Inhibition of Nuclear Factor-κB DNA Binding by Organoselenocyanates through Covalent Modification of the p50 Subunit


Abstract

Most known chemopreventive agents including certain selenium compounds suppress the activation of the nuclear factor κB (NF-κB), but the mechanisms remain largely elusive. Toward this end, we initially showed that the inhibition of NF-κB DNA binding by benzyl selenocyanate (BSC) and 1,4-phenylenebis(methylene)selenocyanate (p-XSC) was reversed by the addition of DTT; this suggests the formation of DTT-reducible selenium-sulfur bonds between selenocyanate moieties and cysteine residues in NF-κB (p50) protein. Furthermore, the inhibitory effect of selenocyanates on NF-κB was not altered in the presence of physiologic level of reduced glutathione (1 mmol/L), suggesting that selenocyanates can also inhibit NF-κB in vivo. Using both matrix-assisted laser desorption/ionization-time of flight and tandem mass spectrometry fragmentation, we showed for the first time that the Cys62 residue in the active site of NF-κB (p50) protein was modified by BSC through the formation of a selenium-sulfur bond. In addition, p-XSC–bound NF-κB (p50) protein was also detected by a radiotracer method. To provide further support, molecular models of both BSC and p-XSC positioned in the DNA binding pocket of the p50 were constructed through the covalent modification of Cys62 of the p50 subunit of NF-κB. [Cancer Res 2007;67(21):10475–83]

Introduction

Collectively, results from epidemiologic studies, laboratory bioassays, and human clinical intervention trials clearly support the protective role of selenium against cancer development (1–3). Although many different mechanisms, including protection against oxidative damage, altered carcinoma metabolism, enhanced immune surveillance, antiangiogenesis, and induction of apoptosis have been proposed to account for the anticarcinogenic effects of selenium, the inhibition of nuclear factor-κB (NF-κB) by selenium-containing compounds has been reported (4–6). The inhibition of NF-κB by selenium, in particular, is appealing because it could result in apoptosis of tumor cells and thus may, in part, account for the chemopreventive activity of selenium compounds (7–10).

NF-κB, identified by Sen et al. in 1986, is one of the major antiapoptotic transcription factors (11). It regulates more than 150 genes including genes involved in tumor progression, such as inducible nitric oxide synthase, cyclooxygenase-2, and proliferative and antiapoptotic cytokines (12). The activity of NF-κB has been found constitutively elevated in human tumors from either hematologic or solid origin, such as melanomas, breast, prostate, ovarian, pancreatic, colon, and thyroid carcinomas, and it contributes to malignant progression and resistance to therapy in most of the major forms of human cancer (13, 14). Five distinct NF-κB subunits have been identified and cloned in mammalian cells including NF-κB1 (p50/p105), NF-κB2 (p52/p100), RelA (p65), RelB, and c-Rel (12). NF-κB1 and NF-κB2 can undergo proteolysis to liberate p50 and p52, respectively. In the cytoplasm of resting cells, the NF-κB subunits can associate to form either homodimers or heterodimers and remain inactive via the association with inhibitory IκB molecules (Fig. 1; ref. 15). On activation by extracellular stimuli such as growth factors, inflammatory cytokines, bacterial products, chemotherapeutic agents, or oxidative stress, NF-κB complexes can undergo phosphorylation of IκB by specific IκB kinases and proteolytic degradation to yield free NF-κB. This, in turn, enters the nucleus and binds to the κB sites of gene promoters to regulate the specific genes typically involved in immune and inflammatory responses and in cell growth control (16–18). Previous studies have shown that the inhibition of constitutive NF-κB activity blocks the oncogenic potential of neoplastic cells in different ways; by sensitizing tumor cells to chemotherapeutic drug-induced apoptosis, by decreasing the highly proliferative rate which characterizes transformed cells, by inhibiting tissue invasiveness and metastatic potential of highly malignant cells, and by suppression of expression of genes involved in carcinogenesis (19, 20). In addition, specific NF-κB (p50) decoy oligonucleotides that can prevent the binding of NF-κB (p50) to the promoter region of target genes can efficiently promote cell death in cancer cells (21). Therefore, NF-κB is an attractive molecular target for cancer therapy and cancer chemoprevention (22); in fact, most known chemopreventive agents suppress the activation of NF-κB (20).

Synthetic organoselenium compounds such as benzyl selenocyanate (BSC) and 1,4-phenylenebis(methylene)selenocyanate (p-XSC) have been developed in our laboratory (structures are shown in Fig. 1), and these compounds are superior cancer-chemopreventive...
agents than other known selenium compounds, as judged by numerous animal models and cell culture systems (4). p-XSC was recently found to reduce the expression of NF-κB and several target genes regulated by NF-κB, such as cyclooxygenase-2, phospholipase A2, and cyclin D1, in non–small cell lung cancer cells (7). In the human colorectal adenocarcinoma HCT-116 cells, p-XSC reduces the binding activity of NF-κB in an electrophoretic mobility shift assay; the inhibition was reversed in the presence of 1 mmol/L DTT, suggesting that the formation of -Se-S- bonds between p-XSC and essential sulfhydryl groups of NF-κB may account for the observed inhibition (5). NF-κB (p50) monomer contains seven cysteine residues. The binding of NF-κB (p50) to its cognate regulatory element has been shown to depend on the integrity of Cys62, which primarily remains in its reduced state (23). This residue is enveloped in a cationic environment that renders the thiol group highly reactive and particularly susceptible to oxidation. The present investigation was conducted to test our novel hypothesis that the chemopreventive selenocyanate-containing compounds BSC and p-XSC may inhibit tumorigenesis through the inhibition of NF-κB via the formation of -Se-S- bonds with cysteine residues in p50 protein, thereby down-regulating its target genes and leading to the inhibition of cell proliferation and induction of apoptosis.

Materials and Methods

Cell culture and treatment. Squamous carcinoma SCC 1483 cells (donated by Dr. Peter G. Sacks, New York University, New York, NY) were maintained in a 1:1 mixture of DMEM/F12 supplemented with 10% fetal bovine serum (FBS) and penicillin/streptomycin (50 μg/mL; ref. 24). Human non–small cell lung cancer cells (NCI-H460) obtained from the American Type Culture Collection were maintained in RPMI 1640 containing 10% FBS and penicillin/streptomycin (50 μg/mL). Both SCC 1483 and NCI-H460 cells were kept in a humidified environment at 37°C with 5% CO2 grown to 70% to 80% confluency, trypsinized with 0.1% trypsin-2 mmol/L EDTA, and plated for experiment use as described below. BSC and p-XSC were synthesized according to published methods (25, 26). The cells were treated with various concentrations of BSC (6.25–200 μmol/L) or p-XSC (0.625–20 μmol/L) dissolved in DMSO in the cell culture medium for 1 h, followed by stimulation with tumor necrosis factor (TNF)-α (2 ng/mL) for 30 min. Control cultures were treated with DMSO alone (not exceeding 0.1% of volume) and were processed similarly.

Preparation of nuclear extracts from cells. Nuclear proteins were prepared with the TransAM nuclear extract kit according to the manufacturer’s protocol (Active Motif North America). In brief, cells were scraped into PBS containing phosphatase inhibitor, centrifuged to remove the supernatant, and then resuspended with 1× hypotonic buffer and kept on ice for 15 min. After the addition of detergent, lysates were centrifuged at 14,000 × g for 30 s at 4°C. The pellets were resuspended in complete lysis buffer and vortexed vigorously. After incubation on ice for 30 min and centrifugation at 14,000 × g for 10 min at 4°C, supernatants were collected and protein concentration was determined by DC Protein assay (Bio-Rad).

Assay of NF-κB (p50) DNA binding activity. The activity of the p50 subunit of NF-κB was analyzed by a 96-well colorimetric ELISA-based procedure using a trans-AM NF-κB (p50) Transcription Factor Assay Kit (Active Motif North America) according to the manufacturer’s instructions. In brief, pure p50 protein (3 nmol/L, Active Motif North America) was incubated with various concentrations of organoselenium compounds at ambient temperature for 30 min. The incubation mixtures or nuclear extracts from cells were then added into a 96-well plate that had been coated with immobilized oligonucleotide containing a consensus-binding site for NF-κB. After 1 h of incubation with agitation, wells were washed thrice with washing buffer [100 mmol/L PBS (pH 7.5), 500 mmol/L NaCl, and 1% Tween 20] and incubated with p50 antibody (dilution 1:10,000 in antibody binding buffer) for 1 h at room temperature. The wells were washed thrice more with washing buffer before incubation with diluted

Figure 1. Schematic representation of our working hypothesis.
horseradish peroxidase–conjugated antibody (dilution 1:1,000 in antibody binding buffer) for 1 h. Wells were further washed thrice before the addition of 100 μL of developing solution. After 5 min of incubation, the reaction was stopped by the addition of 100-μL stop solution and quantified by measuring the absorbance at 450 nm using a microplate spectrophotometer (SPECTRAmax PLUS384, Molecular Devices).

The absorbance is proportional to the amount of NF-κB (p50) bound to the DNA. In each set of experiments, the total binding that occurred without added BSC or p-XSC was normalized to 100%. Inhibition of NF-κB (p50) binding by BSC and p-XSC was analyzed by plotting the percentage of DNA binding against the concentration of organoselenium compound using Sigmaplot for Windows version 10.0. The inhibition was quantified using the four-parameter Hill equation in which $B$ is the amount of DNA binding; $B_{\text{max}}$, the DNA binding without inhibitor; $B_{\text{min}}$, the amount of DNA binding at infinite inhibitor concentration; $C$, the concentration of inhibitor; $IC_{50}$, the concentration required to achieve a 50% inhibition of DNA binding; and $n$, the Hill slope.

$$B = B_{\text{min}} + \left(\frac{B_{\text{max}} - B_{\text{min}}}{1 + \left(\frac{IC_{50}}{C}\right)^n}\right)$$

Detection of BSC-modified p50 by mass spectrometry. The recombinant NF-κB (p50) protein (2 μmol/L) was incubated with BSC (1.67 mmol/L in DMSO) for 30 min at 37°C. Immediately, the reaction mixture was treated with urea (4 mmol/L) and N-ethylmaleimide (0.13 mmol/L) to avoid the rearrangement reaction with the other cysteine residues that had not been modified, and then incubated for an additional 30 min at 37°C. The resulting solution was dialyzed for 1 h against 20 mmol/L Tris-HCl (pH 7.5) in a Slide-a-Lyzer dialysis cassette (7 kDa molecular mass cutoff); this was followed by digestion with Trypsin Gold (Promega) overnight at 37°C with a protein/trypsin ratio of 10:1. The mixture was separated by gradient elution through a Michrom Magic C18 5-μm 100-Å, 0.1%/C$_2$N$_1$60-mm column, with a flow rate of 900 nL/min, provided by an Eksigent Nanoflow LC system. Separation of peptides was achieved by a 30-min gradient of increasing acetonitrile in 0.1% TFA (buffer A: 1% acetonitrile, 0.1% TFA; buffer B: 90% acetonitrile, 0.1% TFA; 0–1 min, 0% B; 1–2 min, 0% → 5% buffer B; 2–5 min, 5% buffer B; 5–30 min, 5% → 55% buffer B; 30–30.5 min, 50% → 0% buffer B). The nanoflow-LC eluent was mixed with an equal volume of matrix-assisted laser desorption/ionization (MALDI) matrix [2 mg/mL α-cyano-4-hydroxycinnamic acid, 2 mg/mL (NH$_4$)$_2$HPO$_4$, 0.1% TFA, 80% acetonitrile] and spotted every 20 s (300 nL eluant + 300 nL matrix solution) directly onto the MALDI target plate using a Probot spotting robot (LC-Packings/Dionex).

After drying, the samples were then analyzed using an Applied Biosystems 4700 Proteomics Analyzer with TOF/TOF ion optics. Both mass spectrometry (MS) and MS/MS data were acquired with a NdYAG laser with 200 Hz repetition rate, accumulating 1,000 laser shots for each MS spectrum in positive ion reflector mode at a laser attenuation setting of 2,600, and between 2,000 and 5,000 laser shots at a laser attenuation setting of 3,650 for MS/MS spectra (accumulation continued until the signal-to-noise ratio for at least six fragment peaks exceeded S/N 60 or 5,000 laser shots had been fired). Both MS and MS/MS data were acquired using a

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Concentration-dependent effects on NF-κB (p50) DNA binding by synthetic organoselenium compounds: SCC 1483 cells + p-XSC (A), H460 cells + p-XSC (B), SCC 1483 cells + BSC (C), and H460 cells + BSC (D). Points, average of three experiments; bars, SD.
recently updated instrument default calibration. Peak lists were submitted to Mascot version 2.0 using GPS Explorer software (ABI) as the front end for data submission, and then manually interpreted using *in silico* generated theoretical fragmentation data produced with the ABI Ion Fragmentation Calculator software, which is part of the 4000 Series Explorer program suite.

**Detection of p-XSC-bound p50 by radiotracer experiment.** NF-κB (p50; 400 nmol/L) was incubated with 12 μmol/L p-[14C]XSC (0.15 Ci, 100 Ci/mol) in 20 mmol/L Tris-HCl (pH 7.0), 0.2 mol/L NaCl, 2 mmol/L MgCl₂, and 10% glycerol for 20 min at room temperature. The reaction mixture was applied to a Superdex 75 HR 10/30 column (GE Healthcare) and was eluted with 20 mmol/L sodium phosphate (pH 7.5), 150 mmol/L NaCl at 0.5 mL/min. The 1-mL fractions were mixed with 10-mL Picofluor 40 (Perkin-Elmer) and the radioactivity was measured with a liquid scintillation counter.

**Molecular modeling of the modification of p50 by BSC or p-XSC.** Three-dimensional model structures of either BSC or p-XSC adducts were positioned in the DNA binding region of the p50 subunit (residues 59–71). In the case of both adducts, a novel residue consisting of the target cysteine derivatized with the ligand (BSC or p-XSC) of interest by a selenium-sulfur linkage was introduced into the parent structure (PDB code 1NFK; ref. 27). CHARMM parameterization of the novel residue was done by semiempirical quantum mechanics calculations using Parametric Model Number 3 (PM3) as implemented by the program GAMESS (28). The composite derivatized structure was then subjected to 10,000 rounds of energy minimization in the NPT ensemble using the program NAMD (29). Model manipulation and image rendering were done by means of Chimera molecular modeling software (30).

**Results**

**Inhibition of NF-κB (p50) DNA binding activity by p-XSC and BSC.** Although p-XSC was reported to reduce the binding activity of NF-κB in colon cancer cells (HCT-116) using electrophoretic mobility shift assay (5), it is not known whether selenocyanate-containing compounds including p-XSC and BSC could inhibit NF-κB DNA binding in other cancer cells. Therefore, both TNF-α–activated oral squamous cell carcinoma SCC 1483 and non–small cell lung cancer NCI-H460 cells were used to evaluate the inhibitory effects of p-XSC or BSC on NF-κB (p50) protein by ELISA. p-XSC clearly inhibited the NF-κB (p50) DNA binding in both cell lines in a dose-dependent manner (Fig. 2 A and B). In contrast, the binding of NF-κB (p50) protein was enhanced by BSC at lower dose (<25 μmol/L), but at higher doses (≥50 μmol/L) a decrease in binding was observed (Fig. 2 C and D).

Using a recombinant p50 protein, we determined whether the inhibition could be due to direct binding of NF-κB (p50) protein to selenocyanates (Fig. 3 A and B). p-XSC and BSC were found to

![Figure 3](https://example.com/figure3.png)

**Figure 3.** Inhibition of recombinant NF-κB (p50) DNA binding by the synthetic organoselenium compounds p-XSC (A and C) and BSC (B and D). Reactions were carried out without reducing agents (●) or with 5 mmol/L DTT (■). The DTT was added during (A and B) or after (C and D) the incubation of the organoselenium compounds with NF-κB (p50). Points, average of two experiments (C and D) or three experiments (A and B; conducted in the absence of DTT); bars, SD.
inhibit p50 DNA binding in a dose-dependent manner in the absence of a reducing agent (DTT), with IC₅₀ of 0.50 ± 0.03 and 2.20 ± 0.12 μmol/L, respectively. The inhibitory effects were time dependent and reached a maximum between 20 and 40 min by both p-XSC (1 μmol/L) and BSC (20 μmol/L; data not shown). This dose-dependent inhibition was eliminated when DTT (5 mmol/L) was present in the reaction mixture. The addition of DTT 30 min after the incubation of selenocyanates and p50 protein and then incubation for another 20 min attenuated the inhibition (Fig. 3C and D). In addition, the inhibitory effects of p-XSC (1 μmol/L) and BSC (20 μmol/L) were partially reversed in a dose-dependent manner by post-addition of DTT (Fig. 4A and B). Collectively, these results suggest that the formation of -Se-S- bonds is responsible for the inhibition of the DNA binding activities of NF-κB (p50) protein. The inhibitory effects by either p-XSC or BSC were unchanged in the presence or absence of physiologic levels of reduced glutathione (GSH; 1 mmol/L; Fig. 4C and D).

**Detection of BSC-induced modification of p50 by MS.** The fact that the addition of DTT can restore the DNA binding activity of NF-κB (p50) protein after treatment with BSC or p-XSC suggests that covalent interactions with thiol groups of cysteine residues in p50 were involved in the interaction of p50 with selenocyanates. Therefore, we attempted to characterize which residue of p50 was chemically modified by organoselenocyanates. Thus, we selected, for practical purpose of obtaining interpretable mass spectra information, the monofunctional selenocyanate BSC. Figure 5A shows the MALDI-time of flight (TOF) mass spectra of a high-performance liquid chromatography fraction obtained by the tryptic digestion of BSC-modified p50.

A peptide from tryptic digestion of BSC treated p50 mixture with an average mass of 1,944.6 was found to be consistent with the theoretical mass of the YVCEGSHGGLPGASSEK (monoisotopic mass of 1,774.8) peptide bearing a benzylselenyl (Se⁸⁰) moiety; in addition, another peptide with an average mass of 1,942.6 that corresponded to the same peptide bearing a benzylselenyl (Se⁷⁸) moiety is also present (Fig. 5A). Neither m/z values were observed in the trypsin-digested p50 mixture without BSC treatment. The observed mass spectrum isotope cluster pattern is consistent with the isotope distribution pattern of the presence of Se-containing peptides. This peptide (m/z = 1,944.6) was subjected to MS/MS fragmentation to precisely map the cysteine residue that was covalently bound to a benzyl selenyl moiety. The results of tandem mass spectrum support the formation of a selenium-sulfur bond between a benzyl selenyl moiety and Cys⁷⁸ of the peptide (Fig. 5B).

**Figure 4.** A and B, effect of DTT on the inhibition of NF-κB (p50) DNA binding by p-XSC or BSC, respectively. C and D, effect of GSH (1 mmol/L) on the inhibitory effects of p-XSC and BSC on NF-κB (p50) DNA binding, respectively. ■, with 1 mmol/L GSH; ●, without GSH. Points, average of two independent experiments.
Figure 5. Detection of BSC-modified p50 protein by MALDI-TOF and MS/MS fragmentation.
A, MALDI-TOF mass spectrum of BSC-bound peptide corresponding to the peptide YVCEGPHGGGLPGASSEK. B, MS/MS spectrum of the peptide (1,944.6 Da) with BSC-modified Cys62. C, MALDI-TOF mass spectrum of BSC-bound peptide corresponding to the peptide MTEACIR.
consistent with the peptide without a selenium-sulfur–containing (C7H8SeS-) moiety. Fragments in the y ion series (y15, y14, y13, y11, y10, and y7) and b11 and a12 are also in agreement with the formation of a selenium-sulfur bond between a benzyl moiety and Cys62 in the peptide. The tandem mass spectrum is entirely consistent not only with the expected peptide sequence but also with the modification of thiol group of Cys62 by BSC, and provides for the first time a chemical evidence that Cys62 of NF-κB (p50) protein was modified by BSC through the formation of a selenium-sulfur linkage.

Another peptide, present with a mass of 993.3, is also found to be consistent with the theoretical mass of MTEACIR peptide bearing a benzylselenyl (Se80) adduct; in addition, the whole mass cluster is also consistent with the isotope effects of the presence of Se-containing peptides; however, we were unable to obtain satisfactory tandem mass spectrum for unequivocal interpretation.

**Detection of p-XSC–bound p50 by radiotracer experiment.** The potential covalent bond between p50 and p-XSC was probed by incubation of the protein with p-[14C]XSC for 20 min. When the reaction mixture was fractionated by gel filtration, a radioactive peak eluted at the elution volume expected for p50. In two separate experiments, the amount of radioactivity that coeluted with the protein indicated that there are 1.6 p-XSC molecules bound to each p50.

**Molecular modeling of the modification of p50 by BSC or p-XSC.** The thiol group of Cys62 is important in DNA recognition by p50; indeed, single-site substitution of this residue with a serine (Cys62Ser mutant) inhibits DNA binding (31). In the crystal structure of p50, the DNA is in contact with both domains of p50 and 12 of the 18 phosphates are involved in hydrogen bonding interactions with the protein, with the six free phosphates residing at the ends of the DNA. Tyr60 and Cys62 interact with the sugar and phosphate backbones, respectively, consistent with the photocross-linking experiments (32) and the observed sensitivity of DNA binding to the oxidation of Cys62 (Fig. 6 A; ref. 33).

To provide further structural evidence that the organoselenium compounds can react with Cys62 and inhibit DNA binding, molecular models of both ligands (BSC and p-XSC) positioned in the DNA binding pocket of the p50 were constructed through the introduction of a novel derivatized residue. Following a number of rounds of whole molecule energy minimization, both adducts seem to be accommodated within the area of interest with limited perturbation to the surrounding protein structure (Fig. 6B and C). Along with the proposed covalent linkage, both ligands could be stabilized by aromatic moiety (π-cloud)–induced van der Waals forces and electrostatic interactions contributed by other amino acid residues in the DNA binding pocket. Available π-stacking interactions between the aromatic components of each ligand and Tyr60 in the binding pocket can provide either T-shaped or off-center parallel displaced stabilized conformations (34). The DNA binding pocket contains a number of charged amino acids, Glu−, Arg+, and Lys+3+4, all of which may contribute to intermolecular electrostatic interactions. This is particularly the case with Lys44 and selenium substituents of the para-substituted ligand (p-XSC), both of which contribute significant areas of charge to the overall electrostatic potential map.

**Discussions**

In this report, we provide data supporting our novel hypothesis and show for the first time that the chemopreventive selenocya-
p-XSC on p50, suggesting that both BSC and p-XSC might also inhibit p50 in vivo, and this needs to be explored in future studies. Both BSC and p-XSC inhibit DNA binding of NF-κB (p50) protein in TNF-α-stimulated oral cancer SCC 1483 and lung cancer non–small cell lung cancer NCI-H460 cells. Numerous previous studies also showed the inhibition of NF-κB in other cancer cells by selenium-containing compounds, such as p-XSC in human colorectal adenocarcinoma HCT-116 cells (5), methylseleninic acid in fibrosarcoma HT1080 cells (35), and selenite in nuclear extracts from human Jurkat T cells and human lung adenocarcinoma NCI-H441 cells (36).

Previous studies that examined numerous inhibitors of NF-κB showed that inhibition of NF-κB activation can occur at several levels: (a) blockage of the extracellular stimulations; (b) interference with a cytoplasmic step in the NF-κB activation pathway by blockage of a specific component of the cascade; and (c) blockage of the nuclear activity of NF-κB by inhibition of translocation to the nucleus or its binding to DNA (37). Among those, the direct inhibition of NF-κB DNA binding by interfering with the DNA binding region in the NF-κB was suggested to be a more effective approach to design specific inhibitors (38). We showed that BSC and p-XSC inhibited the DNA binding of p50 protein through modifications in the DNA binding region in the NF-κB (p50) in vitro, and this may, in part, account for the mechanisms by which these compounds inhibit DNA binding in cell cultures. At lower doses (≤25 μmol/L), BSC unexpectedly increased the DNA binding of NF-κB (p50) protein in both cell lines. However, enhancement of DNA binding does not necessarily indicate that the DNA binding step is specifically altered (37), but it could instead arise from enhancement of extracellular signals by selenium-containing compounds, differences between cell lines, or interaction with other components involved in the NF-κB activation pathway (39, 40). In fact, we did not rule out the possibility that low level of p-XSC (≤1 μmol/L) may also increase the DNA binding of NF-κB (as seen in Fig. 2A) through selenoproteins to control cellular redox potential, which has a different effect on the NF-κB activity than at higher level where the covalent modification mechanisms take place.

It is important to determine in future studies whether the covalent modification of p50 by selenocyanates is responsible for the inhibition observed in lung and oral cancer cell lines used here.

To precisely map the particular cysteine residue that was modified by selenocyanates, we incubated NF-κB (p50) protein with the monofunctional selenocyanate BSC, digested the mixture with trypsin, and analyzed it by both MALDI-TOF and MS/MS fragmentation. We show for the first time that the Cys62 residue in the active site of NF-κB (p50) protein was modified by BSC through the formation of a -Se-S- bond. The thiol group of Cys62 is critical in the DNA recognition by p50 subunit (31, 41–44) and is the only cysteine, among the seven cysteines present in p50, located in the DNA recognition region (44). The formation of -Se-S- bond may be due to an S2 reaction by a nucleophilic attack of the thiol group of Cys62 at the electrophilic selenium moiety of the selenocyanate with displacement of the cyano group; however, an S1 reaction mechanism cannot be excluded.

Using a radiotracer method, we showed that p-XSC also binds to p50 in vitro. To further provide additional support of the modification of p50 protein by selenocyanates, we used a computer modeling approach to examine the potential effect of the binding of both BSC and p-XSC at Cys62 on DNA binding activity. This approach revealed that the modification of p50 protein by BSC or p-XSC could hinder its DNA substrate to enter its DNA binding region. Our results provide structural evidence showing that BSC and p-XSC exert chemopreventive or therapeutic activity, at least in part, through inhibition of the DNA binding of NF-κB (p50) protein by covalent modification of Cys62. This computer-based model may be used as a tool in the design of more effective cancer-chemopreventive selenium compounds.

The rational strategy for the development of chemopreventive or therapeutic agents to inhibit DNA binding of p50 protein has been described (45, 46). In oral squamous cell carcinoma and nasopharyngeal carcinoma, the dominant NF-κB dimer is the p50/p50 homodimer, which was found to be highly expressed in all malignant oral squamous cell carcinoma tissues but only at a moderate level in precancerous lesions. Because the composition of NF-κB regulates transcriptional specificity, a p50/p50 homodimer is likely to mediate NF-κB activity to provide a better survival and proliferative advantage to the tumor cells than the p50/p65 form in oral carcinogenesis. In addition, deletion of the NF-κB p50 subunit results in the inhibition of tumorigenesis in mice given the peroxisome proliferators ciprofibrate and Wy-14,643 (47). Therefore, inhibition of NF-κB (p50) DNA binding may represent an innovative chemopreventive strategy (48, 49). Our results provide a plausible approach to develop more effective chemopreventive agents based on their binding efficiency to NF-κB.

Acknowledgments

Received 7/5/2007; revised 7/26/2007; accepted 8/3/2007.
Grant support: NCI grants R01-CA100924 and P01-CA70972.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
We thank Anne Stanley of the Proteomics/Mass Spectrometry Core Facility, and the Organic Synthesis Facility of the Section of Research Resources, Penn State College of Medicine. We also thank Dr. Peter G. Sacks for providing us SCC 1483 cells.

References

8. McCarty MF, Block AK. Preadministration of high-dose salicylates, suppressors of NF-κB activation, may increase the chemosensitivity of many cancers: an example of proapoptotic signal modulation therapy. Integr Cancer Ther 2006;5:325–28.

Cancer Res 2007; 67: (21). November 1, 2007 10482 www.aacrjournals.org

Downloaded from cancerres.aacrjournals.org on April 29, 2021. © 2007 American Association for Cancer Research.
Modification of p50 by Organoselenium Compounds


Inhibition of Nuclear Factor-κB DNA Binding by Organoselenocyanates through Covalent Modification of the p50 Subunit

Kun-Ming Chen, Thomas E. Spratt, Bruce A. Stanley, et al.


Updated version Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/67/21/10475

Cited articles This article cites 49 articles, 14 of which you can access for free at:
http://cancerres.aacrjournals.org/content/67/21/10475.full#ref-list-1

Citing articles This article has been cited by 4 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/67/21/10475.full#related-urls

E-mail alerts Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, use this link
http://cancerres.aacrjournals.org/content/67/21/10475.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.