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**INTERSTRAND DNA-PLATINUM-DNA/CROSS-LINKS INDUCED  
BY CIS- AND TRANS-DIAMMINEDICHLOROPLATINUM(II)  
AT ELEVATED TEMPERATURE \***

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***Trans-diamminedichloroplatinum(II) (trans-DDP) forms with DNA at 37°C, more numerous interstrand cross-links than cis-DDP in the isolated DNA and DNA in the chromatin complex. An increase in the temperature to 42.5°C had no effect on the interstrand cross-links of DND-Pt-DNA formed by the two isomers, both in DNA and in chromatin.***

Among the coordinative platinum compounds, *cis*-diamminedichloroplatinum(II) (*cis*-DDP) is one of the most active antitumour drugs.

Since hyperthermia combined with several of the commonly applied antitumour drugs, among others with *cis*-DDP, enhances their therapeutic effect [1] it may be assumed that this might be due to the increase in the number of cross-links of the type DNA-Pt-DNA and DNA-Pt-protein [2]. Also the appearance of the tumour properties of *trans*-DDP at elevated temperature might be correlated with increase in the number of DNA-Pt-protein cross-links observed by Murthy *et al.* [3].

The aim of the present work was to examine the effect of hyperthermia (42°C) on the formation of interstrand cross-links of the type DNA-Pt-DNA in DNA preparations and in the chromatin complex with both DDP isomers.

#### MATERIALS AND METHODS

Commercial preparations of DNA from calf thymus, and chromatin isolated from pig peripheral blood lymphocytes were used. Lymphocytes were isolated by the method of Boyum [4] as modified by Geremek & Walter [5],

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cell nuclei as described by Scovel *et al.* [6] and chromatin according to Spelsberg & Hnilica [7].

DNA and chromatin preparations were dissolved in 0.01 M borate buffer, pH 9.5, to a final concentration of 40  $\mu\text{g}$  DNA/ml, and incubated with *cis*- and *trans*-DDP at the  $r_i$  values of 0.025, 0.05, 0.1, and 0.5 ( $r_i$  express  $\mu\text{mol}$ s of Pt introduced per  $\mu\text{mol}$  of DNA) at 37°C or 42.5°C for 2 and 18 h.

The number of the interstrand cross-links formed by DDP was evaluated by the spectrophotometric measurement of ethidium bromide binding to DNA [8-10]. The results are presented as the ratio of the number of interstrand cross-links (Ct) formed to DDP concentration ( $r_i$ ).

### RESULTS AND DISCUSSION

Formation of the interstrand cross-links in isolated DNA preparations depended both on the incubation time and concentration of *cis*- and *trans*-DDP. The number of the DNA-Pt-DNA interstrand cross-links was after 2 h incubation similar for both DDP isomers, whereas on prolonged incubation (18 h) this increase was distinctly greater for the *trans* isomer (Fig. 1) both with the isolated DNA and DNA remaining in the nucleoprotein complex. This is due to a greater tendency of *cis*-DDP to form interstrand cross-links, irrespective of the form in which DNA is present. Lower formation of interstrand cross-links with DNA in the chromatin complex than with isolated DNA, by both isomers comes from the formation of cross-links in DNA-Pt-protein. The similarity of the results obtained for the two DNA preparations after 2 h incubation with *cis*- and *trans*-DDP, is due probably to direct platination of DNA leading to formation mainly of the monofunctional adducts, with but only initiation of bifunctional adducts, which are favoured during prolonged incubation. Slowing down of the rate of cross-links formation with increasing concentration of *cis*- and *trans*-DDP with incubated for 18 h could result from the lower susceptibility to the denaturing conditions due to cross-links formed in DNA-Pt-DNA and protein-Pt protein (Fig. 1).

The results obtained at 37°C and 42.5°C indicated that hyperthermia had no effect on formation of the interstrand cross-links in DNA-Pt-DNA by the two DDP isomers in isolated DNA and in the chromatin complex (Fig. 1 A, B).

Our results differ from those obtained for the cell lines SCC-25 and SCC-25/CP by Herman *et al.* [2], who observed a distinct higher number of cross-links at 42°C than at 37°C, directly after incubation of the cells with DDP and 24 h later, when the repair processes could be operating. Our experiments were performed with isolated structures thus neither metabolism nor repair systems could affect the results.

In our opinion more efficient formation of cross-links with DDP in the cells under hyperthermia is due to the effect of platinum compounds on enzymatic systems.

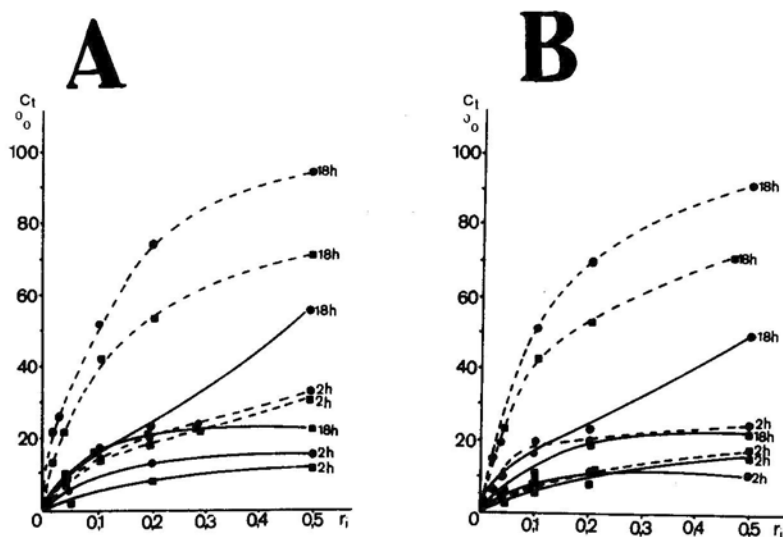


Fig. 1. Interstrand cross-links of DNA-Pt-DNA formed from DNA (●) and chromatin (■) by *cis*- (---) and *trans*- (—) diamminedichloroplatinum(II) after incubation at 37°C (A) and 42.5°C (B)

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