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ESTIMATION OF THE EXTENT OF PLATINATION AND THE SITES OF Pt BINDING AFTER INTERACTION OF *cis*- AND *trans*--DIAMMINEDICHLOROPLATINUM(II) WITH DNA AND CHROMATIN*

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Differences in the mode of binding of *cis*- and *trans*-diamminedichloroplatinum(II) complexes (*cis* and *trans*-DDP) with DNA and chromatin were studied with the use of [^{14}C]methylbromphenvinphos as an alkylating agent which attacts the sites in purines bases involved also in the reaction with *cis*-DDP (Oliński *et al.*, *J. Biochem. Biophys. Meth.*, **7**, 171 - 173, 1983). Methylation of pre-formed DDP-DNA and DDP-chromatin complexes, followed by qualitative and quantitative analysis of the methylation products in DNA hydrolysates, permitted evaluation of the distribution and extent of platination of the bases. No major differences were found between the action of the two DDP isomers on DNA. However, a significant decrease in binding of *trans*-DDP to adenine moieties was observed when the interaction of *cis*- and *trans*-DDP on chromatin was compared.

The effectiveness of *cis*-diamminedichloroplatinum(II) [1] in treating a variety of human malignancies has led to extensive study of its mechanism of action (Roberts & Thomson, 1979; Drobnik, 1983; Marcelis & Reedijk, 1983 and references therein). The antitumour effect of *cis*-DDP is believed to involve its binding to DNA bases but the precise mechanism of its cytotoxicity is far from being established. Though *trans*-DDP reacts essentially equally with DNA, it is biologically inactive (Drobnik, 1983 and references therein). Therefore, the differences in the effectiveness should be due to differences in the type of binding. Thus, it is of particular interest to compare the interaction of both DDP isomers with DNA.

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[1] Abbreviations used: *cis*- and *trans*-DDP, *cis*- and *trans*-diamminedichloroplatinum; PMSF, phenylmethylsulphenylfluoride.

As it has been shown in our previous paper (Oliński *et al.*, 1983), the use of [^{14}C]methylbromphenvinphos is suitable for defining the sites in purine bases involved in the reaction with *cis*-DDP and for determining the extent of platination. Both from the literature data and from our experiments (Roberts & Thomson, 1979; Marcelis & Reedijk, 1983; Oliński *et al.*, 1983) it follows that Pt(II) complexes react with the same sites of a DNA molecule as the alkylating agent. Therefore, a decrease in the methylation rate observed when DNA is incubated with *cis*-DDP prior to the reaction with the alkylating agent permits evaluation of the extent of platination (Oliński *et al.*, 1983).

The purpose of the present work was to apply this method in order to determine the sites and the extent of platination of the bases in DNA after interaction of the two DDP isomers with free DNA and chromatin.

MATERIALS AND METHODS

DNA was isolated from calf thymus by the method of Zamenhof (1953). The RNA content estimated by the orcinol method (Schneider, 1957) as well as protein content estimated by the method of Lowry *et al.* (1951) was lower than 1%. DNA was dissolved in 0.05 M-$NaClO_4$, pH 6.4, at a concentration of 1 mg/ml (such a DNA is further referred to as "naked DNA"). Nuclei were isolated from calf thymus by the hypotonic shock procedure (Wilhelm *et al.*, 1972) and further purified by centrifugation through 0.25 M-sucrose followed by a 0.5% Triton X-100 wash. Chromatin was isolated according to Spelsberg & Hnilica (1971). The isolated chromatin preparations (1 mg DNA/ml) were rehydrated in 0.05 M-$NaClO_4$/0.1 mM--PMSF, pH 6.4. Both DDP isomers were dissolved in 0.05 M-$NaClO_4$, pH 6.4, and hydrolysed for 72 h at 37°C. Interaction of the Pt complexes with DNA or chromatin was allowed to proceed at 37°C for 2 h at Pt/deoxynucleotide ratios (r) of 0.3 - 0.5. After that time the possible unreacted Pt was dialysed off. To get rid of the chromosomal protein, the chromatin samples were digested by proteinase K (0.1 mg/ml) and proteins removed by the procedure of Zamenhof (1953). The ethanol-precipitated DNA was dissolved in 10 mM-Tris/HCl, pH 8.1, digested by RNAase (0.1 mg/ml) and extracted according to Zamenhof (1953). The content of RNA in the DNA samples obtained in this way was lower than 1%, and protein impurities did not exceed 3%. DNA was precipitated with 96% ethanol and dissolved in 0.05 M-$NaClO_4$ at a concentration of 1 mg/ml. To both samples of DNA solution, 10 µl of [^{14}C]methylbromphenvinphos dissolved in 96% ethanol was added to give a final concentration of 1.5 mM, and incubation was continued for another 72 h. DNA was then precipitated with 96% ethanol, unbound radioactivity was washed out with 96% ethanol, and DNA was hydrolysed with 0.5 M-HCl. The Dowex

50W × 2 200 - 400 mesh columns were applied to separate the bases. The bases were loaded on a column (20 × 1 cm) and eluted by the gradient of 0.75 - 4 M-HCl at a flow rate of 0.5 ml/min. Fractions of 5 ml were collected. Samples were then dried, dissolved in 1 ml of 0.1 M-HCl and, after addition of 10 ml of Tritosol, radioactivity was measured on a Technicon scintillation counter.

The control single-stranded DNA was treated in the same way except that no *cis*-DDP was added (cf Oliński *et al.*, 1983).

Reagents. PMSF and *cis*-DDP were purchased from Sigma Chem. Co. (St. Louis, Mo., U.S.A.). Tris and proteinase K were from Serva Feinbiochemica (Heidelberg, F.R.G.). RNAase was a product of Koch-Light Lab. Ltd (Colnbrook, Bucks., England). Dowex 50W × 2 was supplied by Fluka A.G. (Buchs SG, Switzerland). *Trans*-DDP NSC 132558 was from the National Cancer Institute (Bethesda, U.S.A.). [^{14}C]Methylbromphenvinphos was synthesized at the Institute of Radiation Technics (Technical University of Łódź). Other reagents were analytical grade products supplied by POCh (Gliwice, Poland).

RESULTS

Estimation of DDP binding to the bases after the reaction of the drugs with DNA ("*naked DNA*"). Similarly to the results obtained in the previous work (Oliński *et al.*, 1983), we observed a sharp decrease in the methylation ratio of purine bases after the preincubation of DNA with *cis*-DDP (Fig. 1). The extent of methylation of guanine at N(7) and of adenine at N(1) and N(7) dropped to 10, 83 and 61%, respectively (Table 1). These results suggest that 90% of all guanine moieties at the N(7) position and 56% of adenine moieties [17% at the N(1) and 39% at the N(7) positions] were platinated after the reaction of DNA with *cis*-DDP under the conditions of the experiment.

Table 1

The extent of methylation of purines (in percent) after the reaction of platinated DNA (or DNA isolated from platinated chromatin) with [^{14}C] methylbromphenvinphos in vitro

The extent of purine methylation after the reaction of non-platinated DNA with [^{14}C]methylbromphenvinphos was taken as 100%. The results are mean values of 5 determinations ± S.D.

Methylated base	*cis*-DDP		*trans*-DDP	
	DNA	chromatin	DNA	chromatin
1-Methyl guanine	10 ± 2	21 ± 4	15 ± 3	28 ± 4
7-Methyl adenine	61 ± 7	67 ± 4	64 ± 6	83 ± 4
1-Methyl adenine	83 ± 3	88 ± 6	82 ± 3	92 ± 5

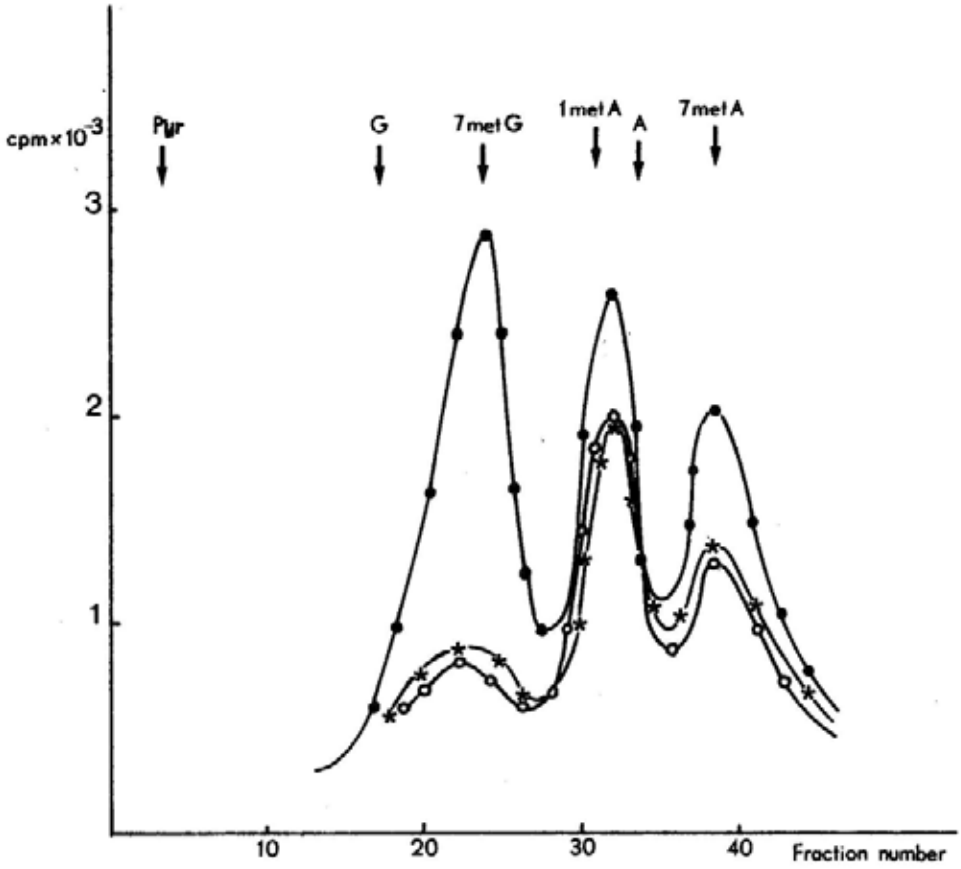

Fig. 1. Dowex 50W chromatography of the control and platinated DNA after the reaction with [^{14}C]methylbromphenvinphos. The elution positions of standards, detected by UV absorption, are marked by arrows. Pyr, pyrimidine nucleotides; G, guanine; 7metG, 7-methylguanine; 1metA, 1-methyladenine; A, adenine; 7metA, 7-methyladenine. DNA after the reaction with: ○, *cis*-DDP; *, *trans*-DDP; ●, control DNA.

After the alkylation of DNA complexed with *trans*-DDP, 15% of guanines at N(7) position and 46% of adenine moieties at N(1) and 82% at N(7) position were methylated (Table 1). This means that the extent and distribution of platination of DNA with *cis*- and *trans*-DDP proved to be very similar.

Estimation of DDP binding to the bases after reaction of the drugs with chromatin. In order to approximate better the conditions prevailing in the living cell, comparative studies were done with chromatin, as described under Methods.

A, cis-DDP. When one compares the results of methylation after the reaction of the *cis*-DDP isomer with chromatin and with "naked DNA",

no major differences are seen in the distribution of methyl groups between various sites (Fig. 2), though the percentage of methylation at all the purine ring nitrogen atoms was somewhat higher in the case of chromatin (Table 1). It can be thus concluded that 79% of guanine and 45% of adenine moieties [12% at N(1) and 33% at N(7)] were platinated.

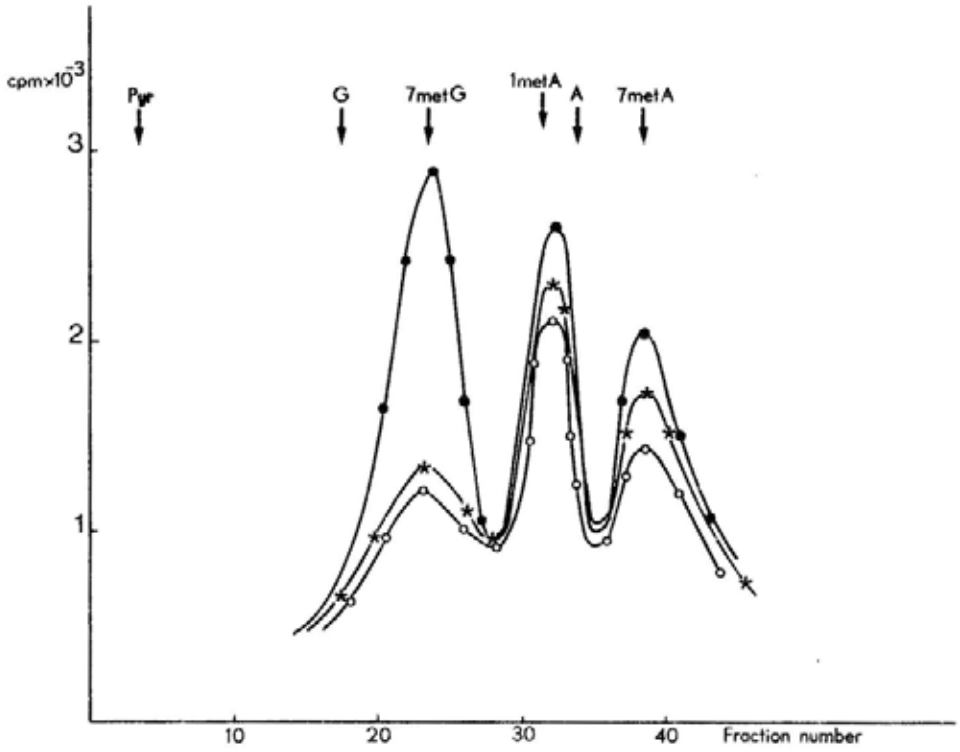

Fig. 2. Dowex 50W chromatography of DNA isolated from control and platinated chromatin preparations, after the reaction with [^{14}C]methylbromphenvinphos. The elution positions of standards are marked by arrows. DNA after the reaction with: ○, *cis*-DDP; ⋆, *trans*-DDP; ●, control DNA. Other designations as in Fig. 1.

B, trans-DDP. The results shown in Table 1 indicate that, under conditions of the experiment, 28% of guanine moieties were methylated at N(7), and 92% of adenine moieties at N(1) and 83% at N(7). Therefore, the extent of platination of the purine bases after the reaction of chromatin with *trans*-DDP was different from that observed after the reaction of "naked DNA" with this compound. There was only a small drop in the platination of guanine moieties. However, greater reduction in the extent of platination of adenines can indirectly be inferred from Fig. 2.

DISCUSSION

The results described confirm our earlier findings concerning the action of *cis*-DDP on DNA (Oliński *et al.*, 1983), and extend them by comparing the action of *cis*-DDP with its biologically inactive isomer, *trans*-DDP. Since the presence of chromosomal proteins in cell nuclei could modify the interaction of Pt(II) complexes with the DNA target, we used also the method described previously (Oliński *et al.*, 1983) to study the interaction of the drugs with chromatin. The literature data as well as our earlier results showed that the most preferred sites in guanine moieties are N(7), and in adenine N(1) and N(7) (Roberts & Thomson, 1979; Marcelis & Reedijk, 1983; Oliński *et al.*, 1983). Up to now there were no literature data available concerning the action of the two DDP isomers in respect of the sites and extent of platination. The results presented here indicate that no differences exist between the binding of the two DDP isomers in respect of the sites of DNA platination.

After incubation of "naked DNA" with the two Pt(II) complexes, the extent of platination was also similar (cf Fig. 2). However, when we compared the binding of the DDP isomers during the reaction with chromatin, some differences did appear (cf Fig. 2 and Table 1). A significant decrease in binding of *trans*-DDP to both adenine positions was observed. Only 25% of adenine moieties were platinated after the reaction of the *trans* isomer with chromatin in comparison with 45% after the interaction of *cis*-DDP. The smaller amount of platinated adenines could be due to greater affinity of *trans*-DDP to chromosomal proteins, leading to less extensive platination of DNA.

Trans-DDP is more likely to bind to sulphur atoms in proteins (Aull *et al.*, 1977) and to form protein-protein or DNA-protein cross-links than *cis*-DDP (Lippard & Hoeschele, 1979; Zwelling *et al.*, 1979; Oliński & Walter, 1984). However, we cannot rule out the possibility that a part of the observed decrease of platination could be a result of blocking of the reactive groups in purine bases by binding with chromosomal proteins. Summing up, the differences observed in the interaction of *cis*- and *trans*-DDP with chromatin may prove helpful for explanation of varied biological activities of these drugs.

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