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Electron Transfer Reaction Mechanism of Cisplatin with DNA at the Molecular Level

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Abstract: The cytotoxicity of cisplatin as a very effective chemotherapeutic anticancer drug is known to arise from its capacity to damage DNA. However, the mechanism of action of cisplatin at the molecular level, in particular, the reaction dynamics of cisplatin with DNA, remains elusive, and the reason why cisplatin binds to the guanine bases rather than to S-donor ligands available in cells remains a mystery. Using time-resolved femtosecond laser spectroscopy, *for the first time*, we reveal the high reactivity of cisplatin with electrons and its preferential electron-transfer reaction with the DNA guanine base. The results not only provide a molecular mechanistic understanding of cisplatin in cancer therapy but may have far-reaching significance for understanding the role of polar molecules such as NH_3 and NH_2 in biological reactions.

Keywords: Anticancer drug; chemotherapy; cisplatin; molecular reaction mechanism; electron-transfer reaction; femtochemistry and femtobiology; time-resolved femtosecond laser spectroscopy

Electron transfer (ET) underlies many reactions in molecular systems of chemical, biological, and biomedical significance.[1−5] Real-time observation of the transition state in an ET reaction may lead to the prediction, understanding, and modification of the course of the reaction. Among available techniques, time-resolved femtosecond (1 fs = 10^{-15} s) laser spectroscopy is the most powerful technique for direct observation of the reaction transition state and reaction dynamics.[6,7]

Cisplatin (CDDP) is one of the most widely used anticancer drugs.[8,9] It is particularly effective in treating testicular and ovarian cancer and increasingly used against cervical, head/neck bladder, and small cell lung cancer. Despite its great success, CDDP has some significant limitations: severe toxic side effects and both intrinsic and acquired resistance. Such outstanding problems have even prompted the call to stop the development of new platinum-based anticancer drugs.[10] Over the past 35 years, there have been many attempts to overcome these limitations and to broaden the range of treatable tumors by designing and testing various CDDP-like complexes. Over 3000 CDDP analogues have been synthesized and screened for anticancer activity, with no more than 30 compounds entering clinical trials and only one approved by the FDA: oxaliplatin, for the treatment of colorectal cancer.[9] Thus, the search for new anticancer drugs by traditional methods has proven to be a difficult and inefficient task. Alternatively, a biochemical modulation of the mechanisms of action of CDDP was proposed to circumvent the resistance and to improve the antitumor activity.[11] However, this strategy requires the knowledge of the precise mechanism of action of the drug.

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(1) Marcus, R. A. Electron-transfer reactions in chemistry—theory and experiment (Nobel lecture). *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1111−1121.

(2) Wan, C.; Fiebig, T.; Kelley, S. O.; Treadway, C. R.; Barton, J. K.; Zewail, A. H. Femtosecond dynamics of DNA-mediated electron transfer. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 6014−6019.

(3) Jortner, J., Boxon, M. Eds. *Advances in Chemical Physics*: *Electron Transfer—From Isolated Molecules to Biomolecules*; Wiley: New York, 1999; Vol. 106.

(4) Qu, X. G.; Wan, C. Z.; Becker, H. C.; Zhong, D. P.; Zewail, A. H. The anticancer drug-DNA complex: Femtosecond primary dynamics for anthracycline antibiotics function. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 14212−14217.

(5) Wang, C. R.; Hu, A.; Lu, Q. B. Direct Observation of the Transition State of Ultrafast Electron Transfer Reaction of a Radiosensitizing Drug Bromodeoxyuridine. *J. Chem. Phys.* **2006**, *124*, 241102−241105.

(6) Polanyi, J. C.; Zewail, A. H. Direct observation of the transition state. *Acc. Chem. Res.* **1995**, *28*, 119−132.

(7) Zewail, A. H. Femtochemistry: Atomic-scale dynamics of the chemical bond using ultrafast lasers (*Nobel lecture*). *Angew. Chem., Int. Ed.* **2000**, *39*, 2587−2631.

(8) Wong, E.; Giandomenico, C. M. Current Status of Platinum-Based Antitumor Drugs. *Chem. Rev.* **1999**, *99*, 2451−2466.

(9) Wang, D.; Lippard, S. J. Cellular Processing of Platinum Anticancer Drugs. *Nat. Rev. Drug Discovery* **2005**, *4*, 307−320.

(10) Reese, D. M. Anticancer drugs. *Nature* **1995**, *378*, 532.

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The cytotoxicity of cisplatin is well-known to arise from its capacity to damage DNA, and the crystal structures of the cisplatin−DNA adducts have been determined.[12−14] But the precise mechanism of action of cisplatin at the molecular level, in particular, the reaction dynamics of cisplatin with DNA, remains elusive, and the reason why cisplatin binds to the guanine bases rather than to S-donor ligands available in cells remains a mystery.[11,15−18]

In studies of the mechanism of action of cisplatin, it has been known that the cytotoxicity of cisplatin arises from its capacity to damage DNA, resulting in cisplatin−DNA adducts.[12−14] Major DNA damage forms induced by cisplatin are 1,2-d(GpG) (65% of the total adducts) and 1,2-d(ApG) (25%) with small percentages of 1,3-d(GpNpG) intrastrand cross-links and interstrand cross-links. Prior to the binding of cisplatin to DNA, the bonds between chlorine atoms and the Pt must be broken in one step or two steps, ending with the *cis*-$Pt(NH_3)_2$ binding to DNA. The hydrolysis of cisplatin has been thought to be the mechanism leading to the formation of reactive aqua complexes, mainly $[PtCl(H_2O)(NH_3)_2]^+$.[11,17] However, it would be hard to understand how $[PtCl(H_2O)(NH_3)_2]^+$ binds to *two* neighboring G bases in DNA. The experimental evidence[19,20] for the hydrolysis of cisplatin, in fact, showed that, under physiological conditions, $[Pt(H_2O)_2(NH_3)_2]^{2+}$, a species that could bind to two neighboring guanine bases, is most difficult to form. More remarkably, it was also estimated that the reaction rate constant for the hydrolysis of cisplatin is only $\sim 10^{-4}\ M^{-1}s^{-1}$ even at a higher temperature (45 °C), at low $[Cl^-]$ concentrations, and in the presence of solvents such as $HClO_4$ and $NaClO_4$, which will only be lower under physiological conditions at 37 °C in water environment.[19] Thus, there may exist an unknown reaction pathway, rather than the hydrolysis, to lead to the chlorine-bond breaks in the interaction of cisplatin with DNA.

It is long known that halogen (Cl, Br, and I)-containing molecules have very efficient dissociative attachment reactions with low-energy electrons to produce a halogen anion and a neutral radical,[21−24] and it has been observed that the presence of NH_3 can cause large enhancements in electron-induced reactions of halogenated molecules.[25,26] A dissociative ET mechanism for the enhancements was proposed:[25,26]

$$e^-{:}(NH_3)_n + ABCl_x \rightarrow [ABCl_x]^{*-} \rightarrow Cl^- + ABCl_{x-1}^{\bullet} \quad (1)$$

where $ABCl_x$ represents a chlorine-containing molecule and $e^-{:}(NH_3)_n$ a presolvated electron (e_{pre}^-) localized in the small polar molecular cluster $(NH_3)_n$. Thus, it is reasonable to speculate that CDDP ($Pt(NH_3)_2Cl_2$), a small molecule in which two NH_3 groups and two chlorine atoms are bound to a Pt atom, should have very efficient dissociative attachment reactions with weakly bound electrons.

In this communication, we report the first time-resolved femtosecond laser spectroscopic study of the molecular reaction mechanism of cisplatin as an effective chemotherapeutic drug. Our results discover the high reactivity of CDDP with weakly bound prehydrated electrons and the preferential ET reaction of CDDP with the DNA guanine base. These results not only reveal the molecular mechanism of action of cisplatin as one of the most effective anticancer drugs

(11) Fuertes, M. A.; Alonso, C.; Perez, J. M. Biochemical Modulation of Cisplatin Mechanisms of Action: Enhancement of Antitumor Activity and Circumvention of Drug Resistance. *Chem. Rev.* **2003**, *103*, 645−662.

(12) Fichtinger-Schepman, A. M. J.; van der Veer, J. L.; den Hartog, J. H. J.; Lohman, P. H. M.; Reedijk, J. Adducts of the antitumor drug cis-diamminedichloroplatinum(II) with DNA−Formation, Identification, and Quantitation. *Biochemistry* **1985**, *24*, 707−713.

(13) Takahara, P. M.; Rosenzweig, A. C.; Frederick, C. A.; Lippard, S. J. Crystal structure of double-stranded DNA containing the major adduct of the anticancer drug cisplatin. *Nature* **1995**, *377*, 649−652.

(14) Huang, H. F.; Zhu, L. M.; Reid, B. R.; Drobny, G. P.; Hopkins, P. B. Solution structure of a cisplatin-induced DNA interstrand cross-link. *Science* **1995**, *270*, 1842−1845.

(15) Barry, M. A.; Behnke, C. A.; Eastman, A. Activation of programmed cell death (apoptosis) by cisplatin, other anticancer drugs, toxins and hyperthermia. *Biochem. Pharmacol.* **1990**, *40*, 2353−2362.

(16) Zdraveski, Z. Z.; Mello, J. A.; Marinus, M. G.; Essigmann, J. M. Multiple pathways of recombination define cellular responses to cisplatin. *Chem. Biol.* **2000**, *7*, 39−50.

(17) Reedijk, J. New clues for platinum antitumor chemistry: Kinetically controlled metal binding to DNA. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 3611−3616.

(18) Nowosielska, A.; Marinus, M. G. Cisplatin induces DNA double-strand break formation in Esherichia coli dam mutants. *DNA Repair* **2005**, *4*, 773−781.

(19) Miller, S. E.; House, D. A. The hydrolysis products of cis-dichlorodiammineplatinum(II) 3. Hydrolysis kinetics at physiological pH. *Inorg. Chim. Acta* **1990**, *173*, 53−60.

(20) Berners-Price, S. J.; Frenkiel, T. A.; Frey, U.; Ranford, J. D.; Sadler, P. J., Hydrolysis products of cisplatin: pK_a determination via [^{1}H, ^{15}N] NMR spectroscopy. *J. Chem. Soc., Chem. Commun.* **1992**, 789−791.

(21) Christophorou, L. G.; McCorkle, D. L.; Christodoulides, A. A. In *Electron-Molecule Interactions and Their Applications*; Christophorou, L. G., Ed.; Academic Press: Orlando, 1984; Vol. 1, Chapter 6.

(22) Dixon-Warren, S. J.; Jensen, E. T.; Polanyi, J. C. Direct evidence for charge-transfer photodissociation at a metal-surface-CCl4/Ag-(111). *Phys. Rev. Lett.* **1991**, *67*, 2395−2398.

(23) Illenberger, E. Electron-attachment reactions in molecular clusters. *Chem. Rev.* **1992**, *92*, 1589−1609.

(24) Rowntree, P.; Sanche, L.; Parenteau, L.; Meinke, M.; Weik, F.; Illenberger, E. Dissociative electron-attachment to condensed and adsorbed halomethanes. *J. Chem. Phys.* **1994**, *101*, 4248−4259.

(25) Lu, Q. B.; Madey, T. E. Negative-ion enhancements in electron-stimulated desorption of CF_2Cl_2 coadsorbed with nonpolar and polar gases on Ru(0001). *Phys. Rev. Lett.* **1999**, *82*, 4122−4125. Giant enhancement of electron-induced dissociation of chlorofluorocarbons coadsorbed with water or ammonia ices: Implications for the atmospheric ozone depletion. *J. Chem. Phys.* **1999**, *111*, 2861−2864.

(26) Lu, Q. B.; Sanche, L. Enhanced Dissociative Electron Attachment to CF_2Cl_2 by Transfer of Electrons Localized in Preexisting Traps of Water and Ammonia Ice. *Phys. Rev. B* **2001**, *63*, 153403−153406.

but may have significance for understanding the role of polar molecules such as NH_3 and NH_2 in enhancing ET reactions in biological systems.

A standard methodology for pump−probe transient absorption measurements was described previously.[5] We used a Ti:sapphire laser system producing 120 fs, 1 mJ laser pulses centered at $\lambda = 800$ nm at a repetition rate of 1 kHz, two optical parametric amplifiers producing pump and probe pulses with wavelengths from visible to IR. The polarization of pump and probe pulses was set at the magic angle (54.7°) to avoid contribution from polarization anisotropy due to orientation motions of molecules. To avoid damage to biomolecules, small pump and probe pulse energies were used, 0.1−0.3 μJ and ~1 nJ, respectively. For the experiment of cisplatin reactions with prehydrated electrons, the latter were produced via two-UV-photon excitation of H_2O at the pump pulse energies (0.15−0.3 μJ) at 318 nm.[5,27] For the experiment of cisplatin reactions with DNA bases, in contrast, a lower pump pulse energy of ≤0.14 μJ was used to avoid the creation of prehydrated electrons. All samples were measured in a 5 mm quartz cell at room temperature and stirred during measurements to avoid product accumulation. Ultrapure water with a resistivity of >18 MΩ/cm obtained directly from a Barnstead Nanopure water system was used, while cisplatin (CDDP) and DNA bases (dGMP, dAMP, dCMP, and dTMP) were obtained from Sigma-Aldrich. A stock solution of 1−5 mM CDDP was made by dissolving the compound into the ultrapure water.

To examine the reactivity of cisplatin with electrons, we first generate weakly bound electrons by a two-UV-photon excitation at 318 nm in water[5,27] and observe their reaction in real time. More specifically, we monitor the ET reaction of CDDP with prehydrated electrons in water:

$$e_p^- + Pt(NH_3)_2Cl_2 \rightarrow [Pt(NH_3)_2Cl_2]^{*-} \rightarrow Pt(NH_3)_2Cl^{\bullet} + Cl^-$$

$$e_p^- + Pt(NH_3)_2Cl \rightarrow [Pt(NH_3)_2Cl]^{*-} \rightarrow Pt(NH_3)_2^{\bullet} + Cl^- \quad (2)$$

The molecular structure and the static UV−vis absorption spectrum of cisplatin are shown in Figure 1. Without any functional groups to absorb UV light, cisplatin has only a very weak absorption band extending up to 350 nm, whose major absorption peak is located at below 200 nm. This makes it difficult to observe the transition state, $[Pt(NH_3)_2Cl_2]^{*-}$ or $[Pt(NH_3)_2Cl]^{*-}$ of the ET reaction. The absorption wavelengths of the transition anionic states of halogenated molecules are generally only slightly larger than those of their ground-state parent molecules,[5] but the signals of the former are much weaker than those of the latter. Thus, by measuring the depletion of the ground-state $Pt(NH_3)_2Cl_2$ in the reaction region, it is easier to observe the reaction in eq 2. The pump and the probe wavelengths used for our

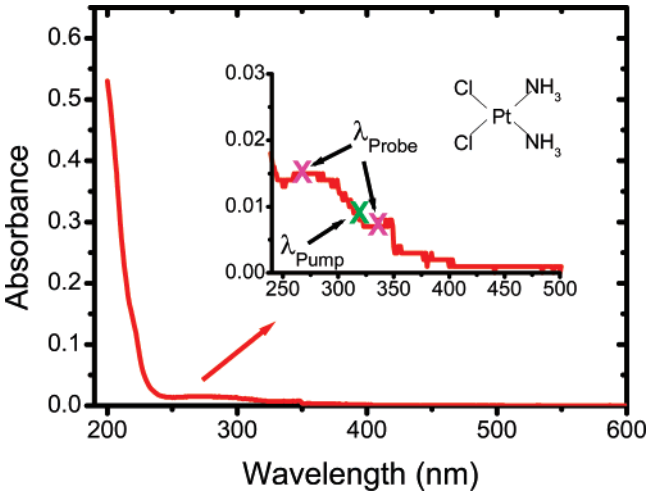

Figure 1. Molecular structure and the static UV/vis absorption spectrum of cisplatin (CDDP) in water. The pump pulse and probe pulse wavelengths used in the pump−probe experiments are indicated in the inset.

femtosecond laser spectroscopic measurements are 318 and 260 nm, as indicated in Figure 1. Figure 2 shows the transient absorption spectra of 2.0 mM CDDP in pure water with various pump pulse energies. As the pump pulse energy is ≤140 nJ/pulse, the results clearly show that no negative transient absorption was observed except the photobleaching near time zero (0 ps) due to the sudden excitation of CDDP by the pump pulse at 318 nm; instead, a small positive signal was observed in the first few picoseconds which was, not surprisingly, due to the CDDP excited state (CDDP*). Interestingly, as the pump pulse energy increased to a certain value so that a considerable amount of electrons could be produced from the two-photon excitation of water, a negative transient absorption signal started to be observed, indicating that CDDP molecules were depleted in the reaction region. A CDDP molecule could be destroyed by one electron (single-electron attachment process) releasing one Cl ion or by a one-step two-electron process releasing two Cl ions. The former process will result in a quadratic dependence of the CDDP depletion on the pump pulse energy, while the latter process will lead to a quadruple dependence. The data in Figure 2A shows between quadratic and quadruple dependences of the negative transient absorption signal on the pump energy, implying that both single-electron and double-electron transfer processes occur. The positive signal arising from the CDDP* state can be removed from the spectra observed at high pump energies since it is solely exhibited at the lowest pump energies and is linearly proportional to the pump energy. After this treatment, the results are shown in Figure 2B: the decay part of the negative signal in the first 20 ps is now attributed to the signal of the transition state of the electron−CDDP reaction, i.e., $CDDP^{*-}$, which overlaps with the negative depletion signal of CDDP molecules. It can also be seen that the rise time of the signal, i.e., the formation time of $CDDP^{*-}$, is well within a picosecond (1 ps = 10^{-12} s) after the electronic excitation by the pump pulse, in agreement with the lifetimes (≤1 ps)

(27) Lu, Q.-B.; Baskin, J. S.; Zewail, A. H. The presolvated electron in water: can it be scavenged at long range? *J. Phys. Chem. B* **2004**, *108*, 10509−10514.

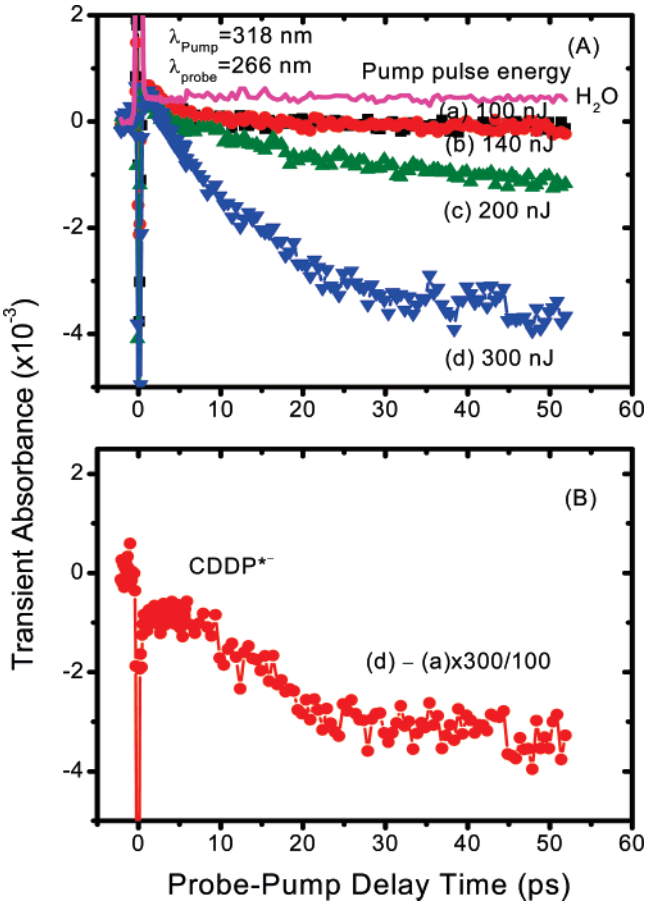

Figure 2. (A) Femtosecond time-resolved transient absorption spectra of 2 mM CDDP in water pumped at 318 nm and probed at 266 nm with various pump pulse energies, where the solid line with a sharp spike at time zero is the spectrum for the pure water obtained at the highest pump pulse energy of 300 nJ. (B) The corrected spectrum (d) in panel A after removal of the signal due to CDDP*.

of prehydrated electrons.[5,27,28] These results provide direct evidence of the high reactivity of CDDP with weakly bound prehydrated electrons in water.

After demonstrating the high reactivity of CDDP with weakly bound electrons, we have a good reason to further propose that, in chemotherapy, CDDP will capture two electrons from two neighboring guanine bases in DNA, since G is the most thermodynamically favored electron donor among four DNA bases.[29] These processes can be expressed as

$$G + Pt(NH_3)_2Cl_2 \rightarrow G^+ + [Pt(NH_3)_2Cl_2]^{*-} \rightarrow G^+ + Pt(NH_3)_2Cl + Cl^-$$

$$G + Pt(NH_3)_2Cl \rightarrow G^+ + [Pt(NH_3)_2Cl]^{*-} \rightarrow G^+ + Pt(NH_3)_2^{\bullet} + Cl^- \quad (3)$$

To examine this mechanism, the experiments were set to measure the reaction of CDDP with electrons *only* from DNA bases such as mononucleotides of guanine and adenine (2′-deoxyguanosine 5′-monophosphate, dGMP, and 2′-deoxy-

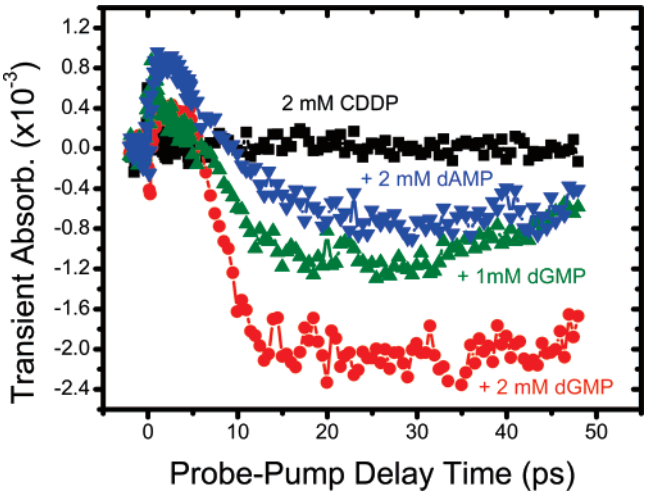

Figure 3. Femtosecond time-resolved transient absorption spectra of the complexes of 2 mM CDDP with DNA bases (dAMP and dGMP), pumped at 318 nm and probed at 330 nm.

adenosine- 5′-monophosphate, dAMP). The results are shown in Figure 3. In this experiment, the probe wavelength of 330 nm was chosen to avoid the direct absorption by DNA bases, which is significant at wavelengths below 300 nm. And the pump pulse energy (≤140 nJ/pulse) was chosen to be so low that no electrons were produced via two-photon excitation of water, as seen in the spectrum for the pure CDDP without the presence of DNA bases. Instead, only reactions of CDDP with electrons from ground-state DNA bases were to be monitored. In this experiment, the ET reaction is facilitated by exciting CDDP into an excited state (CDDP*), but it should be noted that the DNA bases (the electron donors) are kept at their ground states without being excited at the pump wavelength of 318 nm. Similar to the reaction of CDDP with prehydrated electrons, the significant depletion of CDDP (the negative transient absorbance) shown in Figure 3 clearly demonstrates the favored ET reaction of CDDP with guanine, which is significantly stronger than the reaction with adenine. In contrast, no depletion of CDDP and thus no ET reactions were observed for the complexes of CDDP with other two DNA bases T and C. These results provide strong evidence of the mechanism expressed in eq 3. This mechanism gives a direct explanation why Pt anticancer drugs result in preferential binding of the *cis*-Pt-$(NH_3)_2$ to two neighboring G bases in DNA, rather than to S-donor ligands available in the cell, since the latter are well-known electron acceptors. In other experiments, we have also observed an effective ET reaction between ground-state CDDP and a weakly bound electron donor (a photosensitizer

(28) Laenen, R.; Roth, T.; Laubereau, A. Novel precursors of solvated electrons in water: Evidence for a charge transfer process. *Phys. Rev. Lett.* **2000**, *85*, 50−53.

(29) Seidel, C. A. M.; Schulz, A.; Sauer, M. H. M. Nucleobase-Specific Quenching of Fluorescent Dyes. 1. Nucleobase One-Electron Redox Potentials and Their Correlation with Static and Dynamic Quenching Efficiencies. *J. Phys. Chem.* **1996**, *100*, 5541−5553.

excited at 800 nm).[30] These results indicate that the high ET reactivity of CDDP is intrinsically determined by its unique structure and physical properties. As expressed in eqs 1−3, the two NH_3 groups are strong promoters for electron transfer from the source (G bases) to the Cl atom sites in CDDP, leading to the release of Cl^- ions and the binding of the $Pt(NH_3)_2$ unit to DNA.

Moreover, we have further confirmed that the above ET mechanism of cisplatin is responsible for the DNA damage induced by action of CDDP. Namely, we have observed significant enhancements of CDDP-induced DNA damage either by in situ generation of an extra tiny electron source in the DNA−CDDP complex or by photoexcitation of a photosensitizer at 800 nm (to produce an electron donor).[30] These results have indeed provided new mechanistic understandings of the combinational therapies of cisplatin with radiotherapy and photodynamic therapy at the molecular level.[30] In summary, the finding of the extremely high ET reactivity of cisplatin provides new insight into the molecular mechanisms and the efficacy improvement of cisplatin for cancer therapies. More generally, the fundamental understanding of this most widely used anticancer drug will have great significance for developing new antitumor drugs in the future.[17] Moreover, the success in revealing the ET reaction for the mechanism of action of cisplatin at the molecular level may also have far-reaching significance for understanding of many biological reactions triggered by polar molecular groups such as NH_3 and NH_2. It is likely that similar ET reactions may play an important role in causing biological effects such as DNA damage and cell death.

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(30) Lu, Q.-B. Molecular Reaction Mechanisms of Combination Treatments of Low-Dose Cisplatin with Radiotherapy and Photodynamic Therapy. *J. Med. Chem.* **2007**, *50*, 2601−2604.