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ORIGINAL PAPER

Non-empirical quantum chemical studies on electron transfer reactions in *trans*- and *cis*-diamminedichloroplatinum(II) complexes

Janina Kuduk-Jaworska · Henryk Chojnacki · Jerzy J. Jański

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Abstract The search was made for theoretical confirmation of hypothesis that mechanism of cisplatin cytotoxicity is based on dissociative electron transfer (ET) processes. Applying quantum chemical calculations based on super-molecular approach, the reactions mimicking presumed steps of cisplatin activation were evaluated. The electronic structure of model systems: *cis*- and transplatin with free electrons, hydrated electrons, and water, was studied by using density functional (DFT) within the Huzinaga basis set and GAUSSIAN-09 package. The respective energy was evaluated with the use of B3LYP density hybrid functional. The calculations were performed for gas phase and water solution; the solvent effects were studied by using the polarizable continuum model. Analysis of the energetic and structural parameters of cisplatin vs. transplatin behavior in the model systems leads to conclusion: there are two possible ways of cisplatin biotransformation, hydrolysis and hydrated electron impact, dependent on the medium redox state.

J. Kuduk-Jaworska (✉)
Nonpublic Medical College in Wrocław,
Nowowiejska 69,
50–340 Wrocław, Poland
e-mail: janina.kuduk@gmail.com

H. Chojnacki
Institute of Physical and Theoretical Chemistry,
Wrocław University of Technology,
Wyb. Wyspiańskiego 27,
50–370 Wrocław, Poland

J. J. Jański
Faculty of Chemistry, Wrocław University,
F. Joliot-Curie 14,
50–383 Wrocław, Poland

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Introduction

Despite many years of work, the mechanisms underlying anticancer activity of cisplatin (*cis*-diamminedichloroplatinum (II)), and its toxic side effects remain elusive. Thus the better understanding of cellular, molecular and sub-molecular aspects of this drug action is still a true challenge for researchers. The biological activity of cisplatin is determined by kinetic lability and thermodynamic stability at metal-ligand center. After entering the cell, cisplatin undergoes a spontaneous hydrolysis, and the formed aqua products are able to react eagerly with intracellular nucleophiles, among which there is a critical target: genomic DNA [1, 2]. As the motif of cisplatin-DNA adduct, capable of triggering cytotoxic processes leading to cancer cell death, it was postulated the 1,2-d (GpG)-intrastrand cross-linked species. This was assumed from the comparative study of cisplatin with its *trans* stereoisomer, which is anti-cancerous inactive compound, not able to form 1,2-chelated DNA-adduct, probably because of inconvenient structural properties [3].

More recently, it has been discovered many cisplatin metabolic pathways on which the subtle molecular differences between drug and its *trans*-isomer have been traced. In result, there were noticed significant differences in the ability of both isomers to proceed cellular uptake, DNA platination, transduction of DNA-damage signals, arresting cell cycle, and triggering cell death [4]. However, it is still not clear which features of isomers are responsible for drastic differences in their biological effects. Thus, the role

of transplatin as a reference substance at resolution of cisplatin mechanistic problems is still topical.

Regarding mechanistic hypotheses of cisplatin biotransformation, the first process which turn pro-drug into its active forms might be described as SN2 substitution of labile chloride ligands by water molecules [5, 6]. The further steps of in vivo processes were not so clearly postulated, mainly because of difficulties in experimental and theoretical exploration of the interaction between transformed drug and high-molecular, biological targets (DNA, proteins). In general, the chelating binding mode of DNA was concluded from results of studies on the model systems: cis-$[Pt(NH_3)_2Cl(H_2O)]^+$ with biomolecules such as aminoacids, purines, nucleosides and polynucleotides [3, 4, 7–9]. However, the anticipated mechanism has been still a subject of discussion and controversies. Firstly, it is questionable whether the monoaqua-intermediate could selectively and specifically interact with N-donor centers of DNA. The observed preferences of cisplatin to bind DNA-purines over thiols, as well as its ability to chelate DNA at two adjacent *N7* of guanine have been difficult to explain assuming the crucial role of SN2 products. Moreover, the enhancement of metabolic radical levels in biological material after treatment with cisplatin, and strong influence of antioxidants on biological effects became the basis to challenge the SN2 mechanism and to look out for other explanation of cisplatin biotransformation [2, 9–12].

The alternative courses of ligand exchange have been considered for years. Apart from the associative mode of substitution at metal center of chloroam(m)ineplatinum(II) complexes it has been known the concurrent reactions going according to dissociative mechanism, characterized by the three-coordinated, 14-e, intermediate. However, this pathway was rather rejected for cisplatin and its simple analogs, although it was accepted for ligand exchange in organo-metallic complexes [13]. According to Romeo [14] there are some factors which concur in promoting dissociation: (i) high electron density at the metal center; (ii) bond weakening at leaving group due to the *trans* influence of strong sigma-donors; and (iii) the stabilization of three in-plane ligands of intermediate.

Taking into account that electron transfer (ET) processes play an important vital role, the dissociative electron attachment (DEA) was suggested as a possible pathway leading to cisplatin biotransformation. The idea that ET processes might determine the formation of cytotoxic cisplatin species able to chelate DNA, was recently formulated by Lu et al. [15] who then confronted it with experimental observations [16]. Using spectroscopic and femtosecond laser techniques, authors revealed high reactivity of cisplatin with electrons created in this experiment. The results were likely to confirm the role of low-energy electrons (LEE) in the cleavage of Pt-Cl bonds that was assumed as crucial for cisplatin transformation into cytotoxic products. Other authors [17] studied the LEE attachment to cisplatin in processes mimicking chemoradiation therapy. The experiment was carried out in an electron-attachment spectrometer, and resulted data were completed by DFT calculations which persuaded authors to consider the anionic $[Pt(NH_3)_2]^-$ intermediate as reagent able to form cisplatin-DNA adducts, responsible for inhibitory effect. Consequently ET was assumed to be the most direct route in cisplatin-DNA adduct formation during the synchronous combination of cisplatin and radiation.

In present work we undertook the quantum chemical studies on cisplatin "pro-drug into drug" transformation, initiated by electron impact according to description given by Lu and co-authors [15]. But, contrary to studies performed so far [16–18] we do not assume that described reactions are characteristic only of chemoradiation therapy. We think, that Lu's new hypothesis relates also to other conditions in living organisms, as electrons are abundantly produced by various redox systems. Thus, the calculated data are not restricted to interaction of pro-drug with electrons specific for post-radiation state.

The tools and methodology applied here were based on rich literature announcements [19–29] including *ab initio* and DFT studies of electronic structures of cisplatin and its congeners as well as the evaluation of kinetic lability and thermodynamic stability at Pt-ligand center, particularly in reactions with substrates mimicking DNA target [19–21]. One of the important trends in theoretical studies of cisplatin biotransformation became the molecular-dynamic simulation of $[Pt(NH_3)_2Cl_2]$ hydrolysis processes. The results are likely to confirm the associative mechanism of ligand (Cl-) exchange with a trigonal-bipyramidal structure (18-e) of the transition state [7, 8, 23–29]. Thus, it seemed attracting to confront the hydrolysis processes running with or without electron impact, and find the theoretical arguments for hypothetical pathways of cisplatin activation.

Computational procedure

Applying quantum chemical calculations, the model reactions mimicking presumed steps of cisplatin anticancer activation were evaluated. The electronic structure of model systems: cis- and transplatin with free electrons, hydrated electrons, and water, have been studied. The molecular structures with the relevant diagrams of the total energies for substrates, intermediates and products are collected in Tables 1 and 2, and depicted in Figs. 1, 2, 3, 4, 5 and 6.

In our calculations the relative energies of the same number of atoms and electrons were compared. For example, in the case of hydrolysis of *cis*-platin with hydrated $(eH_2O)^-$ electron, this energy is equal to:

Table 1 Mulliken charges (in e) of Pt-containing species and total energies (in atomic units, a.u.) related to the states of reactions **1–8**; calculations obtained within Gas Phase and PCM /solvent models

Reaction components used to calculation	Reaction state	Gas phase [e], Partial charge on Pt	Solvent [e], Partial charge on Pt	Gas phase [a.u.] Total energies	solvent/PCM [a.u.] Total energies
Ic. Cisplatin + 2e-					
cis-$Pt^{II}(NH_3)_2Cl_2$ +2e-	1c1	0.387	0.365	−18354.0703365	−18354.1104432
cis-$[Pt^{I}(NH_3)_2Cl_2]^-$ + e^-	2c1	0.006	−0.050	−18354.0756645	−18354.1829920
$[Pt^{I}(NH_3)_2Cl^-]^0$ + e- + Cl^-	3c1	0.124	0.087	−18354.0024030	−18354.1768288
$Pt^0(NH_3)_2Cl]^-$ + Cl^-	4c1	−0.501	−0.558	−18354.0244630	−18354.2718456
$Pt^0(NH_3)_2$ + $2Cl^-$	5c1	−0.225	−0.275	−18353.9121546	−18354.1842319
It. Transplatin + 2e-					
trans-$Pt^{II}(NH_3)_2Cl_2$ +2e-	1t1	0.380	0.359	−18354.0891905	−18354.117677
trans-$[Pt^{I}(NH_3)_2Cl_2]^-$ + e-	2t1	−0.044	−0.099	−18354.0859179	−18354.1876167
$[Pt^{I}(NH_3)_2Cl^-]^0$ + e- + Cl^-	3t1	0.082	0.058	−18354.0154290	−18354.1816314
$Pt^0(NH_3)_2Cl]^-$ + Cl^-	4t1	−0.596	−0.659	−18354.0035830	−18354.2652804
$Pt^0(NH_3)_2$ + $2Cl^-$	5t1	−0.369	−0.490	−18353.9612303	−18354.2244608
IIc. Cisplatin + $2H_2O$ + $2e^-$					
cis-$[Pt^{II}(NH_3)_2Cl_2]$ + $2H_2O$ + 2e-	1c2	0.387	0.364	−18506.7028124	−18506.9517420
cis-$[Pt^{I}(NH_3)_2Cl_2(H_2O)]^-$ + H_2O + e-	2c2	0.011	−0.030	−18506.8691578	−18507.0717602
$Pt^{I}(NH_3)_2Cl(H_2O)]^{\cdot}$ +H_2O + Cl^- + e-	3c2	0.143	0.107	−18506.7890023	−18507.0665282
$[Pt^0(NH_3)_2Cl(H_2O)_2]^-$ + Cl^-	4c2	−0.418	−0.469	−18506.9784738	−18507.2054740
$[Pt^0(NH_3)_2(H_2O)_2]$ + $2Cl^-$	5c2	−0.281	−0.349	−18506.877592	−18507.1819145
IIt. Transplatin + $2H_2O$ + $2e^-$					
trans-$Pt^{II}(NH_3)_2Cl_2$ + $2H_2O$ + 2e-	1t2	0.380	0.359	−18506.7216375	−18506.9595038
trans-$[Pt^{I}(NH_3)_2Cl_2(H_2O)]^-$ + H_2O + e-	2t2	−0.075	−0.096	−18506.8794150	−18507.0780156
$[Pt^{I}(NH_3)_2Cl(H_2O)]$ + e- + H_2O + Cl^-	3t2	0.101	0.091	−18506.7998136	−18507.0700772
$[Pt^0(NH_3)_2Cl(H_2O)_2]^-$ + Cl^-	4t2	−0.388	−0.426	−18506.9790457	−18507.2181677
$[Pt^0(NH_3)_2(H_2O)_2]$ + $2Cl^-$	5t2	−0.329	−0.394	−18506.9052399	−18507.2115024
IIIc. Cisplatin + $2H_2O$					
cis-$Pt(NH_3)_2Cl_2$ + $2H_2O$	1c3	0.387	0.365	−18506.955363	−18507.0173590
cis-$[Pt(NH_3)_2Cl_2(H_2O)]$ + H_2O	2c3	0.432	0.432	−18506.958158	−18507.0101168
cis-$[Pt(NH_3)_2Cl(H_2O)]^+$ + Cl^- + H_2O	3c3	0.516	0.510	−18506.737112	−18507.0007048
cis-$[Pt(NH_3)_2Cl(H_2O)_2]^+$ + Cl^-	4c3	0.508	0.496	−18506.773572	−18507.0188033
cis-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ + $2Cl^-$	5c3	0.691	0.691	−18506.364600	−18506.9744012
IIIt. Transplatin + $2H_2O$					
trans-$Pt(NH_3)_2Cl_2$ + $2H_2O$	1t3	0.380	0.359	−18506.974188	−18507.0251374
trans-$[Pt(NH_3)_2Cl_2(H_2O)]$ + H_2O	2t3	0.392	0.377	−18506.997170	−18507.0348082
trans-$[Pt(NH_3)_2Cl(H_2O)]^+$ + Cl^- + H_2O	3t3	0.527	0.531	−18506.738179	−18507.0004377
trans-$[Pt(NH_3)_2Cl(H_2O)_2]^+$ + Cl^-	4t3	0.514	0.515	−18506.763185	−18507.0111202
trans-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ + $2Cl^-$	5t3	0.717	0.715	−18506.359081	−18506.9688534

$E = E\left[cis - Pt(NH_3)_2Cl_2\right] + E\left[(eH_2O)^-{}_2\right]$. Thus, the relevant energies of the 5 reaction steps may be expressed as:

$$E[1c2] = E\left[cis{-}Pt(NH_3)_2Cl_2\right] + E\left[(eH_2O)^-{}_2\right]$$
$$E[2c2] = E\left[cis{-}Pt(NH_3)_2Cl_2(eH_2O)\right]^{*-} + E[(eH_2O)^-]$$
$$E[3c2] = E\left[cis{-}Pt(NH_3)_2Cl(eH_2O)\right]^- + E[(eH_2O)^-] + E[Cl^-]$$
$$E[4c2] = E\left[cis{-}Pt(NH_3)_2Cl(eH_2O)_2\right]^{*-} + E[Cl^-]$$
$$E[5c2] = E\left[cis{-}Pt(NH_3)_2(eH_2O)_2\right] + E[2Cl^-].$$

The reactants and products were considered as one molecular system in the singlet or doublet (in the case of anionic systems) electronic ground state. Such molecular systems were considered without evaluation of the interaction energies, i.e., no BSSE was evaluated. This effect is usually not very large, e.g., in the case of the 2t2 complex the corrections to the total energies were of the order of 1 kcal mol^{-1}. This points out that, for both cases the energy profiles of reactions are very similar. We have also compared the reaction total energies and Gibbs free energies and the reaction profiles were the same for both cases.

Table 2 Key geometrical parameters of Pt-containing species formed in reactions 1–8; calculated bond distances in angstroms, bond angles in degrees

Pt-complex	Pt-N	Pt-N	Pt-Cl	Pt-Cl	Pt-O	Pt-O	N-Pt-N	N-Pt-Cl	Cl-Pt-Cl	O-Pt-Cl	O-Pt-N
***cis*-$Pt(NH_3)_2Cl_2$**	2.136	2.136	2.361	2.361			98.5	82.5/179	96.5		
cis-$[Pt(NH_3)_2Cl_2]^-$	2.313	2.313	2.537	2.537			97	77.5/175	108		
$Pt(NH_3)_2Cl^·$	2.171	2.237	2.365				95	177/88			
$[Pt(NH_3)_2Cl]^-$	2.098	3.215	2.331				66	173/107			
$Pt(NH_3)_2$	2.005	3.345					84				
***trans*-$Pt(NH_3)_2Cl_2$**	2.088	2.088	2.382	2.382			180	89/91	180		
trans-$[Pt(NH_3)_2Cl_2]^-$	2.254	2.254	2.547	2.547			180	82/98	180		
$Pt(NH_3)_2Cl^·$	2.124	2.124	2.458				174.5	87/87			
$[Pt(NH_3)_2Cl]^-$	2.085	2.085	3.068				155.5	78/78			
$Pt(NH_3)_2$	2.085	2.085					155				
***cis*-$Pt(NH_3)_2Cl_2$**	2.136	2.136	2.361	2.361			98.5	82.5/179	97		
$[(H_2O)\{$*cis*-$Pt(NH_3)_2Cl_2\}]^-$	2.281	2.341	2.520	2.591	3.247		98	78/78	106		
$[(H_2O)\{$*cis*-$Pt(NH_3)_2Cl\}]^·$	2.178	2.231	2.385	–	3.744		95.6	93			
$[(H_2O)_2(NH_3)\{Pt(NH_3)Cl\}]^-$	2.097	3.394	2.384	–	3.180	3.172		170			
$[(H_2O)(NH_3)\{Pt(NH_3)(H_2O)\}]$	2.055	3.896	–	–	2.181	2.946					105
***trans*-$Pt(NH_3)_2Cl_2$**	2.088	2.088	2.382	2.382			180	89/91	180		
$[(H_2O)\{$*trans*-$Pt(NH_3)_2Cl_2\}]^-$	2.191	2.187	2.639	2.635	3.197		172	92.3	136		
$[(H_2O)\{$*trans*-$Pt(NH_3)_2Cl\}]^·$	2.121	2.132	2.483	–	3.769		178	85			
$[(H_2O)_2(Cl)\{Pt(NH_3)_2\}]^-$	2.097	2.094	3.798	–	3.121	3.230	163				
$[(H_2O)_2\{Pt(NH_3)_2\}]$	2.091	2.091			3.134	3.134	178				
***cis*-$Pt(NH_3)_2Cl_2$**	2.136	2.136	2.361	2.361			98.5	82.5/179	96.5		
Cis-$[Pt(NH_3)_2Cl_2(H_2O)]$	2.022	2.089	2.338	4.052	2.193	–	91	91.5/178		84	94/175
cis-$[Pt(NH_3)_2Cl(H_2O)]^+$	2.076	2.152	2.326	–	2.150		96	86.5/177		89	88/175.5
cis-$[(H_2O)(Pt(NH_3)_2(Cl)(H_2O))]^+$	2.087	2.140	2.348	–	2.107	3.738	96	86/177.5		92	85.5/178
cis-$[Pt(NH_3)_2(H_2O)_2]^{2+}$	2.073	2.073	–	–	2.137	2.137	92				86/178
***trans*-$Pt(NH_3)_2Cl_2$**	2.088	2.088	2.382	2.382			180	89/91	180		
trans-$[Pt(NH_3)_2Cl_2(H_2O)]$	2.057	2.066	2.346	2.382	3.822		178.5	89/92	124		
trans-$[Pt(NH_3)_2Cl(H_2O)]^+$	2.096	2.106	2.315	–	2.211		174	87/86		177	89.5/97
trans-$[(H_2O)Pt(NH_3)_2(Cl)(H_2O)]^+$	2.088	2.105	2.327	–	2.203	3.963	175	87/89		176	90
trans-$[Pt(NH_3)_2(H_2O)_2]^{2+}$	2.093	2.107	–	–	2.110	2.108	180				93

According to the reaction schemes 1 – 8, the calculations have been performed for all considered transition states with full geometry optimization without symmetry constraints and within the Huzinaga basis set with polarization functions [30]. However, diffusion Gaussian functions did not enable good convergence of the optimization process.

In the final procedure the density hybrid functional B3LYP has been used for geometry optimization. The solvent effects were studied by using the polarizable continuum model (PCM) method with the epsilon constant for water assumed to be 78.39. For the same density functional and basis set used in all systems, the calculated Mulliken charges are only a comparable parameter in our interpretation of the reaction mechanism.

Using the GAUSSIAN package [31], the total energies were evaluated for all reagents under consideration and this computational procedure was applied both for the gas phase reaction and for the water solution.

Results and discussion

The motive of this work was the recently proposed mechanism of cisplatin activation based on the non-hydrolytic course of the following reaction paths [15]:

$$ep^- + Pt(NH_3)_2Cl_2 \rightarrow \left[Pt(NH_3)_2Cl_2\right]^{*-} \rightarrow Pt(NH_3)_2Cl^· + Cl^- \quad (1)$$

$$ep^- + Pt(NH_3)_2Cl^· \rightarrow \left[Pt(NH_3)_2Cl\right]^{*-} \rightarrow Pt(NH_3)_2{}^· + Cl^-$$
$$ep^- = \text{prehydrated electron}; \quad (2)$$

Our intention was to perform the computational evaluation of postulated reaction pathways 1 – 2 which begin with the impact of prehydrated (free or weakly

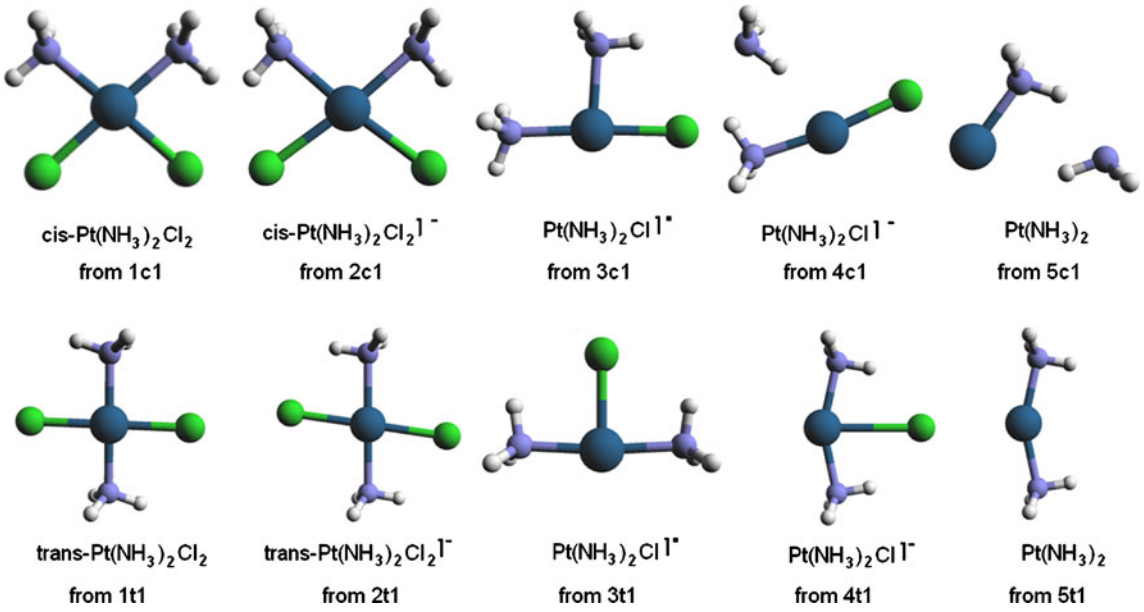

Fig. 1 Optimized structures, intermediates, and products formed in reactions 1–2 of platinum complexes with free electrons

bound) electron on square platinum(II) complex, and lead to the cleavage of Pt-Cl bond(s) and then to formation very reactive [$Pt(NH_3)_2Cl$] radical and [$Pt(NH_3)_2$] species responsible for efficiently damage of DNA. Considering the above theses we have not defined the origin of weakly bound electrons. They might come from various endogenous and exogenous sources such as: (i) metabolic processes (e.g., in mitochondria where electron cascade is created); (ii) biomolecules having weak-bonded electrons (e.g., N-donors); (iii) reducing agents; (iv) UV or ionizing radiation which generate prehydrated electrons [4, 12, 16, 32].

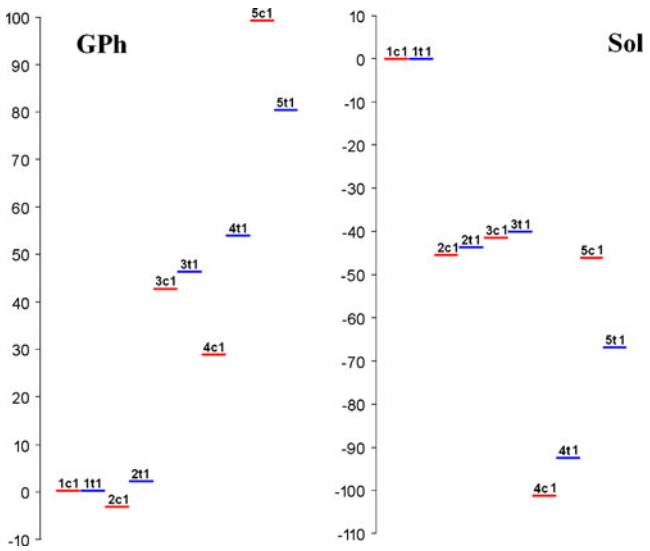

Fig. 2 Reaction profiles for *cis*- and transplatin in paths 1 – 2, calculated using gas phase (GPh) and PCM/solvent models (Sol), [kcal mol^{-1}]

Appreciating the importance of comparative studies on cisplatin and its congeners for the better understanding of mechanism of drug action, we used the isomeric transplatin as reference substrate. Moreover, taking into account that bare free electrons do not exist in real biological systems where water is their indispensable component, we included the H_2O molecules into subjects of calculations. Consequently, there were evaluated three pairs of reagent systems: (i) Ic/It which consist of *cis*- /transplatin and free electrons, related immediately to reactions 1 – 2 of Lu hypothesis; (ii) IIc/IIt which consist of *cis*- /transplatin and hydrated electrons, as was developed in Eqs. 3 – 6; (iii) IIIc/IIIt which present reactions of hydrolysis deprived of electron influence, as was given by Eqs. 7 and 8.

Free electron impact on cisplatin and transplatin

The theoretical investigation of reactions carrying on the systems Ic and It allow to confront the calculated with hypothetical paths of cis- and transplatin transformation initialized by free electron impact. The course of reactions 1–2 was confirmed mainly by structural analysis of resulted species which are depicted in Fig. 1 in a shape of fully optimized structures of substrates, intermediates and products of transformation. The other results characterizing the reaction states and their individual compounds are included in Tables 1 and 2.

Comparing the behavior of isomeric substrates in reactions with free electrons it was noticed that the first stages of transformations, (1c1 → 2c1 → 3c1) and (1t1 → 2t1 → 3t2), proceeded similarly. It means that each complex retains its initial configuration and only negligible

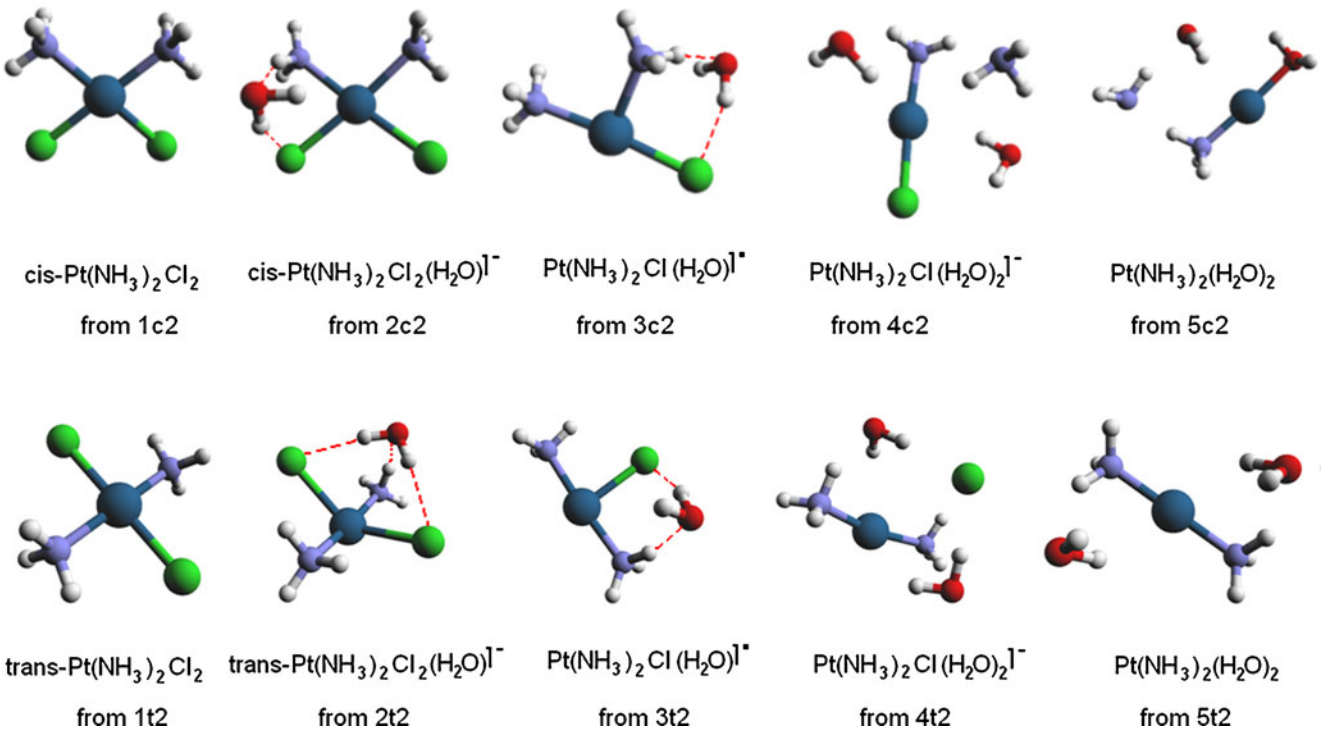

Fig. 3 Optimized structures, intermediates, and products formed in reactions 3–6 of platinum complexes with hydrated electron

changes in the value of angle and bond distances occur (Fig. 1, Table 2). From the 2c1, after the Cl^- ligand releasing and formation of streoisomeric radicals Pt $(NH_3)_2Cl^-$, the original 4-coordinated structures of Pt-complexes have been successively destroyed. Then the second electron impact (Eq. 2) on radical Pt(I)-complexes led to intermediates (4c1 and 4t1) and products (5c1 and 5t1) which inside the pairs differ significantly in the structural properties. For obvious reasons, it was assumed that divergences between results of *cis*- and *trans* reactions are adequate to the different biological effects evoked by both conformers. Consequently, one could believe that biotransformation of cytostatic drug - cisplatin results in compounds able to interact eagerly with DNA, whereas the analogous processes within transplatin do not produce such reactive species. Thus, three compounds: 3c1, 4c1 and 5c1 were considered as activated form of cisplatin, though on this stage we could not definitely indicate which of them is (are) really responsible for the final cytotoxic effect.

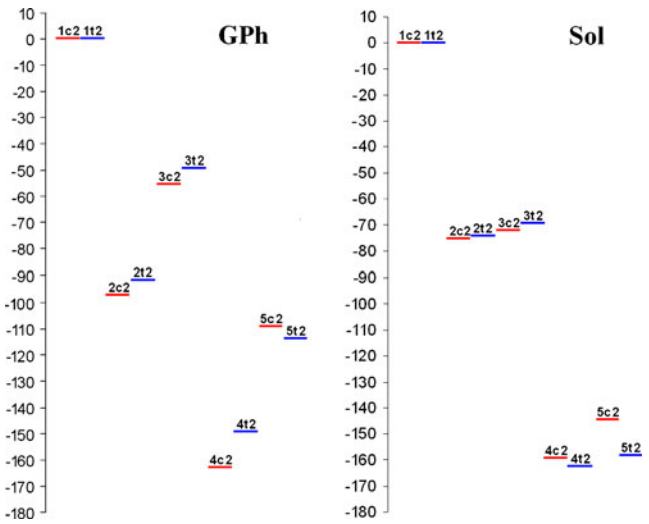

Fig. 4 Reaction profiles for *cis*- and transplatin in paths 3 – 6, calculated using gas phase (GPh) and PCM/solvent models (Sol), [kcal mol^{-1}]

Analyzing the structural data it seemed that crucial for bio-activation might be 3-coordinated Pt-radical species of 3c1, which is an avid electron acceptor, similarly as transplatin intermediate 3t1. However, both radicals differ significantly in their successive courses of reactions. Namely the 3c1 intermediate, after electron attachment is able to lose the NH_3 ligand, leading to formation of anionic 4c1, whereas the Pt-complexes on transplatin pathway do not release the NH_3 ligands. It seems likely that linear location of N-Pt-N bonds makes them very stable while the same location of N-Pt-Cl in 3c1 favors NH_3 abstracting, in accordance with *trans*-effect rule. As a result of different spatial courses the 3t1, contrary to 3c1, is expected to be resistant for ligand substitution and particularly incapable to chelate DNA due to inconvenient stereochemistry.

In a search for other parameters which might correlate with chemical and biological behavior of Pt-compounds there were computed the Mulliken charges and energetic

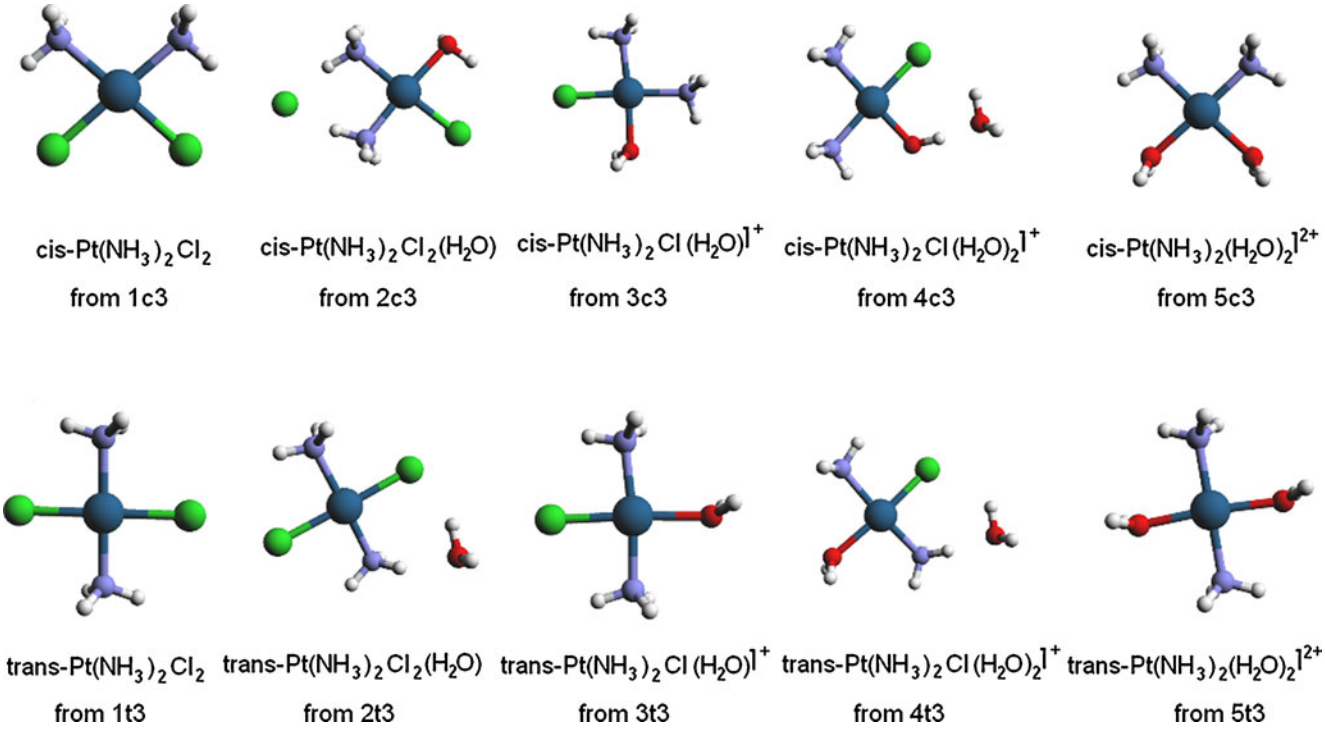

Fig. 5 Optimized structures, intermediates, and products formed in reactions 7–8 of platinum complexes with water

effects associated with the transformation occurring in systems Ic and It (Table 1). Though they differ in values, the Mulliken charges present similar profiles of changes in both confronted, Ic vs. It, paths determined within gas phase and PCM/solvent models. According to predicted courses of Eqs. 1 – 2, the fluctuations of partial charges on Pt atom are appropriate to chemical formula of created compounds. The electron transfer on Pt^{II} complex brings about a reduced central ion ($Pt^{II} \rightarrow Pt^{I}$) which in radical complex, $Pt(NH_3)_2Cl$ (3c1 or 3t1), is able to attach free electron avidly and in reactions 2 evolve into Pt^0 products (4c1 or 4t1), and finally into (5c1 or 5t1).

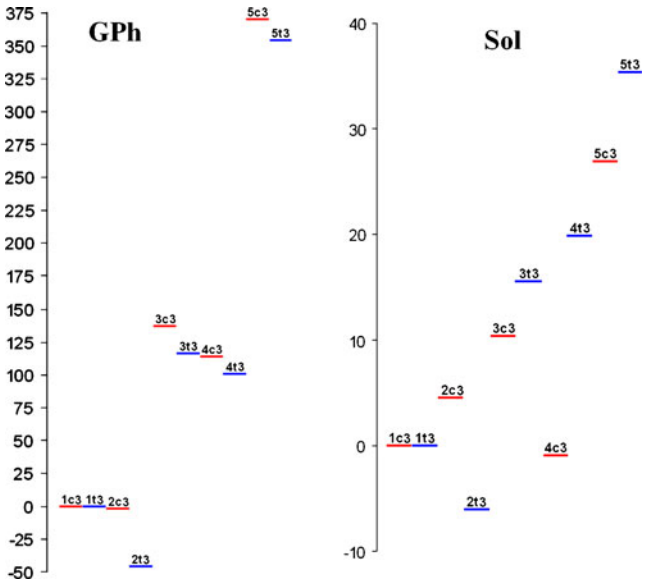

Fig. 6 Reaction profiles for *cis*- and transplatin in paths 7 – 8, calculated using gas phase (GPh) and PCM/solvent models (Sol), [kcal mol^{-1}]

The computed energy effects for Ic/It systems are collected in Table 1, expressed as values of total free energy in a.u., and depicted in Fig. 2 as reaction profiles, calculated in relation to the level of both substrates, and expressed in kcal mol^{-1}. In gas phase model the summary reaction effects are endoenergic for both Ic/It systems, with 99.21 kcal mol^{-1} for difference: E(5c1) – E(1c1), and with 80.26 kcal mol^{-1} for difference: E(5t1) – E(1t1). In PCM/solvent, contrary to gas phase, the summary reaction effects are exoenergic for both Ic/It systems, with −46.28 kcal mol^{-1} for difference: E(5c1) – E(1c1), and with −66.96 kcal mol^{-1} for difference: E(5t1) – E (1t1); the most effective are reaction steps following immediately after electron attachment: 1c1/1t1 → 2c1/2t1 and 3c1/3t1 → 4c1/4t1. The creation of final products: 5c1 and 5t1 are energetically strongly unfavorable (Fig. 2) and thus they should be omitted from potential candidates to bio-active forms. Considering the structural parameters and energy effects the most susceptible to successful interaction with DNA seems to be only 3c1.

Summing up the results of studies carried on reagent systems Ic and It, it was stated the sufficient agreement of calculated data with general assumption of Lu hypothesis [15] represented by the sequence of 1 – 2 reactions. However, it was presumed that more adequate to real biological con-

ditions would be the model enriched by water, bio-molecule of great importance.

Hydrated electron impact on platinum complexes

Seeking after optimal molecular models mimicking the processes of pro-drug biotransformation, it was applied to the systems IIc/IIt reflecting the water-electron impact on both isomeric platinum complexes. It was expected that results would reveal the differences in 3 – 6 reactions courses, and thus help to assess the credibility of commonly accepted or the newly-proposed paths of cisplatin activation. The performed studies showed that hydrated electron impact on substrates 1c2 and 1t2 evolve in two distinct pathways. Despite the significant similarity in ET reaction courses between both, IIc/IIt and Ic/It, systems there are visible the drastic changes in the spatial and energy effects. The following mode of reactions have been obtained:

$$\begin{aligned}&(eH_2O)^- + cis\text{–}Pt(NH_3)_2Cl_2\\ &\rightarrow \left[(H_2O)\{cis\text{–}Pt(NH_3)_2Cl_2\}\right]^-\\ &\rightarrow \left[(H_2O)\{cis\text{–}\left[Pt(NH_3)_2Cl\right]\}\right]^{\cdot} + Cl^-,\end{aligned} \tag{3}$$

$$\begin{aligned}&(eH_2O)^- + \left[(H_2O)\{cis\text{–}Pt(NH_3)_2Cl\}\right]^{\cdot}\\ &\rightarrow \left[(H_2O)_2(NH_3)\{Pt(NH_3)Cl\}\right]^-\\ &\rightarrow [(H_2O)(NH_3)\{Pt(NH_3)(H_2O)\}] + Cl^-,\end{aligned} \tag{4}$$

$$\begin{aligned}&(eH_2O)^- + trans\text{–}Pt(NH_3)_2Cl_2\\ &\rightarrow \left[(H_2O)\{trans\text{–}Pt(NH_3)_2Cl_2\}\right]^-\\ &\rightarrow \left[(H_2O)\{trans\text{–}Pt(NH_3)_2Cl\}\right]^{\cdot} + Cl^-,\end{aligned} \tag{5}$$

$$\begin{aligned}&(eH_2O)^- + \left[(H_2O)\{trans\text{–}Pt(NH_3)_2Cl\}\right]^{\cdot}\\ &\rightarrow \left[(H_2O)_2Cl\{Pt(NH_3)_2\}\right]^-\\ &\rightarrow \left[(H_2O)_2\{Pt(NH_3)_2\}\right] + Cl^-.\end{aligned} \tag{6}$$

The courses of *cis*- and transplatin transformation in the systems IIc/IIt were concluded from the structural analysis of resulted species, including the fully optimized structures of substrates, intermediates and products, as illustrated in Fig. 3. Other data characterizing the compounds are gathered in Tables 1 and 2.

Comparing the geometries of species formed from two isomeric platinum complexes on the parallel reaction steps it was possible to capture the differences in behavior and properties of these compounds. It was found that initial reaction steps of both isomers transformation, (1c2 → 2c2 → 3c2) and (1t2 → 2t2 → 3t2), evoked by a first hydrated electron attack on Pt-reagents, proceeded with retention of configuration at Pt-center. On the further stages, continued by a second time attack of $(eH_2O)^-$, the differentiation of courses and structures occurs together with destruction of Pt-molecules (Fig. 3). Particularly, according to successive reactions 4 (3c2 → 4c2 → 5c2), the attachment of hydrated electron to $[(H_2O)\{cis\text{-}Pt(NH_3)_2Cl\}]^{\cdot}$ leads to ammine loss (probably opposite to Cl) and formation of anionic, two-coordinated chloroammineplatinum(0), $[(H_2O)_2(NH_3)\{Pt(NH_3)Cl)\}]^-$, showing the dispersion interactions with ammine and two water molecules: (Pt-N: 3.39, and Pt-O: 3.18 Å). After Cl-ligand replacement by water, the complex is transformed into aqua(ammine)platinum(0), $[(H_2O)(NH_3)\{Pt(NH_3)(H_2O)\}]$, stabilized by distanced H_2O and NH_3 molecules: (Pt-O: 2.95, and Pt-N: 3.90 Å). Differently, in the case of transplatin (reactions 6, 3t2 → 4t2 → 5t2), the attachment of hydrated electron to $[(H_2O)\{trans\text{-}Pt(NH_3)_2Cl\}]^{\cdot}$ leads to removing of Cl^- ligand and formation of $[(H_2O)_2Cl\{Pt(NH_3)_2\}]^-$, with distanced chloride anion and two water molecules (Pt-Cl: 3.80 and Pt-O: 3.12/3.23 Å). Then after removing of Cl^- from the coordination sphere, the complex is transformed into diammineplatinum(0), $[(H_2O)_2\{Pt(NH_3)_2\}]$, accompanied by two remote water molecules: (Pt-O: 3.13 Å).

Although only the results of reaction 4 and 6 demonstrate clearly differences in *cis*- and transplatin paths, so an intrinsic trigger that releases the specific destruction of individual conformers lies in the various symmetry of the starting Pt-complexes, similarly as was noticed at reactions 1–2. However, contrary to processes in systems Ic/It, the courses in IIc/IIt are modulated additionally by the water. Its role must be appreciated in stabilization of original *cis* and *trans* conformations on the stages 2c2/3c2 and 2t2/3t2 when the subtle chemical differences are initiated by formation of specific hydrogen bonds between complex and H_2O. Considering the H-bond pattern with the H_2O, one can see that in 2t2 intermediate there exist one the O…H-N (2.90 A) and two Cl…H-O bonds (3.46 and 3.43 A) creating unusual in neutral Pt(II) complexes the -Cl…H-O-H…Cl- structure. In isomeric 2c2, similarly as in 3t2 and 3c2, the H_2O is bounded with NH_3 and Cl ligands. The connections of H-bonding have been marked in Fig. 3.

The successive differentiation of structural parameters has been confirmed by computing the Mulliken charges and energetic effects for individual compounds and reaction states; they are summarized in Table 1, and as reaction profiles are depicted in Fig. 4. Though the values of partial charges on Pt atom in systems IIc/IIt differ from that ones of Ic/It, the fluctuations of charges proceed similarly, i.e., in accordance with chemical formula of created compounds during both pathways. It means that electron attachment could evolve the successive ET processes resulting in $Pt^{II} \rightarrow Pt^{I} \rightarrow Pt^{0}$ sequence.

Analyzing the energetic effects of hydrated electrons impact on platinum complexes, there were noticed significant differences when confronted with the action of free electrons. In particular, the summary effects of compared processes in gas phase are opposing: in systems Ic/It both reaction paths were endo-energetic whereas in systems IIc/IIt they are exo-energetic: -109.62 kcal mol^{-1} and −115.15 kcal mol^{-1}, respectively for cisplatin and transplatin pathway. In PCM/solvent, similarly as it was in Ic/It systems, the summary reactions are exo-energetic for both IIc/IIt systems; the effects amount to −144.36 kcal mol^{-1} and −158.05 kcal mol^{-1} for *cis*- and transplatin, respectively.

Summing up the results of computing the systems IIc/IIt, the big role of water in ET processes of transformation of both isomers was shown. Taking into account that energy differences between *cis*- and transplatin reaction courses are more likely insignificant, whereas the structural parameters of species resulted from IIc differ drastically from IIt, one can assume that the radical 3c2 is susceptible enough to interaction with biological targets, especially because of specific spatial capability to chelate the nucleophilic bis-N-donors. The counterpart intermediate, 3t2, being deprived of such properties seems to be incapable of evolving the transplatin cytotoxicity. The concurrent Pt-species of 4c2 and 4t2, because of anionic character, are not good reagents for biological nucleophiles. Considering 5c2 and 5t2, only the first would be structurally convenient to chelate DNA but because of Pt(O) state both seem to be non-active reagents.

To asses more the intrinsic role of electron and water in Pt-complexes biotransformation the theoretical studies on the systems Ic/It and IIc/IIt were completed by using the same tools for evaluation of hydrolytic pathways of *cis*- and transplatin.

Hydrolytic pathways of platinum complexes

Hydrolysis as a process fundamental for cisplatin activation, was a subject of many studies, including the theoretical evaluation of successive reaction courses of drug and its non-active *trans* conformer [7, 21, 28, 33]. In this work we studied the hydrolysis reactions 7–8 in the aim to determine the differences in *cis*- vs. transplatin reactivity, and to confront these courses with the 3–6 reactions evoked by hydrated electron impact on platinum(II) reagents. The computed data are collected in Tables 1 and 2, and depicted in Figs. 5 and 6. The results are substantially in agreement with those computed earlier by different authors [21, 28, 33, 34], and with the following mode of reactions:

$$H_2O + Pt(NH_3)_2Cl_2 \rightarrow \left[Pt(NH_3)_2Cl_2(H_2O)\right] \rightarrow \left[Pt(NH_3)_2Cl(H_2O)\right]^+ + Cl^-, \quad (7)$$

$$H_2O + \left[Pt(NH_3)_2Cl(H_2O)\right]^+ \rightarrow \left[Pt(NH_3)_2Cl(H_2O)_2\right]^+ \rightarrow \left[Pt(NH_3)_2(H_2O)_2\right]^{2+} + Cl^-. \quad (8)$$

The analysis of collected data revealed the differences in structural and energetic properties of intermediates derived from both isomers. The reaction courses are highly stereo-specific, and all intermediates and products retain the starting configurations on each step. The start of differentiation has been characterized by distant axial Cl-ligand (leaving) in 2c3 and distant H_2O-ligand (incoming) in 2t3, (Fig. 5). In result, two different rows of species are formed. The intermediate of 3c3 is likely to be the reactive form of drug; it differs drastically from confronted 3t3, not only in configuration but also in ligands lability. The 3c3 complex must be more susceptible than 3t3 for reaction with biological targets because it contains labile Cl, and very labile H_2O ligands in vicinity positions which would facilitate the substitution by bis-N-donors (DNA pieces) leading to chelates.

Comparing the energy effects in *cis*- and transplatin reactions 7 – 8, the differences in energy values between species on particular stages are sufficient to favor one of the conformers. Consequently, it was possible to distinguish the stages 2c3 → 3c3 and 2t3 → 3t3 as potentially responsible for formation of activated species. The reaction of *trans* derivatives is more energy-consuming, where energy effect (E3t3- E2t3) amounted to 162.44 (in gas phase) and 21.56 (in solvent) kcal mol^{-1}, in comparison to 138.64 and 5.90 kcal mol^{-1} for *cis* derivatives (E3c3 – E2c3). Thus, one can suggest that population of 3c3 and followed 4c3 must be higher than their counterparts of *trans*-conformer. In both systems, the products (5c3 and 5t3) could be excluded from candidates for real active forms because energetically they are strongly unfavorable (Fig. 6).

Respecting the energy effects of IIIc/IIIt in relation to IIc/IIt systems, there were revealed the drastic differences between reaction profiles of hydrolyses (endoenergic, Fig. 6), and processes evoked by hydrated electrons (exoenergic, Fig. 4). It could be concluded that ET processes of cisplatin activation represented by 3 – 4 reactions must be extremely favorable. However, taking into account that in biological milieu, the kinetic parameters more likely than thermodynamic to play a deciding role in drug metabolism, we do not exclude this drug biotransformation on pure hydrolytic way.

Concluding statements

The comparative evaluation of two isomeric Pt-complexes in three reaction systems was computed to test the

hypothesis of electron attachment role in cisplatin transformation toward the active drug. The main conclusions:

- Since rather large molecular systems are under consideration the DFT method seems to be justified.
- Binding energy for two NH_3 ligands for $Pt(NH_3)_2$ complex amounts to 129.7, whereas for $PtNH_3$ system it amounts 76.9 kcal mol^{-1}.
- Cisplatin vs. transplatin differ in their spatial interaction with examined reagents: free electrons, hydrated electrons, and water.
- The differences in reaction courses within water or hydrated electrons might be crucial to evoke the disparities in biological action of both isomers.
- Reactions of *cis*-isomer with free and hydrated electrons are characteristic due to elimination of ammine ligand.
- Intermediates of cisplatin transformation have potential for stereospecific chelation of biological nucleophiles.
- In biological milieu, two paths-ways of cisplatin biotransformation are possible: hydrolysis and hydrated electron impact. Dependent on the redox state of biological surroundings, one of these pathways might be privileged.
- The pure (without electron impact) hydrolysis of cisplatin as well as hydrolysis by hydrated electrons seem to be entitled models of this drug biotransformation.
- The results of in silico calculations correlate well with the Lu et al. hypothesis that cisplatin activation may occur according to dissociative electron attachment mechanism. However, the participation of water should be considered in this process.

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