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Kinetics and mechanism of substitution reactions of the new bimetallic [{PdCl(bipy)}{μ-($NH_2(CH_2)_6H_2N$)}{PtCl(bipy)}]Cl(ClO_4) complex with important bio-molecules

Snežana Jovanović [a], Biljana Petrović [a], Marijana Petković [b], Živadin D. Bugarčić [a,*]

[a] Department of Chemistry, Faculty of Science, University of Kragujevac, R. Domanovića 12, P. O. Box 60, 34000 Kragujevac, Serbia
[b] Department of Physical Chemistry, Institute of Nuclear Sciences "Vinča", University of Belgrade, Mike Petrovića Alasa 12-14, 11001 Belgrade, Serbia

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ABSTRACT

The new dinuclear bimetallic complex, [{PdCl(bipy)}{μ-($NH_2(CH_2)_6H_2N$)}{PtCl(bipy)}]Cl(ClO_4) (bipy is 2,2′-bipyridine), has been prepared and characterized by elemental microanalysis, IR, ^{1}H NMR spectroscopy and MALDI-TOF mass spectrometry. Substitution reactions of the studied complex with selected biologically important ligands such as: thiourea (Tu), L-methionine (L-Met), L-cysteine (L-Cys), L-histidine (L-His) and guanosine-5′-monophosphate (5′-GMP), were studied under the *pseudo*-first order conditions as a function of concentration and temperature using stopped-flow and UV–Vis spectrophotometry. The reactions were monitored in 0.1 M $NaClO_4$ at pH 5.0, in the presence of 40 mM NaCl. All fast reactions were monitored by stopped-flow at three temperatures (288 K, 298 K, 308 K) to determine the activation parameters, while the reactions studied by UV–Vis spectrophotometry were tested only at 298 K. Observed order of reactivity of the used ligands is: Tu > L-Met > L-Cys > L-His > 5′-GMP. Substitution reactions of the investigated bimetallic complex with Tu, L-Cys and L-His were followed by degradation to the corresponding substituted mononuclear complexes of palladium (II) and platinum (II), [Pd(bipy)($Nu)_2$] and [Pt(bipy)($Nu)_2$] (where Nu = Tu, L-Cys, L-His), by releasing of the bridge ligand,1,6-diaminohexane. In contrast, during the substitution reactions with L-Met and 5′-GMP, the structure of starting bimetallic complex was preserved and the process of degradation can be halted. The proposed pathways of the substitution reactions of [{PdCl(bipy)}{μ-($NH_2(CH_2)_6H_2N$)}{PtCl(bipy)}]Cl(ClO_4) complex with all selected ligands were confirmed by ^{1}H NMR spectroscopy at 295 K. Additionally, the two pK_a values of studied diaqua complex, [{Pd(H_2O)(bipy)}{μ-($NH_2(CH_2)_6H_2N$)}{Pt(H_2O)(bipy)}]$^{4+}$, were determined by spectrophotometric pH titration. The large negative values for the entropy of activation, $\Delta S^{\neq}$, support an associative substitution mechanism.

1. Introduction

Although mononuclear platinum anticancer agents hold a major place in the treatment of many forms of cancers, there is a potential to improve response and survival in patients. Development of resistance to therapy and toxic side effects are major problems, which have prompted research into new platinum drugs, displaying different mechanisms of action [1]. Currently, attention is given to platinum compounds with structures distinctly different from cisplatin with an idea that their different nature of interaction with DNA would translate into different spectrum of activity and toxicity profile [2–4]. One such class of compounds is the polynuclear platinum complexes which contain two or more reactive platinum centers firmly linked by variable bridging ligands [5–9]. Polynuclear platinum complexes form DNA adducts structurally distinct from those of cisplatin [10,11]. The first polynuclear platinum complex to enter clinical trials is BBR3464, trinuclear platinum complex with 1,6-diaminohexane as the bridge ligand [12]. This complex has shown promising anticancer activity in a variety of preclinical test systems, including systems with native and acquired resistance to cisplatin [13,14]. Further research in

Abbreviations: 2, 2′-bipy, 2, 2′-bipyridine; Tu, thiourea; L-Met, L-methionine; L-Cys, L-cysteine; L-His, L-histidine; 5′-GMP, guanosine-5′-monophosphate; DHB, 2,5-dihydroxybenzoic acid; MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight.

* Corresponding author. Tel.: +381 (0)34300262; fax: +381 (0)34335040.
E-mail addresses: snezanaj@kg.ac.rs (S. Jovanović), biljanap@kg.ac.rs (B. Petrović), marijanapetkovic@vin.bg.ac.rs (M. Petković), bugarcic@kg.ac.rs (Ž.D. Bugarčić).

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this area leads to the synthesis of many other di- and trinuclear platinum complexes as potential anticancer drugs [15–17].

The long-term application of platinum complexes is leading scientists to examine other metals such as palladium, which could exhibit complementary modes of action. Over the last 10 years several research groups have focused on the application of the palladium complexes as potential anti-cancer agents [18,19].

Another aspect in terms of the application of palladium-based complexes as anticancer agents is related to the introduction of additional transition metals reaching to the possible enhancement in the cytotoxic activity. Heterometallic complexes can lead to difference in interactions with multiple biological targets and therefore could have potential as possible anticancer agents [20,21]. Platinum is the most commonly used transition metal in combination with palladium [22–24]. Thus, many bimetallic di- and trinuclear complexes were found to exhibit significantly anticancer activity against ovarian cancer cell lines in comparison to cisplatin. It is believed that these complexes form a long-range interstrand adducts with duplex DNA that induces global changes in the DNA conformation, unlike cisplatin that forms mainly intrastrand adducts that induces a local kink in a DNA strand [25].

In this study we synthesized new bimetallic dinuclear complex of palladium(II) and platinum(II), [{PdCl(bipy)}{μ-($NH_2(CH_2)_6H_2N$)}{PtCl(bipy)}]Cl(ClO_4), (Fig. 1) and determined pK_a values of the corresponding aqua complex. With the aim to gain insight into possible interactions between dinuclear complex containing two different metal centers (platinum and palladium) with biologically relevant ligands, we studied the substitution reactions of synthesized complex with ligands such as Tu, L-Cys, L-Met, L-His and 5′-GMP. It is well known that the antitumor activity of platinum complexes is a result of the interaction between complex and DNA molecule while resistance and toxic side effects arise from the interactions with sulfur donor biomolecules (proteins and peptides containing L-Cys or L-Met) [2,5,11]. For this reason, it is important the detailed understanding of the reaction mechanism of novel bimetallic complex.

All reactions were studied using different experimental techniques as well as stopped-flow, UV–Vis spectrophotometry and ^{1}H NMR spectroscopy. The structures of the investigated ligands are presented in the Fig. 2.

2. Experimental

2.1. Materials

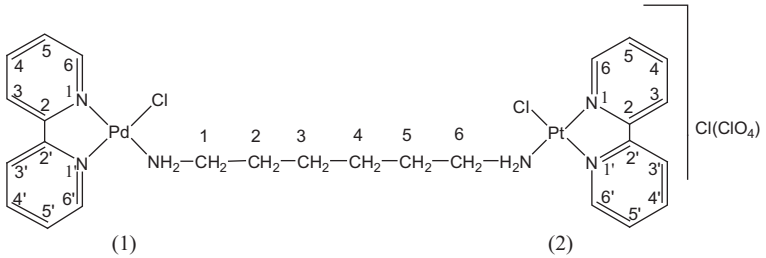
The compounds 2,2′-bipyridine (bipy) (Merck), K_2PtCl_4 (Strem Chemicals), $PdCl_2$ (Acros Organics) as well as nucleophiles, thiourea (Tu) (Fluka), L-cysteine (L-Cys) (Merck), L-methionine (L-Met) (Acros Organics), L-histidine (L-His) (Merck) and guanosine-5′-monophosphate sodium salt (5′-GMP) (Acros Organics), were used without purification. The bridging ligand, 1,6-diaminohexane, was also obtained from Acros Organics. The complexes [$PtCl_2$(bipy)] and [$PdCl_2$(bipy)] were synthesized according to a procedure published in the literature [26,27]. Elemental microanalysis, UV–Vis, IC and ^{1}H NMR spectra were used to evaluate the purity of the complexes.

Matrix for MALDI-TOF mass spectrometry, 2,5-dihydroxybenzoic acid (DHB), and solvent for matrices and complex (methanol) were purchased from Aldrich. Deuterium-oxide (D_2O) (Deutero GmbH, 99.9%) and dimethylformamide (DMF) are commercially available and used as received. All other chemicals were of the highest purity commercially available and were used without further purification. Ultrapure water was used in all experiments involving aqueous solutions.

2.2. Synthesis of complex

The new bimetallic dinuclear complex, [{PdCl(bipy)}{μ-($NH_2(CH_2)_6H_2N$)}{PtCl(bipy)}]Cl(ClO_4), was synthesized by modification of the procedure reported in the literature [25]. The complex [$PtCl_2$(bipy)] (100.0 mg, 0.236 mmol) was dissolved in DMF (10 cm^3) and a solution of $AgClO_4$ (49.1 mg, 0.236 mmol) in DMF (5 cm^3) was added. The mixture was stirred overnight in the dark, at room temperature. The precipitate AgCl was removed by filtration and the resulting pale yellow solution of [PtCl(bipy)(DMF)]ClO_4 was kept in a refrigerator to cool down. A suspension of [$PdCl_2$(bipy)] complex (73.5 mg, 0.220 mmol) in 10 cm^3 of DMF was heated with stirring at 303–313 K for about 30 min. After that, the solution of 1,6-diaminohexane (25.5 mg, 0.220 mmol) in 5 cm^3 DMF was added dropwise. The mixture was stirred at room temperature for 5 h in the dark. The filtrate [PtCl(bipy)(DMF)]ClO_4 was added to the resulting mixture. The clear yellow solution was stirred for 3 h at 323 K and then for 24 h at room temperature. The solution was then evaporated and the residue washed with ether. A light yellow powder was obtained and left to dry in the air. Yield (63.8 mg, 62%). *Anal.* Calc. for $PtPdCl_4O_4N_6C_{26}H_{32}$ (FW = 935.88): C, 33.37; H, 3.45; N, 8.98. Found: C, 33.07; H, 3.80; N, 8.73%. ^{1}H NMR characterization (D_2O, 200 MHz). ^{1}H NMR (δ, ppm): 1.35–1.50 (m, CH_2 C3, C4), 1.60–1.80 (m, CH_2 C2, C5), 2.95–3.06 (m, CH_2 C1, C6), 7.30–7.42 (d, CH H5/H5′(1)), 7.45–7.60 (d, CH H5/H5′(2)), 7.65–7.84 (m, CH H4/H4′(1)), 8.05–8.15 (m CH H4/H4′(2)), 8.17–8.28 m, CH H3/H3′(1)), 8.32–8.40 (m, CH H3/H3′(2)), 8.42–8.48 (d CH H6/H6′(1)), 8.50–8.58 (d CH H6/H6′(2)). IR (KBr, 4000–300 cm^{-1}): 3438 (N–H stretch); 2853, 2927 (CH_2 stretch); 1610 (C=N stretch); 1089 (perchlorate counter ion); 765, 812 (N–H wagging) (Fig. S1).

Solution of the aqua complex was prepared by suspending a known quantity of the [{PdCl(bipy)}{μ-($NH_2(CH_2)_6H_2N$)}{PtCl(bipy)}]Cl(ClO_4) complex in 0.1 M $NaClO_4$ and adding 3 equivalents of $AgClO_4$ (with respect to chloride) [28]. The solution was acidified with $HClO_4$ to pH 2 (for determination of the pK_a values). The mixture was then stirred in the dark over night at 323 K. Formed white



(1) (2)

[{PdCl(bipy)}{μ-($NH_2(CH_2)_6H_2N$)}{PtCl(bipy)}]Cl(ClO_4)

Fig. 1. Structure of the studied complex.