires reduced sulfhydryls for high-affinity binding (3). The OxyR protein (31) of *Salmonella typhimurium* and *Escherichia coli*, when oxidized but not when reduced, can activate transcription of oxidative stress-inducible genes.

For NF-κB, we have not directly addressed the question whether modulation of binding by redox control of the protein is actually involved *in vivo*. There are, however, several lines of evidence consistent with the hypothesis that oxidation–reduction reactions may be used as a general means of cellular signaling and control of cellular metabolism. For example, reducing agents such as 2-ME (33) support the growth of lymphoid cells, whereas depletion of glutathione from T cells inhibits antigen-driven T-cell activation and proliferation (34). Sulfhydryl groups are important in the kinase activity of $p60^{v\text{-}src}$ (35). Further, oxidizing agents such as diamide inhibit microtubule assembly (36). Lastly, investigators have identified a factor denoted ADF that is produced by human T-cell lymphotropic virus I- (37) and Epstein–Barr virus-transformed lymphocytes (38) and by mitogen-stimulated peripheral blood lymphocytes and is homologous to the mammalian thioredoxin known to catalyze thiol reduction reactions (37, 38). ADF can induce IL-2Rα chain expression in some lymphoid cells and it has been hypothesized that ADF influences cellular metabolism by the thiol redox control of regulatory factors. Further studies will be required to determine whether oxidation–reduction is a physiological mechanism for the regulation of NF-κB binding and action and whether it can be used as a means for cellular signaling. In addition, mutagenesis of cysteine residues may provide useful information regarding domains of the protein involved in dimerization and DNA binding.

Note. Since submission of this report, Staal *et al.* (39) have reported that intracellular thiol levels can regulate *in vivo* activation of NF-κB. In contrast, we have directly evaluated effects of oxidation and reduction on NF-κB binding *in vitro*. The data of Staal *et al.* (39) suggest that decreased thiols increase activation of NF-κB whereas our data suggest that oxidation decreases NF-κB binding *in vitro*. Therefore, although both studies are consistent with a potential role for oxidation reduction in the control of NF-κB, they at least superficially disagree in their conclusions. However, because of different methodologies and experimental intent, the studies are not necessarily in conflict. Additional studies are required to elucidate the exact fashion in which NF-κB is influenced by changes in oxidation–reduction.

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