Antitumor Agents

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A Single Slow Electron Triggers the Loss of Both Chlorine Atoms from the Anticancer Drug Cisplatin: Implications for Chemoradiation Therapy**

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Electron attachment to the chemotherapeutic anticancer drug *cis*-diamminedichloroplatinum(II) (cisplatin, CDDP) shows intense resonances at very low energies (close to 0 eV), which result in Pt–Cl bond cleavage. Apart from the products Cl^- and its complement $[Pt(NH_3)_2Cl]^-$, we observe a strong reaction resulting in the cleavage of both Pt–Cl bonds, thereby forming $[Pt(NH_3)_2]^-$. This latter complex can be regarded as the activated intermediate for the formation of CDDP–DNA adducts that inhibit replication. We therefore suggest that in the synchronous in vivo application of CDDP and radiation (chemoradiation), electron transfer is the most direct route in CDDP–DNA adduct formation.

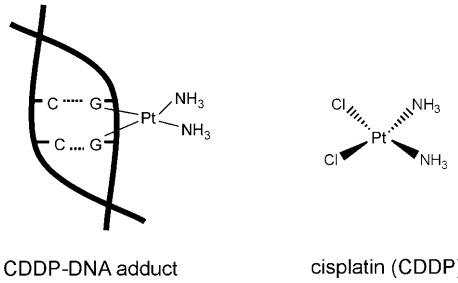

Figure 1. Structure of cisplatin and intrastrand cross-link as the dominant bifunctional binding mode of cisplatin with DNA.

The coordination complex $[Pt(NH_3)_2Cl_2]$ (Figure 1), commonly assigned as cisplatin (CDDP), is one of the most widely used chemotherapeutic anticancer drugs.[1,2] Its cytotoxicity is based on its ability to bind to DNA, thereby inhibiting replication. DNA is a particular target for CDDP within a cellular environment, and CDDP–DNA adducts are formed.[1,3] From the crystal structure it is known that most adducts consist of intrastrand cross-links, preferentially at two adjacent guanine (G) bases. It has also been shown that the combination of both chemotherapy and radiotherapy, that is, chemoradiation therapy, increases the survival of cancer patients compared to those who were treated by radiotherapy alone,[4,5] with the largest effect observed in a synchronous application of the drug and radiation. This effect was attributed to some yet unidentified synergetic interaction between radiation and drug. To date the detailed molecular mechanism of how the CDDP–DNA complexes are formed and many aspects of the mechanisms how these adducts inhibit DNA replication remain under question. For CDDP to bind to DNA, the two Cl–Pt bonds must be cleaved. For adduct formation in vivo, sequential hydrolysis of cisplatin was proposed to yield reactive intermediate aqua complexes of the form $[PtCl(H_2O)(NH_3)_2]^+$ and in the second step $[Pt(H_2O)_2(NH_3)_2]^{2+}$, which could bind to two neighboring G bases.[6,7]

Regarding the synchronous application of drug and radiation, it must be noted that a major amount of energy of the primary radiation quanta is channeled into low-energy (ballistic) electrons (LEEs),[8] and it has become obvious that these LEEs significantly contribute to the damage of DNA.[9] In a recent study, solid films of DNA with and without CDDP[5] bound to guanine were bombarded with electrons at different energies. It was shown that the number of strand breaks in cancer cells is significantly increased in the synchronous action of drug and LEEs. It was suggested that these LEEs trigger bond cleavage by the formation of transient anions and may be the basis of the efficiency of CDDP–radiation therapy.

From experiments applying ultrafast transient absorption spectroscopy[10] it was concluded that CDDP is subjected to electron transfer involving presolvated electrons that were generated by the initial laser pulse. It was suggested that the depletion of CDDP by these presolvated electrons (e_p^-) occurs by the dissociative electron attachment (DEA) process [Eq. (1)]:

$$e_p^- + [Pt(NH_3)_2Cl_2] \rightarrow [Pt(NH_3)_2Cl] + Cl^- \quad (1)$$

The created neutral radical may release the second Cl^- ion in a further DEA reaction, thus forming $[Pt(NH_3)_2]$, which can readily bind to DNA.

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To get information on the intrinsic properties of CDDP with respect to the attack of LEEs, we study herein the interaction of free electrons with gas-phase cisplatin. We find a pronounced DEA resonance at very low energy with the unique feature that it decomposes by the loss of two neutral Cl atoms, thereby forming the $[Pt(NH_3)_2]^-$ ion, which can be viewed as the activated compound to form CDDP–DNA adducts. Further decomposition processes include the loss of Cl^-, Cl_2^-, and $[Pt(NH_3)_2Cl]^-$ ions. While the fact that one electron induces the loss of two Cl atoms is remarkable by itself, it shows that electron attachment or electron transfer can be regarded as the most direct molecular route in the formation of CDDP–DNA adducts.

The experiments were carried out in an electron-attachment spectrometer that has already been described.[11] In brief, an incident electron beam of well-defined energy (full width at half maximum (FWHM) ca. 0.2 eV, electron current ca. 100 nA) generated from a trochoidal electron monochromator[12] orthogonally intersects an effusive molecular beam of cisplatin. The beam emanates from a vessel directly connected to the collision chamber. The overall spectrometer is moderately heated by two in vacuo halogen bulbs. The operating temperature was in the range 164–172 °C (measured by a platinum resistance directly at the vessel), resulting in a vapor pressure of 10^{-7} mbar as recorded by the ionization gauge. For the decomposition temperature we find only one reference stating a value of 270 °C,[13] which is considerably above the evaporation temperature presently used. We therefore assume that CDDP remains intact when it is transferred into the gas phase. In the case of thermal decomposition, we would expect that the relative signal intensity of fragments such as $[Pt(NH_3)_2]^-$ would increase with evaporation temperature at the expense of the Cl^- signal. This is definitely not the case, as the signal ratio $Cl^-/[Pt(NH_3)_2]^-$ is approximately independent on the evaporation temperature. Moreover, data recorded at 130 °C (at very low intensity) did not change this signal ratio. Cisplatin was obtained from Sigma Aldrich at a stated purity of 99.9 %.

Negative ions formed by electron–molecule collisions are extracted from the reaction area towards a quadrupole mass analyzer and detected by a single-pulse counting technique. The yield of the anions is then recorded as a function of the electron energy. The electron energy scale is calibrated by means of the "0 eV" resonance in SF_6. We note that a possible (though unlikely) formation of the parent anion $[Pt(NH_3)_2Cl_2]^-$ (300 amu) is just outside the range covered by the mass spectrometer.

Density functional theory calculations have been performed on neutral and anionic CDDP using the B3LYP hybrid functional in combination with the LANL2DZ effective core potential basis set for the platinum atom and the 6-31G++** basis set for the Cl, N, and H atoms. Geometries of CDDP and fragmentation products have been fully optimized to obtain the bond dissociation energies of all Pt–Cl and Pt–NH_3 bonds. These calculations are not at the level to predict accurate numbers for electron affinities, but they do provide reasonable binding energies in the neutral complex. Calculation of vibrational frequencies yielded thermal corrections to electronic energy values for 298 K. For the calculations, the standard quantum chemical package Gaussian03[14] was used.

Figure 2 and Figure 3 show the yields of the observed anions. While the prominent fragments arising from the cleavage of one or two Pt–Cl bonds are already present at very low energies, the NH_2^- ion is formed at appreciably higher energy and lower intensity. The shape and position of this ion yield resembles that of NH_2^- formation from ammonia.[15,16] A further weak signal at 17 amu is observed at an energy close to 0 eV (not shown here), the origin of which is not clear. From stoichiometry it can be assigned to NH_3^-; a free ammonia, however, cannot bind an excess electron.

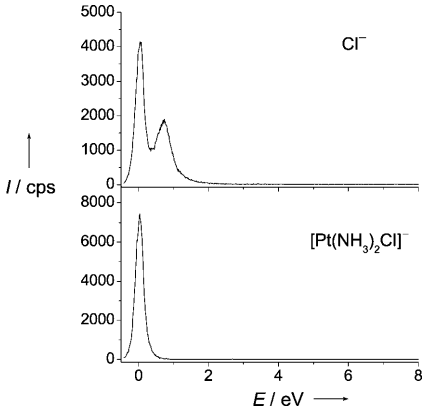

Figure 2. Formation of the complementary ions Cl^- and $[Pt(NH_3)_2Cl]^-$ arising from the cleavage of the Pt–Cl bond.

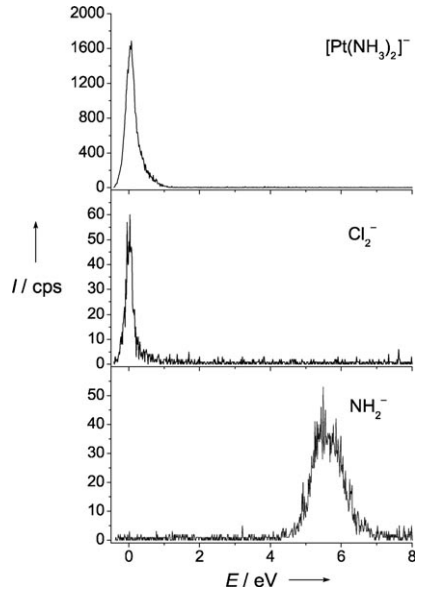

Figure 3. Formation of the complementary fragment ions $[Pt(NH_3)_2]^-$ and Cl_2^- arising from the cleavage of two Pt–Cl bonds and formation of NH_2^-.

Figure 2 shows the fragments arising from the cleavage of a single Pt–Cl bond with the excess charge localized on either of the fragments, as shown in Equations (2a) and (2b):

$$e^- + [Pt(NH_3)_2Cl_2] \rightarrow [Pt(NH_3)_2Cl] + Cl^- \quad (2a)$$

$$e^- + [Pt(NH_3)_2Cl_2] \rightarrow [Pt(NH_3)_2Cl]^- + Cl \quad (2b)$$

The reaction in Equation (2a) corresponds to the reaction in Equation (1) proposed from the electron-transfer experiments in solution mentioned above.[10] Interestingly, the complementary reaction with the excess charge localized on the large fragment is even more intense. It should be noted that the quadrupole mass spectrometer discriminates towards larger masses, so that the true intensity ratio is expected to be even more in favor of $[Pt(NH_3)_2Cl]^-$.

The energy threshold for the single bond cleavage reactions in Equations (2a) and (2b) is given by the Pt–Cl binding energy minus the electron affinity (EA) of the fragment on which the excess charge becomes localized. The calculated Pt–Cl bond dissociation energy is 3.3 eV, and for the removal of the second Cl atom from the relaxed radical $[Pt(NH_3)_2Cl]$ it is only 2.0 eV. Accordingly, the values to remove NH_3 are 1.5 and 1.7 eV, respectively. Taking the known EA of Cl (3.61 eV[17]) it is obvious that the loss of Cl^- is slightly exothermic, and from the fact that the $[Pt(NH_3)_2Cl]^-$ ion is also observed near 0 eV it follows that EA($[Pt(NH_3)_2Cl]$) $\geq$ 3.3 eV.

More interesting is the observation that one electron triggers the cleavage of two Pt–Cl bonds. Again, the complementary reactions are observed [Eqs. (3a) and (3b)]:

$$e^- + [Pt(NH_3)_2Cl_2] \rightarrow [Pt(NH_3)_2]^- + Cl_2 \quad (3a)$$

$$e^- + [Pt(NH_3)_2Cl_2] \rightarrow [Pt(NH_3)_2] + Cl_2^- \quad (3b)$$

with the reaction in Equation (3a) being considerably more intense. It should be noted that under the present conditions (gas pressure, electron intensity) 1) the measurements are performed under single-collision conditions, that is, the probability that a fragment generated by a first electron ($[Pt(NH_3)_2Cl]$) is subjected to a collision with a second electron (generating $[Pt(NH_3)_2]^-$) is negligible, and 2) the presence of radicals $[Pt(NH_3)_2Cl]$ by thermal decomposition (which may undergo DEA to form $[Pt(NH_3)_2]^-$) can be excluded (see above). The cleavage of multiple bonds and formation of new bonds triggered by a single electron has been observed in DEA before,[11,18,19] but only in the case of the DNA and RNA sugars at energies close to 0 eV.[20,21] The energy balance of the reactions in Equations (3a) and (3b) requires the cleavage of the two Pt–Cl bonds, which must be compensated by the Cl–Cl binding energy (2.52 eV[22]) and the electron affinity of the fragment on which the electron becomes localized. With the calculated dissociation energies (5.3 eV) and the EA of Cl_2 (2.38 eV[17]), the threshold of the reaction in Equation (3b) becomes 0.4 eV, while the experimental appearance energy of Cl_2^- is close to 0 eV. This difference may be due to thermal activation of CDDP and some inaccuracy in the calculated binding energies. Conversely, the EA of $[Pt(NH_3)_2]$ is not known, but from the energy balance of Equation (3a), it follows that EA($[Pt(NH_3)_2]$) $\geq$ 2.9 eV.

Figure 4 shows a contour plot of the lowest unoccupied molecular orbital (LUMO) in CDDP, which is most likely involved in LEE attachment to CDDP. It has distinct repulsive σ* character along all coordination axes. Ammonia cannot bind an excess electron so that stabilization of the excess charge has to proceed through cleavage of one or more Pt–Cl bonds. The signal of $[Pt(NH_3)_2]^-$ is more than one order of magnitude larger than that of Cl_2^-, which may result from more favorable energetics of Equation (3a) compared to Equation (3b), which in turn would imply the EA of $[Pt(NH_3)_2]$ to be considerably larger than 3 eV. From entropy arguments, localization of the excess charge is in any case more favorable on $[Pt(NH_3)_2]$ than on Cl_2.

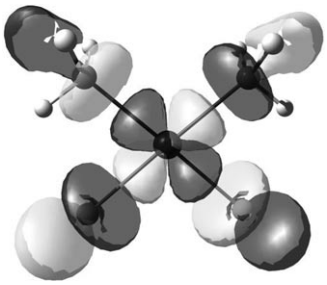

Figure 4. Contour plot of the lowest unoccupied molecular orbital in cisplatin calculated at the B3LYP/6-31G++**/LANL2DZ level of theory.

In summary, gas-phase cisplatin shows pronounced low-energy resonances with the unique feature that a strong reaction is the loss of two Cl atoms. The generated complex $[Pt(NH_3)_2]^-$ can be regarded as the reactive intermediate to form CDDP–DNA adducts that inhibit DNA replication.

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