L-Methionine Inhibits Reaction of DNA with Anticancer *cis*-Diamminedichloroplatinum(II)†

Oldrich Vrana and Viktor Brabec*

Institute of Biophysics, Academy of Sciences of the Czech Republic, Kralovopolska 135, CZ-61265 Brno, Czech Republic

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ABSTRACT: Sufficient evidence has accumulated to identify DNA as the relevant pharmacological target of antitumor cisplatin [*cis*-diamminedichloroplatinum(II)]. This drug is administered intravenously so that before it reaches DNA in the nucleus of tumor cells it may interact with various compounds including sulfur-containing molecules such as L-methionine or the compounds containing these residues. L-Methionine increases the rate of reaction of cisplatin with monomeric guanosine 5′-monophosphate, and it was suggested on the basis of these results previously obtained by other authors that methionine residues could mediate the transfer of platinum onto DNA. We studied in the present work the reactions of the 1:1 complex formed between cisplatin and L-methionine or *N*-acetyl-L-methionine with synthetic, single- and double-stranded oligodeoxyribonucleotides and natural, high molecular mass DNA by using high-pressure liquid chromatography and flameless atomic absorption spectrophotometry. The results demonstrate that both L-methionine and *N*-acetyl-L-methionine decrease the rate of reaction of cisplatin with base residues in natural, high molecular mass DNA. Thus, the possibility that cisplatin bound to methionine residues serves as a drug reservoir available for platination of DNA in the nucleus of tumor cells appears unlikely.

The success of *cis*-diamminedichloroplatinum(II) (cisplatin)[1] and other antitumor platinum complexes in killing tumor cells results from their ability to form on DNA various types of covalent adducts (*1*−*3*). It has been shown (*4*−*6*) that bifunctional cisplatin reacts with DNA in a two-step process forming first monofunctional adducts preferentially at N(7) atoms of guanine residues which subsequently close to various intrastrand and interstrand cross-links (CLs). The second platination site in DNA CLs of cisplatin is most commonly guanine (*7*, *8*). Cisplatin is administered intravenously (*9*). Hence before it reaches DNA in the nucleus of tumor cells, it may interact with various compounds including sulfur-containing molecules (*10*). These interactions are generally believed to play a role in mechanisms underlying tumor resistance to antitumor platinum drugs, their inactivation, and side effects (*11*−*14*). Therefore, interest in the interactions of platinum antitumor drugs with sulfur-containing molecules of biological significance has recently markedly increased.

One such sulfur-containing molecule is L-methionine (Met). The reactions of Met with cisplatin have already been frequently examined. The major products of these reactions have been found to contain S,N-chelated Met, such as $[Pt(Met\text{-}S,N)(NH_3)_2]^+$ and $[Pt(Met\text{-}S,N)_2]$ (*15*, *16*). In addition, it has been demonstrated (*17*) that Met increases the rate of reaction of cisplatin with monomeric guanosine 5′-monophosphate (GMP). It has been suggested on the basis of these results that methionine residues could mediate the transfer of platinum onto DNA in tumor cells, which prompted us to investigate this possibility further. We studied in the present work the reactions of the 1:1 complex formed between cisplatin and Met or *N*-acetyl-L-methionine (AcMet) with single- and double-stranded synthetic oligodeoxyribonucleotides and natural high molecular mass DNA (denatured and double helical) by using high-pressure liquid chromatography (HPLC) and flameless atomic absorption spectrophotometry (FAAS). The results demonstrate that, in contrast to the expectations based on the efficiency of Met to increase the rate of reaction of monomeric GMP with cisplatin (*17*), both Met and AcMet inhibit binding of this antitumor drug to natural, polymeric DNA.

MATERIALS AND METHODS

Met, AcMet, cisplatin, deoxyguanosine 5′-monophosphate (dGMP), NaCN, DNase I from bovine pancreas, nuclease P1 from *Penicillium citrinum*, and alkaline phosphatase from calf intestine were purchased from Sigma-Aldrich sro (Prague, Czech Republic). The stock solution of cisplatin (1 mM in 10 mM $NaClO_4$) was prepared in the dark at 25 °C and used immediately after the complex was dissolved. The synthetic oligodeoxyribonucleotides (20 nucleotides long; Figure 1) were synthesized and purified as described previously (*18*). The concentrations of the single-stranded oligonucleotides are related to the whole molecule of the oligonucleotide (not to its monomeric content) similarly to the concentrations of double-stranded oligonucleotides which are related to the whole duplexes. The oligonucleotide duplex was formed by

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* Corresponding author: tel, 420-5-41517148; fax, 420-5-41240499; e-mail, brabec@ibp.cz.

[1] Abbreviations: AcMet, *N*-acetyl-L-methionine; cisplatin, *cis*-diamminedichloroplatinum(II); CL, cross-link; CT, calf thymus; dGMP, deoxyguanosine 5′-monophosphate; FAAS, flameless atomic absorption spectrophotometry; GMP, guanosine 5′-monophosphate; HPLC, high-pressure liquid chromatography; Met, L-methionine.

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CGGC: 5′-CCTCTCCTCGGCTCTCTTCT-3′
3′-GGAGAGGAGCCGAGAGAAGA-5′

CTGC: 5′-CCTCTCCTCTGCTCTCTTCT-3′
3′-GGAGAGGAGACGAGAGAAGA-5′

FIGURE 1: Sequences of the synthetic oligodeoxyribonucleotide duplexes used in the present study with their abbreviations. The top and bottom strands of each pair are designated top and bottom, respectively, in the text.

heating the mixture of the complementary single-stranded oligonucleotides at equal concentrations at 90 °C for 5 min, followed by incubation at 25 °C for 4 h. Calf thymus (CT) DNA (42% G + C, mean molecular mass ca. 20000 kDa) was prepared and characterized as described previously (*19*, *20*). Denatured CT DNA was prepared by heating the sample of native DNA at 100 °C for 10 min and subsequent rapid cooling on an ice bath.

Reactions of Met or AcMet with cisplatin in the 1:1 mole ratio were studied in 10 mM $NaClO_4$, pH 6, at a final concentration of both reactants of 0.5 mM at 37 °C in the dark if not otherwise stated. The aliquots were removed at various time intervals, quenched by quick cooling on an ice bath, and then warmed to 25 °C immediately prior the acquisition of HPLC profiles. Reactions of the 1:1 complex formed between cisplatin and Met or AcMet with dGMP and single- or double-stranded oligonucleotides (also at a 1:1 mole ratio) were examined in 10 mM $NaClO_4$, pH 6, at a final concentration of the reactants of 0.45 mM at 37 °C in the dark. The efficiency of free cisplatin to bind to CT DNA in the presence of Met or AcMet was examined under similar conditions. Cisplatin was preincubated with Met or AcMet in the 1:1 mole ratio (vide supra) for various periods; then 0.1 mL of these preincubated mixtures was quickly added to 0.9 mL of the solution of CT DNA (0.178 mg/mL) in 10 mM $NaClO_4$ and incubated for an additional 24 h at 37 °C in the dark. The mole ratio of Met and AcMet (or cisplatin) (all at a final concentration of 0.05 mM) to nucleotide phosphates at the onset of incubation with DNA was 0.1 (i.e., DNA was at a final concentration of 0.160 mg/mL). The treatment with 0.2 M NaCN of the product of the reaction of the cisplatin−Met or cisplatin−AcMet complex with dGMP was performed at pH ∼11 at 45 °C for 16 h in the dark. The following enzymatic digestion protocol was used to characterize the platinated oligodeoxyribonucleotides and CT DNA. The samples (50 μg of the oligonucleotides or DNA) were incubated with 72 units 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 units) was added and the incubation continued for additional 4 h at 37 °C. The digested samples containing constituent nucleosides were then heated for 2 min at 80 °C and centrifuged, and the supernatant was analyzed by reverse-phase HPLC. Each analysis was performed four times, and the data varied on average ±1% from their mean.

The HPLC profiles were recorded using the Merck/Hitachi HPLC system, model LaChrom, with a variable wavelength detector and with the following columns and eluents: reactions of cisplatin with Met or AcMet, an ET 125/4 Nucleosil 100-5 C18 column with H_2O as an eluent; reactions of dGMP, a Biospher PSI 120 C18/5 mm, MAC 4.6 × 125 column with 30% methanol/0.1 M ammonium acetate, pH 5.5, as an eluent; the samples of nondigested oligonucleotides, a NUCLEOGEL SAX 1000-8 column with 1 M NaCl/10 mM Tris-HCl, pH 7.4, as an eluent; enzymatically digested oligonucleotides and CT DNA, a Biospher PSI 120 C18/5 mm, MAC 4.6 × 150 column and isocratic elution with 0.1 M ammonium acetate, pH 5.0, in 4% CH_3CN at 1 mL/min flow rate. The concentration of the species separated by HPLC was measured by the integrated area of the corresponding peak in the HPLC profile. FAAS measurements were carried out on a Unicam 939 AA spectrometer with a graphite furnace. For FAAS analysis, DNA was dissolved in 0.1 M HCl. Absorption spectra were measured with a Beckmann DU-7400 spectrophotometer.

RESULTS AND DISCUSSION

Reaction of Cisplatin with Met and AcMet. The time course of reactions of cisplatin with Met at a 1:1 mole ratio was followed by HPLC with detection at 210 nm. The results (not shown) were similar to those obtained in the previous reports (*21*). Two peaks, corresponding to retention times of 2.2 and 3.7 min, were observed at the beginning of the reaction. These peaks were assigned to cisplatin and Met, respectively, via comparisons with chromatograms of these compounds alone and via spiking experiments. Both peaks had decreased in intensity during the reaction and completely disappeared after ca. 16 h. The chromatograms yielded by the aliquots of this reaction withdrawn at various time intervals also revealed a new major peak corresponding to a retention time of 6.7 min. Its integrated intensity increased during the reaction with a half-time of ca. 6 h and reached a maximum after ca. 18 h. Thus, for instance, 8%, 16%, 50%, and 100% of the product corresponding to this new peak were formed after 1, 2, 6, and 24 h of reaction. The fraction corresponding to this new peak (corresponding to a retention time of 6.7 min) was collected, and platinum FAAS and elemental analysis confirmed that this product contained one platinum and one sulfur atom. This finding was in a good agreement with the results published earlier (*15*, *17*) demonstrating that the major product of the reaction of Met and cisplatin in 1:1 mole ratio is $[Pt(Met\text{-}S,N)(NH_3)_2]^+$. The analogous results demonstrating that the major product of the reaction of AcMet and cisplatin in 1:1 mole ratio is $[PtCl(AcMet\text{-}S)(NH_3)_2]^+$ were also obtained.

Reactions of dGMP with Met−Cisplatin and AcMet−Cisplatin Complexes. In further studies reactions of the preformed 1:1 complex between Met and cisplatin with monomeric dGMP in the 1:1 mole ratio were examined. The complex between Met and cisplatin was formed in the reaction of 0.5 mM Met and 0.5 mM cisplatin as described above, and the product was purified by HPLC after 24 h of reaction. Then Met−cisplatin complex was mixed in the 1:1 mole ratio with dGMP, and the time course of this reaction was followed by HPLC. The conditions for this HPLC analysis were chosen so that dGMP and the 1:1 complex between cisplatin and dGMP were eluted at markedly different retention times (peaks G and G-Pt on curve 2 in Figure 2A). The integrated intensity of peak G decreased with time (Figure 2B).

Shortly after the reactants were mixed, a new peak labeled G-Pt-Met was observed (Figure 2A, curve 3), which subsequently increased in intensity at the expense of peak G and attained a maximum after ca. 24 h with the concomitant

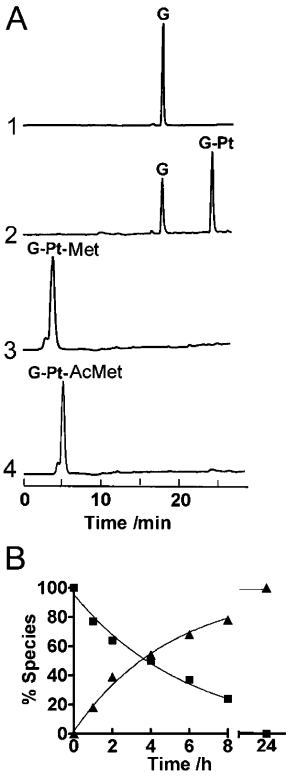

FIGURE 2: (A) HPLC profiles (absorbance at 260 nm versus retention time) of 0.5 mM dGMP (curve 1), 0.5 mM dGMP incubated with 0.5 mM cisplatin for 1 h (curve 2), and 0.45 mM dGMP incubated for 24 h with 0.45 mM 1:1 complex formed between cisplatin and Met (curve 3) or AcMet (curve 4). See the text for other details. (B) HPLC time course of reaction of 0.45 mM dGMP with 0.45 mM 1:1 complex formed between cisplatin and Met. Plot of the concentrations (as percentage of the total species) for dGMP (■) and the product of this reaction corresponding to the peak G-Pt-Met in panel A (▲).

disappearance of peak G (Figure 2A, curve 3, and Figure 2B). The separate HPLC analysis of the aliquots of this reaction withdrawn at the indicated times was also performed using conditions for analysis of the reactions of cisplatin and Met (see Materials and Methods) and revealed a decrease in the intensity of the peak assigned to Met during this reaction (not shown). Its time course (not shown) was similar to that observed for peak G (Figure 2B). The product of the reaction between dGMP and the Met−cisplatin complex was purified by HPLC. Platinum FAAS and elemental analyses revealed that the product contained the same amount of platinum and sulfur atoms. In addition, after treatment of this product with NaCN, dGMP was released as documented via comparisons with chromatograms of dGMP alone and via spiking experiments. Thus, these results were consistent with earlier findings (*17*) indicating that a major product formed on reaction of cisplatin with GMP and Met in a 1:1:1 mol ratio is [Pt(Met-*S*,*N*)(5′-GMP-*N7*)(NH_3)]$^+$ and that the minor product is also *cis*-[Pt-(5′-GMP-*N7*)(Met-*S*)(NH_3)$_2$]$^{2+}$. A surprising finding of these earlier studies was that the reaction of GMP with cisplatin in the presence of Met was even faster than in its absence as a consequence of the fact that the reaction takes a different course than in the absence of Met. It has been demonstrated (*17*) that the latter major product arises via labilization of NH_3 from the intermediate complex [Pt(Met-*S*,*N*)(NH_3)$_2$]$^+$ owing to the high trans effect of sulfur, which is a frequent product formed on reaction of cisplatin with GMP in the presence of Met in a 1:1:1 mole ratio.

It has been suggested (*17*) that in biological systems the Met residue could play a role in the transfer of platinum onto DNA as the amino acid itself or part of a peptide or protein. The eventuality that platinum can also be transferred from the 1:1 Met−cisplatin complex onto dGMP via displacement of monodentate S-bound Met has been examined in the present work using *N*-acetyl-L-methionine, which can only coordinate through the sulfur atom. The 1:1 AcMet−cisplatin complex was mixed in the 1:1 mole ratio with dGMP under the same experimental conditions (Figure 2), and the time course of this reaction was followed by HPLC. The results were similar to those obtained when the reaction of the 1:1 complex of Met−cisplatin was examined (see the text above and Figure 2) with one exception: new peak labeled G-Pt-AcMet corresponded to a retention time of 5 min (Figure 2A, curve 4), which was slightly longer that that corresponding to the peak labeled G-Pt-Met (Figure 2A, curve 3). Hence, these results support the view that under conditions of our measurements dGMP could also form monodentate Met species, such as *cis*-[Pt-(5′-GMP-*N7*)(Met-*S*)(NH_3)$_2$]$^{2+}$, but as shown in the previous study (*17*), this monodentate methionine species is a minor product in the reactions of the amino acid itself but might be a major product in the reactions of peptides or proteins containing Met residue(s).

Reactions of Short Synthetic Oligodeoxyribonucleotides with Met−Cisplatin and AcMet−Cisplatin Complexes. To test the hypothesis that in biological systems the Met residue could play a role in the transfer of platinum onto DNA, we also investigated interactions of the preformed 1:1 complex between Met and cisplatin with synthetic, short single- or double-stranded oligodeoxyribonucleotides by HPLC. Single-stranded oligonucleotide (20-mer, the top, pyrimidine-rich strand of the duplex CGGC in Figure 1 containing two guanine residues at its central part) was incubated with the Met−cisplatin complex, and the aliquots were withdrawn at various times. These aliquots were separated into three parts. The samples of the first series were directly analyzed by HPLC. After only ca. 24 h a small decrease (∼10%) of the integrated intensity of the peak corresponding to the nonmodified oligonucleotide (the peak labeled oligo in Figure 3A) was observed, and a small, new peak (labeled Pt-oligo) was detected on the chromatogram. The integrated intensity of the major peak corresponding to the nonmodified single-stranded oligonucleotide was further slowly reduced with time; for instance, after 72 h its integrated intensity had decreased by ca. 25% (Figure 3B). The samples of the second series of the aliquots were exhaustively dialyzed to remove the Met−cisplatin complex (which remained free in the solution), and the dialyzed samples were analyzed for platinum content by FAAS. The results are shown in Figure 3B and indicate that after 24 h of the reaction only ca. 12% of the total platinum present in the reaction was bound to the oligonucleotides and that after 72 h the amount of platinum bound increased only slightly to ca. 25%. The samples of the third series were enzymatically digested to deoxyribonucleosides, and changes in the HPLC pattern were examined. The peaks corresponding to single nucleosides were unchanged even after 72 h of the reaction except for the peak corresponding to deoxyriboguanosine (not shown).

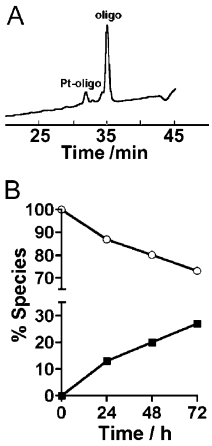

FIGURE 3: Reaction of 0.45 mM single-stranded top strand of the oligodeoxyribonucleotide duplex CGGC (Figure 1) with 0.45 mM 1:1 complex formed between cisplatin and Met. (A) HPLC profiles (absorbance at 260 nm versus retention time) of the products formed after 24 h of this reaction. (B) Time course of the reaction. Plot of the concentrations (as percentage of the total species) for platinum bound to the oligonucleotide (■) and nonmodified oligonucleotide (○).

The integrated intensity of this peak slightly, but significantly, decreased with time. For instance, after 24 and 72 h the integrated intensity of the deoxyriboguanosine peak was ca. 90% and 75% of the initial value, respectively. These results indicate that, in the reaction of the Met−cisplatin complex with the single (top) strand of the short synthetic duplex, CGGC guanine residues were platinated preferentially. Similar results (not shown) were obtained if the Met−cisplatin complex was incubated with the single (top) strand of the duplex CTGC (Figure 1). However, more importantly, no binding of platinum to the oligonucleotide duplexes CGGC or CTGC was noticed either by HPLC or by FAAS analysis even after 72 h of reaction. The results demonstrate that the rate of the reaction of the Met−cisplatin complex with the residues in synthetic, short single-stranded oligonucleotides only 20 nucleotides long is markedly reduced compared to the reaction with monomeric dGMP. A similar result has been described recently (*22*), also demonstrating that the sulfur atom of the platinum−thioether adduct can be substituted by short, single-stranded oligonucleotides. The present work, however, also demonstrates that this reaction is entirely inhibited if the Met−cisplatin complex is incubated with short DNA duplexes only 20 base pairs long. Thus, these results indicate that reaction of cisplatin in the presence of monomeric Met with guanine residues in short oligonucleotide duplexes via labilization of NH_3 from the intermediate complex $[Pt(Met\text{-}S,N)(NH_3)_2]^+$ is unlikely although this reaction may proceed, but with relatively small yields in short single-stranded oligodeoxyribonucleotides.

In biological systems the Met residue could be a part of a peptide or protein and therefore be less likely to chelate. Hence, DNA might be platinated in the presence of peptides or proteins containing Met residues by a different pathway—via displacement of sulfur in the monodentate Met species, such as *cis*-$[PtCl(Met\text{-}S)(NH_3)_2]^+$ by guanine residues in DNA. To test this eventuality, we also investigated interactions of the preformed 1:1 complex between AcMet and cisplatin with oligonucleotide duplexes CGGC and CGTC in the same way as we examined interactions of these duplexes with the 1:1 Met−cisplatin complex (vide supra). Importantly, no binding of platinum to the oligonucleotide duplexes CGGC or CTGC was noticed either by HPLC or FAAS analysis even after 72 h of reaction although a small level of platination was noticed (∼15%) if the duplexes were replaced in these reactions by short single-stranded oligonucleotides (top strand of the duplexes CGGC or CGTC shown in Figure 1). Thus, all of these results support the idea that Met residues, which could be the amino acid itself or part of a peptide or protein, do not play a marked role in the transfer of platinum onto double-stranded DNA as was suggested in earlier reports (*11*, *17*).

The rate of the reaction of the 1:1 Met−cisplatin complex with short single-stranded oligonucleotides is very low compared to the reaction with the monomeric dGMP. As short oligonucleotide duplexes and, as shown below, also high molecular mass, natural DNA (single or double stranded) do not react with the 1:1 Met−cisplatin complex, a small reactivity of short, single-stranded oligonucleotides is hardly relevant to the situation in biological systems containing DNA having a molecular mass higher by several orders of magnitude. Nevertheless, it might be of interest to compare the products of these reactions. The product of the reaction (72 h) of the single-stranded oligonucleotide CTGC with the 1:1 Met−cisplatin complex was purified by HPLC and enzymatically hydrolyzed to deoxyribonucleosides in the same way as described in the Materials and Methods section of this paper. The HPLC profile contained peaks C, G, and T, which were identical to those produced by the nonmodified (control) oligonucleotide and were assigned to deoxyribocytidine, deoxyriboguanosine, and thymidine, respectively, via comparisons with chromatograms of these compounds alone and via spiking experiments. Peak G was only slightly reduced (by ∼20%), indicating that the 1:1 Met−cisplatin complex only reacted with the guanine residue in a small fraction of the molecules of the CTGC oligonucleotide present in the reaction mixture. The single new small peak appeared in the HPLC profile of the digested single-stranded oligonucleotide treated with the 1:1 Met−cisplatin complex. It corresponded exactly to the retention time of the peak of the major product of the reaction of dGMP with the 1:1 Met−cisplatin complex, which was subsequently treated with alkaline phosphatase. Thus, these results are consistent with the view that the products of the reactions of the 1:1 Met−cisplatin complex with dGMP or guanine residues contained in the short synthetic single-stranded oligonucleotides are identical.

Reactions of Natural, High Molecular Mass DNA with Met−Cisplatin and AcMet−Cisplatin Complexes. In the final series of experiments we examined interactions of the 1:1 complex between Met and cisplatin with natural CT DNA in native or denatured form. First, we tested the efficiency of free cisplatin to bind to CT DNA in the presence of Met. Cisplatin was preincubated with Met in the 1:1 mole ratio (vide supra) for various times (0, 1, 2, 6, or 72 h). As has been described above, under the conditions of these experiments cisplatin forms the 1:1 complex with Met (in absence of DNA) with a half-time of ca. 6 h; thus we prepared the 1:1 mixtures of cisplatin and Met that contained a decreasing amount of free cisplatin and a concomitantly increasing amount of the 1:1 Met−cisplatin complex. These preincubated mixtures were added to the solution of native or denatured CT DNA, further incubated for additional 24 h,

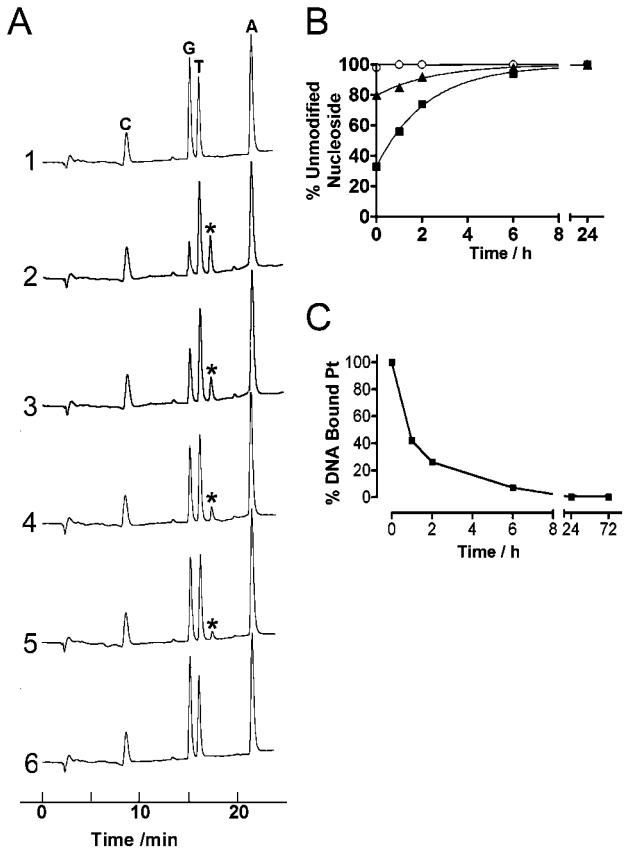

FIGURE 4: Reaction of native CT DNA at a concentration of 0.16 mg/mL with the 1:1 mixture of cisplatin and Met preincubated for various times. The mole ratio of Met (or cisplatin) (both at the final concentration of 0.05 mM) to nucleotide phosphates at the onset of incubation with DNA was 0.1. After addition of the mixture of cisplatin and Met to DNA the resulting solution was incubated for additional 24 h. (A) HPLC profiles (absorbance at 260 nm versus retention time) of the enzymatically digested products. Curves: 1, control DNA in the absence of cisplatin and Met; 2−6, DNA incubated with the 1:1 mixture of cisplatin and Met preincubated for 0, 1, 2, 6, and 72 h, respectively. See the text for other details. (B) Time course of the reaction. Plot of the concentrations (as percentage of the total species) for nonmodified deoxyribonucleosides corresponding to peak G (■), peak A (▲), and peaks C and T (○) in panel A. (C) Time course of the reaction. Plot of the concentrations (as percentage of the total species) for platinum bound to DNA.

precipitated by ethanol, and redissolved in 50 mM sodium acetate, pH 6. One part of these samples was enzymatically digested to deoxyribonucleosides and analyzed by HPLC whereas the other part was analyzed by FAAS for platinum content.

The HPLC profile of enzymatically digested double-helical DNA (control, nonmodified) is shown in Figure 4A, curve 1. It contains four well-separated peaks labeled C, G, T, and A which were assigned to deoxyribocytidine, deoxyriboguanosine, thymidine, and deoxyriboadenosine, respectively, via comparisons with chromatograms of these compounds alone and via spiking experiments. If the 1:1 mixture of Met and cisplatin preincubated for 0−6 h was added to double-helical DNA and further incubated for additional 24 h, the HPLC profiles of the digested DNA contained peaks C and T which were identical to those produced by nonmodified (control) DNA whereas the integrated intensity of peaks G and A was reduced (for instance, after preincubation of 1 h peak G was reduced by ca. 45% and peak A by 17%, curve 3 in Figure 4A and Figure 4B). A new (small) peak labeled by an asterisk on curves 2−5 in Figure 4A was also detected on these chromatograms, which was assigned to *cis*-Pt(NH_3)$_2$d(GpG) via comparisons with chromatograms of this compound alone and via spiking experiments. The reduction of the integrated intensities of peaks G and A was diminished with the increasing time of the preincubation (curves 2−5 in Figure 4A and Figure 4B), and concomitantly the integrated intensity of the peak assigned to *cis*-Pt(NH_3)$_2$d(GpG) (the peak labeled by an asterisk on curves 2−5 in Figure 4A) was decreased. These results can be interpreted to mean that a significant fraction of free cisplatin (not contained in the complex of Met with cisplatin) present in the mixture of cisplatin and Met preincubated for 0−6 h was bound to DNA and that the higher amount of cisplatin was contained in the complex with Met (with increasing time of the preincubation of cisplatin with Met) and the lower amount of cisplatin was bound to DNA. Consistent with this conclusion is also the finding that after 24 h of the preincubation of the 1:1 mixture of cisplatin with Met (when all cisplatin molecules were already contained in the complex with Met) the HPLC profile of the digested DNA was identical to that yielded by the sample of the control (nonmodified) DNA (cf. curves 1 and 6 in Figure 4A).

The other part of the samples of double-helical DNA incubated for 24 h with the 1:1 mixture of Met and cisplatin preincubated for various periods was analyzed by FAAS for platinum content. Consistent with the HPLC analysis of these samples, which were enzymatically digested (Figure 4A,B), the amount of platinum bound to DNA decreased with the growing time of the preincubation of the mixture of Met and cisplatin, and no platinum bound to DNA was found if this preincubation was even 72 h (Figure 4C).

The identical results were also obtained if the same HPLC and FAAS analyses were performed with the samples in which double-helical DNA was replaced by denatured DNA. In addition, no platination of DNA was noticed even if DNA was incubated with the preformed 1:1 Met−cisplatin complex for 10 days. Thus, natural, high molecular mass DNA either in native or denatured form is not platinated if all molecules of cisplatin are contained in the preformed 1:1 complex with Met.

As already mentioned above, DNA might be platinated in the presence of peptides or proteins via displacement of sulfur in the monodentate Met species. Therefore, we also investigated interactions of the preformed 1:1 complex between AcMet and cisplatin with denatured and double-helical CT DNA in the same way as we examined interactions of these DNAs with the 1:1 Met−cisplatin complex (vide supra). Importantly, no binding of platinum to denatured or double-helical CT DNA was noticed after 72 h of reaction. Thus, consistent with the results of the analysis of the reactions in the presence of short, synthetic double-stranded oligonucleotide duplexes, the results obtained in the presence of CT DNA demonstrate that Met residues, either as the amino acid itself or as a part of peptides or proteins, play no significant role in the transfer of platinum onto natural DNA.

CONCLUSIONS

Biotransformation of cisplatin involves various reactions with plasma protein cysteine SH and Met SCH_3 groups.

Irreversible binding of sulfur nucleophiles is thought to be the reason for the toxic side effects of this anticancer platinum drug (*13*). Therefore, the previous finding (*17*) that the reaction of monomeric GMP with cisplatin is faster in the presence of Met than in its absence is somewhat surprising. The reason for this has been proposed to be that the reaction takes a different course. The major product of the 1:1 reaction between cisplatin and Met is [Pt(Met-*S*,*N*)-$(NH_3)_2]^+$ (*15*, *16*). Owing to the high trans effect of sulfur, the NH_3 of this product is labilized so that its displacement by monomeric GMP or dGMP is more favorable. In addition, in biological systems the Met residue could be a part of a peptide or protein and therefore be less likely to chelate. We have shown in the present work that the reaction of dGMP with the 1:1 complex formed between cisplatin and AcMet also results in the platination of this monomeric nucleotide. Thus, hypothetically, DNA might be platinated in the presence of monomeric Met or peptides or proteins containing Met residues via displacement of sulfur in the monodentate Met species by guanine residues in DNA.

The present work has demonstrated that, in contrast to the reactions of monomeric GMP or dGMP, Met, which could be the amino acid itself or part of a peptide or protein, inhibits the reaction of antitumor cisplatin with base residues in natural, high molecular mass DNA. Hence, incorporation of dGMP into the polynucleotide chain makes this reaction apparently less favorable. The reasons for this effect of the polynucleotide chain remain unclear, but owing to the incorporation of dGMP into the polynucleotide chain, the N7 atom of guanine, which is a preferential site of platination, may be less accessible to sterically more demanding complexes formed between cisplatin and Met. This steric demand of these complexes may be even more pronounced in double-helical DNA consistently with a considerably lower extent of the platination of the short, single-stranded oligonucleotides by the 1:1 complexes formed between cisplatin and Met or AcMet in comparison with the corresponding short duplex observed in the present work.

The results of the present work demonstrate that the possibility proposed earlier (*11*, *12*), that cisplatin bound to monomeric methionine or to methionine residues in peptides or proteins could potentially act as a drug reservoir available for platination of DNA in the nucleus of tumor cells, appears unlikely. Thus, the major roles for sulfur-containing compounds in the mechanism of biological activity of cisplatin remain their participation in its toxic side effects and in the processes associated with acquired resistance of certain tumors against cisplatin.

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