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Research paper

Effect of platinum anticancer drugs on the cytochrome *c* conformation

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ABSTRACT

Cytochrome *c* is an important electron transfer carrier in mitochondrial electron transfer and is essential for apoptosis. In the present work we have examined the effect of platinum (II) complexes (cisplatin, transplatin and carboplatin) on the conformation of cytochrome *c* and the state of heme moiety. For this purpose, UV–VIS spectroscopy, circular dichroism (CD) and absorption second derivative spectroscopy methods have been used. Moreover, the amount of platinum per mole of cytochrome *c* and stability of carboplatin–cytochrome *c* system have been determined by ICP-AES and zeta potential measurements, respectively.

The present data has revealed that binding of platinum (II) complexes to cytochrome *c* induces a conformation of the protein with less organized tertiary structure.

Determination of tyrosine exposure by second-derivative spectroscopy indicates changes occurring in the tyrosyl microenvironment, which becomes more polar following the more open conformation compared to that of a native protein.

The reaction of cytochrome *c* with platinum complexes led to the changes in spectral properties, most notably the decreases in intensity of the absorption band at 695 nm, indicating that platinum complexes may affect coordination Met-80 with the heme iron. Under physiological conditions the carboplatin-cytochrome *c* system shows instability, what is desired effect from the pharmacological point of view.

1. Introduction

Cytochrome *c* is an important electron transfer heme protein, that plays a crucial role in apoptotic pathways [1]. The protein is functioning in the respiratory chain in the inner mitochondrial membrane, shuttling electrons from cytochrome *c* reductase to cytochrome *c* oxidase. Extensive chemical modification studies [2,3]indicated a significant electrostatic interaction between the two protein. All the structural and conformational changes of the cytochrome *c* can influence on the electron transport. For many years, the biological function of cytochrome *c* was assumed to be confined to electron transfer from cytochrome c_1 to cytochrome oxidase. However, in 1996 it was found that cytochrome *c*, when released from mitochondria to cytosol, activates a programmed cell death cascade (apoptosis) [4]. During the execution of apoptosis, cytochrome *c* interacts with the phospholipid cardiolipin, forming a complex with peroxidase activity. In the next step, oxidation of cardiolipin induces the opening of permeability pores at the outer mitochondrial membrane, which allows to release of cytochrome *c* from mitochondria into cytoplasm. Subsequently, this process triggers a cascade of events ultimately leading to cell apoptosis [5,6]. Taking into account above process it seems that cytochrome *c* is essential for activation of apoptosis of cancer cells. Moreover, it is known that cytochrome *c* is involved in the aggregation of α-synuclein, which is responsible for Parkinson's disease [7].

cis-Diamminedichloroplatinum(II) (cisplatin), *cis*-diammine(cyclobutane-1,1-dicarboxylate-*O*,*O*′)platinum(II) (carboplatin) and its derivatives are very important chemotherapeutic drugs for various cancer treatment, however, the modes of action and toxicity of these drugs are not well understood. Both drugs have identical mechanism of action, based on selective DNA platination. The essential difference between platinum complexes is a kinetic one. It is known that replacement of two chloride groups in cisplatin by the bulkier bidentate ligand does not change the overall charge of the complex, renders the aquation process of carboplatin far slower than in cisplatin. Although the anticancer treatment by platinum-based drugs causes serious side effects they are still widely used in the clinical practice. The origin of their toxic effects is thought to be related to the ability of platinum ions to form complexes with proteins mainly coordinating to S- or *N*-containing amino acid residues [8]. *trans*-Diamminedichloroplatinum(II)

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(transplatin) does not exhibit a useful pharmacological effect and is not used in clinical practice.

The studies on the interaction between platinum complexes and cytochrome *c* show, that these complexes are highly selective to bind to sulphur-containing Met residue and that protein can be cross-linked selectively via platinum compounds to form stable di-protein complexes. The yield of protein derivatives as well as the likely binding sites for platinum complexes in cytochrome *c* is sensitive to the reaction conditions [9,10]. It has been shown that Met-65 is the primary binding site for the platinum drugs [11]and that histidines might play a role as secondary platinum binding sites [10,12]. It arises from the X-ray structure studies that the cisplatin produces monometallated cytochrome *c* adducts where a $[Pt(NH_3)_2Cl]^+$ fragment is coordinated to Met-65 in a monodentate fashion or a $[Pt(NH_3)_2]^{2+}$ fragment is bound to Met-65 and Glu-61 in a bidentate fashion [11].

A number of advanced biophysical approaches may be used to identify the platinum binding sites. The investigations of the reaction of carboplatin with horse heart cytochrome *c* [12]lead to the formation of stable platinum/protein adducts and may strongly promote carboplatin reactivity. It is also known, that cytochrome *c* may act as reducing agents for the activation of Pt(IV) to a reactive Pt(II) complexes [13].

The interactions of the platinum drugs with cytochrome *c* could be important for a full understanding of the actual mode of action of these anticancer metallodrugs and extending their applications in the areas of biomedicine [14]. Moreover, it is extremely important since this protein is a likely target for anticancer agents being a crucial factor in apoptosis and it is known that cisplatin accumulates in mitochondria causing the release of the protein into the cytosol [15]. The binding of this platinum-based drugs to cytochrome *c* may change structure or conformation of the protein and affect its biological function. In the present work we have examined the effect of cisplatin, carboplatin and transplatin on the conformation of cytochrome *c*, the heme state and stability of formed adducts.

2. Experimental

2.1. Materials and methods

Cytochrome *c* from horse heart was purchased from Fluka Chem. Co. The concentration of the native cytochrome *c* was determined from the absorption coefficient $1.06 \times 10^5\ M^{-1}\ cm^{-1}$ at 410 nm [16]. Cisplatin (*cis*-$[PtCl_2(NH_3)_2]$) and transplatin (trans-$[PtCl_2(NH_3)_2]$) were obtained from Fluka Chem. Co. Carboplatin (*cis*-$[Pt(NH_3)_2$(cyclobutane-1,1-dicarboxylate-*O*,*O*)]) was purchased from Sigma-Aldrich (code P4394). In all the experiments a physiological buffer was used so that the final concentrations were 0.004 M NaH_2PO_4, 0.1 M NaCl and 0.025 M $NaHCO_3$, pH 7.40. All chemicals used were reagent grade. Each experiment was measured after samples incubation at 37 °C for 24 h. This reaction time is enough to achieve the process equilibrium [17] and prepare cytochrome *c*-platinum complexes adducts suitable to perform all experiments.

2.2. UV–VIS spectroscopy

The UV–VIS spectra were recorded at 25 °C on BECKMAN DU-650 spectrophotometer in the spectral range of 270–300 nm and 600–800 nm, using 1 cm cells. Second-derivative spectra were obtained using the software package provided by the manufacturer. Determination of tyrosine exposure in protein by second-derivative spectroscopy was carrying out as described by Ragone et al. [18]. The state of Tyr residues might be evaluated by the second-derivative absorption spectroscopy. It estimates the heights between the second-derivative positive and negative peaks (the arithmetic sums) at 283 vs. 287 nm (a) and 290.5 vs. 294–5 nm (b). The ratio a/b is then a factor reporting the exposure of the tyrosine residues into the water environment.

2.3. ICP-AES measurements

The assays of platinum (II) bound per mole of cytochrome *c* were performed with ARL 3410 ICP-AES Spektrometer. Complexes of cytochrome *c* and platinum (II) compound were prepared by incubation of the reaction mixtures – protein with 10-fold molar excess of proper platinum complex in physiological phosphate buffer pH 7.40. The samples were then chromatographed on the Sephadex G-25 Coarse column eluted with physiological buffer. The concentration of protein in chromatographed samples was estimated by UV–VIS method, taking an extinction coefficient of $\varepsilon_{410nm} = 106\ [mM^{-1}\cdot cm^{-1}]$.

2.4. CD spectroscopy

Circular dichroism (CD) was measured with a JASCO J-715 spectropolarimeter in the range of 190–250 and 300–600 nm, using 0.1 and 1.0 cm cuvettes, respectively. Protein concentration of the samples was 8×10^{-6} M for far-ultraviolet CD studies and 8×10^{-5} M for the range 300–600 nm. The spectra are expressed as MRE (mean residue ellipticity) in $deg\cdot cm^2\cdot dmol^{-1}$.

2.5. Zeta potential measurements

The zeta-potential measurements of the cytochrome *c*-carboplatin system were performed using the Zetasizer (Nano-ZS) from Malvern Instruments and Malvern Zetasizer Software v7.10, by taking the average of five measurements at the stationary level. The measurements were performed in a 750 µl, polycarbonate U-shaped cell with gold-plated electrodes at 310 ± 0.1 K after 24 h incubation at the same temperature and pH 3.50, 7.40, 8.50 buffered solutions (citrate, phosphate and Tris-HCl, respectively). The final concentration of the cytochrome *c* and carboplatin in the samples was 5×10^{-6} M and 5×10^{-6}–5×10^{-5} M, respectively.

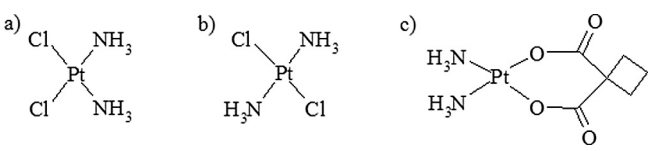
3. Results and discussion

3.1. Structural properties of the cytochrome c modified with platinum complexes

In order to obtain information about the structural perturbation of cytochrome *c* upon binding of platinum complexes (Scheme 1), the UV–VIS spectroscopy, second derivative spectra, circular dichroism (CD), ICP-AES and zeta potential measurements were performed.

3.1.1. Binding of Pt(II) complexes to cytochrome c

The amounts of platinum per mole of cytochrome *c* were determined using the ICP-AES method. Under experiment conditions the



Scheme 1. Selected platinum(II) complexes: cisplatin (a), transplatin (b), carboplatin (c).