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INTERACTIONS OF GOLD COORDINATION COMPLEXES WITH DNA

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Abstract—The interactions of certain gold(I) and gold(III) complexes with isolated plasmid pBR322 DNA were defined and compared to those of *cis*-diamminedichloroplatinum(II), CDDP, using an agarose gel electrophoresis assay. Trichloro(pyridine)gold(III) appeared to bind to DNA as evidenced by its ability to produce dose-dependent changes in the electrophoretic mobilities of (1) closed circular, supercoiled, (2) closed circular, relaxed, and (3) open circular plasmid DNAs. These effects suggest that the gold containing complex induces conformational changes in the plasmid as a result of the compound binding to the DNA and the subsequent unwinding of the double helix and shorting of the DNA. Auranofin [(2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranosato-*S*)-triethylphosphine gold(I)] did not appear to interact with DNA under any conditions. However, its analog chloro(triethylphosphine) gold(I) interacted with DNA at pH 9.5 in borate buffer and produced electrophoretic mobility changes in pBR322 DNA which were different from those produced by the gold(III) complexes that were evaluated. Binding of chloro(triethylphosphine) gold(I) was inhibited by the co-addition of the thiosugar portion of auranofin suggesting preferential binding of the gold moiety to thiosugar, which results in the production of auranofin (or a sugar containing) gold complex and inhibition of gold binding to DNA. The interactions of a number of gold compounds with DNA were also evidenced by their abilities to inhibit the binding of ethidium bromide to DNA. The results from these studies indicate that: (1) gold containing complexes can bind to, and produce conformational changes in, DNA; (2) gold(I) and gold(III) complexes may interact with DNA via different chemical mechanisms to produce different conformational changes in DNA; and (3) certain coordinating ligands in gold complexes (e.g. Cl, Br and SCN) can be exchanged for binding sites on DNA by gold.

During the past several years a renaissance of interest in transition metals as potential therapeutics has occurred. This is clearly the result of two drugs that have had significant impacts on two major, perhaps interrelated diseases: Auranofin, **1** (Ridaura, see Fig. 1), active in the treatment of rheumatoid arthritis [1, 2], and *cis*-diamminedichloroplatinum (II) (CDDP)†, active in the treatment of certain malignancies including testicular, ovarian and head and neck cancers [3].

Introduced more than half a century ago, the organothiolate gold complexes continue to retain an important place in the management of resistant forms of rheumatoid arthritis. Despite the long legacy of clinical use, little is known about the mechanism(s) of action of these compounds as related to their therapeutic effects. During the course of recent studies aimed at understanding the biological mechanism of action of **1** and other gold complexes, a number of investigators have reported on their *in vitro* antiproliferative effects [4, 5]. Our laboratory has reported that **1** possesses potent cytotoxic activity against a number of transformed cell lines *in vitro* and that its potency is similar to that measured for CDDP [6]. Simon *et al.* [7] have reported a significant antitumor effect of **1** in mice inoculated with lymphocytic leukemia P388. Recent studies from our laboratories have confirmed the *in vivo* activity of **1** against P388 leukemia; however, the antitumor activity of the compound is extremely limited as no activity was evidenced in the fifteen other murine tumor models in which it was evaluated [8]. In an effort to identify a gold compound with antitumor activity superior to that of **1**, we have evaluated a diverse series of gold containing complexes in a number of tumor models, and to date a number of gold complexes have demonstrated a greater degree and broader spectrum of activity in animal tumor models than **1** [9].

To define the potential intracellular targets responsible for the cytotoxic and antitumor activity of gold containing complexes, we have initiated studies on the interaction of **1** and other gold complexes with cellular macromolecules both *in vitro* and *in situ* [10]. The evidence that other metal complexes, such as the platinums, with cytotoxic and antitumor activity bind to DNA and produce DNA lesions in cells [11, a review] has prompted our investigation into the DNA binding properties of gold coordination complexes. Inasmuch as gold and platinum complexes share a number of chemical and structural characteristics, and the DNA binding properties of certain platinum containing complexes have been the subject

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† CDDP, *cis*-diamminedichloroplatinum(II); EB, ethidium bromide; HSGlu, glucopyranose; OAc, O-acetyl; Et, ethyl; and nt, nucleotide.

of extensive studies, there is, perhaps, merit in studying gold complexes in a variety of these systems to compare their DNA binding properties with those of platinum complexes. This paper describes our investigation into the interactions of various gold complexes with plasmid pBR322 DNA and calf thymus DNA in comparison with CDDP and defines some of the structure–activity relationships involved in gold coordination complex–DNA interactions.

METHODS

Reagents. Ethidium bromide (EB) and calf thymus DNA were purchased from the Sigma Chemical Co. (St. Louis, MO). Agarose-ME was purchased from Miles Laboratories, Inc. (Elkhart, IN) and *N*-tris-(hydroxymethyl)methylglycine (tricine) was purchased from the Boehringer Mannheim Co. (Indianapolis, IN). T4 DNA polymerase was purchased from BRL Inc., Rockville, MD. CDDP was provided by Johnson Matthey Inc. (West Chester, PA). Gold complexes were obtained from the Smith Kline & French Laboratories (Philadelphia, PA). The gold complexes were 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 [12]. 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). Radiolabeled [^{3}H]calf thymus DNA was prepared using T4 DNA polymerase by the method of Maniatus *et al.* [14].

Agarose gel electrophoresis separation of DNA conformational isomers. pBR322 DNA preparations containing approximately 85, 12 and 3% Forms I (covalently closed, supercoiled), II (open circular, relaxed), 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 tricine, pH 7.1, and 25 mM $NaNO_3$ or 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 metal compounds, the DNA was analyzed by agarose gel electrophoresis as described previously [15]. Form I° pBR322 DNA having a molecular weight and conformation equivalent to Form II pBR322 DNA also can be analyzed by agarose gels containing 1 μg/ml of EB. This is because EB intercalation induces superhelical turns in Forms I° pBR322 DNA and thus separates Form I° from Forms II and III pBR322 DNA [16, 17].

Ethidium bromide–DNA fluorescence assay. Twenty micrograms of calf thymus DNA was incubated with various gold compounds in 25 mM sodium borate, pH 9.5, 25 mM $NaNO_3$ in a total reaction volume of 200 μl. Following a 1-hr incubation at 37°, 100 μl of the reaction mixture was added to 1.89 ml of a solution containing 25 mM sodium borate, pH 9.5, 25 mM $NaNO_3$ and 10 μl of EB (1.0 mg/ml). The amount of EB fluorescence in each reaction mixture 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 fluorescence when incubated with DNA in the absence of EB. The effects of the gold compounds on the EB–DNA fluorescence were recorded as a percent of control [(relative fluorescence in the presence of gold complex/relative fluorescence in the absence of gold complex) × 100].

RESULTS

Interaction of CDDP and gold(I) and gold(III) complexes with pBR322 DNA. Figure 1 shows the generic structures of gold(I) and gold(III) complexes and CDDP. Gold(I) (e.g. Auranofin, **1**) most commonly forms two coordinate complexes in a linear arrangement [18]. In contrast, gold(III) typically forms square planar coordinate complexes [19] (e.g. **2**) similar to those formed by platinum(II), such as CDDP.

Previous reports by Mong *et al.* [13, 15] and others [20, 21] demonstrated that CDDP induces the relaxation of negative supercoils in DNA, the positive supercoiling of closed circular, relaxed DNA, and the shortening of DNA. The effects induced in pBR322 DNA (as estimated by changes in electrophoretic mobility) by CDDP varied depending on the physical configuration of the DNA used in this study. Figure 2 shows that the binding of CDDP to Form I pBR322 DNA resulted in a slight increase in the electrophoretic mobility of the DNA at low CDDP concentrations (CDDP/nt = 0.01). At higher concentrations of CDDP, a significant decrease in the electrophoretic mobility was observed. At a CDDP/nt ratio of 0.5, the electrophoretic mobility of CDDP-bound Form I DNA was restored to the mobility of control Form I pBR322 DNA. At CDDP/nt ratios greater than 0.5, the electrophoretic mobility of CDDP-bound Form I DNA was greater than that of the control Form I. Moreover, at CDDP/

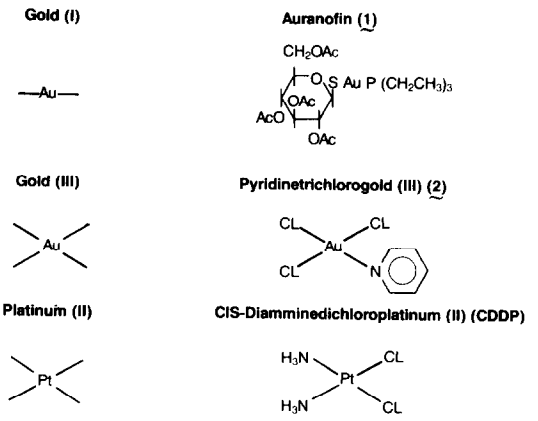


Fig. 1. Structures of gold and platinum containing compounds. Gold complexes are referred to in the text by their corresponding number shown in Table 1.

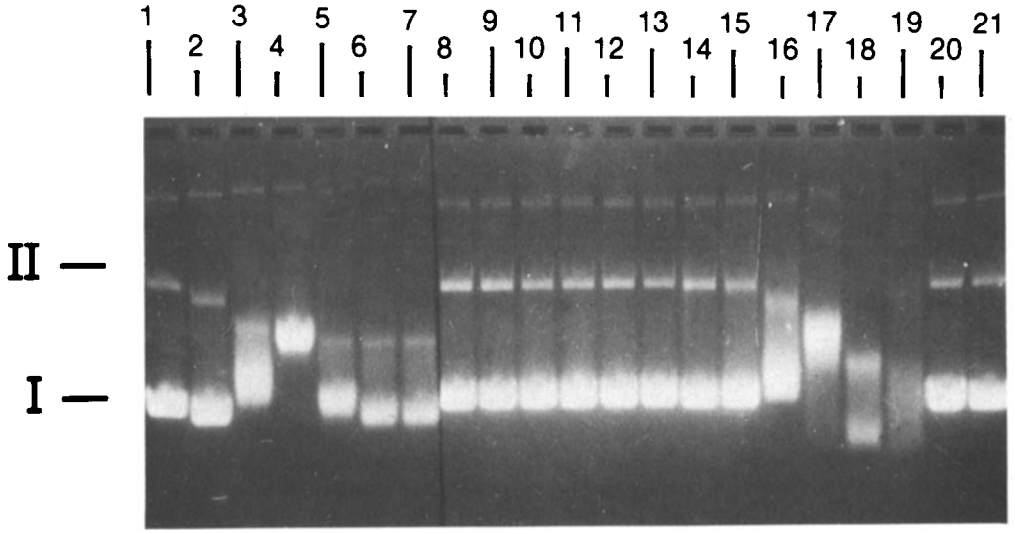


Fig. 2. Conformational changes of pBR322 DNA affected by CDDP, **1** or **2** binding. pBR322 DNA was incubated with increasing concentrations of drug in 25 mM sodium borate (pH 9.5) and 25 mM $NaNO_3$ for 4 hr at 37°. In the case of CDDP, the reaction was in a buffer of 25 mM tricine, pH 7.1, and 25 mM $NaNO_3$. The drug–pBR322 DNA mixtures were electrophoretically separated in 1% agarose gels. Lanes 2–7 contain DNA treated with CDDP, lanes 8–12 contain DNA treated with **1** and lanes 14–19 contain DNA treated with **2**. The drug concentrations are expressed in (drug/nt) ratios as follows: 1, 13, 20 and 21 (0 drug/nucleotide); 2(0.01); 3(0.05); 4(0.1); 5(0.5); 6(1.0); 7(1.5); 8(0.31); 9(0.62); 10(1.25); 11(2.5); 12(5.9); 14(0.15); 15(0.31); 16(0.62); 17(1.25); 18(2.5); and 19(5.9).

nt ratios equal to or greater than 0.5, inhibition of EB standing was observed. These effects on pBR322 DNA induced by CDDP are similar to those reported by Mong *et al.* using PM2 DNA [15].

Binding of trichloro(pyridine)gold(III) **2**, resulted in changes in the electrophoretic mobilities of different forms of pBR 322 DNA similar to those produced by CDDP (Fig. 2). However, a number of subtle differences are evident between the DNA banding patterns produced by the CDDP and **2**. First, higher **2**/nt ratios were required to produce equivalent changes in the electrophoretic mobilities of Forms I and II DNAs. Second, the increase in electrophoretic mobility of Form I DNA produced by low CDDP concentrations (Fig. 2 lane 2) was not observed in the DNA incubated with relatively low concentrations of **2**. Third, at relatively high drug/nt ratios, **2** induced a greater increase in the electrophoretic mobility of Forms I and II DNA than did CDDP.

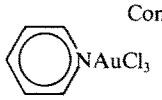
Two other gold(III) containing complexes (**11** and **12**) were tested in this system and were shown to alter the electrophoretic mobility of pBR322 DNA (Table 1). The changes in the electrophoretic mobilities of Forms I and II DNA produced by these compounds were equivalent to those produced by **2**.

Figures 2 (lanes 8–12) and 3 (lanes 17–19) show that the gold(I) complex auranofin (**1**) did not induce any observable changes in the electrophoretic mobilities of either Form I or Form II pBR322 DNA. No binding of **1** to DNA was observed when the drug was incubated with DNA in tricine buffer, pH 7.1, or in sodium borate, pH 9.5 (Table 1). Also, at pH 7.1, Et_3PAuCl, (**3**), did not bind to pBR322 DNA (Table 1). However, when **3** was incubated with DNA at pH 9.5, changes in the electrophoretic mobilities of Forms I and II DNAs were observed which were similar to, but not identical with, those produced by **2** (Fig. 3). The maximal extent to which the electrophoretic mobility of Form I DNA was decreased by **3** was not as great as that produced by **2**. Also, the increase in the electrophoretic mobility of Form I DNA produced by high concentrations of **3** was not as great as that produced by **2**. The binding of **3** to DNA was inhibited by the addition of tetraacetylthioglucose [$HSGlu(OAc)_4$]. When added at a 1 to 1 ratio, $HSGlu(OAc)_4$ completely inhibited the binding of **3** to DNA (Fig. 3, lane 16). The binding of the gold(III) analog of **3** (i.e. **11**) to DNA was also inhibited in the presence of $HSGlu(OAc)_4$. However, in order to completely block the electrophoretic mobility shift in the DNA produced by **11**, a 3-fold molar excess of $HSGlu(OAc)_4$ to **11** was required. Addition of NaCl also appeared to inhibit the binding of **3** and **11** to DNA. The inhibition by NaCl, however, required approximately 100-fold higher concentrations compared to $HSGlu(OAc)_4$ (data not shown).

A variety of other gold(I) and gold(III) containing triethylphosphine containing complexes were assayed in buffer systems containing 25 mM tricine, pH 7.1, or 25 mM sodium borate, pH 9.5, for their abilities to evidence binding to pBR322 DNA in this agarose gel system. The results are summarized in Table 1.

Interactions of gold complexes with different conformational forms of pBR322 DNA. The interactions of **2** with DNA were further characterized using closed circular, relaxed (nonsupercoiled) DNA, Form I°, as substrate. Figure 4 shows the results of

Table 1. Binding of phosphino gold complexes to pBR322 DNA as measured in the agarose gel electrophoresis assay

No.	X	$[CH_3CH_2]_3P$-Au-X DNA binding* 25 mM Tricine† pH 7.1	25 mM Sodium borate‡ pH 9.5
1	$SGlu(OAc)_4$	neg	neg
4	$SCH(CH_2COOH)COOH$	neg	neg
5	SCH_3	neg	neg
6	CH_3	neg	neg
7	CN	neg	neg
8	$P[CH_2CH_3]_3^+ Cl^-$	neg	neg
3	Cl	neg	pos
9	Br	neg	pos
10	SCN	neg	pos
11	Cl_3	pos	pos
12	Br_3	pos	pos
	Compound		
2		pos	pos

* As determined by agarose gel electrophoresis (see Methods): neg, no effect on electrophoretic mobility of DNA at drug/nt ratios ≤ 5; pos, effect on electrophoretic mobility of DNA observed at drug/nt ratio ≤ 5.

† Incubation of gold complexes with DNA was performed in 25 mM tricine buffer, pH 7.1, and 25 mM $NaNO_3$ at 37° for 5 hr.

‡ Incubation of gold complexes with DNA was performed in 25 mM sodium borate, pH 9.5, and 25 mM $NaNO_3$ at 37° for 5 hr.

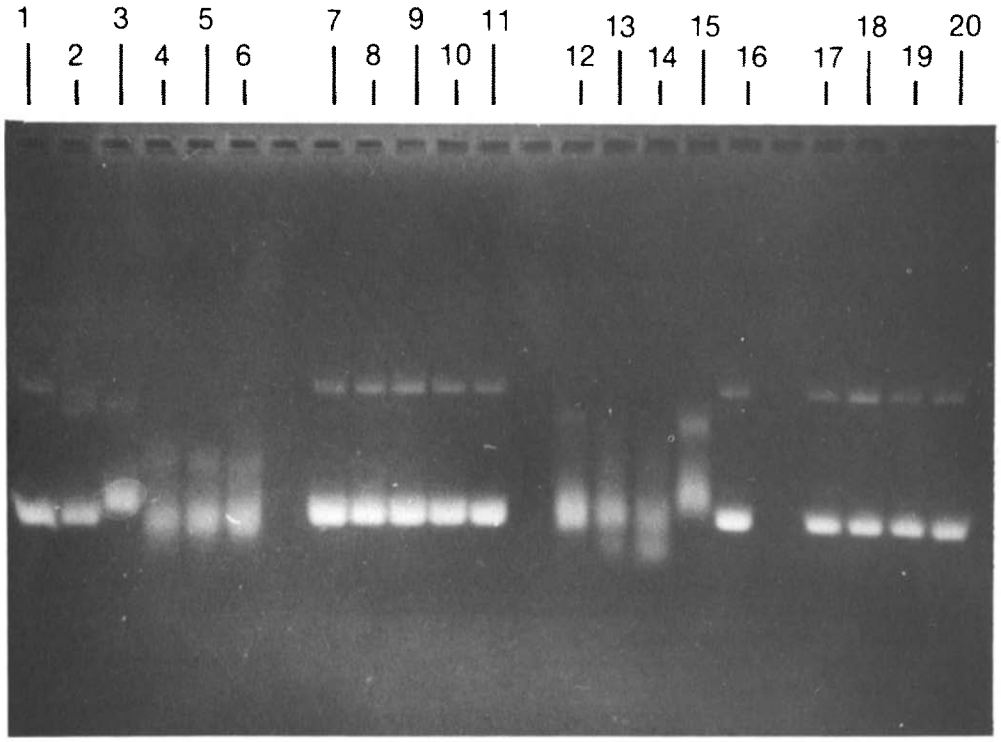


Fig. 3. Conformational changes of pBR322 DNA affected by compounds **1**, **3** and $HSGlu(OAc)_4$. The experimental conditions were equivalent to those in Fig. 1. Lanes 2–6, 7–11 and 17–19 contain DNA treated with **3**, $HSGlu(OAc)_4$ and **1** respectively. Lanes 12–16 contain DNA treated with a combination of both **3** and $HSGlu(OAc)_4$. The drug concentrations are expressed in (drug/nt) ratios as follows: lanes 1 and 20 (0) controls; 2(0.5); 3(1.0); 4(2.0); 5(4.0); 6(8.0); 7(0.5); 8(1.0); 9(2.0); 10(4.0); 11(8.0); 12–16 each were treated with **3** at a drug/nt ratio of 8 and $HSGlu(OAc)_4$ at 12(0.5); 13(1); 14(2); 15(4); 16(8); 17(1); 18(2) and 19(8).

an experiment in which the effects of both **2** and CDDP on Forms I, II and I° DNA were compared. Lanes 7 and 18 (Fig. 4) demonstrate that, as the conformation of Form 1° and Form II DNA are similar, they comigrate in this electrophoresis system. The binding of either **2** or CDDP to the preparation of pBR322 DNA containing predominantly Forms I° and II DNA followed by agarose gel electrophoresis resulted in the identification of two distinct DNA bands (lanes 8–11, 19–22). The faster moving band represents a DNA conformation with greater supercoiling which is being produced as increasing amounts of drugs are bound to Form I° DNA. The slower moving band represents Form II DNA. The gradual increase in electrophoretic mobility of form II DNA at increasing drug/nt ratios may be the result of "DNA shortening" equivalent to that previously described for CDDP [13, 15, 19]. These results suggest that, like CDDP, **2** produced conformational changes in Form I° DNA so that, in addition to being shortened as was Form II DNA, it became positively supercoiled due to the twisting of the DNA molecule and was thus separable from compound **2**-bound Form II DNA by gel electrophoresis.

Effect of gold complexes on the fluorescence induced by EB intercalation into calf thymus DNA. The intercalation of EB into duplex DNA can be observed by measuring the increased fluorescence that is produced upon intercalation of the drug into DNA [22]. The ability of a number of gold compounds to inhibit the measured fluorescence of an EB calf thymus DNA mixture was used as an indirect assay to evaluate the binding of gold compounds to DNA. As shown in Fig. 5, pretreatment of calf thymus DNA with **1** did not inhibit the subsequent fluorescence produced when EB was added to the gold compound–DNA mixture. However, pretreatment of calf thymus DNA with increasing drug/nt levels of **3** produced a dose-dependent inhibition in the fluorescence of EB. This inhibition by **3** was linear up to an inhibition of 80%. To exclude the possibility that the inhibition was the result of the precipitation of the DNA by **3**, the amount of DNA in solution was monitored by using ^{3}H-labeled calf thymus DNA as substrate in the reaction. Prior to the measurement of EB fluorescence, aliquots from the reaction mixtures were taken and the amount of [^{3}H]DNA was measured by scintillation counting. Under reaction conditions identical to those in Fig. 5, the amount of [^{3}H]DNA in solution following incubation with **3** (drug/nt ratios of 0.4 to 2) was equivalent to that measured in the control samples which did not contain the compound (data not shown).

The abilities of a number of gold compounds to inhibit EB–DNA fluorescence are compared in Table 2. These results are consistent with those observed in the pBR322 DNA/agarose gel assay, with **2, 3, 11** and **12** showing evidence of DNA binding. None of the gold compounds tested produced any increase in fluorescence (excitation 530 nm, emission 590 nm)

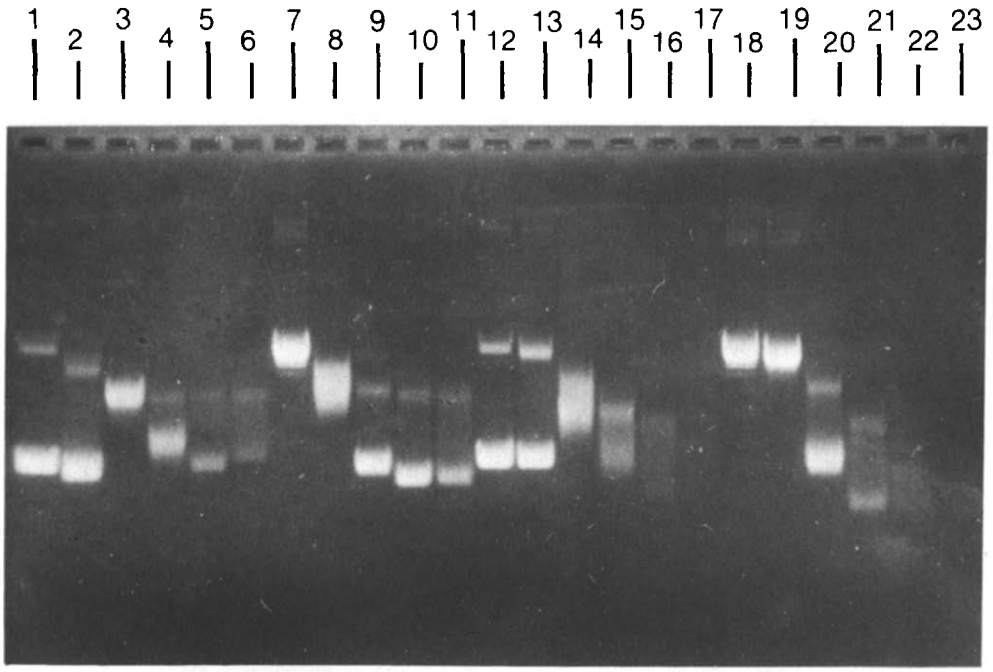


Fig. 4. Conformational changes of Form I and Form I° pBR322 DNA affected by CDDP and **2**. Preparations of either Form I pBR322 DNA (lanes 1–6 and 12–17) or Form I° pBR322 DNA (lanes 7–11) and 18–23) were incubated with either CDDP (lanes 2–6 and 8–11) in 25 mM tricine, pH 7.1, and 25 mM $NaNO_3$ or **2** (lanes 13–17 and 19–23) in 25 mM sodium borate, pH 9.5, and 25 mM $NaNO_3$ for 5 hr at 37° (see text for experimental details). The drug concentrations are expressed in (drug/nt) ratios as follows: lanes 1, 7, 12 and 18 (0) controls; 2(0.01); 3(0.05); 4(0.1); 5(0.5); 6(1.0); 8(0.01); 9(0.05); 10(0.1); 11(0.5); 13(0.31); 14(0.62); 15(1.25); 16(2.5); 17(5.0); 19(0.31); 20(0.62); 21(1.25); 22(2.5); and 23(5.0).

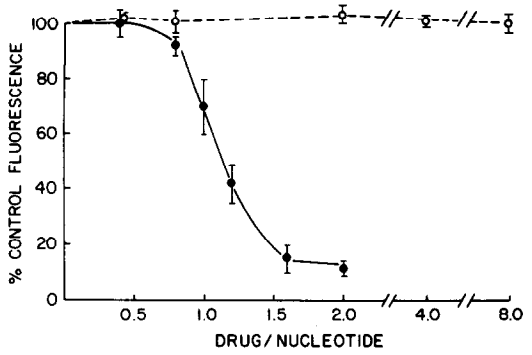


Fig. 5. Effects of **1** (○) and **3** (●) on the fluorescence produced by ethidium bromide binding to DNA. The gold compounds were incubated with 20 μg of calf thymus DNA at increasing drug/nucleotide ratios in 25 mM sodium borate pH 9.5, 25 mM $NaNO_3$ for 1 hr at 37°. One-half of the resulting reaction mixture was then added to a buffer containing EB at a final concentration of 0.5 μg/ml. The amount of EB fluorescence was then measured (see Methods for details). The experimental points were done in triplicate and the points represent the means and the bars represent the standard errors.

above background when added to calf thymus DNA. The inhibition in EB-DNA fluorescence produced by each of the gold compounds was not reversed by the addition of 10-fold excess of EB (50 μg/ml).

DISCUSSION

Covalently closed circular bacteriophage and bacterioplasmid DNAs have been used as models to investigate the interaction of CDDP with DNA. Cohen *et al.* [20] reported that increasing concentrations of CDDP or *trans*-diamminedichloroplatinum decrease and subsequently restore the electrophoretic mobility of bacterioplasmid pSM-1 DNA. Mong *et al.* [15, 23] confirmed the relaxing and supercoiling activity of CDDP on covalently closed circular PM2 DNA using agarose gel electrophoresis and DNA viscometry. It has been postulated that the intrastrand cross-linking [24] or local denaturation (or microloop formation) [21] produced in DNA by CDDP may be the basic mechanism by which the drug generates torsion in the DNA duplex [15, 23]. The resulting torsion would thus be responsible for the dose (CDDP)-dependent shortening of Form II DNA, positive supercoiling of Form I° DNA, and the relaxing and subsequent positive supercoiling of Form I DNA observed by gel electrophoresis. In the present study we have demonstrated that gold complexes can alter the conformation of DNA in a dose-dependent manner. Using supercoiled, relaxed and nicked circular forms of plasmid pBR322 DNA, we have provided evidence that the interactions of gold complexes with DNA may have characteristics similar to CDDP.

Table 2. Inhibition of ethidium bromide–DNA fluorescence by gold complexes*

No.	Compound	IC_{50}† (drug/nucleotide)
11	Et_3PAuCl_3	0.85 ± 0.09
12	Et_3PAuBr_3	0.90 ± 0.04
3	Et_3PAuCl	1.1 ± 0.2
2		0.8 ± 0.2
1	$Et_3PAuSGlu(OAc)_4$	neg‡

* See Methods for details.

† The concentrations of gold complex (drug/nt) required to inhibit EB–DNA fluorescence by 50%.

‡ No inhibition was detected (drug/nt ≤ 8).

While a number of the gold complexes were shown to alter the electrophoretic mobility of DNA in a manner analogous to CDDP, their reactivity with DNA appeared to be significantly less than that of CDDP as measured in this experimental system. For example, higher drug/nt ratios were required for the gold complexes to produce equivalent changes in the electrophoretic mobilities of Forms I and II DNA relative to CDDP (Figs. 2, 3, and 4). This apparent difference in the reactivities of gold and platinum complexes with DNA may be reflective of differences in their respective binding affinities to potential ligands on DNA. The chemistry of platinum(II) amine species (e.g. CDDP) is dominated by the high affinity of NH_3 for the Pt(II) center [25]. Therefore, it is not surprising that DNA has been shown to be a high-affinity substrate for CDDP binding with the various nitrogen atoms contained in the nucleotide bases serving as the binding sites [26]. Gold, on the other hand, can be stabilized by complexation to "soft" or "class b" ligands such as thiolates [27] and phosphines [28]. These soft donor atoms have a high affinity for gold. The high affinity for these atoms may explain the lack of reactivity of **1, 4, 5** and **8** with DNA as evidenced in the agarose electrophoresis assay (Table 1) and the inhibition of binding of **3** to DNA by co-addition of $HSGlu(OAc)_4$ (Fig. 3). In addition, of the gold(I) containing complexes listed in Tables 1 and 2, only those containing a halogen (Cl or Br) or a pseudohalogen (SCN) as one of the coordinating ligands interacted with DNA. Similar binding to DNA by halogen-substituted gold(III) complexes was observed. These results suggest greater lability of the gold—halogen relative to gold—sulfur, gold—phosphine or gold—carbon bonds and an exchange of the halogen ligand for a reactive site on DNA. These data are in agreement with those recently reported by Blank and Dabrowiak [29] in which absorption and circular dichroism spectroscopy were used to show that, when the gold ion possesses an easily displaced ligand, (e.g. Cl^- or Br^-), gold(I) containing complexes are capable of interacting in a non-denaturing fashion, with calf thymus DNA. These authors also showed that auranofin (**1**) did not bind to DNA. These data are consistent with that provided by our studies (Figs. 2, 3 and 5) in which **1** showed no evidence of binding to pBR322 or calf thymus DNA and also correlates with other reports from our laboratory that provide evidence that the antiproliferative action of **1** is not the result of its direct interaction with nucleic acid, chromatin or chromatin metabolism [8, 30].

Our results clearly demonstrate that the binding of gold containing complexes to DNA is not restricted to a single oxidation state of the gold in the

complex. Previous studies have shown that the gold(III) complexes (e.g. $HAuCl_4$) can react with purine and pyrimidine nucleosides to produce a variety of gold–nucleoside complexes in which the gold atom is in the +1, +2 and +3 oxidation state [31]. Other studies have shown that other gold(I) and gold(III) complexes can bind to nucleosides [29, 32], RNA [33] and single- [34] and double-stranded DNA [29, 34, 35]. This study demonstrates for the first time that direct analogs of gold containing complexes in which the gold is either in the +1 or the +3 oxidation state can interact with DNA (**3** vs **11**, Tables 1 and 2). While these and other gold(I) and gold(III) complexes show evidence for the ability to bind to DNA, differences in (1) the DNA electrophoretic patterns produced, (2) the apparent ability of sulfhydryl containing compounds to inhibit the binding of the gold complexes to DNA, and (3) the optimal buffer/pH required for DNA binding by gold(I) and gold(III) complexes may indicate different mechanisms by which complexes containing gold of the two different oxidation states bind to DNA. Previous studies have provided evidence that gold(I) complexes bind to a subset of potential gold(III) complex binding sites on nucleosides [31, 34]. Differences in the geometry of the coordinating ligands in gold(I) and gold(III) complexes [18, 19] may influence which sites on the DNA are available for binding. Differences in the stability of the coordination of specific ligands to gold(I) and gold(III) atoms may also modulate their respective abilities to bind to DNA. To better understand these differences we are currently attempting to chemically define the adducts produced on DNA by certain gold(I) and gold(III) analogs under experimental conditions equivalent to those used in the studies described here.

In conclusion, the reported cytotoxic [4–6, 8] and antitumor [7–9] properties of various gold complexes have directed our attention towards attempting to understand the molecular mechanism(s) by which they produce their antiproliferative effects. This report indicates that certain gold complexes in both the +1 and +3 oxidation state have the ability to interact with DNA *in vitro* and that some of these interactions appear to be analogous with those of CDDP–DNA interactions. Clearly, further studies are required to better define the role of the ligands on the binding of gold containing complexes to DNA and the actual chemical adduct produced on DNA by these gold complexes, and to evaluate the potential biological significance of gold–DNA interactions.

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