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DNA adducts of antitumor *trans*-[$PtCl_2$ (*E*-imino ether)$_2$]

Viktor Brabec*, Oldrich Vrána, Olga Nováková, Vladimír Kleinwächter, Francesco P. Intini[1], Mauro Coluccia[2] and Giovanni Natile[1]

Institute of Biophysics, Academy of Sciences of the Czech Republic, Královopolská 135, CZ-61265 Brno, Czech Republic, [1]Department of Pharmaceutical Chemistry, University of Bari, Via E. Orabona 4, I-70125, Italy and [2]Department of Biomedical Sciences and Human Oncology, Section of General Pathology and Experimental Oncology, Hospital, Piazza Giulio Cesare 11, I-70124 Bari, Italy

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ABSTRACT

It has been shown recently that some analogues of clinically ineffective *trans*-diamminedichloroplatinum(II) (transplatin) exhibit antitumor activity. This finding has inverted the empirical structure-antitumor activity relationships delineated for platinum(II) complexes, according to which only the *cis* geometry of leaving ligands in the bifunctional platinum complexes is therapeutically active. As a result, interactions of *trans* platinum compounds with DNA, which is the main pharmacological target of platinum anticancer drugs, are of great interest. The present paper describes the DNA binding of antitumor *trans*-[$PtCl_2$(*E*-imino ether)$_2$] complex (*trans-EE*) in a cell-free medium, which has been investigated using three experimental approaches. They involve thiourea as a probe of monofunctional DNA adducts of platinum(II) complexes with two leaving ligands in the *trans* configuration, ethidium bromide as a probe for distinguishing between monofunctional and bifunctional DNA adducts of platinum complexes and HPLC analysis of the platinated DNA enzymatically digested to nucleosides. The results show that bifunctional *trans-EE* preferentially forms monofunctional adducts at guanine residues in double-helical DNA even when DNA is incubated with the platinum complex for a relatively long time (48 h at 37°C in 10 mM $NaClO_4$). It implies that antitumor *trans-EE* modifies DNA in a different way than clinically ineffective transplatin, which forms prevalent amount of bifunctional DNA adducts after 48 h. This result has been interpreted to mean that the major adduct of *trans-EE*, occurring in DNA even after long reaction times, is a monofunctional adduct in which the reactivity of the second leaving group is markedly reduced. It has been suggested that the different properties of the adducts formed on DNA by transplatin and *trans-EE* are relevant to their distinct clinical efficacy.

INTRODUCTION

The standard *cis*/*trans* structure–activity relationship of platinum antitumor complexes, exemplified by *cis*-diamminedichloroplatinum(II) (cisplatin), is that only *cis* geometry is therapeutically active. The observed inactivity of the *trans* isomer, *trans*-diamminedichloroplatinum(II) (transplatin), has had a major influence on both the synthesis of new platinum antitumor agents and the mechanistic interpretation of the antitumor activity of cisplatin. It is generally accepted that cisplatin manifests its biological activity through coordination to DNA (1–3). Possible explanations for the different biological activity of the *cis* and *trans* isomers are that *cis* compounds form platinum–DNA adducts which inhibit DNA replication or transcription to a greater extent than those formed by transplatin (5,6), and alternatively, that DNA adducts formed by *trans* compounds may be repaired more efficiently (7).

The nature of the DNA adducts formed by cisplatin and its *trans* isomer has also been explored to explain the differences in biological activity between the two isomers. Cisplatin produces a range of adducts on DNA including bidentate intrastrand cross-links such as 1,2-GG or AG and 1,3-GNG (1–3,8,9) and interstrand cross-links at the GC sequence (10). These lesions result in conformational alterations in DNA and represent blocks to DNA and RNA polymerases (10–13).

Transplatin shows a distinct sequence preference upon binding to DNA, different from that of cisplatin (11,14). Transplatin also produces bidentate intrastrand cross-links, such as 1,3-GNG and interstrand cross-links between complementary guanine and cytosine residues (8,14–16). These lesions of transplatin, however, induce in DNA different conformational alterations than cisplatin (1–4,17,18).

It has been reported in recent contributions that *trans*-[$PtCl_2$(*E*-imino ether)$_2$] complex (*trans-EE*) (imino ether=HN=C(OCH_3)-CH_3; it can have either *E* or *Z* configuration depending on the relative position of OCH_3 and N-bonded Pt with respect to the C=N double bond, *cis* in the *Z* isomer and *trans* in the *E* isomer) is not only more cytotoxic than its congener *cis*-[$PtCl_2$(E-imino ether)$_2$] (*cis-EE*), but is also endowed with significant antitumor

* To whom correspondence should be addressed

activity (19,20). These results strongly imply a new mechanism of action for *trans-EE*. By analogy with the diamminedichloroplatinum(II) isomers, the inhibition of DNA synthesis by *cis-EE* and *trans-EE* (19) implies a role for DNA binding in the mechanism of action. The presence of the imino ether group may result in altered hydrogen bonding and steric effects affecting the kinetics of DNA binding, the structures and/or stability of the adducts formed and resulting local conformational alterations in DNA.

The breaking of the paradigm for structure–activity relationships of platinum antitumor complexes poses new fundamental questions on the mechanism of antitumor activity of platinum complexes. To explain the cytotoxicity of imino ether derivatives, it could be important to examine in detail the DNA binding of the new complexes and compare these results with those previously obtained for other diamminedichloro isomers. Thus, these results could lead to the development of new strategies for the systematic design of platinum antitumor complexes acting by mechanisms different from the presently used agents, and eventually having a different clinical profile of antitumor activity.

This paper describes the results of studies of DNA binding of *trans-EE* with respect to characterization of preferential binding sites in double-helical DNA and the types of the resulting adducts.

MATERIALS AND METHODS

Starting materials

Cisplatin, transplatin and chlorodiethylenetriamineplatinum(II) chloride, [Pt(dien)Cl]Cl, were from Lachema (Brno, Czech Republic). *cis*-EE and *trans*-EE were synthesized as previously described (21). Calf thymus DNA (the content of guanine + cytosine is 42%) was purchased from Sigma and used without further purification. Deoxyriboguanosine (dGuo), DNase I from bovine pancreas, nuclease P1 from *Penicillium citrinum* and alkaline phosphatase from calf intestine were also from Sigma. Thiourea and ethidium bromide (EtBr) were from Merck. Thiourea (^{3}H) was from Amersham.

Preparation of HPLC standards

The standards were prepared by reaction of dGuo with the mononitratomonochloro and dinitrato platinum-iminoether species. Mononitrato platinum-iminoether derivative, *trans*-[PtCl(NO_3)(*E*-imino ether)$_2$] was prepared by dissolving 169 mg (0.41 mmol) of *trans-EE* in 40 ml acetone and treating it with an equivalent amount of $AgNO_3$ (70 mg, 0.41 mmol). After stirring in the dark for 1 h, the reaction mixture was filtered through celite, the solution was dried, the solid residue extracted with ethyl ether and the solution again filtered. By evaporation of the solvent a solid of the desired compound was obtained in ~70% yield. Anal. Calcd for $C_6H_{14}ClN_3O_5Pt$: C, 16.4; H, 3.2; N, 9.6%. Found: C, 17.0; H, 4.4; N, 9.3%. The dinitrato analogue, *trans*-[Pt(NO_3)$_2$(*E*-imino ether)$_2$], was prepared by reaction of a suspension of 99 mg (0.24 mmol) of *trans-EE* in 30 ml of water with two equivalents of $AgNO_3$ (82 mg, 0.48 mmol). After stirring for 16 h in the dark at room temperature (25–28°C), the reaction mixture was filtered through celite, the solvent evaporated and the solid residue redissolved in a minimum volume of methanol and kept to crystallize in a deep freezer. The colorless crystals were separated by filtration of the mother liquor and dried. The desired compound was obtained in ~60% yield. Anal. Calcd for $C_6H_{14}N_4O_8Pt$: C, 15.5; H, 3.0; N, 12.0%. Found: C, 15.4; H, 3.0; N, 12.0%.

Preparation of *trans*-[PtCl(dGuo)(*E*-imino ether)$_2$](NO_3) was done by dissolving 0.043 g (0.1 mmol) of *trans*-[PtCl(NO_3)(*E*-imino ether)$_2$] in 50 ml of water to which a stoichiometric amount of dGuo (26 mg, 0.1 mmol) was added. The solution was stirred at room temperature in the dark for 2 days. After this time the solvent was evaporated under a reduced pressure and *trans*-[PtCl(dGuo)(*E*-imino ether)$_2$](NO_3) was recovered quantitatively. Anal.Calcd for $C_{16}H_{27}N_8O_{10}Pt.H_2O$: C, 25.9; H, 3.9; N, 15.1%. Found: C, 25.9; H, 3.8; N, 14.6%. *trans*-[Pt(dGuo)$_2$(*E*-imino ether)$_2$](NO_3)$_2$ was prepared by suspending 0.015 g (0.032 mmol) of *trans*-[Pt(NO_3)$_2$(*E*-imino ether)$_2$] in 20 ml of water to which 2 eq of dGuo (17 mg, 0.064 mmol) were added. The suspension was stirred at room temperature in the dark for 2 days. After this time a clear solution was obtained, the solvent was evaporated under a reduced pressure and *trans*-[Pt(dGuo)$_2$(*E*-imino ether)$_2$](NO_3)$_2$ was recovered quantitatively. Anal. Calcd for $C_{26}H_{40}N_{14}O18Pt.2H_2O$: C, 29.2; H, 4.1; N, 18.4%. Found: C, 29.8; H, 4.2; N, 18.5%.

Analytical and physico-chemical methods

Ultraviolet spectra were collected on a Beckmann DU-8 spectrophotometer. Fluorescence measurements of DNA modified by platinum in the presence of EtBr were performed using a Perkin-Elmer LS 5B spectrofluorimeter. The excitation wavelength was 546 nm and the emitted fluorescence was measured at 590 nm. The fluorescence was measured at 25°C in 0.4 M NaCl to avoid the second fixation site of EtBr to DNA (22). The concentrations were 0.01 mg/ml for DNA and 0.04 mg/ml for EtBr, which corresponded to the saturation of all intercalation sites of EtBr in DNA (22,23). Flameless atomic absorption spectroscopy (FAAS) measurements were carried out on a Perkin-Elmer 560 instrument with a graphite furnace. For FAAS analysis, DNA was precipitated with ethanol and dissolved in 0.1 M HNO_3. High-pressure liquid chromatographic (HPLC) analyses were performed by using a Perkin-Elmer Series 4 liquid chromatograph equipped with a LCI-100 computing integrator and a Waters μBondapak C_{18} column. Gradient was 0–60% methanol in 0.02 M ammonium acetate, pH 5.5; flow rate was 1 ml/min. Spectra (^{1}H NMR) were obtained with a Bruker AM 300 spectrometer. pH measurements were performed with a CRISON micropH 2002 apparatus.

Modification of DNA by platinum complexes

If not stated otherwise, the platination reactions were performed in 10 mM $NaClO_4$ at 37°C in the dark. If required, the reactions were terminated by adding thiourea to 10 mM and incubating at 37°C for 10 min. The ratio of platinum atoms fixed per nucleotide residue (r_b) was determined by FAAS. Enzymatic digestions of DNA modified by platinum were carried out by using DNase I, nuclease P1, and alkaline phosphatase (8). In a typical experiment, samples (45 μg of DNA) were allowed to react with 72 U of DNase I at 37°C. After 4 h nuclease P1 (40 μg) was added, and the reaction was allowed to continue at 37°C for 18 h. Finally, alkaline phosphatase (39 U) was added and the incubation continued for additional 4 h at 37°C. The samples were then heated for 2 min at 80°C, centrifuged and the supernatant analyzed by HPLC.

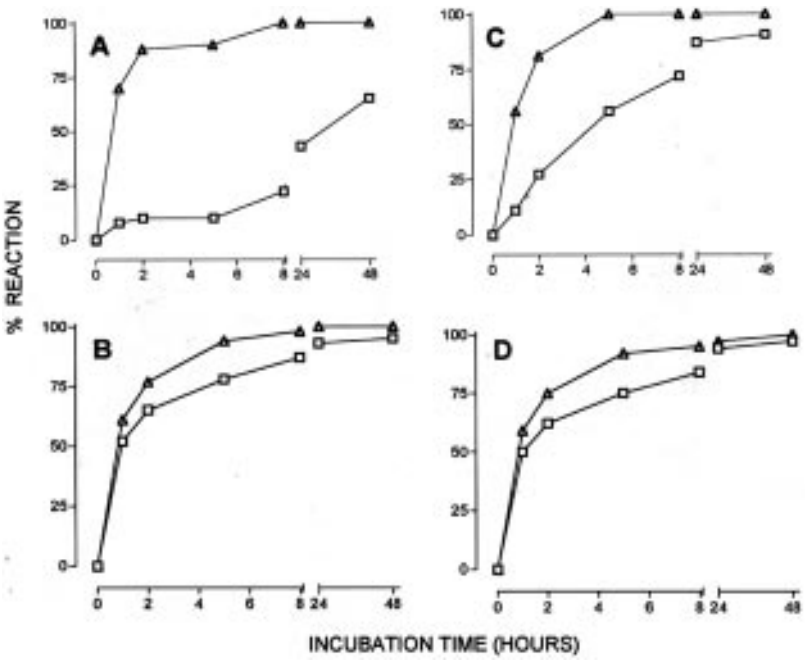

Figure 1. Kinetics of reaction of transplatin (A,C) and *trans*-EE (B,D) with double-stranded DNA (A,B) and thermally denatured DNA (C,D) at r_i = 0.05 in 10 mM $NaClO_4$ at 37°C. DNA concentration was 0.15 mg/ml. Reactions were stopped with (□) or without (Δ) 10 mM thiourea (10 min), and Pt associated with DNA was assessed by FAAS.

Tritiated thiourea binding assay

Calf thymus DNA (double-stranded or thermally denatured) at the concentration of 0.16 mg/ml was incubated with *trans-EE* at various r_i values (r_i is defined as the molar ratio of free metal complex to nucleotide-phosphates at the onset of incubation). At each concentration of *trans-EE*, the aliquots were withdrawn at 10 min, 2, 24 and 48 h time intervals. After the withdrawal, the unbound platinum was immediately removed by a centrifugation (1500 r.p.m., 30 s) through a column of Sephadex G25 (coarse). The r_i values were chosen in the way that the values of r_b determined after this separation step by FAAS were 0.01, 0.05 and 0.2 for each incubation time. Of these solutions, 0.15 ml was added to 0.15 ml of 0.9 mM ^3H labeled thiourea, prepared as described previously (24), having specific radioactivity of 77 MBq/mmol. After 10 min incubation at 37°C, 0.8 ml of 0.15 M NaCl, pH 7.0 was added and 1.0 ml of the resulting solution was layered on a nitrocellulose filter having pores of 0.4 mm in diameter (Synpor, VCHZ Syntezia, Pardubice). In order to remove the unreacted thiourea the filter was washed with 15 ml of 5% trichloroacetic acid. The filters were dried under an infrared lamp, transferred to glass tubes to which 5 ml of toluene scintillator was added. The radioactivity was measured on a LKB Wallac1410 Betaspectrometer (Finland).

RESULTS AND DISCUSSION

Characterization of DNA adducts of *trans-EE* by thiourea

Cisplatin, transplatin and analogous bifunctional platinum compounds bind to DNA in a two-step process, forming first monofunctional adducts, preferentially at guanine residues, which subsequently close to bifunctional lesions (1–4,8,9). Thus, monofunctional adducts are formed in DNA at an early stage of the reaction. Thiourea is successfully used to labilize monofunctional transplatin coordination to DNA (25). The displacement of transplatin takes place via coordination of thiourea *trans* to the base residue. Because of the strong *trans* effect of sulphur, the nitrogen–platinum bond is weakened so that it becomes susceptible to substitution. Importantly, whereas thiourea effectively *trans* labilizes transplatin in monofunctional DNA adducts, bifunctional adducts of this platinum complex are resistant to the thiourea treatment (25).

The initial experiment aimed at characterization of DNA adducts of *trans-EE* was conducted employing thiourea as a probe of DNA monofunctional adducts of *trans*-dichloroplatinum(II) complexes. Double-stranded and thermally denatured DNAs were incubated with transplatin or *trans-EE* at a formal drug to nucleotide ratio r_i = 0.05. At various times the reaction was stopped by ethanol precipitation of the DNA. In parallel tubes, the reactions were stopped by addition of thiourea to 10 mM. These samples were incubated for 10 min at 37°C and then precipitated by ethanol. The DNA was redissolved, and the platinum content was determined by FAAS (Fig. 1).

The modification of double-helical or thermally denatured DNA by transplatin was maximal after ~8 h whereas the modification by *trans-EE* was completed in ~10 h (Fig. 1B and D). In accordance with the previous results (25), thiourea displaced ~90% transplatin from double-helical DNA at early time intervals (1–5 h) (Fig. 1A). At longer incubation times (24–48 h), thiourea was less efficient in removing transplatin from DNA since at these time intervals a large fraction of monofunctional adducts of transplatin had closed to a bifunctional lesion resistant to the treatment with thiourea (25). Thus, after 48 h of the reaction only ~35% transplatin was displaced from double-stranded DNA, which indicates that ~65% monofunctional adducts had evolved to

bifunctional lesions. In a sharp contrast to the behaviour of transplatin, thiourea displaced only 10–20% of DNA-bound *trans-EE* independently of the length of the platination reaction (Fig. 1B). These observations indicate that as compared with transplatin, *trans-EE* forms on double-helical DNA the adducts, which are resistant to the thiourea treatment already at early time intervals. Interestingly, if denatured instead of double-helical DNA was modified by transplatin, ~10–50% adducts were removed by thiourea at early time intervals of the platination reaction (Fig. 1C). If denatured DNA was modified by *trans-EE*, 10–20% platinum was only displaced by thiourea and independently of the time of incubation of this platinum complex with DNA (Fig. 1D). Thus, thiourea was only slightly effective in displacing *trans-EE* adducts from DNA independently of its secondary structure. It was verified that 5–60 min incubations with 10 mM thiourea gave the same results as are those shown in Figure 1.

It is reasonable to suggest that the resistance of DNA adducts of *trans-EE* to the treatment with thiourea is either due to the formation of an extensive amount of bifunctional adducts already at early time intervals or, alternatively, that *trans-EE* forms on DNA monofunctional adducts different from those formed by transplatin. This new type of monofunctional adduct either would not bind readily thiourea or, although binding thiourea, would be resistant to the *trans* labilizing effect of the sulphur donor ligand. The latter eventuality was tested with the tritiated thiourea binding assay. No radioactivity associated with double-stranded or denatured DNAs, both modified by *trans-EE* (r_b = 0.01–0.2), was detected after their treatment with tritiated thiourea. Thus, these results are consistent with the idea that *trans-EE* forms on DNA preferentially either monofunctional adducts which, in contrast to the same type of DNA adducts of transplatin, do not readily bind thiourea, or forms bifunctional adducts already at early times of the reaction of *trans-EE* with DNA. Interestingly, independent ^{1}H NMR experiments performed on *trans*-[PtCl(dGMP)(*E*-imino ether)$_2$] and *trans*-[Pt(dGMP)$_2$(*E*-imino ether)$_2$] complexes have shown that thiourea (10 mM) reacts with the former complex replacing chloride ion and dGMP (the reaction is complete in <10 min at 30°C) while, under similar reaction conditions, it leaves the latter complex unaltered.

Characterization of DNA adducts of *cis-* and *trans-EE* by EtBr fluorescence

In order to differentiate between the latter two eventualities we have employed EtBr as a fluorescent probe. This probe can be used to distinguish between perturbations induced in DNA by monofunctional and bifunctional adducts of platinum compounds (22,23). Binding of EtBr to DNA by intercalation is blocked in a stoichiometric manner by formation of the bifunctional adducts of a series of platinum complexes including cisplatin and transplatin, which results in a loss of fluorescence intensity (22). On the other hand, modification of DNA by monodentate platinum complexes (having only one leaving ligand) results in only a slight decrease of EtBr fluorescence intensity as compared with non-platinated DNA–EtBr complex.

Double-helical DNA was first modified by cisplatin, transplatin, *cis-EE*, *trans-EE* and by monodentate [Pt(dien)Cl]Cl for 48 h. The levels of the modification corresponded to the values of r_b in the range between 0 and 0.1. Modification of DNA by all platinum complexes resulted in a decrease of EtBr fluorescence (Fig. 2). In accordance with the results published earlier (22,23), monodentate

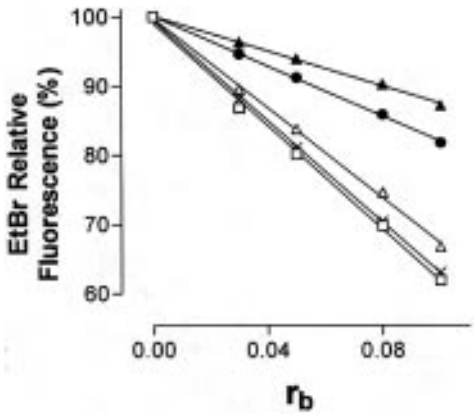

Figure 2. Dependences of the EtBr fluorescence on r_b for DNA modified by various platinum complexes in 10 mM $NaClO_4$ at 37°C for 48 h. (□) cisplatin, (Δ) transplatin, (X) *cis*-EE, (●) *trans*-EE, (▲) [Pt(dien)Cl]Cl.

[Pt(dien)Cl]Cl decreased the fluorescence only to a small extent, whereas the decrease induced by the DNA adducts of cisplatin, transplatin and *cis-EE* was significantly more pronounced. The decrease induced by the adducts of *trans-EE* was markedly less pronounced than that induced by the DNA adducts of other bidentate complexes tested in this work. In fact, the fluorescence intensity was only slightly lower than the fluorescence intensity of DNA modified by the monodentate [Pt(dien)Cl]Cl. This result suggests that *trans-EE* complex forms the DNA adducts which resemble, from the viewpoint of their capability to inhibit EtBr fluorescence, those formed by monofunctional platinum complexes. Importantly, the DNA adducts of *cis-EE* inhibited EtBr fluorescence almost to the same extent as cisplatin. Taken together, the fluorescent analysis is consistent with the idea and supports the postulate that the major DNA adducts of *trans-EE* are monofunctional lesions even after long incubations of DNA with this platinum complex (48 h). On the other hand, under comparable conditions *cis-EE* forms on DNA mainly bifunctional adducts similar to those formed by cisplatin.

Characterization of DNA adducts of *trans-EE* by HPLC analysis of enzymatically digested DNA

To characterize further the coordination mode of *trans-EE*, DNA modified by this platinum complex at r_b of 0.08 for 48 h was enzymatically digested to mononucleosides and analyzed by reversed-phase HPLC. Cisplatin or transplatin exhibit a strong preference for binding to guanine residues in DNA (they also bind in a much smaller extent to other base residues) (1–4,8,9). Therefore, we first characterized the products of the reactions of *trans-EE* with monomeric dGuo by NMR spectroscopy and then used these products as HPLC standards. The ^{1}H NMR spectral data are shown in Table 1. In the case of both standards containing *trans-EE* coordinated to one or two dGuo molecules, *trans*-[PtCl(dGuo)(*E*-imino ether)$_2$]$^+$ or *trans*-[Pt(dGuo)$_2$(*E*-imino ether)$_2$]$^{2+}$ respectively, a major shift was noticed for the H8 protons (0.4–0.6 p.p.m. downfield), which is typical of the N7 coordinated guanines (26,27). The N7 coordination was also supported by the pH dependence of chemical shifts (Fig. 3) and platinum coupling constants of H8 proton (30 and 26 Hz for the mono- and bis-dGuo complexes, respectively).

Table 1. Proton chemical shifts (δ, downfield from trimethylsililpropionic acid, TSP) in D_2O (pH 7.4) of deoxyriboguanosine (dGuo), *trans*-[PtCl(NO_3)(*E*-imino ether)$_2$], *trans*-[Pt(NO_3)$_2$(*E*-imino ether)$_2$] and the complexes of these platinum compounds with dGuo

Compound	H8	C1′H	C2′H_2	C3′H_2	C4′H_2	C5′H_2	O-CH_3	C-CH_3
dGuo	7.98	6.30	2.79	4.62	4.13	3.79		
			2.51					
trans-[Pt(dGuo)$_2$(*E*-imino ether)$_2$](NO_3)$_2$	8.57	6.35	2.78	4.64	4.16	3.81	3.75	2.13
			2.59					
trans-[PtCl(dGuo)(*E*-imino ether)$_2$](NO_3)	8.39	6.32	2.75	4.63	4.15	3.82	3.77	2.44
			2.59					
trans-[Pt(NO_3)$_2$(*E*-imino ether)$_2$][a]							3.87	2.66
trans-[PtCl(NO_3)(*E*-imino ether)$_2$][a]							3.84	2.63

[a]In aqueous solution a rapid displacement of coordinated nitrate ions by solvent molecules takes place.

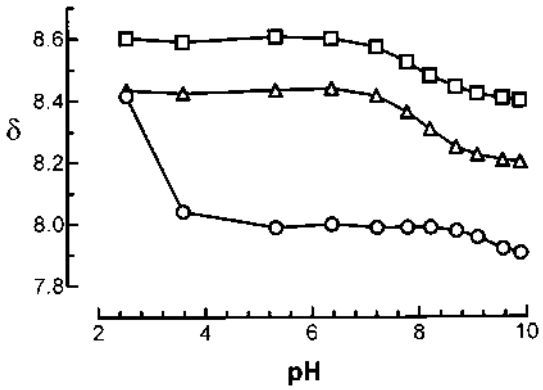

Figure 3. pH dependence of the H8 resonances (δ, downfield from trimethylsilylpropionic acid) of dGuo (○), *trans*-[PtCl(dGuo)(*E*-imino ether)$_2$]$^+$ (Δ) and *trans*-[Pt(dGuo)$_2$(*E*-imino ether)$_2$]$^{2+}$ (□) in the presence of 0.17 M KCl (the salt is required in order to suppress solvolysis of the mono-dGuo species and to keep ionic strength constant).

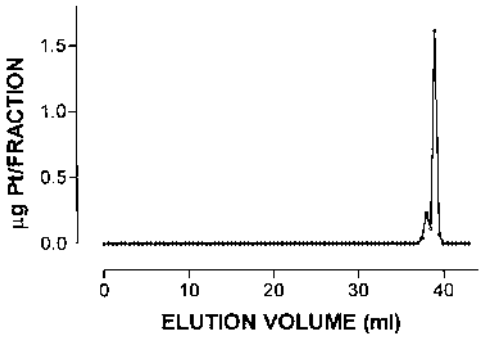

Figure 4. HPLC separations of the enzyme digestion products of 45 μg double-helical DNA modified by *trans*-EE (r_b = 0.08) in 10 mM $NaClO_4$ at 37°C for 48 h. The fractions were analyzed for Pt by FAAS.

HPLC analysis of enzymatic hydrolysates of *trans-EE*-modified DNA was performed by collecting fractions of 0.5 ml in which the platinum content was assayed by FAAS. The profile in Figure 4 shows one major peak derived from the DNA adduct of *trans-EE* which co-eluted with the standard containing one molecule of dGuo per *trans-EE*. A small peak on the left side of this major peak was also observed, which co-eluted with the standard sample containing two molecules of dGuo per *trans-EE*. Importantly, the incubation of the two HPLC standards with the enzymes and in the medium under conditions used to digest the platinated DNA resulted in no change in the elution times of these standards. About 90% platinum was recovered in the two peaks. The remainder represents a smear of platinum and does not correspond to any specific peak. This result is consistent with the view that even after a relatively long platination reaction (48 h) the major DNA adduct of *trans-EE* is a monofunctional adduct formed by coordination of this platinum complex to the N7 position of dGuo residues. In order to prove further the nature of the adducts, the products of the enzymatic hydrolysis of DNA modified by *trans-EE*, which were eluted in both peaks in the HPLC profile shown in Figure 4, were collected (~2 mg) and analyzed by ^{1}H NMR spectroscopy. As expected this analysis revealed a mixture of mono- and bifunctional adducts of *trans-EE* with dGuo in the ratio corresponding to that deduced from the HPLC profile shown in Figure 4.

CONCLUSIONS

The purpose of this study was to examine the effect of substitution of imino ether group for NH_3 in dichloroplatinum(II) complexes on their DNA-binding properties. The results presented here show that imino ether ligands do not alter radically the first step of the binding of bifunctional platinum complexes to DNA, i.e., the formation of monofunctional adducts at N7 position of guanine residues. Importantly, the imino ether substitution, especially in the *trans* geometry, results in a greatly reduced rate of closure of the monofunctional platinum lesions in bifunctional adducts. This result implies an important difference in the nature and frequency of the DNA adducts of *trans-EE* and clinically ineffective transplatin.

Recently, some *trans*-[$PtCl_2$(amine)$_2$] complexes containing sterically hindered planar ligands instead of simple NH_3 groups were reported (28,29). These complexes exhibit greatly enhanced cytotoxicity in tumor cells in comparison with transplatin and in several cases their cytotoxicity was equivalent to that of the clinically used cisplatin. These results along with those obtained for the imino ether derivatives invert the standard *cis*/*trans*

structure–pharmacological activity relationships observed previously for $[PtCl_2(NH_3)_2]$ complexes. The presence of bulky planar amine ligands has been found to enhance strongly DNA interstrand cross-linking capability of the complexes with *trans* geometry. It has been suggested that this enhanced interstrand cross-linking efficiency of *trans*-$[PtCl_2(amine)_2]$ complexes along with specific conformational changes in DNA could be relevant to their enhanced cytotoxicity in tumor cells.

The *trans-EE* complex also exhibits cytotoxicity in tumor cells which was much more pronounced than that of its *cis* congener. However, in contrast with the *trans* complexes of planar amine ligands and transplatin, *trans-EE* appears to form markedly lower amount of bifunctional DNA adducts. Thus, an important feature for biological activity of *trans-EE* is its capability to form in DNA relatively stable monofunctional adducts. Further investigations of conformational alterations induced in DNA by *trans-EE* are warranted to unravel the origin of antitumor activity of platinum complexes with leaving ligands in the *trans* configuration.

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