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Inhibition of sequence independent DNA-binding activity of human recombinant HMGB1 protein with natural triterpenes

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Abstract

DNA-protein interactions regulate pivotal intracellular processes and thus provide an important target for drug discovery efforts. Small molecules remain ineffective inhibitors of DNA binding because of the lack of adequate high-throughput screening procedures, and the paucity of lead molecules for drug design. High Mobility Group protein B1 (HMGB1) is a nuclear DNA-binding protein with no known sequence specificity or enzymatic activity, which plays an important role in chemotherapy-induced apoptosis, tissue regeneration, and inflammation. The natural triterpene glycyrrhizin (GLA) has been reported to be a modest inhibitor of DNA-HMGB1 binding interactions, though the mechanism of inhibition remained unclear. In the present work, we investigated inhibitory effects of GLA and its derivatives (carbenoxolone (CGA) and potassium salt of glycyrrhetinate (GAK)) on DNA-binding activity of HMGB1. Using a newly developed capillary electrophoresis mobility shift assay, we characterized binding of synthetic DNA duplexes with recombinant human HMGB1. Dynamic light scattering and fluorometric experiments demonstrated that the inhibitory effects of these triterpenes on DNA binding depended on their ability to form supramolecular aggregates. GLA and GAK demonstrated inhibitory effect on DNA-HMGB1 binding at concentrations exceeding CMC (100-150μM). GLA inhibited DNA-HMGB1 binding with IC_{50} = 500 μM; 600 μM GAK showed 100% inhibition of DNA-HMGB1 binding. In the presence of GLA, human lung carcinoma cells demonstrated more than 300-fold increased viability when treated with chemotherapeutic drug cytarabine, consistent with the GLA inhibitory activity. The results of our study demonstrated that molecules capable of forming stable aggregates are potential modulators of DNA-protein binding and open avenues to the development of novel classes of physiologically active compounds.

Keywords: DNA-binding proteins, HMGB1, Glycyrrhizin, inhibitors, supramolecular aggregates.

INTRODUCTION

DNA-protein interactions play a critical role in all aspects of genetic activity including transcription, packaging, rearrangement, replication and repair [1]. The ability to modulate these vital interactions is an important area of drug discovery research. DNA-protein interactions are known to include DNA sequence-specific interactions as well as non-sequence specific interactions. Cell permeable small molecule inhibitors of DNA-protein interactions offer a promising strategy for controlling gene expression [2]. However, the design of small molecule inhibitors has presented a number of challenges to medicinal chemists. A major challenge has been the lack of lead molecules on which to base drug design. The paucity of lead compounds has resulted in various approaches that target both the binding proteins and the DNA molecules. For example, synthetic double stranded oligodeoxy nucleotides have been used as decoys to sequester sequence-specific DNA binding proteins. Another approach uses single stranded oligonucleotides (ON), referred to as TFOs (triplex forming oligonucleotides) to target sequence specific sites on duplex DNA [3]. The cyclic peptide echinomycin was identified as an inhibitor of hypoxia-inducible factor-1 binding to DNA [4] while another cyclic peptide was shown to inhibit DNA/AP-1 interactions. A lead hopping strategy based on the cyclic peptide inhibitor of DNA-AP-1 interactions was used to identify non-peptidic small molecule inhibitors. This approach led to non-peptidic inhibitors that were less effective than the starting peptide [5].

Based on the reports described above, attempts to develop peptidic and non-peptidic inhibitors of sequence-specific DNA-protein interactions have met with limited success. Efforts to develop inhibitors of non-sequence specific DNA-protein interactions have been even less fruitful. One such protein, high mobility group protein B1 (HMGB1) is an important DNA binding nuclear protein with no sequence specificity. It is an important therapeutic target that plays an important role in biochemical processes including chemotherapy-induced apoptosis, tissue regeneration, and inflammation. Because antibodies against HMGB1 reverse sepsis and have anti-inflammatory effects, HMGB1 modulation has been suggested as a potential therapeutic strategy for the management of inflammation. Glycyrrhizin (GLA), a natural glycoconjugated triterpene has been identified as an inhibitor of HMGB1 binding to single stranded DNA. GLA was demonstrated to have inhibitory effects on the phosphorylation and DNA-binding properties of HMGB1 at micromolar concentrations [6]. Nuclear magnetic resonance (NMR) and fluorescence studies of HMGB1/ GLA interactions suggest that GLA binds HMGB1 with modest affinity (K_d=156±3 μM), and identified amino acid residues within the HMGB1 polypeptide chain involved in protein-inhibitor interactions [7]. High concentrations have been used to study its effect on the replication of human immunodeficiency virus type 1(HIV-1) in MOLT-4 lymphoblast cells. At high concentrations in the millimolar range GLA was shown to partially inhibit the binding of radiolabeled HIV-1 particles to MT-4 cells[8].

The need for relatively high concentrations of GLA to produce significant biological activity in these assays prompted us to explore the mechanism of the observed inhibitory properties of GLA derivatives. To this end, an *in vitro* screening system was developed based on a quantitative capillary electrophoresis mobility shift assay (CEMSA). This system was used to evaluate DNA-HMGB1 binding in the presence of GLA and two GLA derivatives. The data provided in these experiments indicate that GLA and GAK inhibit DNA-protein interactions at high concentrations suggesting that the inhibition observed in our experiments and reported by others occurs via

interaction with supramolecular aggregates rather than individual triterpene molecules. These preliminary results suggest that molecules capable of forming stable supramolecular aggregates may provide a novel approach for the design of inhibitors of DNA-protein interactions. The approach may be especially useful for targeting non-sequence specific DNA-protein interactions.

MATERIALS AND METHODS

***Reagents*.** GLA (glyzyrrhizic acid ammonium salt, GLA), carbenoxolone disodium (CGA), 18β-glycyrrhetinic acid, 1,6-diphenyl-1,3,5-hexatriene (DPH), 3-(N-morpholino) propane sulfonic acid (MOPS), triethylamine were purchased from Sigma Aldrich (St. Louis, MO,USA). Potassium salt of glycyrrhetinic acid (GAK) was prepared from 18β-glycyrrhetinic acid (an aglycone of GLA): briefly, one g of 18β-glycyrrhetinic acid was suspended in 3 ml water and neutralized with one equivalent of KOH; pH of the solution was controlled with pH meter. Solution was applied on a 12 ml Megabond Elute C18 cartridge (Varian, Palo Alto, CA), pre-activated with 2 ml of deionized water. The column was washed with 4 ml water, and potassium salt of glycyrrhetinic acid was eluted with 2 ml of methanol. Methanol was removed using the rotary evaporator, and the composition of the resulting compound was confirmed by the elemental analysis (within ± 0.4% of the theoretical values). Yield: 79%.

HMGB1 preparation Cloning, Expression, and Purification of HMGB1. cDNA coding for human HMGB1 was generated by reverse transcription and PCR of total mRNA extracted from NALM6 cells using primers GCGCCATGGGCAAAGGAGATCCTAAG and GCGCTCGAGTTATTCATCATCATCATCTTCTTC. The PCR product was cloned in pET28a plasmid (Novagen, NJ) and confirmed by sequencing. Human HMGB1 was expressed in *E. coli* strain BL21(DE3)pLysS (Novagen, NJ), per manufacturer's instructions. Purification of human recombinant HMGB1 was performed using a Mono S HR 5/5 column (GE Healthcare, Piscataway, NJ). Concentrated fractions were dialyzed against buffer F (100 mM HEPES pH 7.9; 100 mM NaCl; 0.5 mM EDTA; 5% glycerol; 5 mM DTT), and concentrated using Centricon 10 (Millipore, Billerica, MA). Glycerol was added to the protein solution to the final concentration 20%; protein was stored at (-20^{o}C). Western blot analysis was used to confirm the immunogenic properties of the recombinant purified protein, as described [9].

DNA duplexes preparation. All 34-mer oligodeoxyribonucleotides with non-natural insert 5-fluoro-2'-deoxyuridine, or containing fluorescein coupled to the 5'-end of all-natural oligonucleotide, were synthesized and purified by TriLink (San Diego, CA). Sequences of the oligodeoxyribonucleotide duplexes are shown in Table 1. Duplexes were prepared by annealing complementary single strands as previously described [10] in buffer A (20 mM Tris-HCl pH 8.0; 5mM $MgCl_2$), or in buffer B (50mM MOPS-triethylamine, pH 7.5; 50mM KCl). DNA duplexes were stored at 4^{o}C.

***Capillary electrophoresis mobility shift (CEMSA) assay*.** CEMSA experiments were performed using a GE1600AX instrument (Agilent Technologies, Santa Clara, CA) equipped with a diode array detector similar to experiments described in Etzkorn et al. [11]. Bare fused silica capillaries (75 μm i.d. x 38.5 cm in length; 30 cm effective length) were purchased from Agilent. Briefly, samples were injected by hydrodynamic injection at 50 mbar for 50 sec, and separated at 25 kV with negative polarity for 30 min. The temperature in the capillary was set to 15^{o}C. Composition

of the analyte was monitored by UV absorbance at 260 and 492 nm using the high sensitivity detection cell. The separation electrolyte was 75 mM MOPS-triethylamine (pH 7.5) filtered through a 0.2 μm filter before use. Samples (40μl) contained 1 μM DNA duplex, or a mixture of 1 μM DNA duplex and 4 μM recombinant HMGB1, or a mixture of 1 μM DNA duplex; 4 μM recombinant HMGB1 and varying concentrations of inhibitors in binding buffer A or B.

Sample mixtures were prepared at 0^{o}C and incubated in buffer A or buffer B at 30^{o}C for 30 min prior to injection. The capillary was washed daily before the series of analyses for 30 min with 0.1 M NaOH, followed by a 15 min wash with de-ionized water and 15 min with running buffer. Before each analysis, the capillary was rinsed for 2 min with 0.1 M NaOH and 2 min with run buffer as described previously [12]. Peak areas were integrated by HP ChemStation software (Agilent Technologies, Santa Clara, CA) and used for quantification of DNA-protein complexes.

Fluorimetric evaluation of critical micelle concentration (CMC). Fluorescence measurements were performed with a SpectraMax M2 fluorescence spectrophotometer (Molecular Devices, Sunnyvale, CA), with the excitation wavelength set at 358 nm and the emission wavelength at 430 nm [13]. All measurements were performed in 1-cm path length quartz cuvettes. For CMC determination, 1μl of 10 mM 1,6-diphenyl-1,3,5-hexatriene (DPH) in THF was added to 2 ml of 0.039-1mM solution of GLA, CGA, or GAK in water; in Buffer A or in buffer B. Before each measurement, the mixtures were incubated for 30 min in the dark at room temperature.

Dynamic Light Scattering. Inhibitors (10mM stock solutions of GLA, CGA and GAK in water) were diluted with filtered buffer A or buffer B. Samples were analyzed on a Malvern Zetasizer Nano (Malvern Instruments Limited, U.K) equipped with a 4mW He-Ne laser at 633 nm. The laser power and integration times were kept constant for all experiments. All measurements were carried out in triplicate with a detector angle of 90^{o} at a temperature of 25^{o}C.

Cell viability in the presence of inhibitors. Human non-small lung carcinoma cells A549 were obtained from the ATCC collection (ATCC, Manassas, VA). A549 cells were maintained in Ham's F12K medium at 40-80% confluency. Cells were treated with 2-10 mM GLA dissolved in F12K medium, and cytarabine (araC) was prepared in water as 1000X stock solution; drug concentration was determined spectrophotometrically (araC, ε_{272}=9,259). For the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium) assay (CellTiter 96 cell proliferation kit, Promega, WI), A549 cells (250 cells per well) were plated into 96-well plates, and cultured for 3 days in 0-50 μM araC. After incubation, MTT reagent was added to each well and endpoint data collected by a SpectraMax M2 microplate spectrophotometer (Molecular Device, CA) according to the manufacturer's instructions.

Statistical analysis The IC_{50} values were calculated using GraphPad Prism (GraphPad Software Inc., CA) by fitting a sigmoid E_{max} model to the cell viability *versus* drug concentration data, as determined in triplicate from three independent experiments. Peak areas of DNA-duplexes and DNA-HMGB1 complexes were integrated by HP ChemStation software (Agilent Technologies, Santa Clara, CA). The statistical analyses were carried out using Student's t test with Statistical software v7.1 (StatSoft, Inc., Tulson, OK).

RESULTS

Capillary electrophoresis mobility shift (CEMSA) assay

To evaluate the inhibitory properties of GLA and derivatives on DNA-protein interactions, we developed a semi-quantitative CEMSA assay. In our experiments, we used human recombinant HMGB1 protein and synthetic DNA duplexes (containing natural and non-natural nucleosides) as binding substrates for HMGB1. The duplexes were prepared from two chemically modified oligodeoxyribonucleotide 34-mers (Table 1). One strand of the duplex contained a non-natural nucleoside (deoxyfluorouridine). The complementary strand contained only natural nucleosides, and was coupled to fluorescein residue at 5'-end. These two strands were annealed and formed a 34-bp substrate FU-DNA for HMGB1 binding with characteristic absorption at 492 nm. We also prepared a control duplex containing only natural nucleosides [14], but this duplex (dsDNA) had low affinity for HMGB1 binding (data not shown); therefore, FU-DNA duplex was used for further experiments.

Table 1. Structure of DNA duplexes used as probes in CEMSA analysis of DNA-protein interactions

##	Sequence of duplex	Abbreviation	length	*λ max*, nm
1	TGAGAACGGAAATTCCTTTCATAGATTTACGAAG **Z**- ACTCTTGCCTTTAAGGAAAGTATCTAAATGCTTC	dsDNA	34	*260/492*
2	TGAGAACGGAAATTCC**U**TTCATAGATTTACGAAG **Z**- ACTCTTGCCTTTAAGGAAAGTATCTAAATGCTTC	FU-DNA	34	*260/492*

Z - fluorescein isothiocyanate (FITC) chromophore group coupled to the 5'-end ; **U** – 5FdU

Incubation of FU-DNA duplex with recombinant human HMGB1 resulted in the formation of a DNA-protein complex, which was detected by CEMSA at 492 nm (Fig. 1). The protocol for CEMSA was similar to the one used for studying AP-1/DNA binding affinity free in solution [11].Two peaks with different migration times were detected; the formation of these complexes was time-dependent; after 4 hr only a peak with retention time 15 min was detected (Figure 1, middle panel). These peaks contained the DNA duplex, as evidenced by absorption at 492 nm, and were formed after addition of HMGB1. Control experiments with other proteins (BSA, GAPDH, HSPA8, and PDIA3) did not show formation of any additional peaks.

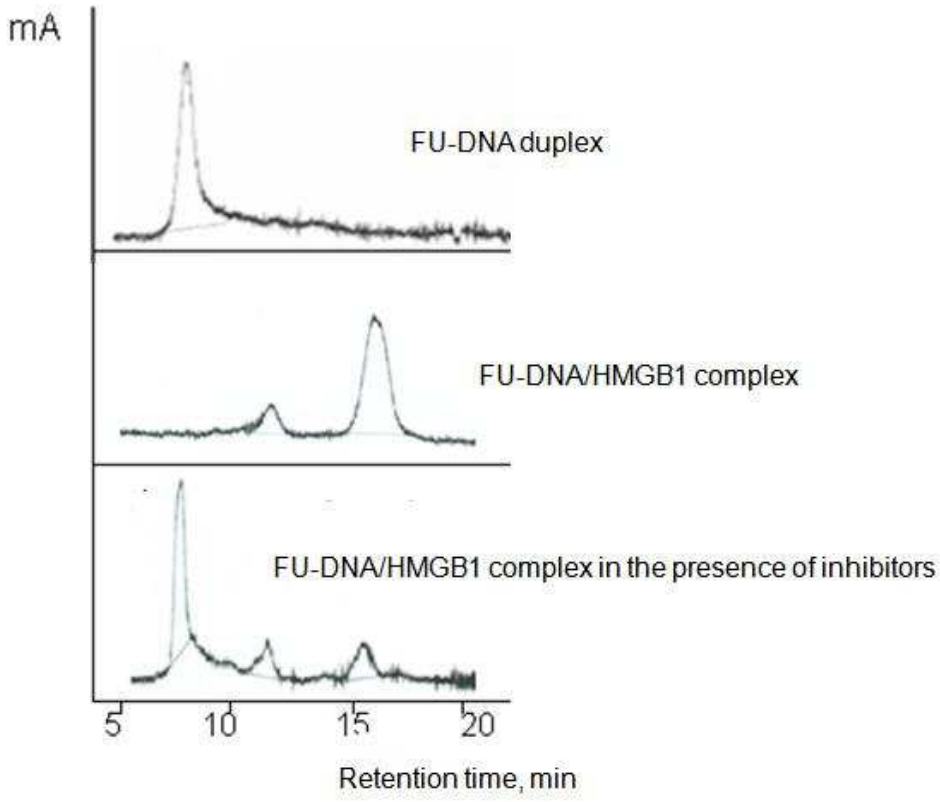

Fig. 1. FU-DNA/HMGB1 complex formation detected by capillary electrophoresis mobility shift assay (CEMSA). CEMSA profiles of separation of reaction mixture containing 1 µM FU-DNA duplex (upper panel), FU-DNA and HMGB1 after 30min incubation with and without inhibitors. CEMSA separation was performed in 75 mM MOPS-triethylamine buffer, pH 7.5.

Inhibition of HMGB1-DNA binding by GLA and derivatives.
The newly developed semi-quantitative CEMSA method was used to evaluate the inhibitory properties of GLA (GLA) and two triterpene derivatives, carbenoxolone (CGA), and glycyrrhetinic acid (GA) (Fig. 2) on HMGB1 binding to FU-DNA.

Increasing concentrations of inhibitors were added to the reaction mixture containing 1µM FU-DNA and 4µM HMGB1; the mixture was incubated for 30-180 min, and then analyzed by CEMSA. The results of the inhibition experiments are shown in (Fig. 3A, B). These experiments were performed under two different conditions: incubation was performed either in Tris-HCl-based buffer A, or MOPS-triethylamine-based buffer B.

Of the three compounds tested, GLA was the most potent inhibitor of DNA-protein interaction in buffer A, with the IC_{50}=493±50 µM. Under the same conditions, CGA and GAK showed no inhibitory effect. In buffer B, a notable inhibitory effect was observed for GAK at concentration exceeding 300 µM; no inhibitory effect was detected in the presence of GLA or CGA.

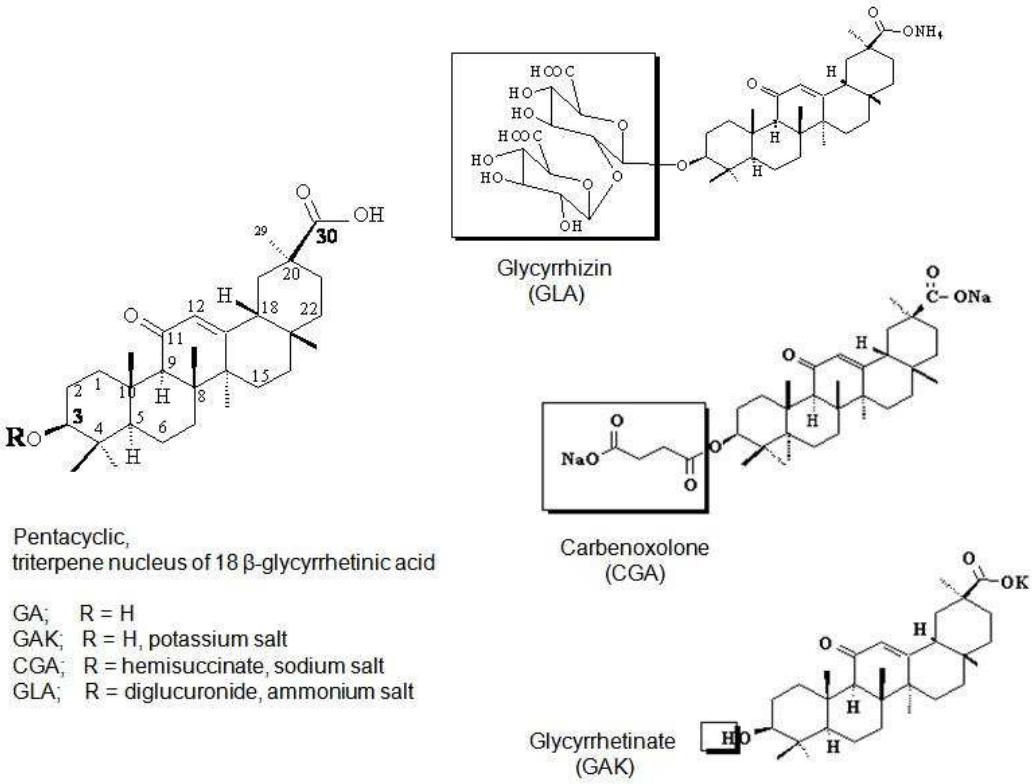

Fig. 2. Chemical structures of GLA (ammonium salt, GLA), carbenoxolone (sodium salt, CGA), and 18β-glycyrrhetinate (potassium salt, GAK).

Micelle formation by GLA and derivatives

The inhibition of DNA-protein interactions observed for GLA derivatives in the experiments described above were not consistent with concentration dependent inhibition by these molecules. In order to investigate aggregate formation as a possible mechanism for the observed inhibition, a fluorometric method was used to determine the critical micelle concentration for the test compounds. Fluorescence studies using DPH as a probe were used to determine the critical micelle concentrations (CMC) as a function of concentration in water as well as in buffer A & B. GLA showed an increase in fluorescence intensity in water as well as in buffer A at concentrations above 300 µM, revealing the formation of micelles/aggregates. Under the same conditions, CGA and GAK showed no increase in fluorescence indicating no molecular aggregation. Similar experiments performed in buffer B demonstrated that only GAK produced an increase in fluorescence (consistent with micelles/aggregate formation) at concentration above 300 µM. GLA and CGA did not show an increase in fluorescence, these compounds did not form aggregates in buffer B.

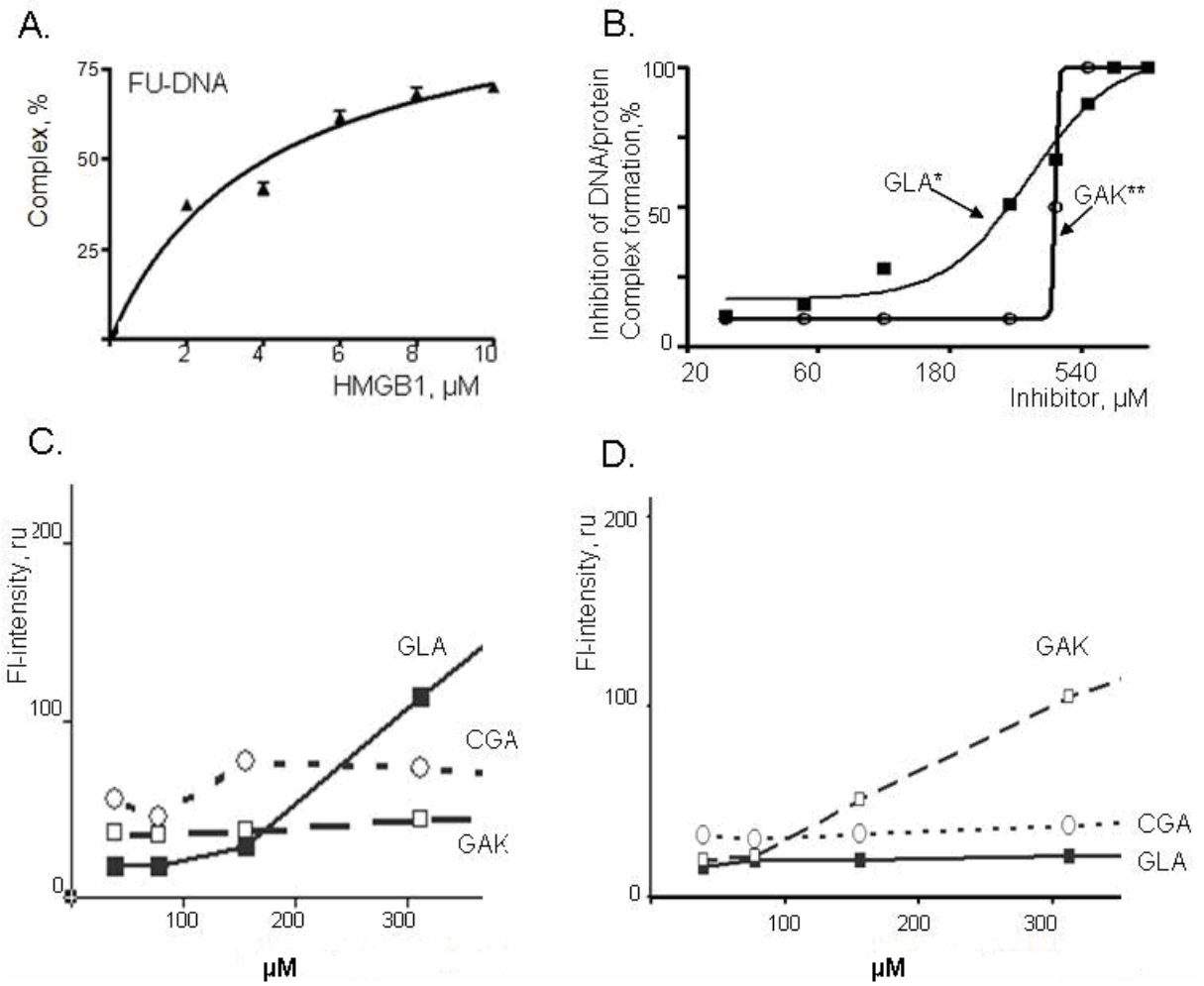

Fig. 3. GLA derivatives inhibit DNA-protein complex formation by formation of supramolecular aggregates. **Panel A:** Accumulation of FU-DNA-HMGB1 complex in buffer A in the absence of inhibitors as function of increasing HMGB1 concentration. **Panel B:** Inhibition of DNA-HMGB1 complex formation with GLA and GAK as a function of increasing concentration of inhibitors under different conditions: GLA*-buffer A; GAK**-buffer B. **Panels C and D:** CMC evaluation of three GLA derivatives (GLA, CGA and GAK) by fluorescent method with DPH as a probe, in water (panel C) and buffer B (MOPS-triethylamine buffer, pH 7.5; panel D).

Further investigation into possible aggregate formation of test compounds involved dynamic light scattering (DLS) experiments of the compounds at various concentrations in water, buffer A and buffer B. DLS analysis of GLA (125-1000 μM) in water revealed the formation of particles with sizes in the submicron range, with strong scattering intensity and a well defined autocorrelation function decaying over the 10-1000 μs time scale (Fig.4A). In buffer A GLA showed strong scattering intensity with autocorrelation function decaying over the 1000-100000 μs time scale.

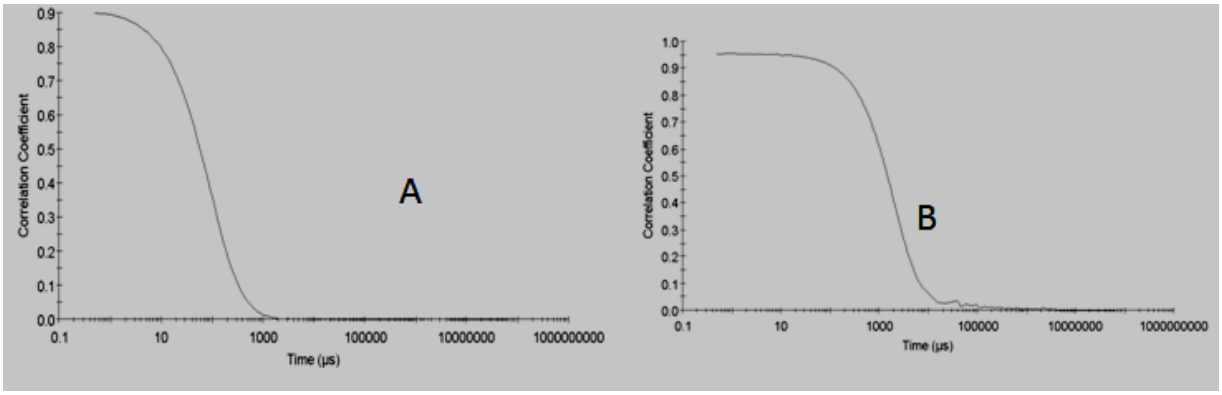

Fig. 4. Representative autocorrelation function of GLA in water (A) and GAK in buffer B (B)

DLS analysis of CGA and GAK in water yielded a low amplitude autocorrelation function that lacked a well-defined decay suggesting the absence of particles in solution. These results were consistent with the fluorescence emission data for these compounds. In buffer B, GAK showed high intensity scatter and an autocorrelation function that decayed over the 1000-100000 µs time scale suggesting the presence of particles in the 1 µm range (Fig. 4B). DLS data for GLA and CGA indicate that no supramolecular aggregates were formed in buffer B.

GLA increases cell viability of carcinoma cells A549 treated with anticancer drug araC.
Cytotoxicity experiments with human lung cancer cells were performed to test the physiological effect of GLA on DNA-protein interactions. Human lung carcinoma A549 cells proficient in p53-dependent DNA damage response pathway were selected as a model system for analysis of cell viability after araC treatment in the presence of GLA. To assess the chemosensitivity of these cells, we used the MTT assay indicative of cytotoxic and cytostatic effects [15].

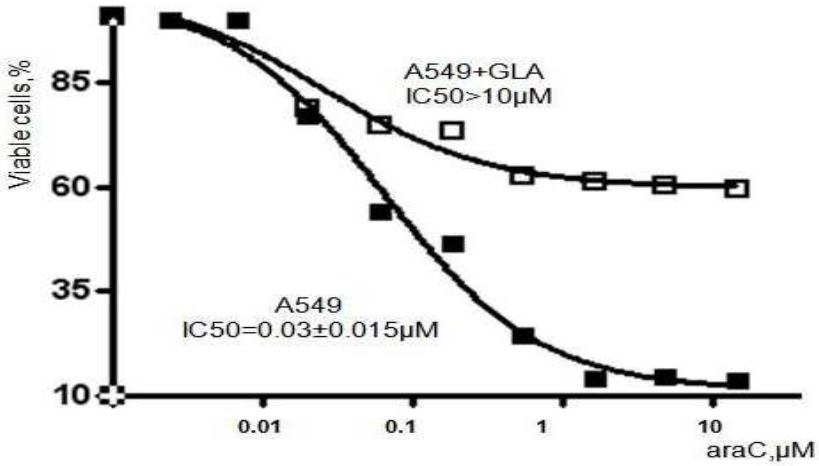

Fig. 5. GLA increases chemoresistance to araC treatment in A549 cells: Y axis indicates the percent of viable cells in the absence (■) or presence (□) of 10 mM GLA dissolved in the medium. Evaluation was performed by MTT assay as described in Materials and Methods.

For genotoxic insult, we used cytosine arabinoside, a drug with well-defined cytotoxicity in A549 cells [9]. After a 48 hr treatment with 0-50 μM araC, we observed more than a 300-fold increase in cell viability in the presence of 10 mM GLA, compared to control cells without GLA

DISCUSSION

The limited number of agents capable of modulating the DNA binding activity hampers the development of novel agents targeted against DNA-protein interactions. Because of their intrinsic physiological activity, natural products represent a valuable, still underused source of lead compounds for modulating complex biological processes [16]. The pioneering work of Sakamoto and co-authors described GLA as an inhibitor of 27-base long single stranded DNA [6]. This work was further extended by Bianchi and collaborators who demonstrated a weak inhibitory effect of GLA on DNA binding functions of HMGB1 [7]. By using fluorescence recovery after photobleaching analysis these authors also demonstrated that GLA reduced the DNA binding by HMGB1 in live cells. Our previous data demonstrated that HMGB1 functions as a component of the DNA damage sensor participating in the early steps of cellular response to DNA damage [9, 17]; in the absence of HMGB1, cells manifest increased viability when treated with anti-cancer drugs.

The focus of the present study was to evaluate the inhibitory properties of compounds which share the pentacyclic triterpene nucleus found in 18β-glycyrrhetinic acid (GA), with respect to HMGB1-DNA binding. A semi-quantitative CEMSA protocol was developed to screen the effects of GLA and derivatives on DNA-protein interactions. Incubation of FU-DNA duplexes with recombinant human HMGB1 resulted in the formation of a DNA-protein complex, which was detected by CEMSA at 492 nm (Fig. 2A). We hypothesize that formation of two DNA-protein complexes with distinct stoichiometry occurred under conditions of our experiment. Formation of two DNA/HMGB1 complexes in CEMSA is consistent with the electrophoretic mobility shift pattern observed in conventional EMSA experiments in the presence of increasing concentrations of recombinant HMGB1 (data not shown).

We evaluated the inhibitory properties of three derivatives: GAK (R = H; potassium salt); CGA (R = succinate monoester; disodium salt); and GLA (R = diglucuronide; ammonium salt). The three test compounds differed in the substituents present at the 3-position of the triterpene scaffold. These structural changes have been reported to result in different physicochemical properties for these molecules and may lead to distinct biological activities [18-19]. In order to examine the ability of these compounds to function as inhibitors of DNA-protein interactions, FU-DNA duplexes were incubated with recombinant HMGB1 in the presence of increasing concentrations of the test inhibitors. The accumulation of FU-DNA-HMGB1 complexes with increasing concentration of HMGB1 was analyzed using the CEMSA assay as described above (Fig. 3A). The results of these experiments demonstrated the inhibitory effect of GLA on DNA binding in buffer A (IC_{50}=493±50uM). Similar experiments with GAK demonstrated a strong inhibitory effect in buffer B at concentrations exceeding 300 μM while no inhibition was observed in the presence of CGA.Two-fold increase of GAK concentration (300-600μM) resulted in a sharp increase (from 0 to 100%) of GAK inhibitory effect.

This abrupt achievement of inhibitory effect of GLA and GAK after a modest increase of concentration suggested a change in the physicochemical properties of the compounds in the solution. Because GLA is a surfactant capable of forming particles (micelles), further experiments were performed to evaluate the ability of the test compounds to form micelles/aggregates. Our fluorescence experiments with GLA, CGA, and GAK in buffers A and B indicated the formation of supramolecular aggregates by GLA in water and buffer A, and by GAK in buffer B (Fig. 3, C-D). Importantly, the CMC values for GLA and GAK determined in fluorescence experiments were consistent with the concentrations that produced the inhibitory effects of these compounds. In parallel with these data, dynamic light scattering experiments demonstrated that inhibitory effects of GLA were prominent in buffer A where molecular aggregates were formed. In contrast, in buffer B GLA neither formed aggregates nor revealed inhibitory properties. On the other hand, GAK demonstrated the ability to form aggregates in buffer B, and inhibited DNA-protein interactions under these conditions. No micelles/aggregates, or inhibitory effects were observed for CGA in the solutions tested. Based on these results, we conclude that the inhibitory properties of GLA and derivatives can be attributed to the aggregates of these compounds, rather than to individual molecules.

To test the physiological effect of GLA on DNA-protein interactions, we performed cytotoxicity experiments with human lung cancer cells. HMGB1 is a component of several DNA repair pathways, and inactivation of HMGB1 in live cells via gene knockdown or RNA interference resulted in increased chemoresistance to anticancer drug araC [9, 17]. In line with our previous findings, an increase in the viability of A549 cells treated with araC was detected in the presence of high concentrations of GLA (1-10mM, Fig. 5). No change in araC cytotoxicity was found in the presence of low concentrations of GLA.

Taken together, these data indicate that triterpene derivatives capable of forming aggregates inhibit the DNA-binding properties of HMGB1. Our findings suggest that compounds capable of maintaining their supramolecular structure at low concentration provide a promising direction for the future development of inhibitors of DNA-protein interactions. Molecular modification of these pentacyclic triterpene derivatives may provide lead compounds that form stable micelles at lower concentrations. Such modifications could include changing the steric bulk, lipophilicity, and/or the polarity of specific triterpene molecules. Our data also suggest that it may be practical to estimate the CMC of newly synthesized inhibitors of DNA-protein interactions.

CONCLUSION

A novel strategy is proposed for inhibiting DNA-proteins interactions with HMGB1. A fast, semi-quantitative method was developed to test DNA-HMGB1 binding, and to evaluate inhibitors of DNA-protein interactions. Using this approach, we evaluated the inhibitory properties of GLA derivatives. GLA and GAK inhibit the DNA-binding activity of HMGB1 under conditions favoring aggregate formation. Compounds with increased micelle stability may represent a novel approach for the development of inhibitors of DNA-protein interactions, and provide useful tools to investigate the role of these proteins in a variety of critical pathways. The inhibitors of DNA-HMGB1 binding may be useful modulators of cancer chemotherapy, or protect healthy cells from toxic chemotherapeutic regimens.

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