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RESEARCH ARTICLE

THEORETICAL STUDIES ON THE HYDRATION OF CISPLATIN AND ISOMERIZATION OF CISPLATIN TO TRANSPLATIN

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ABSTRACT

The study presents the various reaction steps of cisplatin hydrolysis and isomerization to transplatin. The formation of H_2O-cisplation and $2H_2O$-cisplatin on dissociation of –Cl atoms as stable intermediates has been demonstrated. Similar study for transplatin is also carried out to compare the reaction pathway with that of cisplatin and also the relevance of this reaction mechanism for having contrasting anticancer property of these isomers. The formation of solvated cisplatin is favorable, which may produce inefficiency of DNA binding. In addition transformation of cisplatin to transplatin on dissociation of –Cl atom requires energy barrier of 22.7 kcals/mol. The work also discusses the probability of transformation from cisplatin to transplatin on dissociation of –Cl atom.

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INTRODUCTION

Cisplatin emerges as one of the important anticancer drugs and it is clinically used for treatment of several cancers (1-10). It binds with N7 of guanine within duplex dodecamer at the adjacent guanine residues distorting the conformation of major and minor grooves (1). However certain disadvantages of this drug have been noted due to the formation of H_2O-cisplatin complex in aqueous medium on dissociation of -Cl atom from cisplatin. As a result, the efficiency of cisplatin delivery through the cell membrane is reduced because of the various interaction of the cationic cisplatin at the cell membrane[1-6]. Based on the information reported in several literatures the dissociation mechanism of cisplatin is represented in Figure 1, where the reaction steps of -Cl dissociation from cisplatin and subsequent formation of H_2O-cisplatin are shown as simultaneous reactions. Similar reaction steps have been proposed for transplatin (Figure 2). The formation of hydrated form of cisplatin may interfere in DNA binding not only prevent from passing through cell membrane. Again it is also possible that charged cisplatin formed on dissociation of –Cl atom may be important intermediate to undergo isomerization reaction to form another isomer transplatin. Transplatin is considered to be inactive isomer towards cancer cells[7-9]. In view of these aspects the stability of cisplatin in aqueous medium may be investigated, and the possible role of charged cisplatin intermediate to form transplatin may be relevant in assessing anticancer property of cisplatin. The dissociation ability of -Cl atom from cisplatin is essential reaction step since charged cisplatin intermediate may undergo isomerization reaction directly to form transplatin. At the same time, there is possibility of forming H_2O-cisplatin complex as referred in many studies[5-10]. No theoretical studies on the possibility of forming transplatin isomer from cisplatin as per proposed mechanism have been found. The disadvantage of forming H_2O-cisplatin intermediate has been highlighted in many studies, but the generation of another isomer i.e transplatin may be important reaction while examining the efficiency of cisplatin(7-14). The hydrolysis of cisplatin has been predicted as an adverse mechanism in the evaluation of efficacy against cancer cells, also other isomer transplatin is not an anticancer agent. In fact there are several exploratory points from the reaction mechanism of cisplatin. In this case, explicit consideration should be taken on the predominance of hydrolysis step by dissociation of -Cl from cisplatin as well as solvation of intermediate. It may be understood from the sequential reaction steps such as, (a) dissociation of -Cl and (b) subsequent association with water molecule. Again, isomerization pathway from cisplatin to tranplatin isomer on dissociation of chlorine may be highlighted. So, we intend to investigate the two mechanisms, (a) the formation of H_2O-cisplatin, and (b) the isomerization of cisplatin to transplatin. The study may reveal some information on the disadvantages of cisplatin during clinical studies.

The complete quantum mechanical calculations of the reaction steps for both the cisplatin and transplatin should be taken up to extract certain information of drug property. It is also possible to undergo conformational change from ionized cisplatin or transplatin to form a common intermediate after dissociation of -Cl . This area has not illustrated in literatures. This study will focus to describe the possibility of rearrangement from cisplatin to transplatin through these reaction steps. From the results, it may be possible to explain certain adverse effect of cisplatin from the sequential reaction steps.

In addition, redox mechanism is very important for predicting metabolic stability during drug action. The relationship between redox potential and metabolic stability is required in profiling potential drugs. It is a simple approach of analyzing drug like molecules. There are several functionals in biological system which follow redox active mechanism such as electron, proton transfer and protein carboxylation. Complexes may be well documented from the redox potential. In this study, analysis of oxidation energies of cisplatin and transplatin may be done to gain ideas of electronic behavior toward biosystem.

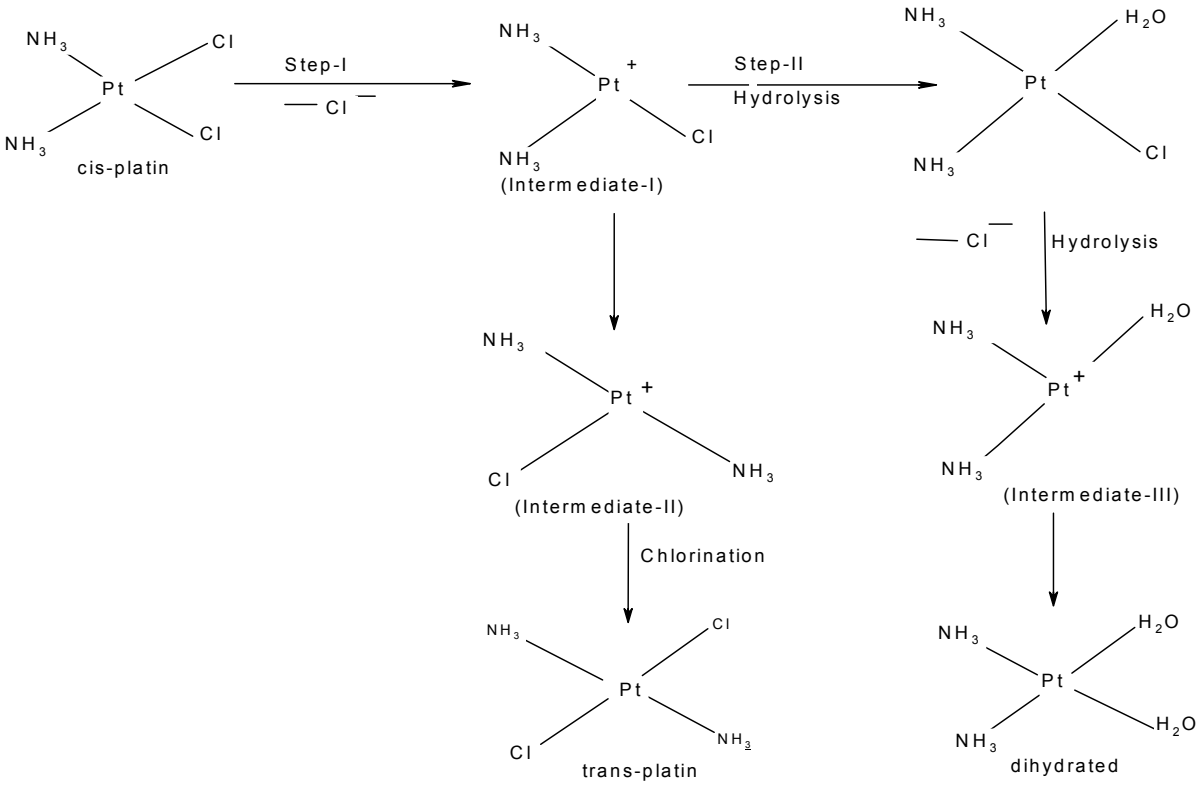


Figure 1. Mechanism of hydrolysis of cisplatin

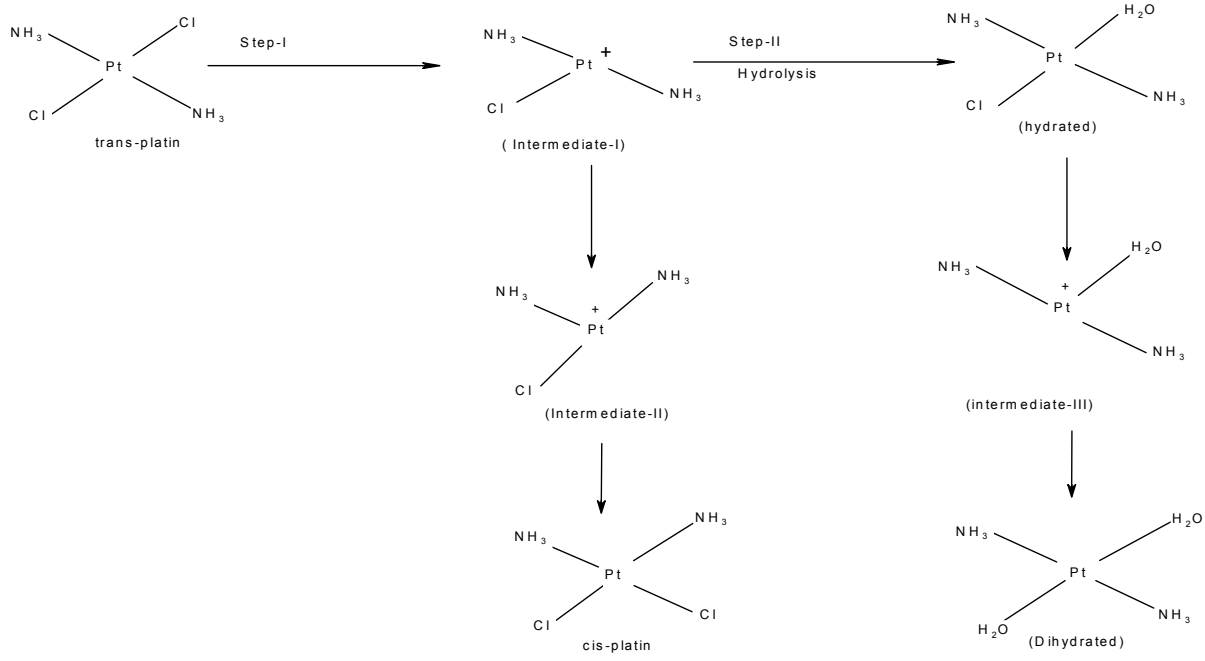


Figure 2. Mechanism of hydrolysis of transplatin

It is applicable for understanding suitable molecules having active component adaptable to biological system. From the electronic properties, it may be possible to predict the mode of action of molecules and corresponding predominant pathway.

MATERIALS AND METHODS

The calculations have been carried out with SDD basis set in the B3LYP method (15). The structures of cisplatin, transplatin and other intermediates are completely optimized. A complete investigation of the reaction pathways through various reaction steps have been carried out (Figures 1 and 2). The energies required for the reaction steps are calculated and other associated changes in the structures of the intermediates are analysed from the optimised geometries. The net charge densities (NPA) obtained from natural population analysis are also computed. The molecular geometry was initially constructed with the help of Gaussview and complete geometry optimization was carried out for both cisplatin and transplatin, and the intermediates involved in these reactions. The primary assumption of studying these two mechanisms separately is that the geometry of the intermediates and also the respective energies of the reaction steps may indicate reasonable information on the cisplatin to transplatin conversion. It is possible to analyse the results of cisplatin water association that may be relevant to the effectiveness of this drug. Here we proposed the essential reaction steps of cisplatin to transplatin conversion also through common intermediate.

RESULTS AND DISCUSSION

Transplatin is not effective anticancer drug. As we know that transplatin can not target DNA effectively and considered to be biologically inactive candidate. From the proposed two reaction pathways, it is possible to describe the more realistic description of these two molecules (Figures 1 and 2). The computed Pt coordinated bond lengths and net charge densities (NPA) on Pt and other bonding atoms of cisplatin are shown in Table 1, the corresponding values for transplatin are given in Table 2. The variation of bonding distances and NPA of intermediates shown in the reaction steps of cisplation are shown in Tables 3-7.

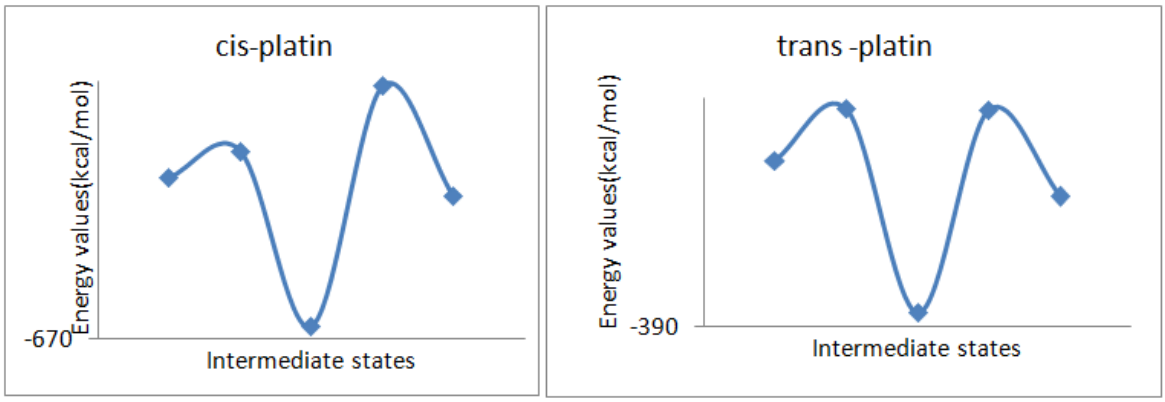
The association of water molecule in step II as given by the energy difference of the molecules involved for this reaction. The major changes in the geometry after dissociation of –Cl is due to the rearrangement of - NH_3 and -Cl in the optimized geometry of intermediate. We have observed stabilizing interaction of the hydrated complex in step II. The energy values for –Cl atom dissociation and association of water may be compared for both cisplatin and transplatin (Table 8). The Ru-O distances are shown in hydrated complexes of cisplatin and bond distances are given in Tables 5-7. The stabilizing interaction in reaction steps II and III support the formation of hydrated intermediates. It is possible that the -NH_3 may change the configurational space after dissociation of -Cl. The energy values obtained from B3LYP functions with SDD bases set shows the substantial variation of energies of intermediates in the reaction steps. Similar to cisplatin, the intermediate I of transplatin exists on dissociation of –Cl , and the energy of the reactions and energies of each of the intermediates are shown in Table 8. The. Since the hydration of intermediate-I after dissociation of Cl is predicted, the formation of stable hydrated form is clarified from the energy values of this reaction step. Moreover the conformation of -NH_3 are changed quite significantly to provide enough space for interaction with water molecule. Obviously, cisplatin and transplatin should pass through similar intermediate on dissociation of –Cl atom because the conformations of the intermediate I of the reaction pathways of cisplatin and transplatin usually converge to the most favored geometry. The relative energies profile of intermediates involved in reaction pathways of cisplatin and transplatin are shown in Figures 3 and 4. The present studies on cisplatin and transplatin are taken to explain why cisplatin is bioactive in nature but not transplatin. The only difference is the geometrical structures in these complexes is that the configuration of two chlorine atoms. The electron transfer abilities for these isomers are also analysed from the one electron oxidation energies (Pt-II to Pt-III) of cisplatin and transplatin are shown in Table 9. The oxidation energies of both these isomers are almost equal and the net charge density obtained from NPA analysis shows no drastic variation for Pt atom in these complexes. The NPA charges on certain atomic centres of cisplatin and transplatin are considered because of their importance in biochemical interaction, particularly Pt to the N7 of guanine.

Table 1. Computed bond lengths between Pt-coordinated atoms and NPA charges on the atoms co-ordinated to platinum(Pt) in cisplatin complex along with the free ligand (NH_3)

Bond length between Pt and coordinated atom pm	NPA charges on atoms co-ordinated with Pt		NPA charges on atoms of free NH_3 ligand.	
	Atom	Charges	Atom	Charges
Ru-N_2 = 2.156	N_2	-0.930	N	-1.278
Ru-N_4 = 2.158	N_4	-1.156		
Ru-Cl_3 = 2.444	Cl_3	0.255		
Ru-Cl_5 = 2.444	Cl_5	0.166		
	Pt	0.645		

Table 2. Computed bond lengths between Pt-coordinated atoms and NPA charges on the atoms co-ordinated to platinum(Pt) in transplatin complex along with the free ligand (NH_3)

Bond length between Pt and coordinated atom pm	NPA charges on atoms co-ordinated with Pt		NPA charges on atoms of free NH_3 ligand.	
	atom	Charges	Atom	Charges
Ru-N_4 = 2.117	N_4	-1.074	N	-1.278
Ru-N_5 = 2.165	N_5	-1.063		
Ru-Cl_2 = 2.445	Cl_2	0.158		
Ru-Cl_3 = 2.472	Cl_3	0.099		
	Pt	0.651		



(a) (b)

Figure 3. Variation relative energies of intermediates of (a) Cisplatin(b) Transplatin

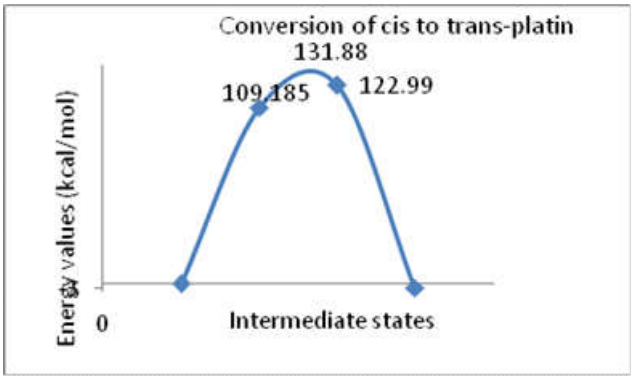


Figure 5. Conversion of Cisplatin to Transplatin

Table 3. Computed bond length between Pt-coordinated atom and NPA charges on the atoms co-ordinated to platinum(Pt) in cisplatin intermediate-I along with the free ligand (NH_3)

Bond length between Pt and coordinated atom A^0	NPA charges on atoms co-ordinated with Pt		NPA charges on atoms of free NH_3 ligand.	
	atom	Charges	Atom	Charges
Pt-N_2 = 2.112	N_2	-0.485	N	-1.278
Pt-N_4 = 2.114	N_5	-0.457		
Pt-Cl_5 = 2.430	Cl_5	0.056		
	Pt	0.855		

Table 4. Computed bond length between Pt-coordinated atoms and NPA charges on the atoms co-ordinated to platinum(Pt) in cisplatin intermediate-II complex along with the free ligand (NH_3)

Bond length between Pt and coordinated atom A^0	NPA charges on atoms co-ordinated with Pt		NPA charges on atoms of free NH_3 ligand.	
	atom	Charges	Atom	Charges
Pt-N_2 = 2.040	N_2	-0.374	N	-1.278
Pt-N_4 = 2.305	N_4	-0.384		
Pt-Cl_3 = 2.429	Cl_3	-0.142		
	Pt	1.147		

Table 5. Computed the bond length between Pt-coordinated atoms and NPA charges on the atoms co-ordinated to platinum(Pt) in cisplatin intermediate-III complex along with the free ligand (NH_3)

Bond length between Pt and coordinated atom A^0	NPA charges on atoms co-ordinated with Pt		NPA charges on atoms of free NH_3 ligand.	
	atom	Charges	Atom	Charges
Pt-N_2 = 2.112	N_2	-1.292	N	-1.278
Pt-N_4 = 2.113	N_4	-1.162		
Pt-O_3 = 1.931	O_3	-0.727		
	Pt	0.898		

The ability of two –NH3 to donate electron towards Pt for these two isomers are not equal. It indicates that the stabilities of cisplatin and transplatin also affect the one electron oxidation of Pt II to Pt III. Hence the configuration of –NH_3 groups, and the stabilities of the complexes produce resultant effect on the oxidation energies of cisplatin and transplatin.

The oxidation energies of Pt-II to III is very important to comparing the coordination ability of Pt of these isomers towards N7 of guanine. Although the complete structure of transplatin bonded with DNA is not available, the oxidation energy is computed for comparison with that of cisplatin. Oxidation energy may not be the concrete reason for the

Table 6. Computed bond lengths between Pt-coordinated atoms and NPA charges on the atoms co-ordinated to platinum(Pt) in cisplatin hydrated complex along with the free ligand (NH_3)

Bond length between Pt and coordinated atom A^0	NPA charges on atoms co-ordinated with Pt		NPA charges on atoms of free NH_3 ligand.	
	atom	Charges	Atom	Charges
Pt-N_2 = 2.114	N_2	-0.610	N	-1.278
Pt-N_5 = 2.112	N_5	-0.422		
Pt-Cl_6 = 2.430	Cl_6	0.147		
Pt-O_4 = 1.935	Pt	0.652		

Table 7. Computed bond lengths between Pt-coordinated atoms and NPA charges on the atoms co-ordinated to platinum(Pt) in cisplatin dihydrated complex along with the free ligand (NH_3)

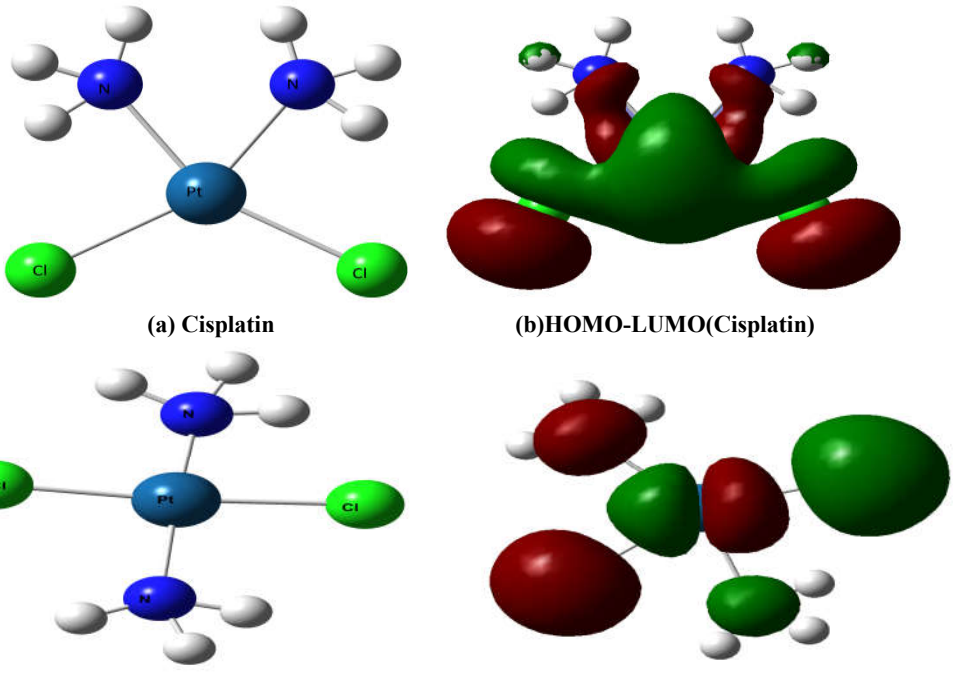
Bond length between Pt and coordinated atom A^0	NPA charges on atoms co-ordinated with Pt		NPA charges on atoms of free NH_3 ligand.	
	atom	Charges	Atom	Charges
Pt-N_2 = 2.114	N_2	-1.197	N	-1.278
Pt-N_5 = 2.116	N_5	-1.108		
Pt-O_4 = 1.937	O_4	-0.723		
Pt-O_6 = 1.936	O6	-0.796		
	Pt	0.710		

Table 8. Energy of formation of intermediates (ΔE) for Cisplatin and Transplatin aquation

Complexes/ Molecules	ΔE (kcal/mol)	Complexes/ Molecules	ΔE (kcal/mol)
Cisplatin intermediate-I	109.185	Transplatin intermediate-I	122.990
Cisplatin hydration	- 621.225	Transplatin hydration	-358.302
Cisplatin intermediate-II	379.010	Transplatin intermediate-II	121.107
Cisplatin dihydration	- 80.32	Transplatin dihydration	-84.085

Table 9. Oxidation energies and HOMO-LUMO gap(ΔE) of cisplatin and transplatin

Complex	Oxd. State	Energy difference, kcal/mol	ΔE
Cisplatin	Pt(II)		0.2695
	Pt(III)	446.152	
Transplatin	Pt(II)		0.0579
	Pt(III)	448.662	



(a) Cisplatin **(b)HOMO-LUMO(Cisplatin)**

(c) Transplatin **(d) HOMO-LUMO (Transplatin)**

Figure 5 (a) Structure of Cisplatin (b) HOMO-LUMO (Cisplatin) (c) Structure of Transplatin (d) HOMO-LUMO (Transplatin)

difference of biological properties of these isomers. Hence forth we have explored the hydrolysis of cisplatin on dissociation of -Cl atom from cisplatin and transplatin. The sequential reaction steps given in Figures 1 and 2 have been studied, hence the energies of formation of the several intermediates are calculated (Table 8). Initially it is essential to examine the reaction step I where -Cl atom dissociated to form intermediate I. The values for both the isomers are somewhat

different. The results indicate that oxidation of Pt indirectly links with the coordination ability with N7 of guanine. As a result, both the isomers should have similar behaviour. Eventually the progress of the sequential reaction steps are concerned, the formation of intermediate I of cisplatin is more favourable than that of transplatin. This observation also somewhat provides how the coordination of cisplatin should be efficient than transplatin. In turn, the solvation energy of intermediate II is found to be large negative value for cisplatin than transplatin. It shows that the cisplatin Pt-II ion can exists a stable species in aqueous solution. We have further extended the study after dissociation of second -Cl atom from both cisplatin and transplatin and subsequently solvation energies are calculated with water molecules (Table 8). The dissociation of second -Cl is not a feasible pathway, however we extend to study the hydration with two water molecules since hydration of cisplatin is well known adverse pathway in cisplatin drug administration. The results shown in Table 8 for cisplatin and transplatin are significantly different, but hydration with second water molecule is less feasible than the monohydrated forms of cisplatin and transplatin. From the solvation energy, it is possible to understand the reason of inefficient drug action of cisplatin. The formation of stable intermediate II(H_2O-Cisplatin) may be the criteria for this adverse effect in drug action. However transplatin also can form less stable hydrated complex with than cisplatin. Another aspect to explain the inefficiency of cisplatin may be due to transformation of cisplatin to transplatin from intermediate I. There may be fair chance of transforming the configuration of intermediate I to due to large accessible conformational space occupied by two -NH_3 and one -Cl. It is likely that transplatin can generate same intermediate II. The potential energy plot for the transformation of cisplatin to transplatin through intermediate I is shown in Figure 5. Hence the possibility of forming ineffective isomer transplatin may be another pathway, since transplatin is more stable isomer than cisplatin.

Considering all the possible aspects, the only information procured is the solvation of cisplatin(intermediate I) might be the reason for the inefficiency of cisplatin in drug administration. But another characteristic feature of cisplatin compared to transplatin is that Pt-Cl bond may not dissociate efficiently for binding with DNA. So the energy of formation of intermediate I for transplatin is slightly more than that of cisplatin. Under physiological condition, transplatin may not release –Cl atom before binding with DNA. It may be related to the inactivity of transplatin. On the other hand , we have explored the Frontier orbital diagrams around cisplatin and transplatin. The shapes of the electron densities of cisplatin and transplatin and HOMO-LUMO gaps are very different(Figure 6, Table 9). The association of transplatin with DNA is not known, so it is not possible how the variation of electronic behavior of transplatin from cisplatin affect the binding with DNA. The results only provide the difference of electronic behavior of transplatin from cisplatin. On the other hand solvation of cisplatin is a favorable step on dissociation of –Cl atom. In this case dissociation of –Cl and subsequent binding of Pt with N7 of guanine is the acceptable pathway of forming cisplatin-DNA complex. Here H_2O association of Intermediate I is quite favorable and the formation of H_2O-cisplatin complex is possible. On the contrary for the conversion of cisplatin to transplatin, Intermediate I must attain additional energy barrier of 22.7 kcal/mol to change the conformation to transplatin. It is also quite possible since intermediate I may consequently convert to the more stable conformation i.e transplation.

Conclusion

The investigation so far carried out on the proposed mechanism clearly shows the stabilizing interaction between H_2O and cisplatin on dissociation of –Cl atom. Based on the findings, aquation of cisplatin may be one of the adverse effect during drug action. Transplatin follows similar energy profile of intermediates as assigned in the proposed mechanism. The distinctive feature is that cisplatin acquires more negative solvation energy than that of transplatin, and also dissociation of –Cl from transplatin requires more energy than cisplatin. Inspite of contrasting anticancer property of cisplation and transplatin, oxidation energies are not very different but the variation of HOMO-LUMO gap is quite significant. The isomerization reaction from cisplatin to transplatin on dissociation of –Cl atom is another possible mechanism.

REFERENCES

Patricia M Takahara, Christin A Frederick and Stephen J Lippard, Crystal Structure of anticancer drug cisplatin bound to duplex DNA, *J. Am. Chem. Soc.* 1996,118,49,12309-12321.

Ghosh, S. Cisplatin: The first metal based anticancer drug. *Bioorg Chem.* 2019, *11*, 88:102925.

Muggia, F.M.; Bonetti, A.; Hoeschele, J.D.; Rozencweig, M.; Howell, S.B. Platinum antitumor complexes: 50 years since Barnett Rosenberg's discovery. *J. Clin. Oncol.* 2015, *33*, 4219–4226.

Dasari, S.; Tchounwou, P.B. Cisplatin in cancer therapy: Molecular mechanisms of action. *Eur. J. Pharmacol.* 2014, *5*, 364–378.

Amable, L. Cisplatin resistance and opportunities for precision medicine. *Pharmacol. Res.* 2016, *106*, 27–36.

Jo, Y.; Choi, N.; Kim, K.; Koo, H.J.; Choi, J.; Kim, H.N. Chemoresistance of cancer cells: Requirements of tumor microenvironment-mimicking in vitro models in anti-cancer drug development. *Theranostics* 2018, *8*, 5259–5275

Davies, M.S.; Berners-Price, S.J.; Hambley, T.W. Slowing of cisplatin aquation in the presence of DNA but not in the presence of phosphate: Improved understanding of sequence selectivity and the roles of monoaquated and diaquated species in the binding of cisplatin to DNA. *Inorg. Chem.* 2000, *39*, 5603–5613.

Eastman, A. Cross-linking of glutathione to DNA by cancer chemotherapeutic platinum coordination complexes. *Chem. Biol. Interact.* 1987, *61*, 241–248.

Rocha, C.R.R.; Silva, M.M.; Quinet, A.; Cabral-Neto, J.B. Menck CFM. DNA repair pathways and cisplatin resistance: An intimate relationship. *Clinics* 2018, *73.*

Rebillard, A.; Lagadic-Gossmann, D.; Dimanche-Boitrel, M.T. Cisplatin cytotoxicity: DNA and plasma membrane targets. *Curr. Med. Chem.* 2008, *15*, 2656–2663.

Sancho-Martínez, S.M.; Prieto-García, L.; Prieto, M.; López-Novoa, J.M.; López-Hernández, F.J. Subcellular targets of cisplatin cytotoxicity: An integrated view. *Pharmacol. Ther.* 2012, *136*, 35–55.

Song, I.S.; Savaraj, N.; Siddik, Z.H.; Liu, P.; Wei, Y.; Wu, C.J.; Kuo, M.T. Role of human copper transporter Ctr1 in

the transport of platinum-based antitumor agents in cisplatin-sensitive and cisplatin-resistant cells. *Mol. Cancer Ther.* 2004, *3*, 1543–1549.

Kim, E.S.; Tang, X.; Peterson, D.R.; Kilari, D.; Chow, C.W.; Fujimoto, J.; Kalhor, N.; Swisher, S.G.; Stewart, D.J.; Wistuba, I.I.; et al. Copper transporter CTR1 expression and tissue platinum concentration in non-small cell lung cancer. *Lung Cancer* 2014, *85*, 88–93.

Öhrvik, H.; Logeman, B.; Turk, B.; Reinheckel, T.; Thiele, D.J. Cathepsin protease controls copper and cisplatin accumulation via cleavage of the Ctr1 metal-binding ectodomain. *J. Biol. Chem.* 2016, *291*, 13905–13916.

(a) Gaussian 03, Revision B.04, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, J. A. Montgomery, Jr., T. Vreven, K. N. Kudin, J. C. Burant, J. M. Millam, S. S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G. A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J. E. Knox, H. P. Hratchian, J. B. Cross, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, P. Y. Ayala, K. Morokuma, G. A. Voth, P. Salvador, J. J. Dannenberg, V. G. Zakrzewski, S. Dapprich, A. D. Daniels, M. C. Strain, O. Farkas, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. V. Ortiz, Q. Cui, A. G. Baboul, S. Clifford, J. Cioslowski, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, C. Gonzalez, and J. A. Pople, Gaussian, Inc., Pittsburgh PA, 2003. 2. GaussView 3.0, Gaussian, Inc., Pittsburgh PA, 2003. (b) Gaussview, 2003.

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