The Effect of Glutathion on the Reaction of *cis*- and *trans*-Diamminedichloroplatinum(II) with DNA

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Differential pulse polarography study was used to investigate the influence of glutathion on *cis*- and *trans*-DDP induced alterations of DNA structure. Though the applied concentration of glutathion has no effect on the reaction of DNA with *cis*-DDP, it greatly modifies the reaction with *trans* isomer. This may be an important reason for the ineffectivness of the *trans*-DDP as an antitumor drug.

Introduction

The effectiveness of *cis*-diamminedichloroplatinum (II) (*cis*-DDP) in treating a variety of human malignancies has led to extensive study of its mechanism of action [1–5]. The antitumor effect of *cis*-DDP is believed to involve its binding to DNA but the precise mechanism of its biological activity is far from being established [1–5]. Though *trans*-DDP reacts essentially equally with DNA it is clinically inactive and less cytotoxic than *cis*-DDP [1, 5, 6]. When reacting with DNA both isomers can produce a complex variety of lesions including monofunctional adducts, inter- and intrastrand cross-links but the *trans* isomer is sterically restricted in the type of intrastrand cross-links it could feasibly produce [3, 5, 7].

Since monofunctional adducts only form transiently during the reaction of Pt complexes with DNA [7, 8] bifunctional adducts are believed to be responsible for the toxic action of *cis*- and *trans*-DDP [5, 9]. However, evidences exist that bifunctional adducts formed by *cis*- and *trans*-DDP are made in two step-reaction. At first monoaquated diamminechloroplatinum appeared which forms a monodentate DNA adducts. These adducts subsequently rearrange to form bifunctional complexes [5, 7, 8].

The thiol containing compounds could also serve as a possible binding sites for Pt complexes [5, 10–13]. Among these compounds glutathion the most aboundant endogenous thiols compound has been shown to play a role in modifying the reaction between DNA and Pt complexes both *in vitro* and *in vivo* [12, 14, 15]. Glutathion may influence the reaction by two mechanisms: firstly by direct binding of the drugs, secondly by quenching DNA-Pt-monoadducts before they can rearrange to bifunctional adducts [5, 11, 12]. Since rearrangement of *cis*- and *trans*-DDP induced monofunctional adducts into bifunctional complexes may take a different time [7, 9, 11], it is conceivable that glutathion may modify the reactions of the both isomers with DNA in different way.

Conformational changes induced by bifunctional adducts may be detected by means of differential pulse polarography (d.p.p.) [16, 17]. Thus in this raport we used the method to investigate the influence of glutathion on *cis*- and *trans*-DDP imposed alterations of DNA structure.

Material and Methods

DNA was isolated from calf thymus by the method of Zamenhof [18]. The RNA content estimated by the orcinol method [19] as well as protein content estimated by the method of Lowry [20] was lower than 1%. DNA was dissolved in 0.01 M $NaClO_4$, pH 6.4 at a concentration of 1 mg/ml. Both DDP isomers were freshly dissolved in 0.01 M $NaClO_4$, pH 6.4. Interaction of the Pt complexes with DNA was allowed to proceed at 37 °C for 24 h at Pt/deoxynucleotide ratio (r) = 0.001. In some experiments DNA was incubated with *cis*- or *trans*-DDP for 1 h at 37 °C, then glutathion was added to achieve molar ratio of DNA : PT : glutathion; 1 : 0.01 : 0.5 and incubation was continued

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up to 24 h. After that time the samples were exhaustively dialyzed against 0.01 M $NaClO_4$ at 4 °C to remove possible unreacted Pt compounds. DDP measurements were carried out with an apparatus Polarographic Analyzer PA 3 using a mercury dropping electrode, according to Palecek [21, 22]. For polarographic measurements the samples (400 μg/ml) were transferred to the medium of 0.3 M ammonium chloride, 0.01 M Tris/HCl buffer, pH 7.0 [16, 17].

Results

Cis- or *trans*-DDP was reacted with DNA for 24 h at 37 °C, $r = 0.01$. After the incubation all of the platinum is bound on the DNA [7]. To detect any DDP induced conformational alteration we used differential pulse polarography, the method particularly suitable for characterization of local distortion of the DNA [16, 17]. Fig. 1 and 2 illustrate the action of both DDP isomers with DNA. Binding of *cis*-DDP to DNA caused a marked increase of peak II, but peak III was absent. Appearance and height of peak II correspond to the presence of double-stranded ("premelted") regions in DNA molecules [16, 17, 21, 22]. On the other hand the reaction of DNA with *trans*-DDP induces the formation of denaturated, single stranded segments, what is reflected in the appearance of peak III. These results are in very good agreement with the data of Brabec, Vrana and Kleinwachter [16, 17].

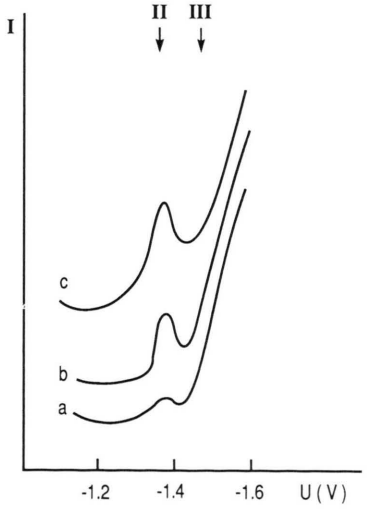

Fig. 1. Differential pulse polarograms: a) native, control DNA; b) DNA after the reaction with *cis*-DDP; c) DNA after the reaction with *cis*-DDP in the presence of glutathion. I, current; U(V), voltage.

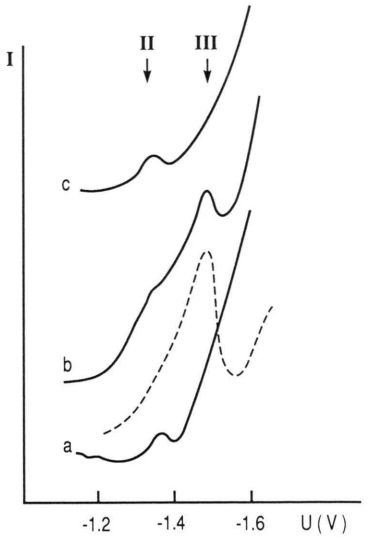

Fig. 2. Differential pulse polarograms: a) native, control DNA; b) DNA after the reaction with *trans*-DDP; c) DNA after the reaction with *trans*-DDP in presence of glutathion. Dashed line represents the polarogram of control thermally denaturated DNA. I, current; U(V), voltage.

Experiments were also performed to investigate the ability of glutathion to prevent formation of adducts responsible for the local distortion in DNA. DNA was incubated with *cis*- or *trans*-DDP for 1 h at 37 °C, then glutathion was added to achieve molar ratio of DNA:Pt:glutathion 1:0.01:0.5 and incubation was continued up to 24 h.

Though the applied concentration of glutathion has no effect on the reaction of DNA with *cis*-DPP, it greatly modifies the reaction with *trans* isomer. The presence of glutathion in the reaction mixture prevents *trans*-DDP induced denaturation of DNA (Fig. 2).

Discussion

The results presented in this study demonstrate greater disruption of DNA structure upon binding of *trans*-DDP in comparison with its *cis* isomer. When reacting with DNA both the DDP isomers

produce monofunctional adducts and interstrand cross-links. A major adduct formed *in vitro* subsequent to the interaction between *cis*-DDP and DNA is an intrastrand bidentate adducts on adjacent guanines [1–3, 5]. These adducts caused only weak conformational alterations in DNA [2, 17] manifested in the increase of peak II (Fig. 1). On the other hand *trans*-DDP is sterically restricted in the type of intrastrand cross-links; it is unable to cross-link neighbouring bases in DNA strand. Since DDP induced interstrand cross-links are rather a rear event [3, 5] and the amount of bifunctional adducts after the reaction of *trans*-DDP and DNA is relatively high [23], these adducts should comprise cross-links between non adjacent guanines. Formation of cross-links between two guanines separated by a third base requires a relatively large distortion of DNA [2]. Thus, such a distortion may be responsible for an appearance of peak III on our D.P.P. (Fig. 2). (Monofunctional adducts induced in DNA no conformational changes detectable by differential pulse polarography [17].

The *trans*-DDP induced alternation of DNA structure are largely reversed by an addition of natural cellular thiol glutathion (Fig. 2).

Immediately after reaction with DNA both DDP isomers initially form monofunctional adducts, which subsequently rearrange to bifunctional adducts [5, 7, 8].

In monofunctional *trans*-DDP adducts second chloro ligand hydrolizes as fast as the chloride from DNA adduct of the *cis* isomer [8]. However, because of the orientation of the labile ligands, *trans*-DDP cannot chelate adjacent nucleobases and monofunctional adducts can persist much longer [7, 9, 12].

Among sulfur-containing compounds glutathion has been reported to be very reactive with DDP [11, 12]. Therefore the presence of glutathion in incubation mixture enables the persistant *trans*-DDP induced monofunctional adducts to react rapidly with sulfhydryl group of glutathion preventing them from rearranging to bifunctional adducts.

However, we cannot rule out the possibility that the effect of glutathion is probably a scavenging effect on free glutathion which should react much faster with *trans*- than with *cis*-DDP [5, 13].

The results suggest that the quenching of *trans*-DDP induced monofunctional adducts and (or) inactivation of *trans*-DDP by physiological concentration of glutathion may be an important reason for the ineffectiveness of the compound as an antitumor drug.

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