The Influence of Cysteine on the Reaction of d-Guanosine with *cis*-Diamminedichloroplatinum (II)

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Mono- and bifunctional complexes of *cis* DDP and d-guanosine were isolated on Sephadex G-25. The presence of cysteine in incubation mixture resulted in the vanishing of bifunctional complex of d-guanosine and the appearance of d-guanosine-cis DDP-cysteine complex in the eluted peak. It is also possible that the eluted peak contained no homogeneous product of the reaction.

Introduction

cis-Diamminedichloroplatinum (II) (*cis*-DDP) has emerged as an important chemotherapeutic agent with activity in a wide variety of human neoplasms. It is assumed that DNA is the target of the Pt-compounds, and that their biological effect is caused by the reaction with this cellular macromolecule [1]. Three kinds of DNA lessions are though to be formed during the treatment of cell with Pt-compounds, namely intrastrand DNA cross-links, interstrand DNA cross-links and chelation products [2–4]. In studies correlating DNA damage produced by platinum complexes with their biological effects, cytotoxicity appeared to correlate best with inter- and intrastrand crosslinking [5, 6]. The investigation of DNA reaction with *cis*-DDP *in vitro* has indicated that the latter one preferentially binds to the guanine bases and the cross-linking of these bases *via* N7 is an event responsible for the formation of both kinds of DNA damages [1, 7, 8]. Sulfur analogs readily serve as targets for nucleophilic attack by *cis*-DDP, and several sulfur-containing compounds are capable of blocking the formation of *cis*-DDP-DNA complexes and reducing cytotoxicity of *cis*-DDP [9–11]. Here we have applied a system for the separation of d-guanosine platinated derivatives and by the same method we have examined the influence of cysteine on the formation of *cis*-DDP-d-guanosine complexes.

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Materials and Methods

Deoxyriboguanosine (Sigma Ch. C.), *cis*-DDP (Sigma Ch. C.) and cysteine hydrochloride (Merck) were dissolved in 0.05 M $NaClO_4$, pH 6.2.

d-Guanosine solutions were incubated at 37 °C, for given period of time, after addition of freshly dissolved *cis*-DDP and cysteine, to achieve the certain Pt(d-Guo)Cys HCl ratio.

Sephadex G-25 (Pharmacia Fine Chemicals) columns (90 × 1.5 cm) were applied to separate the obtained reaction products. The procedure of elution was performed using 0.9% NaCl solution, pH 6.2, with a flow rate of 1 ml/1 min. Five ml fractions were collected.

After evaporation and mineralization of samples, platinum concentrations were measured by the stannous chloride method, determining absorption at 405 nm [12].

The estimation of cysteine in an isolated fraction, was done with ninhydrine reagent, determining absorption at 570 nm.

Results and Discussion

The standard of d-guanosine was eluted from Sephadex G-25 column in a sharp single peak. After 72 h incubation with *cis*-DDP in molar ratio 1:1, two additional peaks appeared with simultaneous disappearing of the peak characteristic for d-guanosine standard (Fig. 1). It suggests a complete reaction of nucleoside with the drug. When the d-guanosine *cis*-DDP molar ratio was changed into 2:1 the only product of the reaction was eluted in peak I what suggested that in this position bifunctional complex of d-guanosine and *cis*-DDP was eluted. The amounts of the nucleoside and *cis*-DDP having been calculated it became evident that in peak I bifunctional and in peak II monofunctional adducts of d-guanosine and *cis*-DDP were eluted. The UV spectra of both the complexes differ considerably, when compared with the standard (Fig. 2) with the λ_{max} for both complexes at 260 nm. To study the possible protective role of cysteine on the platination of d-guanosine we analyzed the

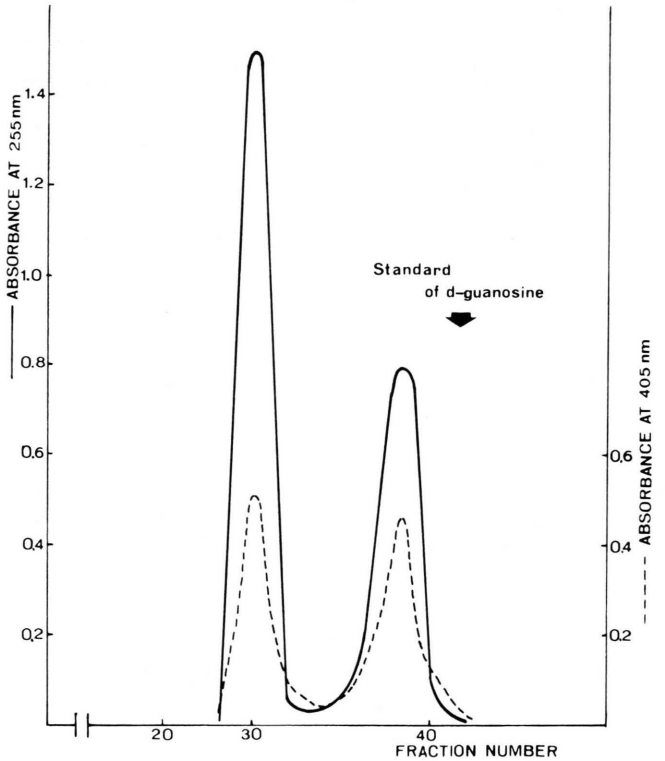

Fig. 1. Chromatography of d-guanosine and derivatives eluted from Sephadex G-25 after the reaction of the nucleoside with *cis*-DDP.

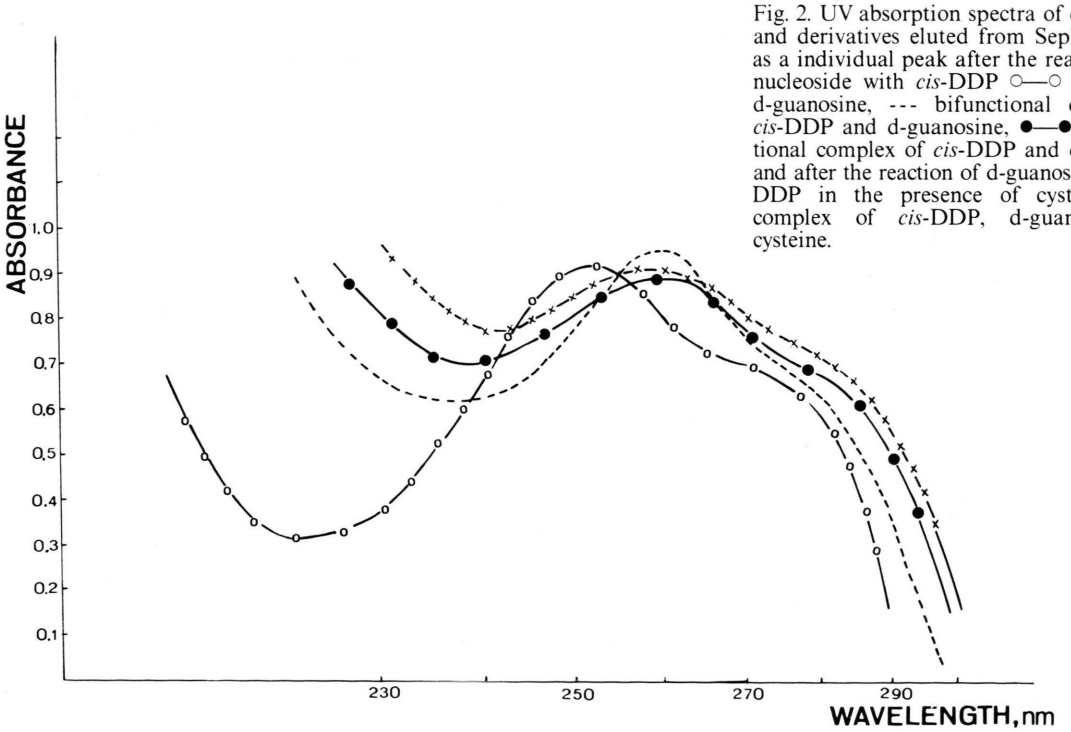

Fig. 2. UV absorption spectra of d-guanosine and derivatives eluted from Sephadex G-25 as a individual peak after the reaction of the nucleoside with *cis*-DDP ○—○ standard of d-guanosine, --- bifunctional complex of *cis*-DDP and d-guanosine, ●—● monofunctional complex of *cis*-DDP and d-guanosine and after the reaction of d-guanosine with *cis*-DDP in the presence of cysteine ×—× complex of *cis*-DDP, d-guanosine and cysteine.

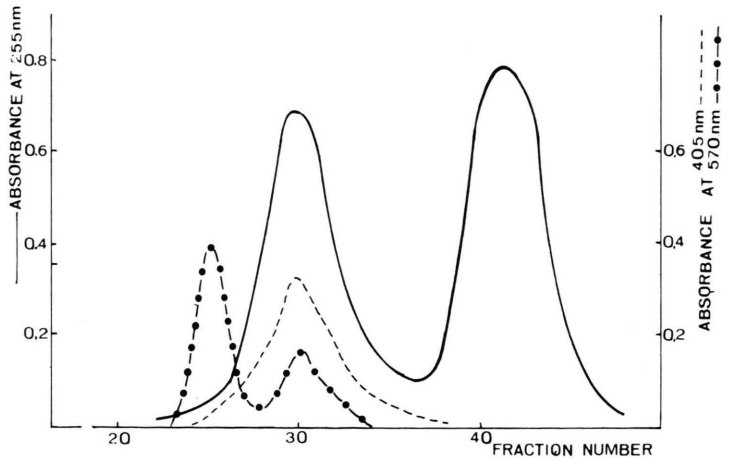

Fig. 3. Chromatography of d-guanosine and derivatives eluted from Sephadex G-25 after the reaction of the nucleoside with *cis*-DDP in the presence of cysteine.

Table I. Spectral properties for d-guanosine and platinum complexes of d-guanosine.

Complex	λ_{max}	λ_{min}	$\frac{A_{\lambda_{max}}}{A_{\lambda_{min}}}$
d-guanosine	252	225	4.0
d-guanosine-*cis* DDP = 2:1	260	235	1.65
d-guanosine-*cis* DDP = 1:1	260	232	1.3
d-guanosine-*cis* DDP-cysteine = 1:1:1	258	238	1.2

reaction products of the nucleoside with the drug which were incubated together with cysteine. After 72 h of incubation in molar ratio of d-guanosine (*cis*-DDP) cysteine 1:1:1, separation of the reaction products on Sephadex G-25 column yielded two peaks with d-guanosine presence. The position of elution for peak II was the same as for d-guanosine standard. UV spectrum proved it to be unchanged d-guanosine. About 50% of all d-guanosine moieties were eluted in this peak, what suggests that this amount of the nucleoside has not reacted with *cis*-DDP when cysteine was present in incubation mixture. Although the position of elution for peak I was the same as for the bifunctional complex of *cis*-DDP and d-guanosine, both the UV spectrum and the calculated amount of Pt suggested the presence of some other product (Fig. 3, Tab. I). The presence of cysteine was also detected in this peak. Calculated molar ratio of cysteine:Pt:d-guanosine in the complex eluted in peak I was 0.4:1:1. The elution of the complex and its UV characteristic suggest that it is a bifunctional complex of d-guanosine, cysteine and *cis*-DDP. The lower amount of cysteine detected in the complex might be a result of an equal reactivity of all its coordination sites (−SH, −COOH, $-NH_2$) what overshadows the estimation of cysteine amount. It is also possible that the peak contained no homogeneous product of the reaction. For instance along with the complex of *cis*-DDP-cysteine-guanosine, the adduct of cysteine-*cis*-DDP as well as bifunctional complex of d-guanosine-*cis*-DDP might be eluted. Nevertheless the results presented here pointed out that cysteine has considerably limited production of bifunctional complex of *cis*-DDP and d-guanosine which can be potential lethal product of the action of the drug on the cell [5, 6].

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