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Experimental and theoretical studies on the complexes between cisplatin and guanidinoacetic acid

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ABSTRACT

Cisplatin, *cis*-dichlorodiammineplatinum(II) is one the most employed antineoplasic drugs, exhibiting activity for different types of tumors. Cisplatin resistance can be related to its reduced uptake or retention, causing the decrease of the platinum–DNA adduct. This is attributed to cisplatin binding to other cellular components, causing its deactivation or damage to its normal biochemical pathway. Several studies with a focus on elucidating cisplatin binding to compounds present in cells have been conducted. In the present study, the interaction of cisplatin with one potential O and N-donor ligand, guanidinoacetic acid, was investigated, an amino acid found at high concentrations in the kidney and liver, organs where the drug accumulates. *In vitro* GAA and cisplatin interaction was studied in aqueous medium at the same ionic strength (0.1 mol L^{-1} KCl) and temperature (36.5 °C) as fluids of the body. Potentiometric and NMR (^{1}H and ^{195}Pt) analyses have been done, with the determination of stability constants and theoretical calculations for the principal structures involved. Results showed that GAA may be one of the potential biomolecules of cisplatin since, under the conditions of temperature and ionic strength of the body at around pH 7, part of cisplatin interacted with GAA to form $[Pt(NH_3)_2(HGAA)_2]^{2+}$. Optimization of this species, $[Pt(NH_3)_2(HGAA)_2]^{2+}$ was carried out using the GAUSSIAN 03 package with the B3LYP function in combination with LANL2DZ (6–311G(d,p)), yielding a planar sphere coordination around Pt(II) and also O–H intramolecular interactions in the complex. The results on speciation for cisplatin/GAA systems could be of great interest since GAA may be one of the potential binding species of cisplatin, especially in the study of 5:1 cisplatin/GAA ratio (found in rats, for example).

1. Introduction

Cisplatin, *cis*-dichlorodiammineplatinum(II) (Fig. 1a), is one the most employed antineoplasic drugs, exhibiting activity for different types of tumors, including cancers of the ovaries, testes, solid tumors of the head, sarcomas and also for soft tissues, bones and muscle [1,2]. Although effective, cisplatin has several undesirable side effects, including nephrotoxicity, ototoxicity, neurotoxicity, decreased immunity to infections, and gastrointestinal disorders. This has prompted research into novel compounds containing platinum and other metals such as palladium, in an effort to attain lower toxicity, less resistance and fewer side effects. Other platinum complexes such as carboplatin, oxaliplatin, nedaplatin [3], lobaplatin and JM216 [4] have been synthesized and evaluated in clinical trials. Among these complexes, carboplatin offers major advantages over cisplatin and has achieved worldwide use. In addition, platinum(II) and palladium(II) complexes with amino acids have also been investigated as potential cytotoxic agents [5,6].

Currently, cisplatin is commonly employed in chemotherapy, in combination with other chemical compounds, either to overcome its resistance or to ameliorate side effects [2]. Examples of these compounds include paclitaxel, doxorubicin, gemcitabine, tegafur-uracil, vitamin D [2] and also other natural substances such as honey venom [7].

Cisplatin's mechanism of action in the body has been extensively discussed. There is a consensus that cisplatin enters cytoplasm passively, where its chloride ligands are displaced by water ligands and hydrolyzed to form a complex that is a potent electrophile able to interact with nitrogen atoms in nucleic acids or sulphydryl groups from proteins [2]. The resultant cationic hydrolyzed platinum complexes bind to the purine bases of DNA, preferably to N7 from guanine, impairing its duplication and causing apoptosis [2].

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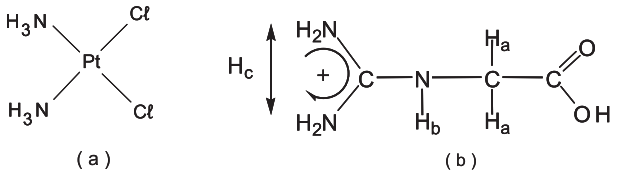

Fig. 1. Structures of cisplatin (a) and GAA showing hydrogen atoms analyzed by ^{1}H NMR (b).

However, besides attacking DNA of cancer cells, cisplatin can also interact with proteins and enzymes, damaging some biological mechanisms and causing side effects. One such side effect is nephrotoxicity, a phenomenon which has yet to be fully explained. It has been shown, for instance, that glutathione-cisplatin adducts, which are more toxic than cisplatin, play a key role in inducing nephrotoxicity [8–10].

On the other hand, cisplatin resistance can be related to its reduced uptake or retention, causing the decrease in the platinum–DNA adduct. By binding to other cellular components, cisplatin can become subject to deactivation or damage to its normal biochemical pathway. Several studies with a focus on elucidating cisplatin binding to compounds present in cells have been conducted. Theoretical studies have shown that N-donor ligands were preferred to bind cisplatin over S-donors mainly due to electrostatic reasons rather than orbital interactions [11].

In the present study, *in vitro* interaction of cisplatin with one potential O and N-donor ligand, guanidinoacetic acid, was investigated, an amino acid found at high concentrations in the kidney and liver, organs where the drug accumulates. It has also been reported that levels of urinary guanidinoacetic acid are affected by cisplatin, showing a marked reduction after cisplatin administration in urinary tract cancer patients. This effect has been attributed to the nephropathy caused by the drug [12,13].

Guanidinoacetic acid (Fig. 1b) is significantly involved in renal metabolism, since it is mainly synthesized in the kidneys and subsequently excreted in urine or methylated for creatine production [13,14]. For this reason, GAA is regarded as a more sensitive marker for chronic renal dysfunction [15]. Moreover, GAA is an endogenous convulsant and involved in other biological processes such as plasma cholesterol level [16] and thyroid dysfunction [17]. High levels of GAA in the brain, blood, urine and cerebrospinal fluid have been observed in patients with the genetic deficiency in the enzyme that methylates it, a dysfunction known as guanidinomethyltransferase deficiency (GAMT-D) [18–20].

Our group has investigated and studied the properties of GAA for some years, with research focusing on GAA interaction or coordination to metal ions of biological interest. Initial studies explored GAA complexes in aqueous solutions with Mn(II), Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Pb(II) [21], in which an α-nitrogen and oxygen bidentate coordination were predominantly observed. Copper(II) ternary complexes with GAA and a second amino acid (glutamic acid, aspartic acid or glycine) were also investigated, with the presence of guanidino-carboxylate intramolecular interactions observed [22]. GAA complexes have also been synthesized: a copper(II) complex was synthesized with GAA and a ligand derived from it, N^{1}-carboxilatometilguanidino-oxidoacetato-Oag [23] and a paddle-wheel copper(II) dimeric compound, $Cu_2(GAA)_4(NO_3)_2$, (tetrakis(μ-guanidinoacetic acid-k2O:O′)bis[(nitrato-kO) copper (II)]) [24], with anti-ferromagnetic interaction [25].

Our group has also reported interesting reactions of GAA during the *in situ* synthesis of some of its complexes, such as desamidination [26] and deamidination [27].

The objective of the present study was to investigate *in vitro* GAA and cisplatin interaction in aqueous medium at the same ionic strength (0.1 mol L^{-1} KCl) and temperature (36.5 °C) as fluids of the body. These interactions were investigated using potentiometric and NMR (^{1}H and ^{195}Pt) analysis. In addition, stability constants and theoretical calculations for the principal structures involved were also determined.

2. Experimental

All reagents were used without further purification. Potassium tetrachloroplatinate and 0.1 mol L^{-1} standard nitric acid solution, 0.1 mol L^{-1} standard sodium hydroxide solution, as well as standard buffer solutions were obtained from Merck, guanidinoacetic acid from Aldrich, potassium chloride and potassium nitrate from Vetec, and cisplatin from Quiral Química do Brasil.

2.1. Potentiometric analysis

Potentiometric titrations were carried out using a Titrino 751 GPD Metrohm apparatus equipped with an autoburette with a combined glass electrode, previously calibrated for each titration. Electrode calibration was performed with pH 4.0, 7.0 and 9.0 standard buffer solutions. All titrations were performed in an inert atmosphere under a controlled temperature of 36.5 °C (±0.5) using a thermostatic water bath. The titrant used was a CO_2 free 0.1 mol L^{-1} sodium hydroxide solution. The ionic strength was kept constant at 0.1 mol L^{-1} using KCl solution. Concentrations of GAA and platinum solutions used in all titrations were 2×10^{-3} mol L^{-1} and 1×10^{-2} mol L^{-1}, respectively. The Pt(II)/GAA ratio analyzed was 1:2. Regarding cisplatin, 1:2 cisplatin/GAA and 5:1 cisplatin/GAA ratios were analyzed. This latter ratio was studied to allow a better comparison with the ratio of these two substances in living systems estimated from the data reported in the literature for the kidneys of neoplasic mice [28]. All Pt(II)/GAA and cisplatin/GAA solutions were kept at a constant temperature of 36.5 °C with stirring for 2 days before potentiometric analysis due to the slow kinetic rates of Pt(II) complexation reactions [29]. Determination of the stability constants of the complexes and distribution of the species was carried out using the HYPERQUAD and HYSS [30] programs, respectively.

In addition, a comparison between theoretical and experimental potentiometric curves was performed to evaluate the proposed model of species, using the refined stability constants and the HYSS Program [31].

Platinum hydrolysis constants were also determined, based on analyses of more than ten titrations using a 10^{-2} mol L^{-1} $PtCl_4^{2-}$ aqueous solution (~500 points) at the same conditions of 36.5 °C temperature and 0.1 mol L^{-1} ionic strength (KCl) for all other potentiometric analysis. The hydrolyzed constants of platinum(II) were necessary to propose the model of the complex species to be formed.

2.2. NMR analysis

NMR spectra were recorded on a DRX-600 Bruker (^{1}H 600 MHz) and a DRX-300 Bruker (^{1}H 300 MHz, ^{195}Pt 64.52 MHz) using 5 mm NMR quartz tubes. The parameters for ^{1}H NMR analysis were as follows: suppression of the water signal, lock with D_2O, 128 accumulations, spectral window of 7 kHz, DSS (sodium-2,2-dimethyl-2-silapentane-5-sulfonate) as internal reference and temperature of 36.0 °C. For the Pt/GAA system at pH 7–8, a different temperature of 6 °C was also analyzed. The concentration of both GAA and cisplatin solutions was 2×10^{-3} mol L^{-1}. The electrolyte used was KCl. Pt(II)/GAA solutions were previously stirred for at least 24 h at 36.5 °C.