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INTER-STRAND CROSS-LINKS AND SINGLE-STRAND BREAKS PRODUCED BY GOLD(I) AND GOLD(III) COORDINATION COMPLEXES

CHRISTOPHER K. MIRABELLI*, JOSEPH P. ZIMMERMAN, HENRY R. BARTUS, CHIU-MEI SUNG and STANLEY T. CROOKE

Department of Molecular Pharmacology, SK&F Laboratories, Philadelphia, PA 19101, U.S.A.

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Abstract—The ability of gold coordination complexes to bind to DNA and produce inter-strand cross-links in DNA was assessed in an assay system based on the fluorescence properties of the DNA intercalative dye, ethidium bromide. Results from these studies using a variety of gold(I) and gold(III) complexes suggest that the ability of gold complexes to bind to and produce inter-strand cross-links in DNA is not dependent on the oxidation state of gold in the complex but is influenced by the nature of the coordinating ligands. Those complexes in which the gold was ligated through one or more weakly coordinating ligands showed evidence for DNA binding. However, only those complexes with two or more of these relatively weak coordinating ligands produced inter-strand cross-links. Both the amount of binding to and cross-linking of DNA by these compounds were decreased by treatment of the gold–DNA complex with 2-mercaptoethanol and other thiol containing agents. As shown by agarose gel electrophoresis, 2-mercaptoethanol caused a dissociation of the gold–DNA complexes and a regeneration of closed circular superhelical pBR322 DNA. DNA strand breakage also resulted from treatment of a number of gold–DNA complexes with 2-mercaptoethanol; this was observed with the gold compounds which were shown to produce inter-strand cross-links in DNA. The amount of DNA strand breakage produced by treatment of gold–DNA complexes with 2-mercaptoethanol was influenced by the initial conformation of the DNA; gold–DNA complexes which resulted from the binding of gold compounds to covalently closed superhelical DNA were more sensitive to the breakage induced by 2-mercaptoethanol treatment than those complexes in which closed circular, relaxed DNA was used as substrate. The DNA breakage was not reduced in partially anaerobic conditions or by free-radical scavengers, suggesting that it is not mediated by oxygen. The results are discussed with respect to the potential for the interaction of gold complexes with intracellular DNA and chromatin and their biological implications.

A number of gold coordination complexes are used clinically in the treatment of rheumatoid arthritis. Recent studies have also shown that some gold containing compounds may have potential as antineoplastic agents as evidenced by their activity in experimental tumor systems *in vitro* and *in vivo* [1–4].

The precise molecular mechanism by which the gold complexes produce their antineoplastic effects is unclear at present; however, those gold complexes which demonstrate *in vivo* antitumor activity also have been shown to be highly cytotoxic to tumor cells *in vitro* [5, 6]. Another series of metal complexes, the platinums, also contain numerous examples of compounds that are highly cytotoxic and have *in vivo* antitumor activity. Numerous studies using both isolated DNA and tissue culture cells have shown that these platinum complexes can interact with DNA and have implicated DNA interactions and the resulting DNA damage as the primary mechanism by which these platinum compounds kill tumor cells [7, review]. Therefore, it would seem reasonable to suggest that some gold containing compounds may interact with DNA and that these interactions may be involved in mechanisms by which they kill tumor cells. To investigate this hypothesis, we have begun to characterize the interaction of a variety of gold coordination complexes with a variety of DNA structures. In a companion paper [8], we have shown evidence that a variety of gold(I) and gold(III) complexes can produce conformational changes in covalently closed plasmid DNA that are similar to those produced by *cis*-diamminedichloroplatinum(II) (CDDP†). Biochemical and electron micrographic studies of CDDP–DNA adducts have shown that CDDP can produce inter- [9–12] and intra-molecular cross-links in DNA [13–15] and that these cross-links are responsible for the conformational changes produced in DNA by the drug [16–19]. As the DNA cross-linking capability of platinum complexes would appear to be directly related to their cytotoxic and antitumor activities [20, 21], we have investigated the potential cross-linking properties of a variety of gold complexes. This report further defines the interaction of gold complexes (see Fig. 1) with DNA and demonstrates that certain structural classes can produce DNA inter-strand cross-links as well as DNA strand breaks.

* Send correspondence to: C. K. Mirabelli, L 511, SK&F Laboratories, 1500 Spring Garden St., P.O. Box 7929, Philadelphia, PA 19101.

† Abbreviations: CDDP, *cis*-diamminedichloroplatinum(II); EB, ethidium bromide; SOD, superoxide dismutase; nt, nucleotide; DABCO, 1,3-diazabicyclo(2.2.2) octane; and Tiron, 4,5-dihydroxyl-1,2-benzenedisulfonic acid disodium salt.

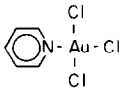
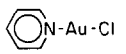
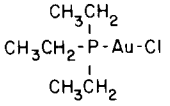
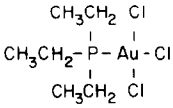
Compound No	Structure
1	
2	
3	
4	

Fig. 1. Structures of gold(I) and gold(III) complexes used in this study. Gold complexes are referred to in the text by their corresponding number shown here.

METHODS

Reagents. Ethidum bromide (EB), catalase, dimethyl sulfoxide, Na_2EDTA, KI, mannitol, Tiron, Tris-base and calf thymus DNA were purchased from the Sigma Chemical Co. (St. Louis, MO). Agarose-ME and bovine erythrocyte superoxide dismutase (SOD) were obtained from Miles Laboratories, Inc. (Elkhart, IN). *N*-Tris(hydroxymethyl)methylglycine (tricine) was purchased from the Boehringer Mannheim Co. (Indianapolis, IN). CDDP was provided by Johnson Matthey Inc. (West Chester, PA) and dissolved in distilled water to a concentration of 2 mM. Gold complexes were obtained from Smith Kline & French Laboratories (Philadelphia, PA) and dissolved in 100% ethanol to a concentration of 2 mM immediately before use in experiments.

DNA isolation and purification. pBR322 DNA was isolated from *Escherichia coli* JA221 as described previously [22]. Form I° (closed circular, relaxed) pBR322 DNA was obtained by calf thymus topoisomerase relaxation of Form I pBR322 DNA [13]. Calf thymus DNA was dissolved in 25 mM Tris, pH 7.4, 10 mM NaCl and 1 mM EDTA. The DNA solution was then sonicated, followed by two phenol extractions. The concentration of DNA was determined optically (εM_{258}, 6600).

EB fluorescence assay to detect DNA binding and cross-linking. This assay is similar to that described by Lown *et al.* [23]. Calf thymus DNA (20 μg) was incubated with drug in a 200 μl volume of buffer containing 25 mM sodium borate, pH 9.5, and 25 mM $NaNO_3$. After 1 hr at 37°, the reaction mixture was divided into two aliquots, and both were added to 1.89 ml of buffer containing 50 mM NaH_2PO_4, pH 11.7, and 0.4 mM EDTA. One sample was kept on ice while the other was incubated at 95° for 5 min and then chilled on ice for 5 min. Ten microliters of EB (1.0 mg/ml) was then added to each sample and allowed to equilibrate to room temperature for 20 min [24]. The amount of EB fluorescence in both samples was determined (excitation wavelength, 530 nm; emission wavelength, 590 nm) using a Perkin Elmer spectrofluorometer. All reactions were performed in triplicate. None of the gold complexes tested showed any increase in relative fluorescence above background when incubated with DNA without addition of EB. The effect of the gold compounds on the EB–DNA fluorescence was recorded as a percent of control [(relative fluorescence in the presence of gold complex/relative fluorescence in the absence of gold complex) × 100].

Agarose gel electrophoresis separation of DNA conformational isomers. pBR322 DNA preparations containing approximately 85, 12 and 3% Forms I (closed circular, supercoiled), II (open circular), and III DNA (double-strand broken, linear), respectively, were used to investigate the drug-induced electrophoretic mobility changes. One microgram of DNA was incubated with the compounds in a buffer of 25 mM sodium borate, pH 9.5, and 25 mM $NaNO_3$ (final reaction volume of 20 μl) for 5 hr at 37°. Immediately after incubation with the compounds, the DNA was analyzed by agarose gel electrophoresis as described previously [18, 19].

Densitometric tracing of agarose slab gels containing drug bound pBR322 DNA. After the slab gels were stained with EB and photographed, the pBR322 DNA bands separated by electrophoresis were scanned with a Beckman DU-8 scanning densitometer (Beckman Instruments, Inc., Palo Alto, CA) as described previously [25].

RESULTS

Binding and cross-linking of calf thymus DNA by gold complexes. The "snap-back" assay developed by Lown *et al.* [23] is an assay based on the fluorescence properties of the dye EB, by which DNA inter-strand cross-links can be assayed. This system has been used for the detection of cross-links produced by nitrosoureas [26, 27] and mitomycins [28] and platinums [24]. In this assay system, DNA inter-strand cross-links provide nucleation sites and render the complementary strands renaturable when the DNA is transferred from a denaturation to a renaturation condition such as heat to cold or alkali to neutral pH buffer. The renatured double-strand DNA region can bind EB, and thus the extent of EB-induced fluorescence reflects the degree of cross-linking.

Figure 2A shows the results of such an assay using CDDP and calf thymus DNA. When the DNA was incubated with concentrations of CDDP in which the drug/nucleotide (nt) ratio was below 0.1, no inhibition of fluorescence was observed. However, at CDDP concentrations greater than 0.1 d/nt, the EB fluorescence was reduced in a concentration-dependent manner. This inhibition in EB fluorescence is probably the result of decreased EB binding to the DNA as a result of the binding of CDDP to DNA and thus directly blocking the EB binding and/or producing local sites of denaturation in the DNA [29] and thereby decreasing the amount of double-stranded DNA available for EB intercalation and fluorescence. If, on the other hand, the CDDP–DNA complex was first incubated in the denaturation buffer (see Methods) at 95° for 5 min and then rapidly cooled to 0° for 5 min before addition of EB, a

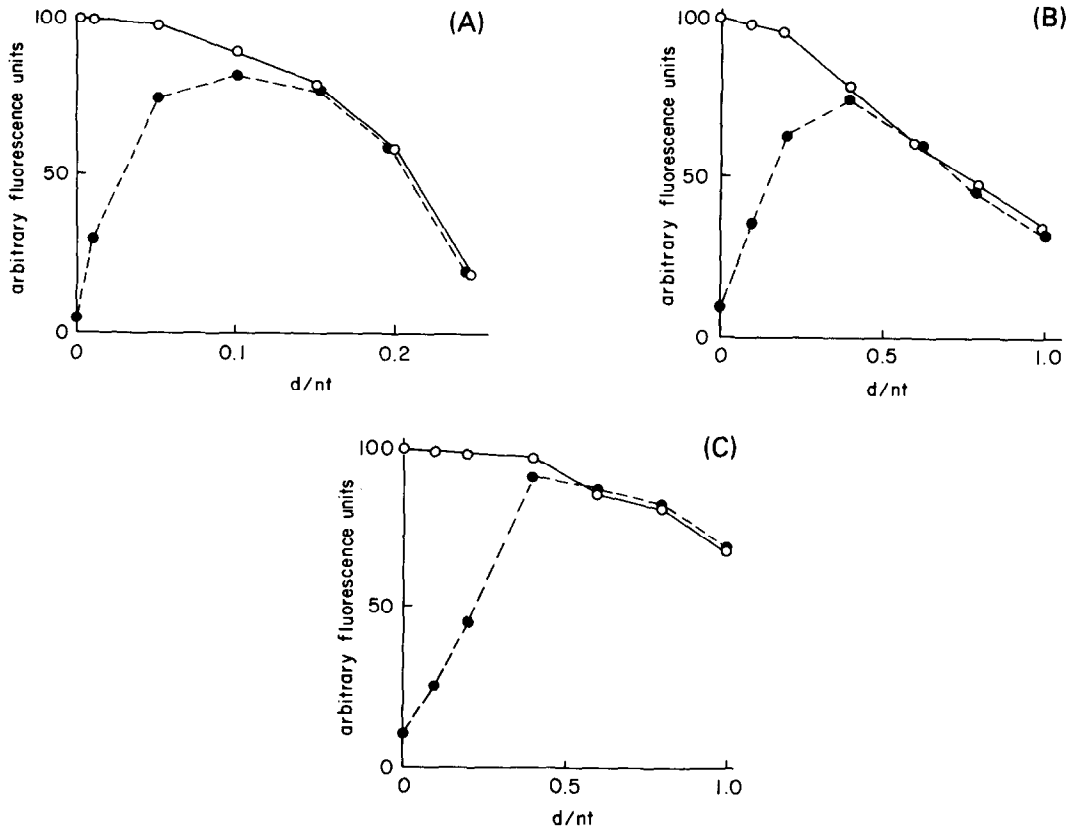


Fig. 2. Binding and cross-linking of DNA by CDDP (A), **1** (B), and **2** (C) as measured in the EB fluorescence/snap-back assay. Calf thymus DNA was incubated with increasing concentrations of the compounds for 60 min. Ethidium bromide was then added to aliquots of the reaction mixture or to samples in which denaturation buffer had been added and then heated to 95° for 5 min and then cooled to 0° for 5 min. The amount of EB fluorescence was measured at an excitation wavelength, 530 nm; emission wavelength, 590 nm. Fluorescence is expressed as arbitrary units. Open symbols (○) correspond to the nondenatured samples, and closed symbols (●) correspond to the denatured samples. Each data point is the mean of three experimental determinations; standard errors were ± 5 arbitrary units.

different pattern of changes in EB fluorescence was produced as a function of CDDP concentration. As seen in Fig. 2A under these conditions, in the absence of any CDDP, the EB produced relatively little fluorescence (10 ± 5% of the fluorescence produced using nondenatured DNA). However, at increasing concentrations of CDDP, an increase in EB fluorescence was measured. A maximal increase in EB fluorescence was observed at a CDDP/nt ratio of 0.1. At d/nt ratios of CDDP of 0.15 and greater, the amount of EB fluorescence measured before and after denaturation of the DNA was equivalent. These results suggest that CDDP interacted with DNA and produced inter-strand cross-links. Similar evidence for the production of DNA cross-links has been reported for bis(isoproylamine)-*trans*-dihydroxy-*cis*-dichloroplatinum(IV) [24] and certain bifunctional alkylating agents [26–28].

As shown in panels B and C of Fig. 2, both pyridinetrichlorogold(III) and pyridinechlorogold(I) complexes (**1** and **2** respectively) showed evidence of binding to DNA and producing cross-links in the DNA. Also note that **1** inhibited the EB fluorescence in both the nondenatured and denatured DNA samples to a greater degree than **2** at d/nt ratios of ≥ 0.5. The structure–activity relationships involved in DNA binding and DNA cross-linking were further explored with a series of gold–phosphine complexes. Figure 3A shows that, as reported in the companion paper [8], triethylphosphine gold(I) chloride (**3**) appeared to interact with DNA, as evidenced here by its ability to inhibit EB fluorescence. However, this compound did not produce inter-strand cross-links in the DNA (Fig. 3A). Triethylphosphine gold complexes in which the gold is ligated to a sulfur containing moiety [(2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranosato-*S*)-triethylphosphine gold (I)], or another phosphine moiety [chloro-bis(trethylphosphine)gold(I)], were evaluated and shown not to have any effect on EB fluorescence either before or after denaturation (data not shown). The gold(III) analog of **3**, (triethylphosphine)trichlorogold(III) (**4**), inhibited EB fluorescence and produced inter-strand cross-links in DNA (Fig. 3B). These results demonstrate that the ligands complexed to gold can affect the affinity of these compounds for DNA and/or their ability to produce inter-strand cross-links in DNA.

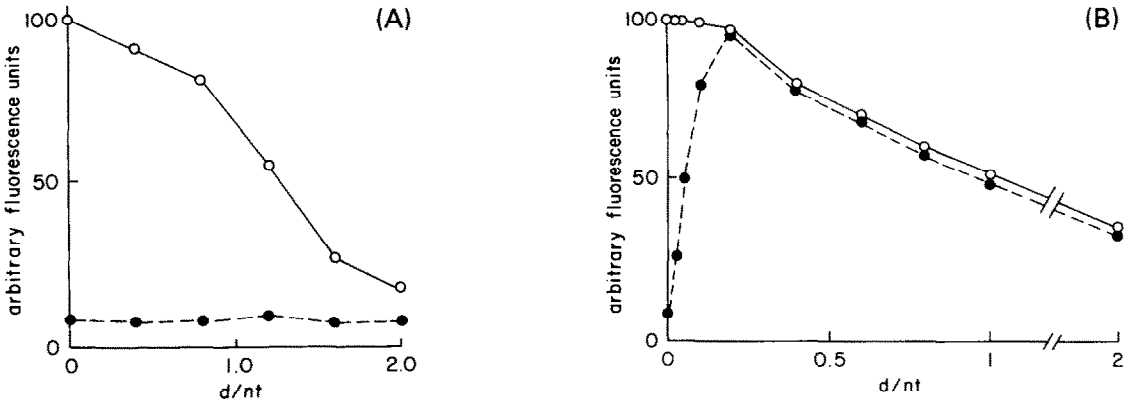


Fig. 3. Binding and cross-linking of DNA by **3** (A) and **4** (B). Experimental details are as described in the legend of Fig. 2; (○) corresponds to nondenatured samples, and (●) corresponds to denatured samples.

In the companion paper [8], we have demonstrated that the interactions of gold(I) and gold(III) complexes with DNA were inhibited by thiol containing compounds. The effect of thiols on gold–DNA interactions was explored further. In these experiments, 20 mM 2-mercaptoethanol was added after the 1-hr incubation of the gold complexes with the DNA. As shown in Fig. 4, 2-mercaptoethanol inhibited the effects of **1** on the EB fluorescence both before and after DNA denaturation. 2-Mercaptoethanol also reversed (1) the inhibition of EB fluorescence by **3** before denaturation, and (2) the inhibition of EB fluorescence before denaturation and the enhancement of EB fluorescence after denaturation by **2**. A 20 mM concentration of 2-mecaptoethanol had no effect on the effects of CDDP in this assay system (data not shown).

Binding and breakage of pBR322 DNA by gold complexes. Experiments analogous to those described above using 2-mercaptoethanol were conducted in which closed circular, supercoiled DNA (plasmid pBR322 DNA) was used as substrate instead of calf thymus DNA. In these experiments, the pBR322 DNA was incubated with the gold complex for up to 5 hr and then 20 mM 2-mercaptoethanol was added for 1 hr. The DNA was then analyzed by agarose gel electrophoresis. The results of an experiment using **1** are shown in Fig. 5. As shown in lanes 1–6, **1** produced changes in the electrophoretic migration pattern of pBR322, consistent with those reported in the preceding paper [8], reflecting changes in the conformation of the plasmid produced by binding of the compound to the DNA. However, addition of 2-mercaptoethanol to the **1**-DNA incubation mixture prior to electrophoresis produced an altered DNA mobility pattern in which three effects were apparent. First, the forms of DNA

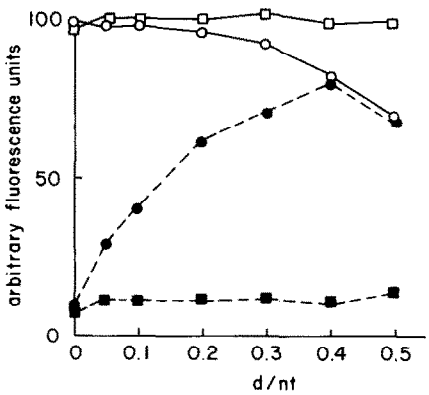


Fig. 4. Effect of 2-mercaptoethanol on the binding and cross-linking of **1** to DNA. Experimental details are as described in Fig. 2 except that each **1**–DNA reaction mixture was divided into two sets into which 20 mM 2-mercaptoethanol was added to one set; (circles, no 2-mercaptoethanol added; squares, 2-mercaptoethanol added). Open symbols correspond to the nondenatured samples, and closed symbols correspond to the denatured samples.

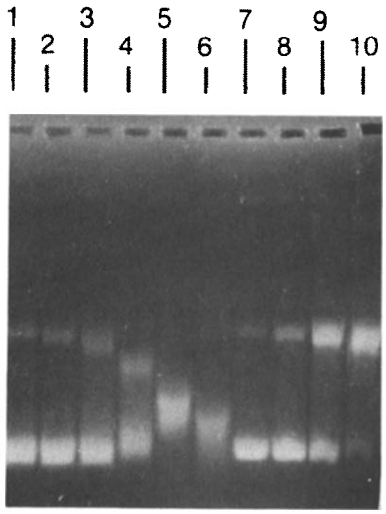


Fig. 5. DNA strand breaks produced by treatment of Au–DNA complexes with 2-mercaptoethanol. pBR322 DNA was incubated with **1** in a buffer containing 25 mM sodium borate (pH 9.5) and 25 mM $NaNO_3$ at 37° for 5 hr in the absence (lanes 1–6) or presence (lanes 7–10) of 20 mM 2-mercaptoethanol during the final hour of incubation. (See text for experimental details.) The drug concentrations are expressed in (drug/nt) ratios as follows: 1(0); 2(0.1); 3(0.2); 4(0.5); 5(1.0); 6(1.5); 7(0.2); 8(0.5); 9(1.0); and 10(1.5).

with electrophoretic mobility intermediate of Form I (covalently closed, supercoiled) and Form II (open, circular) produced by compound **1** (lanes 2–6) were no longer present in the 2-mercaptoethanol-treated DNA (lanes 7–10). Second, there was a drug concentration-dependent increase in the amount of form II evidenced in lanes 7–10. This latter effect indicates that the treatment of the **1**–DNA complex with 2-mercaptoethanol resulted in the production of single-strand breaks in the DNA and that the amount of breakage was directly related to the amount of compound associated with the DNA. Third, the amount of staining of the DNA by EB was enhanced following treatment of the **1**–DNA complexes with 2-mercaptoethanol (Fig. 5, lanes 4–6 and 8–10 respectively). This observation suggests that the inhibitory effect of **1** on EB intercalation into pBR322 DNA is reversed by 2-mercaptoethanol and is consistent with the results from experiments using calf thymus DNA (Fig. 4). Similar results were observed when **2** was incubated with pBR322 DNA, followed by 2-mercaptoethanol treatment. Figure 6 shows the loss in Form I and the production of Form II as a function of the concentration of **2** as determined from densitometric scans of an agarose gel containing DNA treated with **2** followed by 2-mercaptoethanol treatment.

The effect of 2-mercaptoethanol on the **3**–DNA complex is shown in Fig. 7. Treatment of the DNA–drug complex formed by **3** (lanes 2–6) with 20 mM 2-mercaptoethanol resulted in a reversion of the DNA to its native superhelical state, reversal of the inhibitory effect of **3** on EB intercalation into DNA, but no evidence of the production of form II DNA (lanes 7–10). This suggests that 2-mercaptoethanol resulted in dissociation of **3** from DNA but, in contrast to **1** and **2**, DNA breakage was not produced during the process under the experimental conditions employed. However, treatment of the triethylphosphine gold(III) trichloride (**4**)–DNA complex with 2-mercaptoethanol resulted in single-strand break production analogous to that evidenced for **1** and **2** (data not shown).

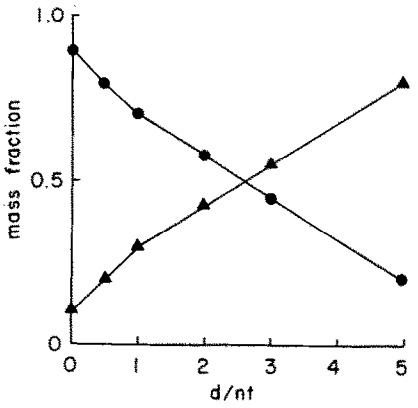


Fig. 6. Mass fractions of DNA conformational isomers produced by treatment of pBR322 DNA–**2** complexes with 2-mercaptoethanol. See text for experimental details. Data points were obtained from densitometric scans of agarose gels following electrophoretic separation of reaction products. Form I DNA (●); Form II DNA (▲).

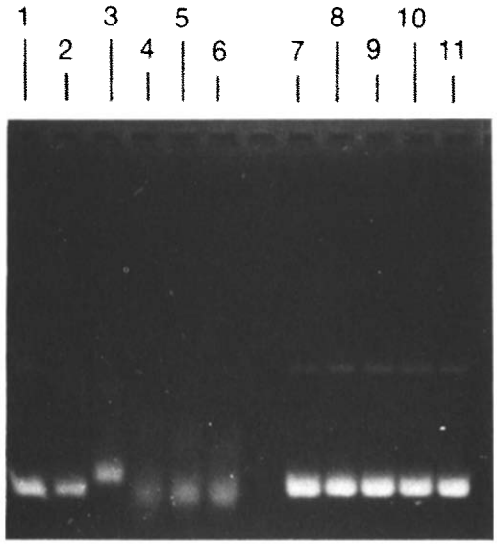


Fig. 7. Conformational changes of pBR322 DNA affected by **3** in the absence or presence of 2-mercaptoethanol. pBR322 DNA was incubated with **3** under conditions similar to those described in Fig. 2, in the absence (lanes 1–6) or presence (lanes 7–11) of 2-mercaptoethanol during the final hour of incubation. The drug concentrations are expressed in (drug/nt) ratios as follows: 1 and (11 (0), controls; 2(0.5); 3(1); 4(2); 5(4); 6(8); 7(1); 8(2); 9(4); and 10(8).

Treatment of CDDP–DNA complexes with 2-mercaptoethanol under conditions equivalent to those described above had no effect on the electrophoretic mobility of the CDDP–DNA complexes and did not produce single-strand breaks (data not shown).

The effects of 2-mercaptoethanol on a gold–DNA complex were compared using preparations of either Form I or Form I° (closed circular, relaxed) DNA as a substrate for **1** binding (Fig. 8). In the preceding paper [8], we have shown that **1** altered the electrophoretic mobility of Form I° of DNA. The gel system shown in Fig. 8 contained 1 μg/ml of EB. Under those conditions, both Forms I and I° pBR322 DNA are rewound into positive supercoils by the saturating concentration of EB [30, 31] and migrate to equivalent positions in agarose gels. As evidenced in Fig. 8, at equivalent **1**/nt ratios, treatment of the **1**–DNA complexes with 2-mercaptoethanol produced more single-strand breaks when the DNA was initially Form I (lanes 2–5 and 7–10) than when it was Form I° DNA (lanes 12–15 and 17–20). Densitometric analysis indicates that the Form I DNA was approximately 2.5-fold more sensitive to the production of single-strand breaks than form I° DNA when incubated under these conditions (Fig. 9).

Effects of partial anaerobic conditions and free radical scavengers on DNA breakage. The 2-mercaptoethanol-induced breakage of **1**–DNA complexes was studied under reduced oxygen pressure. As a control for these experiments, bleomycin was tested under partial oxygen pressure. Previous studies have shown that oxygen is a necessary cofactor for the degradation of DNA by bleomycin [32, 33]. Using our standard reaction conditions (as described in Fig. 5) 20 nM bleomycin produced a

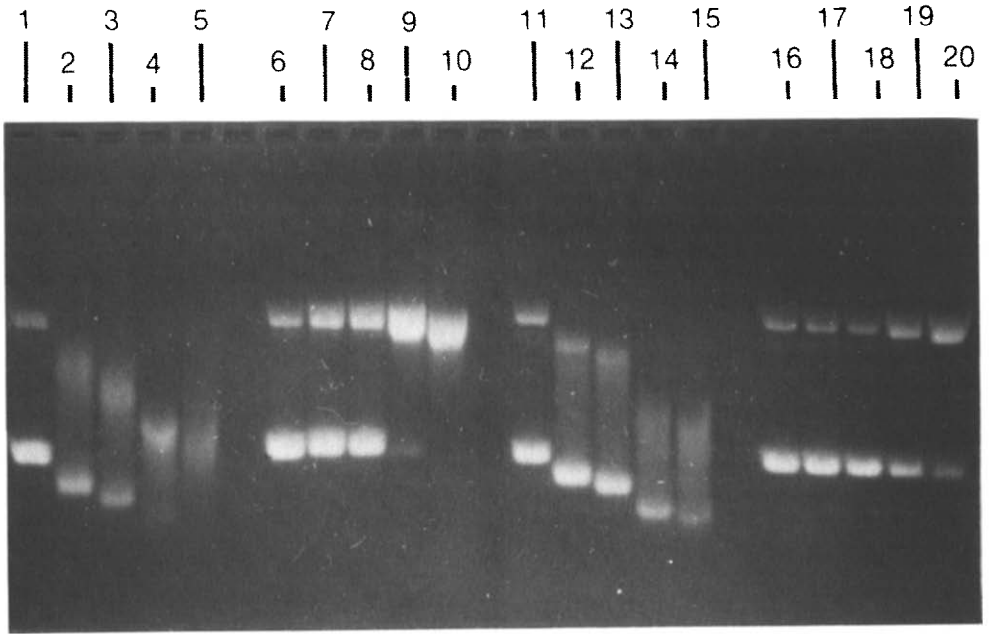


Fig. 8. Conformational changes and DNA strand breaks produced in Form I or Form I° pBR322 DNA by **1** in the absence or presence of 2-mercaptoethanol. Preparations of Form I pBR322 DNA (lanes 1–10) and Form I° pBR322 DNA (lanes 11–20) were incubated with **1** under conditions similar to those described in the legend of Fig. 5 in the absence (lanes 1–5 and 11–15) or presence (lanes 6–10 and 16–20) of 25 mM 2-mercaptoethanol during the final hour of incubation. The reaction mixtures were electrophoresed in gels containing 1 μg/ml of EB. The drug concentrations are expressed in (drugs/nt) ratios as follows: lanes 1, 6, 11 and 16 (0), controls; 2(0.5); 3(1); 4(2); 5(4); 7(0.5); 8(1); 9(2); 10(4); 12(0.5); 13(1); 14(2); 15(4); 17(0.5); 18(1); 19(2); and 20(4).

50% reduction in Form I DNA. Under conditions of reduced oxygen pressure, 20 nM bleomycin did not produce any reduction in Form I DNA. However, the breakage of Form I DNA by 100 μM of 1 (52% reduction in Form I DNA) was not inhibited when the reactions were conducted under partially anaerobic conditions (54% reduction in Form I DNA). This suggests that molecular oxygen is not involved in the DNA breakage produced by treatment of **1**–DNA complex with 2-mercaptoethanol. This suggestion is further supported by the finding that DABCO (a singlet oxygen scavenger), Tiron and SOD (superoxide free radical scavengers), catalase (a hydrogen peroxide scavenger) and KI and mannitol (hydroxyl radical scavengers) did not inhibit the breakage induced by 2-mercaptoethanol. EDTA (a metal chelator) also had no effect on the 2-mercaptoethanol-induced DNA breakage of the compound **1**–DNA complex.

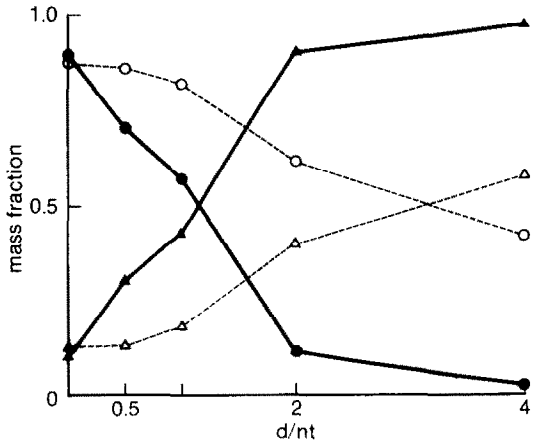


Fig. 9. Mass fractions of DNA conformational isomers after treatment of Form I, or Form I°, DNA–**1** complexes with 2-mercaptoethanol (see text for experimental details). Data were obtained from densitometric scans of the lanes of agarose gel shown in Fig. 8. Closed symbols represent the Form I (●) and Form II (▲) pBR322 DNA produced following treatment of the **1**–Form I pBR322 DNA mixture with 2-mercaptoethanol. Open symbols represent the Form I (○) and Form II (△) pBR322 DNA produced following treatment of the **1**–Form I° pBR322 DNA mixture with 2-mercaptoethanol.

DISCUSSION

The results presented in this and the preceding paper [8] clearly establish that gold coordination complexes have the ability to interact with duplex DNA *in vitro*. This interaction is displayed by the abilities of various gold complexes to change the conformation of DNA, block the binding of an intercalative dye to DNA, produce DNA inter-strand cross-links and/or produce single-strand nicks in DNA in the presence of 2-mercaptoethanol. The interactions with DNA described would appear to be of a covalent or coordinate nature in that the conformational changes that are produced are retained following electrophoretic separation in aga-

rose gels (Figs. 5, 7 and 8) and that these effects and the production of DNA inter-strand cross-links can be inhibited and reversed by the action of thiol containing compounds. The interactions of both gold(I) and gold(III) coordination complexes with DNA are affected by the characteristics of the ligands coordinated to the gold in the complex. The data reported in the preceding paper and here would suggest that those gold complexes in which at least one of the ligand groups is associated with the gold through a nitrogen atom and/or a halogen can interact with DNA. Presumably this interaction is the result of exchange of the suitably labile ligand group with a reactive site on the DNA. Previous studies have identified the probable binding sites for gold(III) complexes to be N(1)/N(7) of adenine, N(7) and/or C(6)O of guanine, N(3) of cytosine and N(3) of thymidine [34] and for gold(I) complexes to be N(7) of guanine and cytosine [35, 36]. The reported binding sites for gold(I) would, therefore, suggest that the inter-strand cross-linking sites for gold(I) complexes may be limited to dG:dC base pairs in duplex DNA.

Our studies provide evidence that a subset of those gold complexes which bind to DNA also have the ability to produce inter-strand cross-links in DNA. The results obtained using **1** and **2** also indicate that the ability of gold complexes to produce inter-strand cross-links in DNA is not restricted by the oxidation state of gold in the complex. The inter-strand cross-links produced in the DNA following treatment with **2** may be the result of (1) a change in the oxidation state of the gold (from +1 to +3) upon its interaction with DNA, or (2) the exchange of both the chloride and the pyridine ligands by the gold(I) for reactive sites on both strands of the DNA. These data support previous reports indicating the potential for gold(I) and gold(III) complexes to produce cross-links in DNA [35, 37].

Both *cis*- and *trans*-platinum complexes can form inter-strand cross-links in DNA. However, intra-strand cross-links occur predominantly as a consequence of CDDP treatment of DNA [29]. The geometry of the ligand configurations in *cis*- and *trans*-platinum appear to explain these apparent differences in their respective DNA binding properties. Therefore, while both the linear and the square planar coordination of ligands to gold in gold(I) and gold(III) complexes, respectively, could allow for the production of inter-strand cross-linking, it may be that only those gold(III) complexes with leaving groups in the *cis* configuration have the potential to form intra-strand cross-links. Definitive physical and biochemical proof of these hypothesized differences in the inter- and intra-strand cross-linking capabilities of gold(I) and gold(III) complexes is not yet available. However, differences noted in the electrophoretic mobility patterns induced by gold(I) and gold(III) complexes support the hypothesis. For example, it has been postulated that the local denaturation [38] or microloop formation [16] induced by the intra-strand cross-links produced by CDDP provides the basic mechanism(s) that generates torsion and thus results in the unwinding of supercoiled plasmid DNA [18, 19]. We have shown that gold(I) and gold(III) complexes produce different patterns of electrophoretic mobility changes in pBR322 DNA [8] and that the patterns induced by the gold(III) complexes are analogous to those induced by CDDP. For example, **1** produced changes in the mobility of pBR322 DNA similar to those produced by CDDP ([8], and Fig. 5 in this paper), whereas **2** (the gold(I) analog of **1**) produced mobility changes in the DNA dissimilar to those produced by CDDP and **1** (data not shown).

The DNA strand-breaking activity demonstrated for a number of gold(I) and gold(III) complexes has not been reported previously. This DNA breakage appears to (1) be exclusively single stranded, (2) require the presence of a gold-induced inter-strand cross-link, (3) be related to the dissociation of the gold complex from the DNA, and (4) be affected by the degree of supercoiling in the DNA to which the gold complex initially binds.

The single-stranded nature of the DNA strand breakage produced by certain gold(I) and gold(III) compounds is evidenced by the production of form II DNA and concomitant reduction of Form I DNA upon treatment of the DNA–gold complexes with 2-mercaptoethanol. No form III DNA (linear, double-strand broken DNA) was apparent in these gels (Figs. 5 and 8). Figure 8 provides additional evidence that the strand breaks are single stranded. This gel was run with EB so that the Form I° and Form II could be separated. These data show that the DNA migrating to the position of Form II DNA in Fig. 8 was indeed a nicked (Form II) and not a covalently-closed, relaxed form of DNA. The potential for double-strand breaks being undetected because of a gold mediated cross-link at or near a double-strand break site is unlikely as the 2-mercaptoethanol treatment of the DNA–gold complex not only produced single-strand breaks but also appeared to remove the gold complex from the DNA as evidenced by the electrophoretic mobilities of the DNAs relative to their mobilities before 2-mercaptoethanol treatment.

These studies suggest that there is a direct correlation between the ability of a gold compound to produce cross-links in duplex DNA and its ability to produce single-stranded breaks in DNA upon treatment with 2-mercaptoethanol. Although the precise mechanism for the breakage is unclear at present, several observations provide preliminary insights. First, as described above, the treatment of DNA–gold complex with 2-mercaptoethanol was associated with the apparent removal of some or all of the gold complexed to the DNA. This was observed for those gold complexes which induced subsequent strand breaks as well as those for which no strand breakage was observed. Also, the breakage was not observed when the 2-mercaptoethanol was added at the same time as the gold complex to the DNA. These two experimental results suggest that the breakage was the direct result of the reaction of 2-mercaptoethanol with the adduct formed by gold and DNA. This reaction may be thiol mediated as other thiol containing agents such as dithiothreitol and glutathione also produced the strand breaks (data not shown). The effect of non-thiol containing reducing agents on gold–DNA complexes is currently under investigation in our laboratory. Second, the breakage is probably not O_2 dependent and is not

mediated by an oxygen radical mechanism as the breakage was not inhibited by lowering the oxygen tension or by the addition of oxygen free radical scavengers. Third, the results shown in Figs. 8 and 9 indicate that the interactions of gold complexes with DNA may also be affected by the conformation of the DNA substrate. The increased sensitivity of the **1**–Form I DNA complex (relative to the **1**–Form I° complex) to single-strand breaks induced by 2-mercaptoethanol may be the result of a greater affinity of **1** to Form I DNA. Binding affinities of various gold compounds to Forms I, I°, II and III DNA are being measured currently in our laboratory to determine whether or not these various forms of DNA differ in their capacity to bind gold compounds. However, the possibility also exists that the conformation of the DNA directly modulates the response (i.e. strand-breakage) to the subsequent dissociation of the gold–DNA complex by 2-mercaptoethanol. This hypothesis is supported by the data in Figs. 8 and 9 as well as our recent observation that the single-strand breaks did not occur in response to treatment of linear DNA–gold complexes with 2-mercaptoethanol.* In these experiments, **1** was shown to complex to a 5′-^{32}P end-labeled restriction fragment of pBR322. However, while 2-mercaptoethanol dissociated the gold–DNA complex, no strand breakage was detected by high resolution polyacrylamide gel electrophoresis.

If the thiol-induced strand breakage is indeed a consequence of the superhelical conformation of the DNA, then a mechanism may be proposed whereby the intracellular DNA damage induced by gold complexes could be localized to specific sites in the chromatin. For example, the altered structures of chromatin in areas surrounding active genes have been shown to be maintained by continuous DNA supercoiling and that in the absence of this superhelical tension active chromatin reverts to "transcriptionally inactive" ground state [39, 40]. Therefore, these areas of continuous DNA supercoiling may serve as target sites at which gold-induced DNA strand breakage could occur. This process might involve the binding of a gold complex to DNA, the production of a cross-link in the DNA, and the eventual removal of the gold complex from the DNA by a sulfhydryl containing DNA binding protein (e.g. DNA polymerase, topoisomerase II, etc.), with those binding sites which are under superhelical tension (active chromatin) serving as DNA strand break points. It is interesting to note that other metals with varying degrees of cross-linking potential (Hg, Cr, Co, Ag, and Ni) have been shown to produce DNA strand breakage in cells [41–43]. We have identified recently a class of bis(diphenylphosphine) ethane gold complexes which possess potent *in vitro* and *in vivo* antitumor activity [3] and produce significant DNA strand breakage in tumor cells treated with the compounds.† These compounds will thus serve as probes to validate these hypothesized mechanisms for gold–chromatin interactions which have resulted from our evaluations of the interactions of a variety of gold complexes with isolated DNA.

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* C. K. Mirabelli and E. Sternberg, unpublished observation.

† C. K. Mirabelli and S. Dean, unpublished observation.

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