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The Degradation Pathways in Chloride Medium of the Third Generation Anticancer Drug Oxaliplatin

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We have investigated the degradation reactions, in chloride medium, of the third generation drug oxaliplatin using density functional theory. Our calculations confirm that this drug should be administered in chloride free solutions, and we have ascertained the main biodegradation products upon chloride binding, which are essential to establish the active compounds reacting with DNA. In addition, detailed knowledge of these platinum complexes is fundamental for correct elimination procedures in wastewater treatments.

Introduction

Cisplatin (*cis*-diamminedichloroplatinum(II)) is a successful anticancer drug,[1,2] yet it was nearly discarded in the early 1970s because of its gastrointestinal and renal toxicities. When it was established that these side effects could be alleviated by antiemetic drugs and intense hydration of the patient, cisplatin became a fundamental drug in testicular, ovarian, bladder, lung, head, neck, and cervical cancers.[3] Most patients with metastatic testicular cancer will be long-term survivors thanks to cisplatin-based chemotherapy. However, cisplatin exhibits two severe limitations: potentially long-term side effects such as oto-,[4] neuro-,[5] and nephrotoxicity[6] and drug resistance which prevents some of the patients from achieving long-term remissions. In order to overcome these limitations, many structural analogues have been investigated.[7−10] Oxaliplatin[11] (1,2-diaminocyclohexaneoxalate−platinum) is active against some tumors that are primarily resistant to cisplatin and carboplatin (a second generation platinum drug) being the first antineoplastic agent to exhibit activity against metastatic colorectal cancer.[12,13]

It is thought that platinum complexes exert their cytotoxic action in a similar manner to alkylating agents by causing inter- and intrastrand cross-links with DNA, inhibiting its synthesis and inducing apoptotic cell death.[14−16] Much research has been done in this area, and it has been proposed that these drugs interact with DNA following several steps: aquation of the platinum complex, preassociation with the DNA, monofunctional adduct formation, closure of the bifunctional adduct, and distortion of the DNA and recognition of this distortion.[17] Oxaliplatin is normally administrated by intravenous infusion, and it is biotransformed essentially by water and nucleophiles such as Cl^- and HCO_3^-.[18] It has been established that oxaliplatin's degradation in aqueous media depends on chloride concentration as well as the pH. However, in contrast to cisplatin, the decay of oxaliplatin is promoted by increasing the concentration of chloride with [Pt(DACH)Cl_2] (DACH stands for 1,2-diaminocyclohexyl) being the major product.[18−20] Some studies account that oxaliplatin undergoes extensive biotransformation (17 products have been reported) leading to the degradation of the parent drug into a variety of platinum containing products.[21,22] $[Pt(DACH)OxCl]^-$, a transformation product from oxaliplatin, upon chloride binding, has been identified, and in the presence of chloride, oxaliplatin rapidly degrades initially to this compound. It supports the recommendations that oxaliplatin should not be mixed with solutions containing chloride. On the other hand, the slower degradation seen subsequently must be due to the fact that the $[Pt(DACH)OxCl]^-$ is converted back to oxaliplatin by an intramolecular reaction. At pH 7.4 and 37°, this takes place within an hour;[18] moreover, the rate of formation of [Pt(DACH)Cl_2] is slow.

Given that oxaliplatin is administered by infusion solutions, it is very important to ascertain its degradation reaction in the presence of chloride ions. In addition, analysis of patient urine suggests that oxaliplatin is present in wastewater as a variety of biotransformed products.[22] The investigation of the ecotoxicity of oxaliplatin and the development of elimination procedures during sewage treatment requires a complete knowledge of all degradation products of this drug.

Although different theoretical studies already exist on the aquation reactions of platinum anticancer compounds,[23−27] no investigation has been carried on the chloride binding processes. In this communication, we present, for the first time, the acid and neutral degradation reactions for oxaliplatin reactions with chloride. The reaction is expected to take place in a two-step process, as illustrated in Scheme 1.

Methods

The considered reactions were investigated using density functional theory (DFT), with the B3LYP[28] functional, as implemented in Gaussian 03.[29] Gas phase optimizations were carried out under neutral conditions as well as in acid (one oxygen atom on oxaliplatin's carboxylate group protonated) and in strong acid (both carboxylate oxygen atoms protonated). All structures were optimized with the 6-31G(d) basis set on all atoms except the platinum atom, which was described by the

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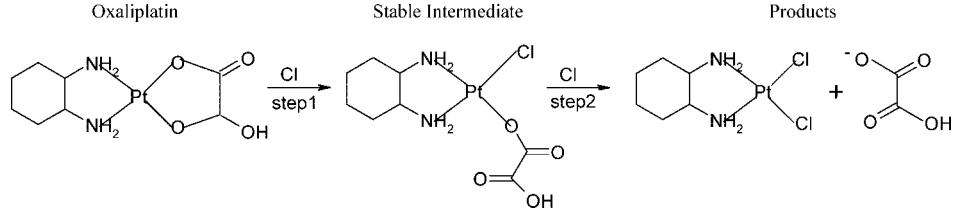
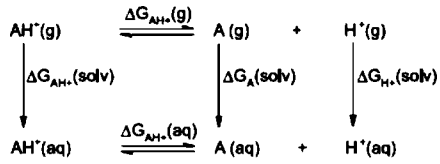
SCHEME 1: Investigated Reaction Paths for Chloride Binding to Oxaliplatin under Acid Conditions



quasi-relativistic Stuttgart−Dresden pseudopotential[30] with pseudo-orbital basis set augmented by a set of diffuse functions—α_s = 0.0075, α_p = 0.013, and α_d = 0.025—and polarization functions—α_f = 0.98. In order to confirm proper convergence to equilibrium and transition state geometries, vibrational frequency analysis was done based on analytical second derivatives of Hamiltonian at this level of theory. For all transition states, intrinsic reaction coordinate (IRC) calculations were used to verify corresponding reactant and product structures. The final energies were obtained with single point energy calculations on optimized structures using the 6-31++G(2df,2pd) basis set, and the water environment modeled using the conductor-like polarized continuum model (CPCM).[31] Klamt radii were used for constructing the solute cavity.[32] Similarly, the platinum valence basis set was augmented with diffuse (α_f = 0.46) and polarization (α_g = 1.21) functions. Potential energy profiles were estimated from total electronic energies at the 6-31++G(2df,2pd) level (with solvent effects included) adding zero point energy (ZPE), enthalpy, and Gibbs corrections at room temperature (298.15 K). Previous studies on platinum-based compounds[27] have shown an excellent agreement between experimental and calculated data, and for this reason, the present level of theory has been considered appropriate.

For the calculation of the absolute pK_a, the following thermodynamic cycle was used:



The G_{H^+}(g) and ΔG_{H^+}(solv) terms are −6.28[33] and −263.98 kcal/mol,[34] respectively, and a term $-RT\ln(24.46)$ was added to take into account the transformation of concentration units in the aqueous phase (atm to mol dm^{-3}).

Results

As a first step of our investigation, we considered the neutral process. The reaction should begin with the addition of a chloride ion and the consequent ring-opening process. We tried to locate the transition state for step 1 under neutral conditions, but in spite of the many attempts performed, it was not possible to find this structure. In addition to DFT, the Hartree−Fock level of theory using the 6-31G(d) basis set was also employed in the search of this saddle point. However, also in this case, it was not found on the potential energy surface (PES). This situation is not new, and theoretical calculations done on cisplatin from Mu-Hyun Baik et al. reveal that not all structures can be located on the PES.[35] Thus, we have considered an alternative path in which the attack of a chloride ion on the platinum center leads to the opening of the DACH ring. We were able to locate the pertinent stationary points on the potential energy surface and observed that this step requires 30.93 kcal·mol^{-1} activation energy and presents an endothermic reaction heat of 10.95 kcal·mol^{-1}. Since it is well-known that the *N*,*N*-chelates of Pt(II) are thermodynamically more stable than *O*,*O*-chelates, we think that the barrier to open the Pt−O bond under chlorine attack should be lower than that found for the Pt−N bond breaking.

For the step 2 reaction, we have located all the stationary points on the PES. The initial complex and the transition states lie at 5.98 and 36.24 kcal·mol^{-1}, respectively, above the reactant energies. The reaction is found to be endothermic by 15.45 kcal·mol^{-1}. On the basis of our results, we can reasonably hypothesize that the second chlorination step should have a barrier greater than the first ones.

In order to establish the conditions in which oxaliplatin can be administered as well as the degradation products, it is essential to investigate these reactions also under acidic conditions. For this, we have assumed that the oxaliplatin's oxalato group will be protonated (under physiological conditions, to some extent, it can exist in protonated form and will be present in normal urine). In order to have information on pK_a values

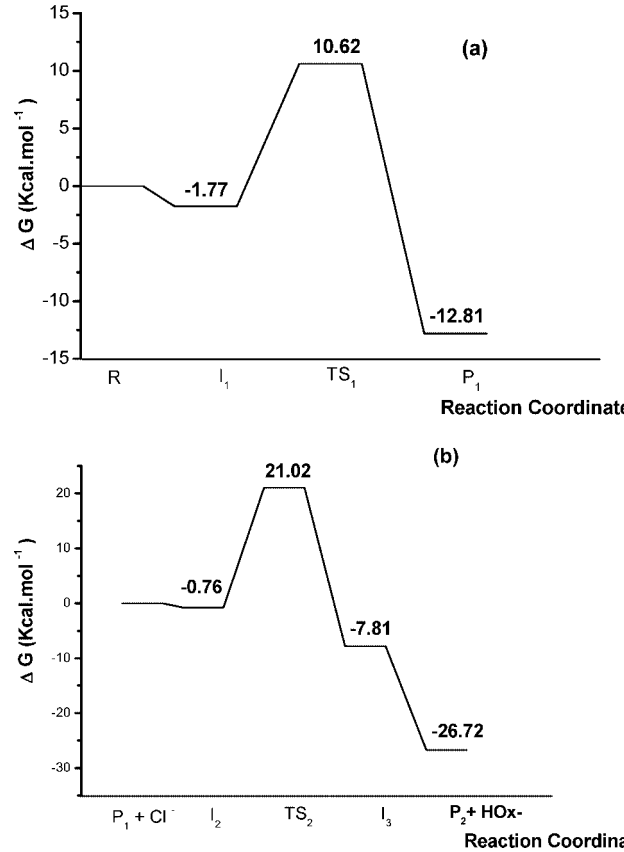


Figure 1. Potential energy surface for (a) the addition of the first chloride ion to oxaliplatin (step 1) and for (b) the addition of chloride ion to the [Pt(DACH)(HOx)Cl]$^-$ (step 2), under acidic conditions.

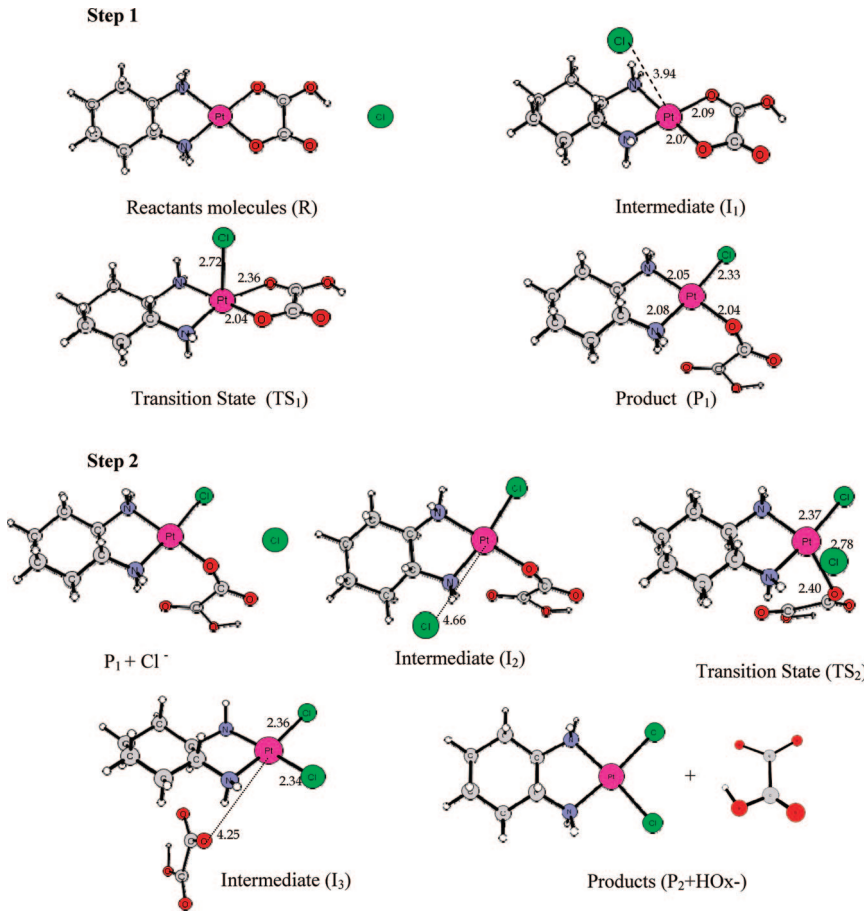


Figure 2. Optimized structures of the stationary points along the PES of step 1 and step 2 reactions. The indicated values are bond distances in angstroms.

and on the reliability of the method in predicting this parameter, we have computed these values for the oxalic acid and oxalate (for which the experimental values are known) and for the corresponding oxaliplatin complex. The theoretical values for oxalic acid (H2Ox) and H-oxalate (HOx−) are found to be 0.98 and 4.81, respectively. The experimental counterparts are 1.23 and 4.27, respectively. The agreement is quite satisfactory, and the experimental trend is well reproduced. In analogy, for $[Pt(DACH)H2Ox]^{+2}$ and $[Pt(DACH)HOx]^{+}$ complexes, the calculated pK_a is −1.52 and 6.37, respectively. The increase of the pK_a value of HOx− upon binding the platinum(II) moiety is essentially due to the greater O−H bond polarization in the HOx system, as evidenced by the computed atomic net charges. Furthermore, we note a decrease of the C−OH bond distance in going from the HOx− to $[Pt(DACH)HOx]^{+}$ complex.

The potential energy surfaces for the first and second chlorination reactions (steps 1 and 2 reported in Scheme 1) under acid conditions are reported in parts a and b of Figure 1, respectively. The optimized geometrical structure for the reactants, transition states, and products are depicted in Figure 2.

The reaction starts with the attack of the chloride ion on the platinum center and the consequent formation of a weak complex (I_1) that lies at 1.77 kcal·mol^{-1} below the reactant energies. The Pt−Cl bond distance is 3.94 Å, and the geometrical parameters of the initial oxaliplatin are essentially unchanged. In the transition state, located at 10.62 kcal·mol^{-1}, the platinum H-oxalate bond (Pt−HOx) is stretched to 2.36 Å, while the chloride platinum bond (Pt−Cl) is 2.72 Å. The reaction proceeds with the scission of the Pt−Ox bond from the transition state TS_1 and by the formation of the product (P_1) that lies at 12.81 kcal·mol^{-1} below the reactants (R).

The product from step 1 is the [Pt(DACH)(HOx)Cl] with oxalate ligand bonded by only one oxygen atom.

The second chloride addition begins with the approach of the Cl^- ion that is found further away from the platinum center than in the previous reaction. The attack of the chloride ion gives rise to the intermediate (I_2) which is found at 0.76 kcal·mol^{-1} below the reactants and is characterized, as in the corresponding complex of step 1, by a long Pt−Cl bond distance (4.66 Å). In the transition state structure (TS_2), the Pt−Cl bond is 2.78 Å and the Pt−O bond is 2.40 Å. The computed imaginary frequency for the transition state is 124i cm^{-1}, and the following IRC optimization clearly shows the formation of the Pt−Cl bond and the scission of the Pt−O one. For such reaction, the activation barrier is 21.02 kcal·mol^{-1}.

An intermediate complex is then observed (I_3) at 7.81 kcal·mol^{-1} below the reactants which proceed without any barrier, to the separated products (P_2 + HOx). The exothermicity is 26.72 kcal·mol^{-1}.

In addition, we have considered the possibility that the chlorine attack could break the Pt−N bond. For the first chlorination, we have located the intermediate complex and the transition state at −0.80 and 23.97 kcal·mol^{-1} with respect to the reactants, respectively. The reaction is endothermic by 9.93 kcal·mol^{-1}. As expected from much experimental evidence, the Pt−N bond breaking requires a higher energy than that demanded for the Pt−O bond scission.

Considering the lowest energy path, it is clear that the formation of the [Pt(DACH)(HOx)Cl] (step 1) is strongly favored over the formation of [Pt(DACH)Cl_2] (step 2).

Finally, we have also considered the strong acid condition with both carboxylate groups protonated. The results indicate that, under these conditions, the decomposition of oxaliplatin into [Pt(DACH)Cl_2] takes place with a calculated activation barrier of 15.53 kcal·mol^{-1} for step 1 and 17.19 kcal·mol^{-1} for step 2. Step 1 is exothermic by 15.53 kcal·mol^{-1} and step 2 by 15.01 kcal·mol^{-1}.

Conclusions

In summary, we have explored the decomposition of oxaliplatin in the presence of chloride using density functional theory with the B3LYP functional. Under neutral conditions, we have seen that reaction, according to step 1, is not observed on the potential energy surface due to the lack of the localization of the transition state for the Pt−O bond breaking. On the other hand, under acidic conditions, the presence of chloride ion leads to the decomposition of oxaliplatin. We were also able to establish that the most favorable reaction leads to the decomposition of oxaliplatin following steps 1 and 2 with the formation of [Pt(DACH)Cl_2]. The first step for this reaction is the ring-opening process with a calculated activation barrier of 10.62 kcal·mol^{-1} accompanied by a 12.81 kcal·mol^{-1} energy release. The final product is obtained by overcoming a 21.02 kcal·mol^{-1} activation barrier, which makes it the rate limiting process, and is exothermic by 7.81 kcal·mol^{-1}. In addition, as expected, both processes leading to the detachment of the DACH ligand require a big amount of energy and are endothermic. For this reason, the formation of $[PtOxCl_2]^{2-}$ and $[Pt(HOx)Cl_2]^{-}$ is not favored both kinetically and thermodynamically.

This study is of importance for the clinical use of oxaliplatin, since it is essential to identify the active form of the drug reacting with DNA. It also concurs with the previous experimental findings that oxaliplatin should be administered in chloride free solutions and that acidification leads to fast chloride reaction with this drug. In addition, we were able to ascertain the most likely products that can be found in wastewaters. Urine is normally acidic (pH $\approx$ 6),[36] and we have established that, under these conditions, oxaliplatin is degraded into a variety of products.

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