Histone H3 and Heat Shock Protein GRP78 Are Selectively Cross-Linked to DNA by Photoactivated Gilvocarcin V in Human Fibroblasts

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ABSTRACT

Gilvocarcin V (GV) is an antitumor antibiotic with a coumarin-based aromatic structure that promotes protein-DNA cross-linking when photoactivated by near-UV light. We have now identified several proteins that are selectively cross-linked to DNA in human fibroblasts by photoactivated GV, using NH2-terminal amino acid sequencing and Western blot analysis of the purified cross-linked proteins. The selectively cross-linked proteins are histone H3 and GRP78, a heat shock protein belonging to the heat shock protein-70 family. The hydrophobic leader sequence is missing from the cross-linked GRP78, suggesting that only the processed form of heat shock protein-70 is cross-linked to DNA. It is primarily the phosphorylated form of histone H3 that is cross-linked to DNA. Gel retardation analysis from four different GV-treated human fibroblast cell lines revealed two distinct shifted bands, and subsequent immunoblotting confirmed in situ that the slower and the faster bands, respectively, contained GRP78 and histone H3 cross-linked to DNA. The selective cross-linking of these particular proteins is dependent on UV irradiation in the presence of GV, which may help to clarify the unique molecular mechanism of this potent antitumor agent.

INTRODUCTION

Thegilvocarcins were originally isolated from Streptomyces gilvotanalen fermentation medium (1, 2), and they harbor a common chromophore composed of four fused planar rings, including a coumarin backbone (Fig. 1). The antitumor and virus-killing efficiency of gilvocarcins depend on the alkyl group in the C-8 position (3–5). It has been shown that gilvocarcin with a vinyl-group in this position, gilvocarcins depend on the alkyl group in the C-8 position (3–5). It has been shown that gilvocarcin with a vinyl-group in this position, GV,3 has enhanced biological activity (5–7). Biological activities such as cell killing, prophage induction, mutagenesis, and DNA strand break formation in both bacteria (6, 7) and mammalian cells (9, 10) require photoactivation of the gilvocarcin by UVA, UVB, or visible light (6, 7, 9). Very low concentrations of photoactivated GV are biologically active, and the cellular target for GV includes DNA (11, 12). A covalent photoduct of GV to DNA has been isolated in vitro and identified as a thymine-GV adduct (13, 14). We have previously used a modified Southern blot analysis to detect interstrand DNA cross-linking (15) in specific DNA sequences. We found that the GV lesions represented DNA-protein cross-linking rather than interstrand DNA cross-linking in several genes studied (16).

The objective of the present study was to identify the specific protein(s) cross-linked to DNA by photoactivated GV. Identification of these proteins may help to clarify the molecular mechanism of this potent antitumor antibiotic.

MATERIALS AND METHODS

Cell Culture and Photoactivated GV Treatment. Four normal human diploid fibroblast cell lines were used: (a) GM637 and GM38 (Human Genetic Mutant Cell Depository, Camden, NJ); and (b) NHDF and NHLF (Takara, Osaka, Japan). Cells were grown at 37°C in modified Eagle’s essential medium supplemented with 10% fetal bovine serum at 37°C in a humidified atmosphere containing 5% CO2. The culture medium was changed twice weekly. Preparative GV was dissolved in DMSO and stored at −20°C. Monolayer cells growing in 82-mm culture dishes were washed twice with cold PBS (2.68 mM KCl, 1.47 mM KH2PO4, 8.06 mM Na2HPO4·7H2O, and 0.137 mM NaCl). The cells were then treated with 0.3 μg/ml GV in 2 ml of PBS. After a 5-min equilibration in the dark at 4°C, a portion of the cell culture was irradiated with fluorescent “black light” (HP15L; ATTO, Kusatsu, Japan) at 1.0 kJ/m2/min for 15 min; control cells were covered with a light shield and were not irradiated. The cells were then washed three times with cold PBS and lysed for DNA preparation.

Preparation and Separation of Proteins Cross-Linked to DNA and NH2-Terminal Amino Acid Sequencing. Proteins cross-linked to DNA were prepared using potassium-SDS precipitation, which uses the difference between SDS and potassium in protein precipitation properties (17, 18). Cells were scraped with a rubber policeman, washed three times in ice-cold PBS, and then pelleted and resuspended in ice-cold PBS to a concentration of 5 × 106 cells/ml. After centrifugation at 3000 × g for 10 min, approximately 2 × 106 cells were lysed in 25 ml of 20 mM Tris·HCl (pH 7.5), 2% SDS, and 1 mM phenylmethanesulfonyl fluoride. The lysed sample was vortexed for 20 s at 4°C and then pelleted and resuspended in ice-cold PBS to a concentration of 5 × 107 cells/ml. After centrifugation at 3000 × g for 10 min at 4°C, the cell pellet was dispersed and suspended in 5 ml of 20 mM Tris·HCl (pH 7.5) and 100 mM KCl by pipetting five times. The sample was heated for 15 min at 65°C, chilled on ice, and centrifuged at 3000 × g for 10 min, followed by repetition of the washing step three times. The resulting pellet, which is enriched with proteins associated with DNA, was resuspended in 5 ml of sterile water and then used for the following two analyses. Because the pellet contained nonspecific proteins precipitated by SDS, it was dissolved in 20 mM Tris·HCl (pH 7.5)/50 mM NaCl and then immunoprecipitated with an antihuman double-strand DNA antibody using protein A-Sepharose beads. The immunoprecipitate was suspended in 20 mM Tris·HCl (pH 7.5), kept at 65°C for 20 min, and then passed through a protein A-Sepharose affinity column to remove the antibody. The obtained eluant was condensed using a lyophilizer and then mixed with an equal amount of the SDS sample buffer of Tris-glycine SDS-PAGE (19). Separation and precise calibration of low molecular weight proteins were conducted using the Tris-tricine gel system SDS-PAGE (20). Marker proteins for calibration were as follows: (a) phosphorylase b, 94 kDa; (b) albumin, 67 kDa; (c) ovalbumin, 43 kDa; (d) carbonic anhydrase B, 30 kDa; (e) trypsin inhibitor, 20.1 kDa; (f) a-lactalbumin, 14.4 kDa; (g) myoglobin I and III, 10.7 kDa; and (h) myoglobin I, 0.82 kDa (Amersham Pharmacia, Buckinghamshire, United Kingdom). Separated proteins were detected by Coomassie Brilliant Blue R-250 (Bio-Rad) staining.

In the scaled-up experiment for amino acid sequencing, it was sometimes difficult to resuspend the pellet due to an excess amount of SDS and potassium salts. To remove these, the suspension was dialyzed overnight against 1 liter of sterile distilled water. This reduced the amount of residual undissolved material and also removed nonspecific SDS-protein precipitate. The final precipitate was dissolved in 10 mM Tris·Cl (pH 7.5) and 1 mM EDTA and applied to a limit filtration column (Amersham Pharmacia) to trap proteins with molecular masses larger than 100 kDa. The same volume of SDS sample buffer used in the Laemmli gel system was added to the final pass-through fraction of the

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3 The abbreviations used are: GV, gilvocarcin V; HSP, heat shock protein. 

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above-mentioned column, boiled for 5 min, and applied to a 10% SDS-PAGE gel. After electrophoresis at 15 mA constant current for 4 h, the gel was soaked in a 3-(cyclohexylamino)propanesulfonic acid blotting buffer [10 mM 3-(cyclohexylamino)propanesulfonic acid and 10% methanol] for 15 min. The gel was then electroblotted onto a polyvinylidene difluoride membrane (Immobilon PSQ, Millipore) using a semidry blotting apparatus (21). The blot filter was then stained with Coomassie Brilliant Blue R-250. After washing by vigorous vortexing in 50% methanol, the stained band was subjected to Edman degradation using a Procise TM Model 492 peptide sequencer (Applied Biosystems).

Preparation of High Molecular Weight DNA without Proteinase K Treatment and Gel Retardation Analysis Using Modified Immunoblotting. Approximately $2 \times 10^6$ washed cells were lysed at 37°C for 16 h with 5 ml of lysis buffer consisting of 10 mM Tris-Cl (pH 8.0), 1 mM EDTA, and 0.5% SDS. To avoid loss of DNA-linked protein, DNA samples were prepared without proteinase K treatment; instead, sodium deoxycholate was added to 0.2 mM (final concentration) of the lysate. The lysate was then extracted with phenol, phenol/chloroform, chloroform/isooamyalcohol, and ether. Precipitated DNA was then dissolved in TE buffer [10 mM Tris-Cl (pH 8.0) and 1 mM EDTA] and treated with 100 µg/ml pancreatic RNase A. To avoid artifactual effects due to visible light exposure, these procedures were conducted under yellow light, and all samples were shielded by aluminum foil. A portion of high molecular weight DNA prepared from each of four human fibroblast cell lines was exhaustively digested with EcoRI (Takara) and then loaded in the parallel well of neutral agarose gel (15). The separation profile was detected and photographed using a camera-ready UV transilluminator (Kodak). Transfer to a sheet of Hybond C nitrocellulose membrane (Amersham), which has binding capacities for both DNA and proteins, was then performed using a modified method in which 0.05 M NaOH was used for denaturation, an electroblotter (ATTO) was used for transfer instead of osmotic blotting, and blotting was completed within 1 h. After washing the Hybond C filter with three changes of PBS for 15 min each, it was subjected to the immunoblot detection procedure described below.

Antibodies. Anti-GRP78 goat polyclonal antibody (Santa Cruz Biotechnology) was raised against a peptide corresponding to amino acids 7–20 of human histone H3 with phosphorylated serine at residue 10.

Fig. 1. Structure of gilvocarins.

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\begin{align*}
R : \text{alkyl group} \\
\text{CH}_2 (\text{vinyl}) & \quad \text{Gilvocarin V} \\
\text{CH}_3 (\text{methyl}) & \quad \text{Gilvocarin M}
\end{align*}
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Fig. 1. Structure of gilvocarins.

Detection and Purification of Proteins Cross-Linked to DNA by Photoactivated GV in Human Diploid Fibroblasts. Proteins cross-linked to DNA were prepared from control cells, cells treated with GV without UVB irradiation, or cells treated with photoactivated GV.

![Fig. 2. Preparation of cross-linked proteins to DNA induced by photoactivated GV in human fibroblasts (GM637).](image)

A separate experiment was conducted using the SDS-PAGE Tris-glycine gel system. Sample prepared from control cells (Lane 1), sham-irradiated cells with GV (Lane 2), or UVB-irradiated cells with GV (Lane 3) was mixed with the same amount of the SDS sample buffer, boiled for 5 min, and loaded on wells for SDS-PAGE. After electrophoresis, the gel was stained with Coomassie Brilliant Blue R-250. B, precise separation of the low molecular mass cross-linked protein using the SDS-PAGE Tris-tricine gel system. Samples were the same as those described in A, except that each sample was passed through a limit filtration column (Ultrafree 30) to remove proteins with molecular mass higher than 30 kDa and that the passed-through sample was mixed with the same amount of the sample buffer of Tris-tricine SDS-PAGE at 37°C for 15 min instead of boiling. Left, migration positions of high molecular weight (A) or low molecular weight (B) calibration markers in each gel system. Arrowhead, migration position of the 78-kDa protein in Tris-glycine SDS-PAGE; arrows, the positions of the 13-kDa protein in both gel systems.

Fig. 3. Preparation of cross-linked proteins for NH₂-terminal amino acid sequencing. Approximately 60 pmol (total) of the prepared cross-linked protein were separated by either Tris-glycine SDS-PAGE to isolate the 78-kDa protein (A) or Tris-tricine SDS-PAGE to isolate the 13-kDa protein (B). Each gel was then transferred to an Immobilon PSQ membrane and stained with Coomassie Brilliant Blue. The protein band corresponding to each molecular mass was cut out and subjected to Edman degradation analysis. Left, migration positions of molecular mass markers. Arrowheads, migration position of the 78-kDa protein (A) and the 13-kDa protein (B), respectively.
Table 1  Edman degradation profile of the 78-kDa protein cross-linked by photovativated GV

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SDS-PAGE separation profiles of the three samples were obtained from 1.5 × 10^5 cells of each sample as shown in Fig. 2. No cross-linked protein was detected in the control samples within the sensitivity limits of Coomassie Brilliant Blue staining (Fig. 2A, Lane 1). The sample obtained from cells treated with 0.3 μg/ml GV and in sham-irradiated conditions exhibited a trace amount of a 13-kDa band (Fig. 2A, Lane 2). The sample obtained from cells treated with 0.3 μg/ml photovativated GV exhibited two distinct bands of 78 kDa and ~10 kDa in the Tris-glycine gel system SDS-PAGE (Fig. 2A, Lane 3). Each band appeared monodisperse. Because the migration position of the smaller band in Tris-glycine SDS-PAGE is beyond the range of proportional distribution in the molecular mass, the sample was passed through a filtration limit column to remove proteins with molecular masses exceeding 30 kDa and then separated by using Tris-tricine system SDS-PAGE to more accurately determine the molecular mass of the smaller protein band (Fig. 2B). The molecular mass of the smaller cross-linked protein is 13 kDa, judging from its migration position in relation to the protein markers (Fig. 2B, Lane 3).

NH₂-terminal Amino Acid Sequencing of Two Cross-Linked Proteins. To identify the two distinct proteins detected in the above-mentioned analysis, a larger-scale experiment was performed. GM637 cells (5 × 10^6) treated with 0.3 μg/ml photovativated GV were used as a starting material, and approximately 60 pmol (total) of the 78-kDa protein were isolated by Tris-glycine SDS-PAGE (Fig. 3A), and the same amount of the 13-kDa protein was isolated by Tris-tricine SDS-PAGE (Fig. 3B), blotted onto a polyvinyliden difluoride membrane, and visualized by Coomassie Brilliant Blue staining. The results of each sequential Edman degradation profile expressed as the recovery of each cycle in pmol for the 78-kDa protein band are presented in Table 1, and the results of each sequential Edman degradation profile expressed as the recovery of each cycle in pmol for the 13-kDa protein band are presented in Table 2.

Deduced Amino Acid Sequence Alignment with a Sequence Identified in the Protein Database. A homology search of two protein databases, PIR and SWISS-PROT, using each amino acid sequencing result in Table 1 showed that the NH₂-terminal 17-amino acids of the 78-kDa protein exhibit the highest homology with the amino acid sequence of the 13-kDa protein, which is consistent with the results of the Edman degradation analysis.
acid sequence of the 78-kDa protein was identical to that of the GRP78 protein, without the hydrophobic leader sequence (Fig. 4), and that the NH$_2$-terminal 17-amino acid sequence of the 13-kDa protein completely matched that of the mammalian histone H3.

Western Blot Analysis of Two Proteins Cross-Linked by Photoactivated GV. To further identify the prepared cross-linked proteins, these proteins were separated by either Tris-glycine or Tris-tricine SDS-PAGE, and each gel was blotted onto a polyvinylidene difluoride membrane sheet. Cross-linked protein on the membrane was then detected immunologically by Western blot analysis using anti-GRP78 antibody (Fig. 5A) or anti-phosphorylated histone H3 antibody (Fig. 5B). The major 78-kDa band was detected in all preparations of cross-linked human fibroblast proteins derived from three different sources (GM637, NHDF, and NHLF) on treatment with photoactivated GV (Fig. 5A, Lanes 2–4). In cells treated with sham-irradiated GV, however, only a trace amount of the protein band was detected (Fig. 5A, Lane 5). In all three samples treated with photoactivated GV, a trace amount of the 70-kDa band was detected (Fig. 5A, Lanes 2–4). Although the nature of this protein band is unknown at present, this protein seems to cross-react with the epitope in 24–43 amino acid residues at the NH$_2$-terminal portion of GRP78, against which the polyclonal antibody was raised. No cross-reactive band signal was detected using anti-phosphorylated histone H3 antibody. Although the 13-kDa band is detected most sensitively and specifically by this antibody, another antibody that recognizes overall histone H3 detects the band quite insensitively (data not shown). Note that other band signals corresponding to histone H2A, H2B, and H4 were not detected.

Gel Retardation Analysis of GV-treated Fibroblasts from Various Sources and Immunoblot Detection in Situ. To obtain direct evidence that the two identified proteins prepared by the SDS-potassium precipitation method are actually unique as DNA-cross-linked proteins in GV-treated human fibroblasts, we used native agarose gel separation of endonuclease-cleaved high-molecular weight DNA prepared without proteinase K treatment. Using EcoRI to cleave the DNA, separation of DNA fragments by neutral agarose gel electrophoresis effectively revealed two distinctive retarded bands in all four human fibroblast DNA samples tested (Fig. 6A). To minimize the denaturing effect for the epitopes expressed in the cross-linked proteins, a modified blotting procedure to a nitrocellulose filter membrane was used as described above. This enabled identification in situ of the cross-linked proteins in all four human fibroblasts as GRP78 (Fig. 6B) and histone H3 (Fig. 6C). Neither retarded bands nor these immunoreactive protein bands were detected in control samples prepared with proteinase K (Fig. 6, Lanes 1) or in those treated in the absence of GV (Fig. 6, Lanes 2) or UVB (Fig. 6, Lanes 3).

DISCUSSION

Two independent methods were used for identification of the selectively cross-linked proteins: (a) preparation of enriched DNA-cross-linked proteins followed by both amino acid sequencing and immunological detection; and (b) in situ identification of the DNA-cross-linked proteins by immunological detection. The first method left some uncertainty regarding the relationship of the two prepared proteins to the two retarded bands originally found in the GV-treated fibroblasts (16). The second method has inherent weakness regarding the stability of epitope(s) of protein that is detected immunologically. Fortunately, identification of the faster-moving band as histone H3, a protein rich in basic amino acids, was successful, possibly due to its stability in alkali (Fig. 6C). However, immunological detection of the slower-moving band as GRP78 appears as a weak single band signal (Fig. 6B). This suggests that some epitopes expressed on GRP78 are alkali-labile, and only the stable epitopes are detected by the anti-GRP78 polyclonal antibody. Taken together, the results indicate that the primary target proteins in human fibroblasts for DNA-to-protein cross-linking induced by photoactivated GV are GRP78 and histone H3. Failure to detect these retarded bands in samples prepared with proteinase K and in those treated under incomplete conditions for the cross-link formation also supports the significance of the gel retardation analysis.

GRP78, a Stress-70 (HSP-70) Family Protein Lacking the Hydrophobic Leader Sequence, Is a Target Protein for DNA-to-Protein Cross-Linking Induced by Photoactivated GV. HSPs are synthesized when cells are exposed to hyperthermia or to certain toxic chemicals (22). The most abundant and well conserved of these is the stress-70 (HSP-70) protein family. GRP78, a member of this family that is abundantly expressed in the lumen of the endoplasmic reticulum, was originally found independently as an immunoglobulin heavy chain-binding protein in myeloma cells (23) and as a glucose regulated protein whose synthesis rate is increased by glucose starvation or...
a variety of stress conditions (24). Like other HSP-70 proteins, the likely function of GRP78 binding protein is to recognize unfolded polypeptides and proteins and, by inhibiting intra- or intermolecular aggregation, maintain them in a state competent for subsequent refolding and oligomerization (25). The GRP78 protein is also directly or indirectly involved in the translocation of secretory precursors across the endoplasmic reticulum membrane (26).

The SDS-PAGE profile of the 78-kDa protein appears as a single homogeneous protein band (Fig. 2), and the NH2-terminal amino acid sequencing result also indicates that the cross-linked protein is GRP78 protein without the hydrophobic leader sequence, which is always found in the lumen of endoplasmic reticulum. HSP-70 family proteins are, in general, expressed in the mammalian cell nucleus and cytosol. The leader sequence has been clearly shown to be a tissue-specific determinant of subcellular distribution (27), Munro et al. (28) transfected COS cells with GRP78 cDNA, with or without the 5′ sequence that encodes the hydrophobic leader sequence, and then analyzed the subcellular GRP78 distribution in cells. Cells transfected with the cDNA containing the hydrophobic leader sequence exhibited a typical granular expression pattern in cytosol, suggesting specific expression in the endoplasmic reticulum. However, cells transfected with GRP78 cDNA lacking the hydrophobic leader sequence exhibited a typical nuclear distribution similar to that of endogenous HSP-70 protein and that of *Drosophila* HSP-70 when expressed in the same cells (29). Note that in this study, GRP78 with the leader sequence was not detected as a GV-cross-linked protein, as verified by NH2-terminal amino acid sequencing (Table 1). However, the reason for the selective cross-linking of GRP78 with DNA by this DNA-damaging agent remains unknown. Because it has been shown that the moiety reactive to DNA in photoactivated GV is the C-8 position of the vinyl group (30), the other reactive moiety that bridges the GRP78 protein and photoactivated GV may be certain amino acid residues with photoactivatable aromatic side chains, such as tryptophan. Photoactivation by UVB irradiation may then induce both reactive moieties of GV and particular amino acids of proteins, and the specific proteins that are closely associated with DNA will be cross-linked.

Histone H3 as Protein DNA Cross-Linked by Photoactivated GV. The nucleosome consists of DNA wound around a histone octamer containing two molecules each of core histones H2A, H2B, H3, and H4. The histone octamer plays a passive role in efficient DNA packaging, an important role in DNA replication and transcription (31, 32). Secondary modification of histones, such as acetylation and phosphorylation, is closely associated with these roles (33). Specific cross-linking of histone H3, but not of other histones, is associated with the molecular characteristics of photoactivated GV. Thus, the interaction is not simply a nonspecific one occurring in amine-rich proteins such as histones. One explanation could be that the distance and three-dimensional position between DNA and the reactive group of histone H3 matches well with the molecular structure of photoactivated GV. Of the four core histones, H3 and H4 can form nucleosome-like structures by themselves, and both are evolutionarily conserved. The NH2 terminus of histone H3 extends 10–20 amino acids further from the nucleosome core (34). In the histone-DNA cross-linking analysis, histone H3 and histone H4 show different cross-linking patterns along the nucleosomal DNA, in which histone H3 but not histone H4 (35) associates with DNA that is both entering and exiting the nucleosome, suggesting that histone H3 associates with the entry and exit DNA or linker DNA in its vicinity. Interestingly, phosphorylation of the NH2-terminal domain of histone H3 is correlated with activation of *jun* and *fos* (36). Because the antibody used in our analysis detects the phosphorylated form of histone H3 (Figs. 5B and 6C), this finding suggests a possible relevance of the antitumor activity of photoactivated GV.

Formaldehyde preferentially cross-links histones to DNA (37) because amine-rich proteins are the favored substrates for formaldehyde cross-linking to amine positions of unpaired DNA bases. (37, 38). Although the group of the photoactivated GV that is reactive to the target protein has not yet been identified, it must differ from the activated vinyl group at the C-8 position that reacts with DNA (30). The cross-linking formation mechanism thus appears to differ from that of the formaldehyde-histone cross-linking, which involves the same reactive group present in both DNA and protein.

DNA-to-Protein Cross-Linking, DNA Damage with Potent Biological Activity, and Significance of Target Protein Identification. Formaldehyde, *cis*-platinum, and trivalent chromate are known to be carcinogenic in animals, and each induces DNA-to-protein cross-links (39–41). Although the biological significance of the lesions is not fully understood, several properties suggest that DNA-protein cross-links have the potential to cause mutations. These lesions are known to be relatively long-lived and, consequently, may be present during DNA replication (42); the lesion size may be a hindrance to enzymes that participate in DNA replication and repair, resulting in blockage on the DNA template. DNA processing has been shown to be blocked in *in vitro* systems by immobile proteins on DNA.
The biological activity of photoactivated GV *in vitro*, such as in prophase induction and antitumor activity, is expressed at extremely low concentrations and UV doses (7) compared with those for other coumarin-based, UVB-activated compounds, such as the psoralens (30). This activity cannot be ascribed simply to DNA lesions, single-strand breakage, or intercalation (30). Also, unlike psoralens, this compound does not induce interstrand DNA cross-linking (16). It therefore appears that the most potent DNA damage induced by photoactivated GV is DNA-protein cross-linking. Given the specific interaction of photoactivated GV and GRP78, it is particularly interesting to note that a benzoquinone-based antitumor antibiotic, geldanamycin, specifically interacts with cytosol HSP-90. The antitumor activity of geldanamycin is due to the inhibition of tyrosine kinase activity, as seen in the Src family kinases. However, it does not directly bind Src kinase. It has been speculated that the mechanism is indirect and involves the interaction with HSP-90 that regulates the Src protein (45). Geldanamycin apparently binds to the pocket required to form the complex of HSP-90 and the target protein. The molecular action of photoactivated GV might also involve a protein that interacts with GRP78 and participates in signal transduction. It may be important to analyze the three-dimensional structure of GRP78 to search for a pocket for photoactivated GV. Otherwise, one could suppose that it is the covariant cross-linking of these principal target proteins *per se* that is responsible for GV activity and that the mechanism of action does not relate to the specific roles of these proteins in the cell.

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Histone H3 and Heat Shock Protein GRP78 Are Selectively Cross-Linked to DNA by Photoactivated Gilvocarcin V in Human Fibroblasts

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