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Hydrolysis of the Anticancer Drug Cisplatin: Pitfalls in the Interpretation of Quantum Chemical Calculations

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Abstract: All three hydrolysis reactions of the anticancer drug cisplatin, *cis*-$[Pt(NH_3)_2Cl_2]$, including the acidity constants (pK_a) of the aqua complexes have been compared using a combined density functional theory (DFT) and continuum dielectric model (CDM) approach. The calculations predict very similar activation barriers (25−27 kcal/mol) and reaction free energies (0−2 kcal/mol) for each of the three hydrolysis reactions. The predicted relative free energies of both Pt(II) and Ru(II) anticancer complexes agree well with available experimental values. However, our calculated data strongly disagree with several recent computational studies that predicted the second and third hydrolysis to be thermodynamically highly unfavorable and thus would have ruled out the involvement of *cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ and *cis*-$[Pt(NH_3)_2(OH_2)(OH)]^{+}$ in the mode of action of the drug. This controversy can be resolved by the fact that former computational predictions of activation and reaction free energies in solution were based on second-shell reactant adducts and product adducts, which are the correct endpoints of the intrinsic reaction coordinate in vacuo but artifacts in aqueous solution.

Objective

Aiming to predict potentially active species in the mode of action of the anticancer drug cisplatin (*cis*-$[Pt(NH_3)_2Cl_2]$),[1] many quantum chemical studies have focused on the hydrolysis of one or both platinum−chloro bonds of the drug (Figure 1).[2−4] Most computational work arrived at the conclusion that both the second[2i] and third[2j] hydrolysis are strongly endothermic and thus neither *cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ nor *cis*-$[Pt(NH_3)_2(OH_2)(OH)]^{+}$ are involved in the anticancer activity of cisplatin. Such conclusions are traditionally[2b,c] based on the calculated energy of the transition state (TS) and a product adduct (PA) relative to the energy of a reactant adduct (RA). In RA and PA, a water molecule and chloride, respectively, are located in the second coordination shell of the metal (Figure 2). Because the intrinsic reaction coordinate[5] calculated in vacuo does end at such adducts and the activation barriers reported in most papers appear to be in good agreement with experimental values, recent studies on cisplatin hydrolysis and related reactions in aqueous solution have uncritically inherited this strategy.

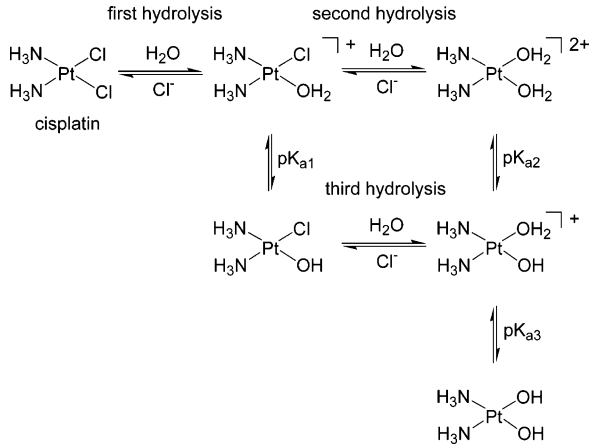

Figure 1. Cisplatin hydrolysis.

To compare for the first time all three hydrolysis reactions of cisplatin including the acidity constants (pK_a) of the aqua complexes, we have performed a combined density functional

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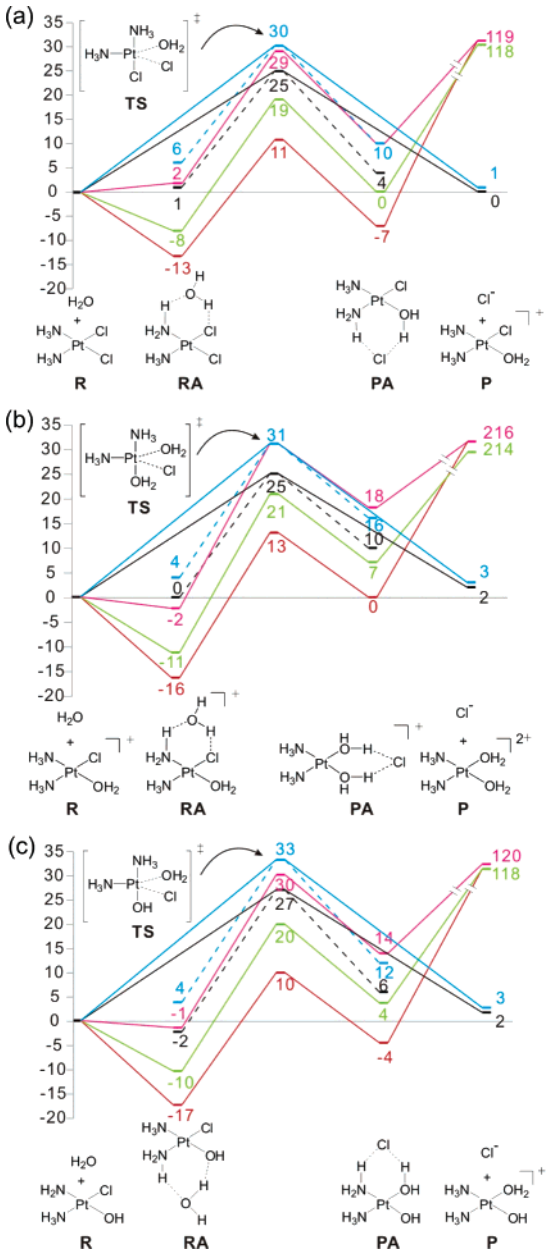

Figure 2. Calculated reaction profile (in kcal/mol) for the (a) first, (b) second, and (c) third hydrolysis of cisplatin. Red: Energies at B3LYP with small basis set in vacuo. Green: Improved energies with large basis set in vacuo. Purple: Free energies in vacuo. Blue: Free energies in solution, with Poisson−Boltzmann calculations. Black: Free energies in solution, with Poisson−Boltzmann calculations, Wertz correction included. Dashed lines: Reactant adduct (RA) and product adduct (PA) are used as a reference in aqueous solution.

theory (DFT) and continuum dielectric model (CDM) study. Our calculations suggest second-shell adducts to be artifacts from the calculations in vacuo, calling for a critical reassessment of former computational results.

Results and Discussion

Figure 2a displays the calculated reaction profile at the B3LYP level[6,7] for the *first hydrolysis* of cisplatin, including the separated reactants (R), the reactant adduct (RA), the transition state (TS), the product adduct (PA), and the separated products (P). The energy of the TS relative to the reactants (R) increases by 8 kcal/mol when instead of a common double-ζ basis set (red) a triple-ζ basis set is used (green), indicating that the values reported in some former works are altered by severe basis-set superposition errors. Entropic corrections at room temperature (purple) increase the relative energy of RA, TS, and PA by 10 kcal/mol. Consideration of solvation free energies (blue) with Poisson−Boltzmann calculations decreases the reaction free energy from 119 to 1 kcal/mol and increases the free energy of RA from 2 to 6 kcal/mol.[8] Hence, the reactant adduct (RA) would be predicted in solution to be significantly less stable than the separated reactants (R), casting doubt on the physical basis of taking RA as the reference state. Note that a Car-Parrinello study with a larger number of explicit solvent molecules did not give evidence for such adducts,[9] i.e., the attacking water molecule comes from bulk solution.

The calculated activation free energy (Figure 2a, blue) for the first hydrolysis (30 kcal/mol) relative to the separated reactants (R) is larger than experimental values (24 kcal/mol).[10,11] We believe that the continuum dielectric models do not properly consider the changes of solvation entropy in bimolecular reactions. According to Wertz and others,[12] various molecules lose a constant fraction (∼0.5) of their entropy, when they are dissolved in water. Therefore, the solvation entropy of each species including that of the TS may be assumed to be half of the entropy in vacuo with the opposite sign. With this empirical correction (Figure 2a, black),[13] the predicted activation barrier (25 kcal/mol) is in good agreement with the experimental values. Furthermore, the reactant adduct (RA) is now (Figure 2a, black) approximately as stable as the separated reactants (R). This result is very convincing, because RA and R represent the same metal complex dissolved in water. Note that the experimental activation barrier would be reproduced as well by a poor approach (red) that (i) uses inappropriately the reactant adducts (RA) as the reference, (ii) suffers from basis-set superposition errors, (iii) neglects entropic corrections, and (iv) neglects solvation effects.

Analogous calculations for the *second hydrolysis* (Figure 2b, black) arrive at relative free energies for the reactants (set to 0), TS (25 kcal/mol), and products (2 kcal/mol) that are remarkably similar to those of the first hydrolysis step. In contrast, the second hydrolysis reaction would have been predicted to be 12 kcal/mol endothermic, if the reactant adduct (RA) and product adduct (PA) had been taken into account (Figure 2b, blue, dashed lines). This result would have suggested *cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ not to be involved at all in the mode of action of cisplatin. Such an interpretation would have ignored the result that the product adduct (PA) is 8 kcal/mol less stable than the separated products (P), i.e., a fully solvated chloride ion is significantly more stable than a chloride in the second coordination shell of the aqua complex.

As an alternative to the second hydrolysis, *cis*-$[Pt(NH_3)_2(OH_2)Cl]^{+}$ may be deprotonated first, and then the Pt−Cl bond of *cis*-$[Pt(NH_3)_2(OH)Cl]$ may be hydrolyzed, herein denoted *third hydrolysis* (Figure 2c, black). For the third

Table 1. Comparison of Calculated and Experimental Activation Free Energies (ΔG_a; in kcal/mol) and Reaction Free Energies (ΔG_r; in kcal/mol) for the Hydrolysis of Pt−Cl Bonds Anticancer Complexes and Absolute pK_a Values of the Aqua Complexes[a]

metal complex		calc	exp	exp ref
cis-$[Pt(NH_3)_2Cl_2]$	ΔG_a	24.9	23.8; 24.1	c; d
cis-$[Pt(NH_3)_2Cl_2]$	ΔG_r	0.1	4.2; 3.6	c; d
cis-$[Pt(NH_3)_2(OH_2)Cl]^+$	ΔG_a	25.3	23.3	d
$[Ru(Ar)(en)Cl]^+$ [b]	ΔG_a	20.7	21.4	b
$[Ru(Ar)(en)Cl]^+$ [b]	ΔG_r	1.1	3.2	b
cis-$[Pt(NH_3)_2(OH_2)Cl]^+$ (pK_{a1})	pK_a	7.8	6.41	e
cis-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ (pK_{a2})	pK_a	8.3	5.37	e
cis-$[Pt(NH_3)_2(OH_2)(OH)]^+$ (pK_{a3})	pK_a	9.5	7.21	e
$[Ru(Ar)(en)(OH_2)]^{2+}$ [b]	pK_a	9.8	7.71	b

[a] A difference of 1 pK_a unit reflects a free energy difference of $RT\ln 10 = 1.36$ kcal/mol. [b] en = 1,2-diaminoethane. Ar = η^6-benzene (calc), η^6-biphenyl (exp). Reference 14b. [c] Coe, J. S. *MTP Int. Rev. Sci.: Inorg. Chem., Ser. 2* **1974**, 45. [d] Perumareddi, J. R.; Adamson, A. W. *J. Phys. Chem.* **1978**, *72*, 414. [e] Reference 14a.

hydrolysis, we predict an activation free energy (27 kcal/mol) that is slightly higher than the barriers for the first two hydrolysis steps, indicating that *cis*-$[Pt(NH_3)_2(OH_2)(OH)]^+$ may form more likely via deprotonation of the second hydrolysis product. The theoretical prediction of the pK_a values of the three aqua complexes of cisplatin[14a] presented in Table 1 corroborates the remarkable absolute accuracy of ~4 kcal/mol of the quantum chemical approach, while the relative accuracy appears to be even better.

The former unisonous prediction of a strongly endergonic second and third hydrolysis—the most recent papers suggested reaction free energies of 12[2i] and 8 kcal/mol,[2j] respectively—would strongly contradict the experimental detection of diaqua and hydroxo species more than two decades ago.[15] Today it is still controversial whether *cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ and *cis*-$[Pt(NH_3)_2(OH_2)(OH)]^+$ are responsible for the anticancer activity, in addition to *cis*-$[Pt(NH_3)_2(OH_2)Cl]^+$.[16] For instance, the rate constants for the reaction of cisplatin derivatives with GG and AG moieties of double-stranded oligonucleotides suggest the diaqua species to be the actually active species.[16] The current work is the first theoretical study on cisplatin hydrolysis that supports this possibility, together with recent theoretical studies on the reactivity of cisplatin hydrolysis products with the nucleobases.[17] The question as to whether reactant adducts play a role in DNA binding remains controversial.[17−19] In this context, it is interesting to note the experimental detection of weak noncovalent interactions of *cis*-$[Pt(NH_3)_2(OH_2)_2]^{2+}$ and oligonucleotides prior to the reaction,[20] but their structure in aqueous solution and their impact on the rates of binding to DNA in this medium has not yet been clarified.

Computational Details

The geometries of molecules and transition states (TS) were optimized at the gradient-corrected DFT level using the 3-parameter fit of exchange and correlation functionals of Becke (B3LYP),[6] which includes the correlation functional of Lee, Yang, and Parr (LYP),[7] as implemented in Gaussian 98.[21] The LANL2DZ ECP's[22] and valence-basis sets were used at platinum, and the 6-31G(d,p) basis sets were used at the other atoms.[23] This basis-set combination is denoted II. Vibrational frequencies were also calculated at B3LYP/II. The structures reported are either minima (NIMAG = 0) or transition states (NIMAG = 1) on the potential energy surfaces. Improved total energies were calculated at the B3LYP level using the same ECP and valence-basis set at the metal, but totally uncontracted and augmented with Frenking's set of f functions,[24] together with the 6-311+G(3d) basis sets at chlorine and the 6-311+G(d,p) basis sets at the other atoms. This basis-set combination is denoted III+. Activation and reaction free energies (ΔG_a, ΔG_r) were calculated by adding corrections from unscaled zero-point energy (ZPE), thermal energy, work, and entropy evaluated at the B3LYP/II level at 298.15 K, 1 atm to the activation and reaction energies (ΔE_a, ΔE_r), which were calculated at the B3LYP/III+//II level. We found a good agreement between B3LYP and CCSD(T) relative energies (see the Supporting Information), which is not unexpected.[2j,25] Additional calculations were performed using the Stuttgart-Dresden-Bonn ECP[26] and improved basis sets,[27] which gave relative energies very similar to those obtained using LANL2DZ.

Solvation free energies G_{solv}^{ϵ} of the structures optimized at the B3LYP/II level were calculated by Poisson−Boltzmann (PB)[8] calculations with a dielectric constant ϵ of the dielectric continuum that represents the solvent. The PB calculations were performed at the B3LYP level using the LACVP** basis set on platinum, the 6-31+G* basis on oxygen, and the 6-31G** basis set on the other atoms as implemented in the Jaguar 5 program package.[28] The continuum boundary in the PB calculations was defined by a solvent-accessible molecular surface with a set of atomic radii for H (1.150 Å), C (1.900 Å), N (1.600 Å), O (1.400 Å), S (1.900 Å), Cl (1.974 Å), and Pt (1.377 Å).[29] pK_a predictions were carried out using a thermodynamic cycle,[30] $\Delta G^{\epsilon} = \Delta G^1 + G_{solv}^{\epsilon}(H^+) + G_{solv}^{\epsilon}(A^-) - G_{solv}^{\epsilon}(A)$ and $pK_a^{\epsilon} = \Delta G^{\epsilon}/RT\ln 10$, where ΔG^1 and ΔG^{ϵ} are the reaction free energies of the reaction, $AH \rightarrow A^- + H^+$, in vacuo and at a dielectric constant $\epsilon = 80.37$ for water, respectively, $G_{solv}^{\epsilon}(X)$ is the solvation free energy of species AH or A^- at ϵ obtained via PB calculations, R is the ideal gas constant, and T is the temperature (298.15 K). Experimental values have been used for the hydration free energy $G_{solv}^{\epsilon}(X)$ of small molecules and ions.[31] We believe that continuum dielectric models do not consider properly the changes of solvation entropy in bimolecular reactions; comparisons with experimental values indicate that reactions of platinum complexes and palladium complexes (unpublished) are systematically about ~6 kcal/mol too high. According to Wertz and others,[12] various molecules lose a constant fraction (approximately 0.5) of their entropy, when they are dissolved in water. All free energies in solution except that of the H^+ ion in solution were modified by an entropic term that is half (0.5) of the entropy in vacuo, with the opposite sign. This empirical correction has led to predicted pK_a values of platinum aqua complexes as well as reaction and activation free energies for the hydrolysis of metal complexes that are in good agreement with experimental values (Table 1).

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Supporting Information Available: Calculated relative energies and free energies in vacuo and aqueous solution for the hydrolysis of the Pt−Cl bonds of cisplatin, *cis*-[Pt$(NH_3)_2Cl_2$], *cis*-[Pt$(NH_3)_2(OH_2)Cl]^+$, and *cis*-[Pt$(NH_3)_2(OH)$-Cl] (Table S-1). This material is available free of charge via the Internet at http://pubs.acs.org.

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