Regulation of Human Apolipoprotein A-I Gene Expression by Gramoxone*

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To induce oxidative stress, HepG2 cells were exposed to a compound known as gramoxone. This compound undergoes a one-electron reduction to form a stable free radical which is capable of generating reactive oxygen species. We demonstrated that exposure of HepG2 cells to gramoxone (0.1 mM) resulted in a 2-fold decrease in apoA-I mRNA with no significant change in apolipoprotein AI; ARE, antioxidant response element; GST, glutathione S-transferase Ya subunit (21) and the rat glutathione S-transferase (GST) Ya subunit (21) and the

Epidemiological studies have demonstrated that lowering low density lipoprotein-cholesterol (LDL-C)1 or raising high density lipoprotein-cholesterol (HDL-C) reduces cardiovascular risk (1). However, among myocardial infarction survivors, greater than one-half have normal lipid levels, suggesting that factors other than lipoprotein profiles contribute to the disease process (2). One such factor appears likely to be the oxidation of LDL (3–5). Oxidized LDL has been implicated in the formation of foam cells and thus may play an important role in the etiology of atherosclerosis (6, 7). In contrast, oxidized HDL is not avidly taken up by macrophages, does not lead to foam cell formation (8), and may actually inhibit endothelial cell-mediated LDL modification (9, 10). HDL is also capable of protecting against LDL peroxidation in vitro (8, 10, 11). Recently, the antioxidative activity of HDL has been demonstrated in vivo (12). These properties suggest another protective role for HDL (in addition to its involvement in "reverse cholesterol transport") in reducing atherosclerotic risk.

Reduced levels of plasma HDL are observed in cigarette smokers (13, 14). However, the mechanisms responsible for the decrease are not known. During cigarette smoking, the oxidation of polycyclic aromatic hydrocarbons produces free radicals (15). The presence of quinone and hydroquinone complexes in the particulate phase of cigarette smoke can result in generation of reactive species such as superoxides and hydrogen peroxide. If a metal catalyst is present, hydroxyl radicals will also form. Consequently, the smoker has a higher free radical burden and a lower HDL level than the nonsmoker and it has been suggested that this may contribute to the smoker’s higher risk of developing atherosclerosis (16).

In this study, we determined whether oxidative stress could affect the synthesis of apoA-I, the major protein constituent of HDL. To induce oxidative stress, the human hepatoma cell line, HepG2, was exposed to gramoxone (also called paraquat and methyl viologen). This compound is a quaternary dipyridyl that is not metabolized but undergoes a one-electron reduction to form a stable free radical (17, 18). Redox cycling of the free radical decreases the levels of reducing equivalents in the cell and it is the critical biomedical event in gramoxone toxicity (19). Gramoxone has no other known mechanism of cytotoxicity (20).

The present study demonstrates that exposure of HepG2 cells to gramoxone resulted in a 2-fold reduction of apoA-I mRNA, with no significant effect on apolipoprotein AI; ARE, antioxidant response element; GST, glutathione S-transferase; HDL, high density lipoprotein; XRE, xenobiotic response element.

1 The abbreviations used are: LDL, low density lipoprotein; apoA-I, apolipoprotein AI; ARE, antioxidant response element; GST, glutathione S-transferase; HDL, high density lipoprotein; XRE, xenobiotic response element.
NAD(P)H quinine reductase genes (22). The match involves the motif, 5'-puGTGACNNNGC-3' (where pu is a purine and N is any nucleotide), corresponding to a putative antioxidant response element (ARE) (21, 22). One copy of the element with one mismatched nucleotide is present in the apoA-I promoter between nucleotides −142 and −132 relative to the transcription start site of the gene. Transient transfection studies demonstrate that the putative ARE is necessary for gramozone-mediated induction of human apoA-I gene expression. By performing gel mobility shift experiments, we found that exposure of HepG2 cells to gramozone resulted in increased binding of nuclear proteins to the ARE. UV cross-linking experiments identified two polypeptides of approximately 100 and 115 kDa. Taken together, the data suggest that the mechanism(s) by which gramozone affects apoA-I mRNA levels occur(s) at both transcriptional and post-transcriptional levels.

EXPERIMENTAL PROCEDURES

Materials—Complementary pairs of oligonucleotides were synthesized using the Beckman Oligo 1000 DNA Synthesizer according to the manufacturer’s instructions. The following oligonucleotides (and their complementary strands) were used: 5'-GAGCGGACAGCTG-3'; mutated ARE, 5'-CAGCCCACTTGTAGTATG-3'; GST-ARE, 5'-CTAATGGTGACAAACG-3'; xenobiotic response element (XRE), 5'-AGTGTGCCTACAGCTG-3'.

Cell Culture and Drug Treatments—The human hepatoma cell line, HepG2, was obtained from the American Type Culture Collection (Rockville, MD). Cells were grown in T75 flasks containing 20 ml of Eagle’s minimal essential medium supplemented with 10% fetal bovine serum as described previously (23). Freshly confluent monolayers were washed twice with minimal essential medium and then incubated with fresh medium for 0–8 h in the absence or presence of gramozone, dissolved in phosphate-buffered saline, ranging from 0.1 μM to 10 μM. In some experiments, gramozone and cycloheximide were added to cells to give final concentrations of 0.1 μM and 10 μg/ml, respectively. Whole cells, gramozone and cycloheximide were added to cells to give final concentrations of 0.1 μM and 10 μg/ml, respectively. Where noted, HepG2 cells were also treated with or without gramozone and in the presence of actinomycin D (1 μg/ml), for various time periods as described in the figure legends. Cell viability was routinely monitored by trypan blue exclusion and lactate dehydrogenase leakage as described previously (24). In all experiments the number of dead cells never exceeded 5% of the total number of cells.

RNA Isolation and Detection—Total RNA was isolated using the acid guanidinium thiocyanate-phenol-chloroform extraction method described by Chomczynski and Sacchi (25). RNA detection and quantitation were determined by slot blot analyses. For slot blots, multiple RNA samples (0.5–5.0 μg) from cells cultured under a variety of conditions were denatured with formaldehyde and applied to wells of a slot blot apparatus (Bio-Rad) onto Zeta-Probe GT membranes. Blots were prehybridized in a solution of 5× SSPE, 5× Denhardt’s, 0.1% SDS, and apoE cDNA probes as described previously (26). Detection of catalase mRNA levels was essentially under the same conditions as described for apolipoprotein mRNA (26). The catalase cDNA probe was obtained from the American Type Tissue Culture Collection (ATCC). All results were normalized using densitometric analyses of slot blots probed with radiolabeled oligo(dT) to correct for loading variations.

Nuclear Run-off Transcription Assay and Preparation of Nuclear Extracts—Nuclei were prepared according to the procedure of Bar-talena and co-workers (27). An in vitro nuclear run-off transcription assay was carried out as described (27) with minor modifications (24). Procedures for nuclear run-off transcription assays and preparation of nuclear extracts from HepG2 cells have been described in detail previously (28).

Bandshift Assays—For bandshift assays, nuclear extracts (1.0 μg) were incubated with 100 μg of poly(dI-dC) in binding buffer containing 5 mM dithiothreitol and 5 mM ZnCl₂ on ice for 30 min. Then 2 fmol (10,000 cpm) of 5'-end labeled synthetic oligonucleotides corresponding to either the apoAI-ARE or GST-ARE were added to the reaction mixtures and incubated on ice for another 30 min. Procedures for bandshift assays have been described in detail previously (29). Competition assays were performed by adding the unlabeled competitor DNA 15 min prior to the addition of either labeled apoAI-ARE or GST-ARE as indicated in the figure legends.

Ultraviolet Irradiation Cross-linking Experiments—Ultraviolet (UV) cross-linking experiments were carried out as described by Wu et al. (29) with minor modifications. Briefly, the binding reactions were first carried out as described above for bandshift assays, except that the reaction was scaled up 25-fold. The binding reactions were irradiated on ice for 30 min with a 254-nm wavelength ultraviolet source (Stratalinker). Equal amounts of 2 × SDS sample buffer were added to the irradiated reactions. The samples were then heated at 90 °C for 10 min and thereafter processed on a radiolonomat/SDS denaturing gel by the method of Laemmli (30). The gel was dried and autoradiographed.

Preparation of Luciferase Constructs—Two GeneLight™ vectors (Promega, Life Technologies) were used: pGL2-Basic (pGL2-B) and pGL2-Promoter (pGL2-P). A 491-base pair DNA fragment of the human apoA-I promoter between nucleotides −491 to +1 was generated by polymerase chain reaction amplification described in detail previously (28). The sequence of this DNA fragment was confirmed by DNA sequencing. The fragment was inserted into the XhoI site of the pGL2-Basic vector, upstream of the luciferase gene. This plasmid is hereafter referred to as pGL2(apoAI-491-luc). Plasmid pGL2(apoAI-250-luc) was constructed by releasing a DNA fragment (−491 to −251 of the apoA-I promoter) from pGL2(apoAI-491-luc) using Smal I. The vector was gel purified and re-ligated.

Plasmid pGL2/apoAI-250 mutant ARE/luc was prepared by a polymerase chain reaction-based protocol as described by Morrison and Desrosiers (31). To generate this mutated ARE plasmid, two sets of primers were used. One set of primers, designated GL and LUC, was hybridized to specific regions of the plasmid pGL2. The other set of primers which covered the nucleotides −491 to −250 was used with the primers FOR and REV. The primers GL (5'-GTATCTATGATGTACTGATG-3') and REV (5'-GATCATACACTCAATGGGGCTGGG-3') were complementary to the noncoding strand of DNA while primers FOR (5'-CCCATTTAGGTTGATGACCG-3') and LUC (5'-GGGCGTCTACATTACC-3') were complementary to the coding strand of DNA. The plasmid pGL2/apoAI-250/luc was used as the polymerase chain reaction template. Amplification was carried out as described previously (28). The DNA fragment generated was then digested with Smal I and HindIII and the resulting DNA fragment was purified from an agarose gel. The purified DNA fragment was cloned into the Smal I and HindIII sites of the pGL2-Basic (Promega Inc.) and used to transform competent Escherichia coli cells. DNA was prepared from individual clones by the alkaline lysis miniprep procedure and the entire DNA insert including the area of mutagenesis was sequenced using the sequencing version 2.0 system (U. S. Biochemical Corp.).

A series of pGL2-P vectors containing apoAI-ARE, GST-ARE, and mutated ARE were also constructed. pGL2-P was digested with the restriction enzymes KpnI and Nhel I. The restriction digestion was electro-phoresed and the digested plasmid was gel purified. Synthetic oli-gonucleotides corresponding to the putative ARE from the apoAI promoter (apoAI-ARE), the consensus GST-ARE, and mutated ARE were inserted individually into the KpnI and Nhel I of the linearized pGL2-P vectors. All synthetic oligomers contained a 5'-KpnI and a 3'-NhelI site to facilitate unidirectional cloning into the pGL2-P vector. The sequence of all pGL2-P constructs was confirmed by DNA sequencing.

Transfection and Luciferase Assay—The human hepatoma cell line, HepG2, was maintained as monolayers on 100-mm plates in minimal Eagle’s minimal essential medium supplemented with 10% fetal bovine serum. Transient DNA transfections were performed by the calcium phosphate precipitation procedure described by Gorman and co-workers (32) and detailed previously (28). The cells were then cultured in the absence or presence of gramozone (0.1 μM) ranging from 0 to 8 h.

To determine the effect of gramozone on liver-specific transcription factors the HepG2 cells were harvested by washing three times in phosphate-buffered saline and assayed for luciferase activity as described in the Luciferase Assay Kit Technical Manual (Promega, Inc.) (33). This assay has also been described in detail previously (28). In all transfections, 5 μg of an internal control plasmid (pSGαlacZ) containing the E. coli Lac Z gene under the control of the SV40 early promoter and enhancer, was included to correct for differences in transfection and enhancing efficiency. Transfected cells were harvested as described (28) and β-galactosidase activities in the cell lysates determined (28). The pGL2-promoter vector which contains a SV40 promoter is used as a reference for both transfection and luciferase assays. All luciferase activities are reported as mean ± S.E. Significance of group differences was determined by Student’s t test, using two-tailed p values.

RESULTS

Modulation of Steady State Apolipoprotein mRNA Levels by Gramoxone—The effects of gramozone on levels of apolipoprotein mRNA were examined by slot blot analysis using the level of total poly(A)+ RNA determined by oligo(dT) hybridization to control for variation in RNA loading. Exposure of HepG2 cells
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Fig. 1. Kinetic studies of apoA-I, apoB, and apoE mRNA levels in response to gramoxone. Newly confluent HepG2 cells were grown in media containing 10% fetal bovine serum. Cells were then cultured in the presence of gramoxone (0.1 μM) for various time periods. Total RNA was isolated at 0, 1, 2, 4, 6, and 8 h. The levels of apoA-I, apoB, and apoE mRNAs were determined by slot blot analysis as described under “Experimental Procedures.” All results were normalized using densitometric analyses of slot blots probed with radiolabeled oligo(dT) to correct for loading variations. Steady state levels of apoA-I, apoB, and apoE mRNAs from untreated cells were arbitrarily set at 100%. Results are mean ± S.E. of four experiments.

Analysis of Gramoxone Temporal Response Profiles—Time course studies of the effect of gramoxone on apoA-I mRNA levels in HepG2 cells were performed to determine whether or not the decline in steady state levels of apoA-I mRNA could be seen earlier than 8 h. After 2 h of exposure to gramoxone, apoA-I mRNA levels decreased to 50% of control values and this decrease was maintained for the duration of the remaining 6 h (Fig. 1). No significant differences in apoB and apoE mRNA levels were observed when HepG2 cells were cultured in the presence of gramoxone over the 8-h period. To ensure that the cells were under oxidative stress, the levels of catalase mRNA were also determined. Exposure of HepG2 cells to gramoxone resulted in a 4- and 10-fold increase in steady state levels of catalase mRNA at 6 and 8 h, respectively. This induction could be suppressed by simultaneously exposing the cells to 1% (v/v) dimethyl sulfoxide, a free radical scavenger (data not shown).

Analysis of the Effect of Gramoxone on Transcription of the ApoA-I Gene and Half-life of ApoA-I mRNA—The 2-fold decrease in apoA-I mRNA levels could be the result of either a decrease in rates of transcription or an increased degradation of the apoA-I mRNA. Therefore, we measured the transcription rate of the apoA-I gene using isolated nuclei from HepG2 cells cultured in the presence of gramoxone for 0–8 h. Nuclear run-off assays indicated that the rate of transcription of apoA-I gene increased approximately 2-fold between 4 and 8 h after gramoxone treatment (Fig. 2).

To determine if the rate of apoA-I mRNA degradation also changed in response to gramoxone treatment, the turnover of apoA-I mRNA was determined in the presence of actinomycin D (1 μg/ml) with and without gramoxone. The half-lives obtained for apoA-I mRNAs following the addition of actinomycin D in the absence or presence of gramoxone were approximately 12.5 ± 1.5 and 3.0 ± 0.4 h, respectively (Fig. 3). Thus, there was a 4-fold increase in the rate of degradation of apoA-I mRNA when HepG2 cells were subjected to oxidative stress.

Transient Transfection Studies—To further investigate the increase in transcription detected by nuclear run-off assays, transient transfection experiments were carried out using a series of pGL2-derived luciferase reporter plasmids. As shown in Fig. 4, both pGL2(apoAI-491)luc and pGL2(apoAI-250)luc constructs showed a significant 2-fold induction of luciferase activity in HepG2 cells cultured in the presence of gramoxone for 8 h. We hypothesized that the 2-fold increase in apoA-I promoter activity could involve a potential ARE detected by sequence comparison with the GST Ya subunit and NADP(H) quinone reductase genes. To test this hypothesis, we constructed the pGL2(apoAI-250 mutant ARE) in which the entire ARE consensus sequence was replaced (G → T, C → A, T → G, A → C). Results from these studies demonstrated that pGL2-(apoAI-250 mutant ARE) showed no increase in luciferase activity in response to gramoxone treatment. To confirm that the ARE was able to confer responsiveness to gramoxone, transient transfection experiments were carried out using a series of pGL2-P/luc constructs. The control vector pGL2-P/luc demonstrated no change in luciferase activity in response to gramoxone treatment. Plasmids with one copy of the ARE derived from the apoA-I or GST promoters pGL2(apoAI-ARE)/luc and pGL2-
(GST-ARE)/luc inserted upstream of the SV40 promoter, displayed a 4-fold increase in luciferase expression relative to the control vector. This suggests that the ARE may enhance the basal rate of transcription of the reporter gene. Furthermore, the luciferase activity of pGL2/apoAI-ARE/luc and pGL2/GST-ARE/luc was increased by an additional 2-fold in the presence of gramoxone. However, a plasmid containing one copy of the mutant apoAI-ARE, pGL2P/apoAI-mutant ARE/luc, had a basal rate of expression similar to the control vector and displayed no responsiveness to gramoxone.

**Protein-DNA Interaction at the ARE of the Human ApoA-I Proximal Promoter—** Mobility shift experiments were performed to further examine the mechanism by which the ARE confers responsiveness to gramoxone. A double-stranded oligonucleotide (apoAI-ARE), corresponding to the apoA-I promoter between nucleotides −149 and −130, was end-labeled with 32P and analyzed for its ability to bind to nuclear proteins isolated from HepG2 cells cultured in the presence and absence of gramoxone. As shown in Fig. 5A, a retardation complex was detected with extracts prepared from control HepG2 cells. After 1 h of gramoxone treatment, the levels of this complex increased 2-fold. This induction was not blocked by inhibiting protein synthesis with cycloheximide (data not shown). In nuclear extracts from gramoxone-treated HepG2 cells, binding to the labeled apoAI-ARE was efficiently blocked by competition with 50–100-fold molar excess of unlabeled apoAI-ARE and to a lesser extent with unlabeled GST-ARE. No competition was observed with up to 100-fold molar excess of the XRE or a mutant ARE oligomer.

In contrast, when mobility shift assays were carried out using the GST-ARE as a probe, similar levels of binding activity were observed using nuclear extracts isolated from control and gramoxone-treated HepG2 cells (Fig. 5B). Binding to the labeled GST-ARE probe was also effectively inhibited by competition with 50–100-fold molar excess of unlabeled GST-ARE. However, unlabeled apoAI-ARE was not able to block the formation of the protein-DNA complex efficiently. Both negative control oligomers (XRE and mutant ARE) showed no competi-

**Relative Luciferase Activity**

|          | Control | Gramoxone |
|----------|---------|-----------|
| pGL2(apoAI-491)luc: | 2.3±0.1 | 4.5±0.2 * |
| pGL2(apoAI-250)luc: | 2.4±0.1 | 4.8±0.1 * |
| pGL2(apoAI-250-mutant ARE)luc: | 2.1±0.1 | 2.2±0.2 |
| pGL2(apoAI-ARE)SV40/luc: | 3.8±0.2 | 7.9±0.2 ** |
| pGL2(apoAI-mutant-ARE)SV40/luc: | 1.1±0.1 | 1.2±0.2 |
| pGL2(GST-ARE)SV40/luc: | 4.0±0.2 | 8.2±0.2 ** |
| pGL2-Promoter Vector: | 1.0±0.1 | 1.0±0.1 |
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Identification of the Trans-acting Factor by UV Cross-linking Experiments—UV cross-linking studies indicated binding of two polypeptides of apparent molecular masses of approximately 115 and 100 kDa to the apoAI-ARE in both untreated and gramoxone-treated nuclear extracts (Fig. 6A), with the 100-kDa species being the predominant one bound in both extracts. Densitometry demonstrated that gramoxone treatment increased in labeling of the 115- and 100-kDa polypeptides by approximately 71 and approximately 105%, respectively. Neither polypeptide was detected when binding reactions were supplemented with a 100-fold molar excess of unlabeled apoAI-ARE (Fig. 6B).

UV cross-linking experiments were also carried out by using labeled GST-ARE as a probe to determine whether or not the proteins with similar apparent molecular masses were bound. As shown in Fig. 6C, four polypeptides of approximately 21, 28, 57, and 98 kDa were labeled using gramoxone-treated nuclear extracts and a GST-ARE. Similar results were also observed when control nuclear extracts were utilized (data not shown). Densitometric analyses indicated that the 57-kDa protein was the predominant species bound, followed by the 21-, 98-, and 28-kDa protein bands. Binding of these four polypeptides to the labeled GST-ARE probe was efficiently blocked by competition with a 100-fold molar excess of unlabeled GST-ARE.

Discussion

It has been reported that hyperoxic conditions increase steady state mRNA levels of catalase, Cu/Zn-superoxide dismutase, and glutathione peroxidase in human endothelial cells (34, 35). In HepG2 cells, we found that oxidative stress induced by treatment with gramoxone resulted in a 10-fold increase in steady state levels of catalase mRNA and a 2-fold increase in levels of Cu/Zn-superoxide dismutase mRNA (data not shown). Consistent with the proposed mechanism of action of gramoxone, this response was eliminated by the addition of the free radical scavenger, Me₃SO (34). In contrast, gramoxone treatment decreased the steady state levels of apoA-I mRNA 2-fold without affecting the levels of mRNAs for other major apolipoproteins such as apoB and apoE. This down-regulation of apoAI mRNA in response to oxidant treatment suggests that decreased apoA-I synthesis may contribute to the observed reduction in plasma HDL seen in cigarette smokers (13–16).

Our studies demonstrate for the first time that oxidative stress may act by selectively decreasing hepatic apoA-I mRNA levels. Although the molecular mechanisms by which gramoxone modulates apoA-I are not completely understood, we have provided evidence that the 2-fold reduction in apoA-I mRNA level results from a combination of a 4-fold increase in apoA-I mRNA degradation and a 2-fold increase in apoA-I gene transcription. This apparently paradoxical effect on mRNA synthesis and stability is not without precedent. For instance, apoA-I gene transcription decreases during chronic hyperthyroidism while the hepatic abundance of apoA-I increases 3-fold by a mechanism that involves stabilization and/or more efficient processing of the nuclear apoA-I mRNA precursors (36, 37). This observation has prompted the suggestion that apoA-I gene transcription may be subject to feedback regulation and that degradation of nuclear apoA-I RNA could have a positive effect on apoA-I gene transcription (36, 37). While the suggestion remains a hypothesis, the data presented here are also consistent with the possibility that the increase in transcription is a compensatory response to the decrease in apoA-I mRNA levels. In addition to the perturbations of thyroid hormone status, dietary cholesterol and saturated fat can also affect apoA-I gene expression at both transcriptional and post-transcriptional levels (38–42).

In addition to the proposed autoregulation of apoA-I transcription described above, apoA-I gene expression could be directly responsive to oxidative stress since the apoA-I promoter does contain a potential ARE, although the sequence differs by one nucleotide from the putative ARE (21, 22). Bandshift assays demonstrate that both untreated and gramoxone-treated HepG2 nuclear extracts contain factors that bind specifically to the ARE and these factors can be induced by gramoxone treatment. The increase in protein-DNA complex formation was apparent within 1 h of gramoxone exposure and was not blocked by inhibiting protein synthesis, suggesting that the increased binding activity was attributable to modification of a pre-existing factor. UV cross-linking experiments identified two proteins with apparent molecular masses of approximately 100 and 115 kDa (Fig. 6A). Although present in control HepG2 nuclear extracts, gramoxone treatment resulted in an increase in binding of both proteins. Our data differ from a report by Nguyen and Pickett (43) which indicates that proteins UV cross-linked to the GST-ARE have apparent molecular masses of approximately 28 and 45 kDa and that the DNA binding activity of these proteins are not increased by t-butylhydroquinone treatment in HepG2 cells. To examine this discrepancy, we carried out UV cross-linking experiments using labeled GST-ARE as a probe together with either control or gramoxone-treated nuclear extracts. Our results demonstrated...
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![Image](https://example.com/image.png)

**Fig. 6.** UV cross-linking analysis of the apoAI-ARE and GST-ARE binding nuclear proteins. Double-stranded apoAI-ARE and GST-ARE were labeled with [32P]-P and used in binding reactions under the conditions of the gel mobility shift assays. After UV-irradiation for 30 min, equal amounts of 2 × SDS sample buffer were added to the reactions, heated, and applied to a 8% SDS-polyacrylamide gel. UV cross-linking experiments were carried out using labeled apoAI-ARE as a probe (panels A and B). – and + represent nuclear extracts isolated from untreated or gramoxone-treated HepG2 cells, respectively (panel A). In panels B and C, only nuclear extracts isolated from gramoxone-treated HepG2 cells were utilized. Competition studies were performed using [32P]-labeled apoAI-ARE (panel B) and GST-ARE (panel C) together with 100-fold molar excesses of unlabeled apoAI-ARE and GST-ARE, respectively. α, β, γ, and δ correspond to apparent molecular masses of approximately 98, 57, 28, and 21 kDa, respectively (panel C).

In summary, we have identified gramozone-inducible nuclear proteins which bind specifically to the ARE region of the human apoAI gene. These protein-DNA interactions appear likely to be involved in the mechanism by which oxidant or antioxidant-inducible trans-acting nuclear factors modulate apoAI gene transcription. Taken together, our data demonstrate that gramozone affects hepatic apoAI mRNA abundance by both transcriptional and post-transcriptional mechanisms.

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