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Structural features and cytotoxic activities of [Ru(AA-H)(dppb)(bipy)]PF_6 complexes

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ABSTRACT

Amino acid/diphosphine ruthenium complexes, of general formula [Ru(AA-H)(dppb)(bipy)]PF_6 [AA = amino acid: glycine (gly), L-alanine (ala), L-tyrosine (tyr), L-methionine (met), L-leucine (leu), L-valine (val), L-serine (ser), L-tryptophan (trp), and L-lysine(lys); dppb = 1,4-bis(diphenylphosphino)butane; bipy = 2,2′-bipyridine], were synthesized and characterized. The X-ray structures of the glycine and leucine complexes showed that the amino acids are coordinated to the metal center through the carboxylate and amine groups. The complexes, except for the glycine ligand, form two different conformational isomers, as suggested by X-ray structure determination of the complex with leucine, and shown by $^{31}P\{^1H\}$ NMR analysis. The cyclic and differential pulse voltammograms of the complexes exhibited an oxidation potential (Ru^{II}/Ru^{III}) close to 1.0 V, against Ag/AgCl. Toxicity tests against the MDA-MB-231 and DU 145 human tumor cell lines and the Ehrlich mouse cell line indicated a high degree of cytotoxicity for the amino acid complexes. The intrinsic binding constants (K_b) for the amino acid complexes were determined and found to be in the range of 1.40×10^4–$1.80 \times 10^4\ M^{-1}$, suggesting weak interactions of the complexes with DNA.

1. Introduction

Until now, metal-based drugs used in cancer treatment have been restricted to Pt compounds, with DNA as the main target. Alternative compounds containing Ru, Ga and Ti have become the subject of intense investigation in cancer research [1,2]. Ruthenium compounds are of particular interest as they should have a mechanism of action different from that of Pt antitumor agents. Ruthenium should also offer reduced toxicity, since its chemistry similar to that of iron, making it able to bind biomolecules such as transferrin [3]. Thus, there are some ruthenium complexes with remarkably low general toxicity, in preclinical or clinical trials, which show efficacy against several kinds of cancer and metastases [4–7]. Recently our research group has published some results showing that ruthenium/diphosphine complexes are promising as potential anticancer drugs; and here we present some ruthenium/diphosphine/amino acid complexes that also displayed good cytotoxic activity against the tumor cells MDA-MB-231, DU-145 (human), and Ehrlich (mouse), but lower activity against healthy cells (L-929, mouse) [7].

In the literature, there are some reports on compounds with the general formula [Ru-(diimine)$_2$(AA-H)]$^+$ (diimine = 1,10-phenanthroline or 2,2′-bipyridine; AA = optically active amino acid), for which diastereoisomeric pairs have been isolated, under conversion reactions in the light, according to the equation [8–12]:

$$\Delta - [Ru(diimine)_2(AA-H)]^+ \rightleftarrows \Lambda - [Ru(diimine)_2(AA-H)]^+ \quad (1)$$

Here, we wish to describe a class of Ru(II)-amino acid complexes that, unlike those diimine/ruthenium complexes, do not suffer light-catalysed inversion of their diastereomers.

The design of the [Ru(AA-H)(dppb)(bipy)]PF_6 [dppb = 1,4-bis(diphenylphosphino)butane; AA-H = deprotonated aminoaacid] complexes took into account the fact that phosphines are π acceptor ligands that stabilize the ruthenium(II) ion and that amino acids, as biomolecules, are well recognized by human or

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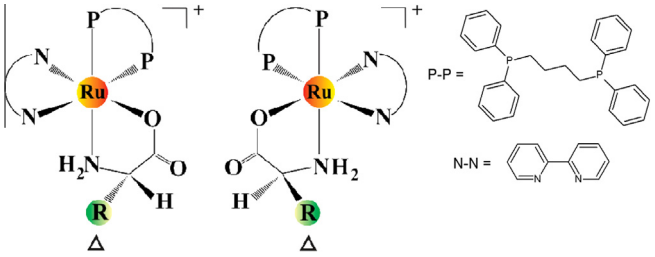

Fig. 1. Diastereomers of [Ru(Ala)(dppb)(bipy)]PF_6, derived from the chirality of the glycinato ion.

mouse cells, making the complexes attractive to them. The 2,2′-bipyridine was used to complete the coordination sphere of the metal center, and also to help to stabilize the complexes. This kind of complex, [Ru(AA-H)(dppb)(bipy)]PF_6, can exist as two diastereomers, as in Fig. 1.

Thus, one aim of this work was also to show that the diastereomers (Λ and Δ) of the Ru(II)/diphosphine/amino acids complexes exhibit cytotoxic activities against the MDA-MB-231 and DU 145 human tumor cell lines and the Ehrlich mouse cell line

2. Experimental

2.1. Chemicals

All manipulations were carried out under purified argon using standard Schlenk technique. Reagent grade solvents were appropriately distilled and dried before use. The $RuCl_3.xH_2O$, NH_4PF_6, 1,4-bis(diphenylphosphino)butane (dppb), 2,2′-bipyridine (bipy), highly polymerized CT-DNA and Tris–HCl (5 mM Tris–HCl and 50 mM NaCl, pH 7.4). were purchased from Aldrich. The concentration of the DNA nucleotides was determined by UV absorption spectroscopy, using the molar absorption coefficient (ε = 6600 M^{-1} cm^{-1}) at 260 nm [13]. The stock solution was stored at 4 °C. The amino acids glycine (gly), L-alanine (ala), L-tyrosine (tyr), L-methionine (met), L-leucine (leu), L-valine (val), L-serine (ser), L-tryptophan (trp), and L-Lysine(lys), were purchased from Strem. The starting complex, *cis*-[$RuCl_2$(dppb)(bipy)], was prepared as described in the literature [14].

2.2. Apparatus

Elemental analyses (C, H and N contents) were recorded in an EA1108 Fisions Instrument CHNS microanalyzer, at the Microanalytical Laboratory of Universidade Federal de São Carlos (São Carlos, Brazil). Cyclic voltammetry (CV) experiments were carried out at room temperature in CH_2Cl_2 containing 0.100 M tetrabutylammonium perchlorate (TBAP Fluka Purum) using a BAS-100B/W Bioanalytical Systems Instrument. The working and auxiliary electrodes were stationary Pt foils; the reference electrode was Ag/AgCl, in a Luggin capillary probe, a medium in which ferrocene is oxidized at 0.43 V (Fc+/Fc). The voltammograms were performed at a scan rate of 0.100 V s^{-1}, at 25 °C. The IR spectra of the complexes were recorded on a FTIR Bomem-Michelson 102 spectrometer in the 4000–200 cm^{-1} region using solid samples pressed in CsI pellets. The electronic spectra were recorded in CH_2Cl_2 solutions on a Hewlett–Packard diode array model 8452A spectrophotometer. $^{31}P\{^1H\}$ NMR experiments were recorded on a BRUKER 9.4 T spectrometer (400 MHz for hydrogen frequency) in CH_2Cl_2, using a capillary containing D_2O.

All the UV–Vis spectra for DNA-complex interactions were collected with the HP 8452A diode array spectrophotometer. Solutions of CT-DNA and complexes were scanned in a 1 cm quartz cuvette. All absorbance measurements were carried out with a fixed concentration of DNA (4.5×10^{-3} mol L^{-1}), while the complex concentration varied from 1.0×10^{-5} mol L^{-1} to 3.5×10^{-5} mol L^{-1}. The intrinsic binding constants of interaction of the complexes with DNA were calculated by the neighbor exclusion model [15]. It was not possible to dissolve any of the complexes directly in aqueous phosphate buffer, but all the complexes were very soluble in DMSO, and the presence of 1–2% DMSO proved sufficient to solubilize them in either pH 7.4 phosphate buffer or PBS. In these solutions all complexes were stable for at least 72 h. A Rayonet reactor with seventeen lamps (RPR-4190 or RPR-5750) was used to test for the possible photochemical reaction.

2.3. X-ray crystallography

Orange crystals of the isolated complexes [Ru(gly)(dppb)(bipy)]PF_6·3/2(H_2O) (**1**) and [Ru(leu)(dppb)(bipy)]PF_6·1/2(CH_3OH) (**2**) were grown by slow evaporation of methanol/diethyl ether solutions. The crystals were mounted in an Enraf–Nonius Kappa-CCD diffractometer with graphite monochromated Mo Kα (λ = 0.71073 Å) radiation. The final unit cell parameters were based on all reflections. Data were collected with the COLLECT program [15]. Integration and scaling of the reflections were performed with the HKL Denzo-Scalepack suite of programs [16]. Gaussian or semi-empirical corrections from equivalents absorption were carried out [17]. The structures were solved by direct methods with SHELXS-97 [18]. The models were refined by full-matrix least squares on F^2 by means of SHELXL-97 [19]. All hydrogen atoms were stereochemically positioned and refined with the riding model. The thermal ellipsoid representations, at 50% probability, shown in Figs. 2 and 3 were prepared with ORTEP-3 for Windows [20]. Disordered anions and lattice solvent molecules have been omitted from the figures for clarity. This disordered species were treated as sum of occupancy for F atoms. The collected data and some experimental details are summarized in Table 1.

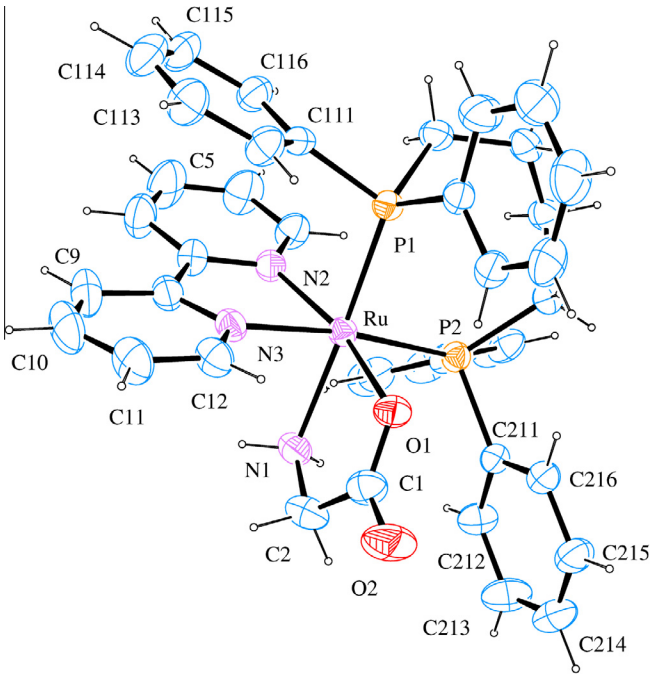

Fig. 2. ORTEP view of the cation [Ru(gly)(dppb)(bipy)]$^+$ with labeled atoms at the 50% probability level.