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The effect of platinum derivatives on interfacial properties of DNA

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The interaction of DNA modified by the binding of various platinum compounds with an electrically charged mercury surface was studied by means of linear sweep voltammetry. It was found that DNA and its adducts with antitumour active *cis*-diamminedichloroplatinum(II) (*cis*-DDP) on the one hand and antitumour inactive *trans*-diamminedichloroplatinum(II) (*trans*-DDP) and diethylenetriaminechloroplatinum(II) chloride (dien-Pt) on the other were unwound due to their adsorption on the negatively charged mercury surface polarized to the potentials of a narrow region around −1.2 V (vs. saturated calomel electrode). The modification of DNA by bifunctional platinum compounds (*cis*- and *trans*-DDP) resulted in a substantial lowering of the extent of this interfacial conformational rearrangement, the modification by *trans*-DDP being more effective. The modification of DNA by monofunctional dien-Pt influenced the unwinding of DNA on the mercury surface only negligibly. It has been concluded that in particular interstrand cross-links induced by platinum compounds in DNA are responsible for the effect of these drugs on the extent of the interfacial unwinding of DNA. This conclusion is in good agreement with the view that among the lesions induced in DNA by platinum compounds, the interstrand cross-links are of less significance from the point of view of the antitumour efficacy of these inorganic drugs.

1. Introduction

Studies aimed at clarifying the selectivity of cancer chemotherapy have established that some tumour cells, but not normal cells, have nucleic acids associated with the cell surface [1,2]. It has been shown that these cell-surface-associated nucleic acids can suppress a variety of immunological reactions of cells [2,3]. This immunosuppression can be abrogated by treatment with DNase I [2,4] and also by antitumour active *cis*-diamminedichloroplatinum(II) (*cis*-DDP) [5]. Both treatments (with DNase I and *cis*-DDP) resulted in the removal of DNA from the cell surface [3,6]. These observations support Rosenberg's suggestion [2,4] that a source of selectivity of cancer chemotherapy could be a modification of the host immunological reaction: it has been suggested that the low immunogenicity of nucleic acids may, by the trivial mechanism of masking of antigens, serve to decrease the antigenicity of tumour cells. In this context the question arises as to whether *cis*-DDP-induced abrogation of immunosuppression could also be connected with the alterations induced by the binding of this drug in properties of nucleic acids associated with the cell surface. Analyses of interactions of nucleic acids modified by platinum complexes with electrically charged surfaces are thus of interest.

The interfacial properties of nucleic acids have been extensively studied using various adsorbents [7–9]. The results so far obtained in this field of nucleic acid research have revealed that adsorbed molecules of double-helical nucleic acids can be significantly destabilized or even unwound or denatured on negatively charged surfaces [7,8,10]. In

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this work an attempt was made to determine how this interfacial phenomenon is affected by the modification of DNA by various platinum compounds.

The interactions of isolated mammalian DNA modified by *cis*-DDP, and its *trans* isomer (*trans*-DDP) and diethylenetriaminechloroplatinum(II) chloride (dien-Pt) with a chemically inert surface of mercury were examined. The latter surface can be considered, similarly to many recent papers (for reviews see, e.g., refs. 7 and 10–12), as a model of biological surfaces. Although the surface of mercury obviously far from resembles a biological surface, metal and biological surfaces may have some similar properties, in particular physical (for instance, the parameters of the electric double layer). The properties of the layer next to the mercury surface can easily be investigated by the methods of electroanalytical chemistry, especially by voltammetry [7,10–12]. As regards the use of *trans*-DDP in our work, it is well established that this isomer of *cis*-DDP exhibits cytotoxicity but no antitumour activity [4,13]. Because of the similarity of the structures and chemistry associated with the two isomers on the one hand, and the highly different therapeutic efficacy against cancer on the other, comparative studies with the two isomers may be helpful for revealing unique properties of *cis*-DDP relevant to its antitumour activity. Moreover, interfacial properties of DNA adducts with dien-Pt, which can be bound to DNA only monofunctionally and exhibits no antitumour activity [14], were investigated as well.

2. Materials and methods

Polydisperse linear fragments of calf thymus DNA were isolated and characterized as in our previous papers [7,15]. *cis*-DDP, *trans*-DDP and dien-Pt were synthesized and characterized at the institute for Pure Chemicals (Lachema, Brno, Czechoslovakia). All platinum compounds and DNA were dissolved in 0.01 M sodium perchlorate and used immediately.

Interaction of the platinum complexes with DNA was performed as described earlier [16]. The composition of Pt-DNA adducts was characterized by the number of platinum atoms fixed per nucleotide residue, r_b. The amount of platinum bound to DNA was determined polarographically and spectrophotometrically [17,18]. The control DNA or the adducts of DNA with *cis*-DDP, *trans*-DDP and dien-Pt were then transferred to a medium of 0.1 M sodium phosphate (Na_2HPO_4/NaH_2PO_4) plus 5 mm sodium perchlorate, pH 7.1, by adding an equal amount of 0.2 M sodium phosphate, pH 7.1, just before electrochemical or spectrophotometrical analysis. These samples were then subjected to linear sweep voltammetry (LSV) at a hanging mercury drop electrode (HMDE) or to absorbance measurements.

LSV was used for detecting changes in properties of the mercury/solution interface. Differences in values of currents appearing at more negative potentials related to the electrocapillary maximum (around -1.5 V) recorded by means of LSV were employed for investigating the changes in DNA conformation induced by its interaction with the electrode, as described earlier [7,10,12]. Briefly, DNA or its adduct with the platinum complex was adsorbed at the electrode during the waiting time t_k (unless stated otherwise t_k was 60 s), when the HMDE was kept at the initial potential E_i (its value was always more positive than -1.45 V). The LSV at the HMDE was performed at a concentration of DNA or its adduct with platinum complex such that the surface of the electrode was fully covered at $t_k = 60$ s. This means that any changes in the diffusion coefficient of DNA induced by the modifications by platinum complexes or by denaturation had a negligible effect on the LSV response obtained. After the time t_k, the forward sweep, which was a single negative rapidly and linearly changing ramp potential (scan rate 1 V s^{-1}), was applied to the HMDE (fig. 1). It has been shown [7,10,12] that data providing evidence for the surface denaturation of DNA as a consequence of its interaction with the electrode can be provided by the appearance of the voltammetric peak III (fig. 2A). In the medium of 0.1 M sodium phosphate plus 5 mM sodium perchlorate, pH 7.1, LSV peak III (fig. 2A) occurred most probably as a consequence of two processes at identical or almost identical potentials: the faradayic reduction of bases present in single-stranded

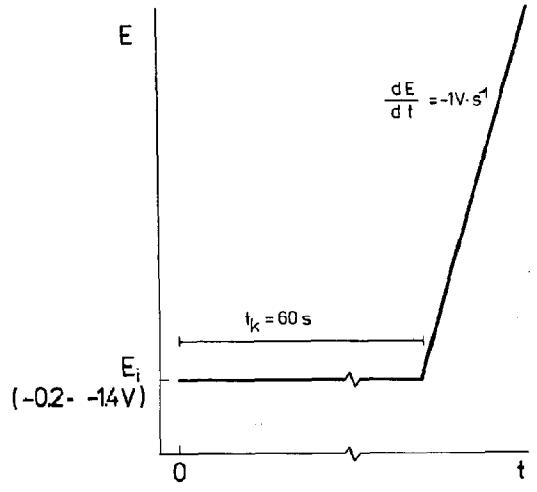

Fig. 1. Signal applied to the HMDE during linear sweep voltammetric experiments carried out in this study.

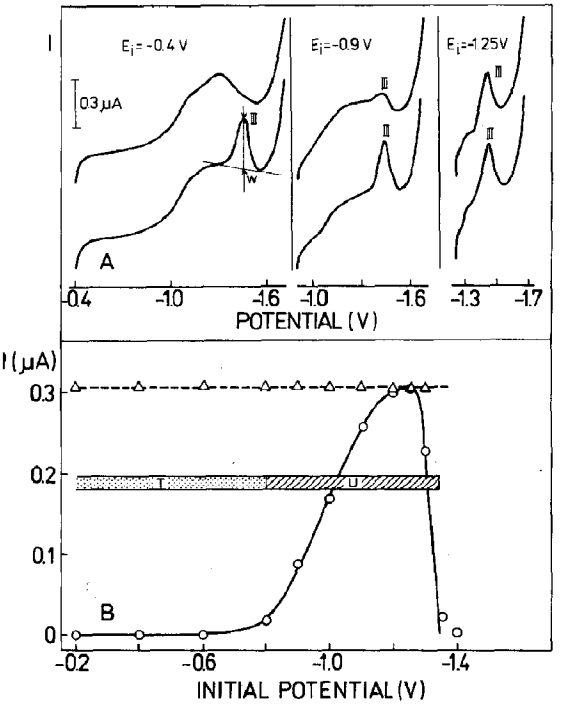

Fig. 2. Linear sweep voltammetry at the HMDE of samples of double-helical and thermally denatured DNAs. Medium: 0.1 M sodium phosphate plus 5 mM sodium perchlorate (pH 7.1). DNA concentration, 100 μg/ml; scan rate, 1 V s^{-1}; waiting time t_k, 60 s. (A) Voltammograms: (upper curves) samples of double-stranded DNA, (lower curves) samples of thermally denatured DNA; initial potentials E_i are indicated in the upper parts of individual panels. (B) Dependence of LSV peak III height of the samples of double-stranded (○ ——— ○) and thermally denatured (△— — —△) DNAs on initial potential E_i. In this paper the value of x (see a lower curve at $E_i = -0.4$ V) was taken to represent the height of LSV peak III.

segments of DNA and an adsorption/desorption process in which these segments participated [7,12]. Other details of this method can be found in our previous papers [7,12]. LSV was carried out with a PARC model 174A polarographic analyzer in connection with a PARC model 303A static mercury drop electrode; the surface area of the mercury drop was 1.72×10^{-2} cm^{-2}. All potentials quoted in the present paper are given vs. the saturated calomel electrode at 25°C. All electrochemical measurements were carried out with an electrochemical cell maintained at 25°C. The other details of our LSV measurements can be found elsewhere [7,12].

Absorption spectroscopic and denaturation-renaturation measurements were carried out as described in our recent papers [16,18] using a Beckman DU8B apparatus.

3. Results and discussion

3.1. Properties of DNA adducts with platinum complexes in the bulk of solution

The conformation of the adducts of DNA with platinum complexes at low levels of binding ($r_b \leq 0.02$) has already been described with the aid of differential pulse polarography and measurements of circular dichroism spectra [16,18]. The results indicate that the binding of antitumour active platinum compounds including *cis*-DDP resulted in minor conformational non-denaturation distortions in DNA with the double-stranded structure preserved. On the other hand, an attack by antitumour inactive platinum compounds including *trans*-DDP and dien-Pt induced more severe alterations, which had the character of denaturation of longer regions of the DNA molecule.

The aim of this paper is to describe the extent of denaturation of DNA and its adducts with

platinum compounds on the surface of a mercury electrode. The LSV measurements with these samples could not be performed directly in the medium in which the adducts were formed. It was therefore necessary to transfer these samples into a medium suitable for this sort of measurements as mentioned in section 2. Various media were tested and 0.1 M sodium phosphate, pH 7.1, seemed suitable.

Information about the stability of the secondary structure of platinum-DNA adducts was first obtained in the bulk of solution by means of measurements of the course of thermal denaturation. It is obvious that denaturation of the adducts of DNA with platinum compounds induced by their adsorption on the surface of mercury is affected by the stability of these adducts in the bulk of solution. As follows from the melting temperature values T_m obtained in the medium used in the LSV measurements (table 1), the binding of antitumour active *cis*-DDP decreased the stability of DNA molecules with increasing r_b. On the other hand, the stability of DNA was slightly increased by the binding of antitumour inactive *trans*-DDP and dien-Pt.

If the thermally denatured samples were cooled slowly, cooperative transitions characterizing renaturation were observed for the adducts of both isomers with DNA. It has been shown [16] that these renaturation effects are caused in particular by interstrand cross-links formed upon binding of the two bifunctional platinum compounds. The relative level of interstrand cross-linking was therefore evaluated on the basis of the magnitude of the cooperativity in the renaturation transition. For this purpose we used the empirical parameter $\Delta h/w$ determined from the renaturation curves (where Δh is the height of the cooperative renaturation transition and w its 2/3 width). Thus, for non-modified DNA, which does not renature, w is very high and $\Delta h/w$ tends towards zero. Other details of this approach have already been published [16].

The maximum values of $\Delta h/w$ and the corresponding values of r_b for adducts of the three platinum compounds are given in table 1. A markedly higher level of interstrand cross-linking is induced by binding of *trans*-DDP in comparison with that of *cis*-DDP. On the other hand, no cooperative transition during renaturation of the adducts of DNA with dien-Pt was in accord with the inability of this monofunctional platinum complex to form interstrand cross-links.

Table 1

Denaturation and renaturation properties of adducts of DNA with platinum compounds in 0.1 M sodium phosphate plus 5 mM sodium perchlorate (pH 7.1)

Platinum compound	ΔT_m (°C) [a] at $r_b = 0.02$	Maximum renaturation	
		$(\Delta h/w)$ [b]	r_b
cis-DDP	−1.6	0.0213	0.005
trans-DDP	0.2	0.0376	0.005
Dien-Pt	0.3	0	⩽ 0.02

[a] $\Delta T_m = (T_m)_{DNA\text{-}Pt} - (T_m)_{DNA}$. Melting temperature of control non-modified DNA ($(T_m)_{DNA}$) was 89.8 °C.

[b] Empirical renaturation parameter $\Delta h/w$ is defined in our previous paper [16]. In the table its maximum values are given together with the corresponding r_b. For non-platinated DNA, $\Delta h/w = 0$.

The results describing the denaturation properties of DNA adducts with platinum complexes in bulk of solution containing 0.1 M sodium phosphate plus 5 mM sodium perchlorate, pH 7.1, were qualitatively identical to those already published for other media [16,19]. In this communication we therefore do not report on these observations in detail.

3.2. The conformation of DNA and its adducts with platinum complexes in the interface

As already shown earlier [7,10,12] extensive denaturation of DNA on the surface of the mercury electrode is observed if this electrode is polarized to potentials in the narrow region around −1.2 V (about −0.9 to −1.3 V; region U) (fig. 2B). This surface denaturation can be demonstrated by a marked increase in LSV peak III (fig. 2). It was interesting that the maximum increase in LSV peak III in region U and thus the maximum extent of surface denaturation were observed in the case of control non-platinated samples of DNA (fig. 3 and table 2). In this paper the extent of surface denaturation of DNA or its adducts with platinum complexes has been defined as the ratio

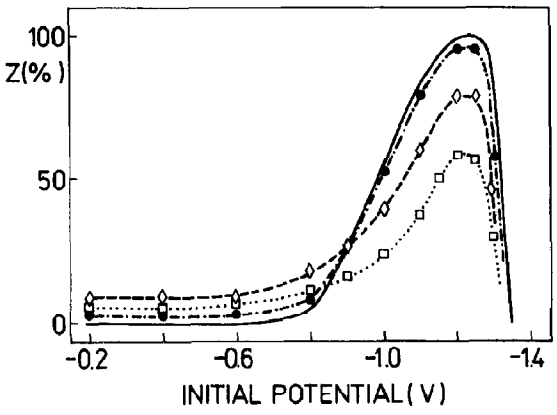

Fig. 3. Dependence of the extent of surface denaturation Z of double-stranded DNA and its adducts with platinum complexes at $r_b = 0.02$ on initial potential E_i: (———) non-platinated control DNA, (◇———◇) *cis*-DDP-DNA adduct, (□···□) *trans*-DDP-DNA adduct, (●–·–·–●) dien-Pt-DNA adduct. Z is defined as the ratio of the height of LSV peak III yielded by double-helical DNA sample or its adduct with the platinum complex to that of thermally denatured DNA obtained under the same experimental conditions multiplied by 100. Other conditions of the LSV experiments were identical to those in fig. 2.

of the LSV peak III yielded by a double-helical sample at the initial potentials of region U to the height of the LSV peak of thermally denatured DNA obtained under the same experimental conditions, multiplied by 100.

Modification of DNA by both *cis*- and *trans*-DDP resulted in a significant lowering of the LSV peak III obtained for initial potentials of the region U, which indicates a decrease in the extent of surface denaturation. This observation was somewhat surprising, particularly for the adducts of DNA with *cis*-DDP, which had lower stability to thermal denaturation in the bulk of solution than control non-platinated samples of DNA (table 1). In this context one must bear in mind which factors contribute to the overall stability of DNA adducts with platinum compounds: the stabilizing effect of the positive charge of platinum moieties and of interstrand cross-links on the one hand and the destabilizing effect of conformational distortions on the other. It thus seems evident that during the course of thermal denaturation (in the bulk of solution) the action of the destabilizing effects of the secondary structure distortions prevailed in the case of the adducts of DNA with *cis*-DDP while stabilizing factors were stronger in the case of the adducts of DNA with *trans*-DDP.

Table 2

The extent of surface denaturation Z[a] of DNA and its adducts with platinum complexes at the HMDE polarized to −1.25 V for 60 s[b]

Platinum compound	Z (%) at r_b		
	0.005	0.01	0.02
Non-modified DNA		100	
cis-DDP	97	93	78
trans-DDP	92	76	59
Dien-Pt	98	96	96

[a] Z is defined in the same way as in fig. 3. The conditions of the LSV experiments were identical to those in fig. 2.

[b] Medium: 0.1 M sodium phosphate plus 5 mM sodium perchlorate (pH 7.1).

The behaviour of the adducts of DNA with *cis*-DDP and *trans*-DDP during the course of the surface denaturation on the negatively charged surface of mercury (in region U) was quite different. The stabilizing factors prevailed in the case of both adducts, although these factors asserted themselves more markedly in the case of the adducts with *trans*-DDP. The stabilizing effect of the positive charge of platinum moieties is evidently identical in the case of both isomers. It is thus apparent that the contribution to the increase in the stability of platinum-DNA adducts to the surface denaturation observed in this work arose from the interstrand cross-links. This type of DNA modification therefore appears to be the main candidate for the factor determining the distinct interfacial behaviour of the DNA adducts with the two isomers observed in this work (fig. 3 and table 2).

The conclusion on the importance of interstrand cross-link formation in the extent of surface denaturation is also supported by our LSV results obtained for DNA adducts with monofunctional dien-Pt. As already mentioned, this monofunctional antitumour inactive platinum complex does not form interstrand cross-links in DNA upon binding. Thus, when surface denaturation of the adducts of DNA with dien-Pt was investigated by

means of LSV under the same conditions as in the case of the adducts with bifunctional *cis*-DDP and *trans*-DDP, the changes in height of LSV peak III and thus the changes in extent of surface denaturation on the mercury polarized to the potentials of region U were negligible. For instance, at $r_b = 0.02$, peak III obtained for the DNA adduct with dien-Pt at $E_i = -1.25$ V was only approx. 4% lower than that of the control, unmodified DNA sample, compared with a 22% decrease in the case of the adduct with *cis*-DDP and even 41% in the case of the adduct with *trans*-DDP (fig. 3). Moreover, it has been shown [17,18] that the character and extent of the alterations in secondary structure of DNA in the bulk of solution induced by the attack of *trans*-DDP and dien-Pt are rather similar. The LSV results obtained in this work with dien-Pt-DNA adducts thus support the view that conformational distortions induced in DNA by the binding of platinum compounds at low r_b in the bulk of solution have no substantial effect on the extent of surface denaturation on mercury polarized to potentials around -1.2 V.

The LSV experiments were also performed under different conditions, namely, in the medium of Britton-Robinson buffer, pH 9.85, when its ionic strength was adjusted by means of KCl to a value of 0.5. This medium, to which DNA was transferred just before analysis, is also very suitable for investigations of surface denaturation of DNA at the mercury electrode [10], although the pH is rather far from physiological values. The latter medium was used to confirm our results obtained with 0.1 M sodium phosphate, pH 7.1, as the supporting electrolyte because the LSV peaks obtained in alkaline medium displayed a less complex nature, having only a non-faradayic character [10]. Evaluation of the data obtained at pH 9.85 resulted in conclusions that were identical to those made on the basis of the data obtained under the more physiological conditions of a neutral solution of sodium phosphate.

4. Conclusions

The results described here reveal that DNA modified by *cis*-DDP is unwound at a negatively charged surface to a lesser extent than the control non-platinated DNA. This finding is connected in particular with the presence of interstrand cross-links in the adducts. However, the adducts of DNA with antitumour inactive *trans*-DDP contain more interstrand cross-links, and are thus unwound on the surface to a still smaller extent than those with antitumour active *cis*-DDP. It is therefore apparent that mere interstrand cross-links induced in DNA by platinum complexes cannot be responsible for the antitumour activity of this sort of inorganic drug, even if this activity were connected in some way with the unwinding of DNA on the surface of tumour cells. The results in this paper also support the view that the abrogation of immunosuppression of tumour cells observed after treatment with *cis*-DDP is not connected with a process in which the interfacial unwinding of DNA associated with the cell surface might play a decisive role.

This paper does not answer the rather crucial question of whether a source of selectivity in cancer chemotherapy is connected with a modification of the host immunologic reaction, as has been proposed by Rosenberg [2,4]. If, however, this hypothesis gains more experimental support, then the results reported in this paper could contribute to clarifying the mechanism of selectivity of this kind of chemotherapy.

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