Ultrafast Electron Transfer

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Real-Time Observation of a Molecular Reaction Mechanism of Aqueous 5-Halo-2′-deoxyuridines under UV/Ionizing Radiation**

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Electron transfer (ET) underlies many processes in molecular systems of chemical, biological, and environmental significance and is therefore a subject of continued interest.[1–6] 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 transition states of chemical reactions.[7,8] In particular, this technique is suitable for studying ultrafast electron transfer (UET) reactions in chemical and biological systems.[2,9,10]

5-Halo-2′-deoxyuridines (XdUs, X = Cl, Br, and I), such as bromodeoxyuridine (BrdU) and iododeoxyuridine (IdU), are potential radiosensitizing agents. Replacement of thymidine in DNA by BrdU or IdU has long been known to enhance DNA damage and cell death induced by ionizing radiolysis and UV photolysis.[11–14] In addition, BrdU and IdU have also been employed to probe protein–nucleic acid interactions by inducing DNA/RNA–protein photocross-linking.[15] As potential sensitizers for radiotherapy of cancer, BrdU and IdU have been tested in several Phase I–III clinical trials.[16] However, the clinical results were not satisfactory, and therefore no XdUs have been approved for clinical use. This may be related to the fact that the mechanism of the radiosensitivity enhancement is not well understood. In the action of halouracils (XUs) it was believed that the first and critical step is the dissociative attachment (DA) of a radiation-generated hydrated electron (e_{hyd}^-) to an aqueous XU, leading to the formation of an anion and a highly reactive radical $U^\bullet$: $e_{hyd}^- + XU \rightarrow XU^{*-} \rightarrow X^- + U^\bullet$.[17,18] This seems reasonable as gaseous halogenated molecules, such as XUs and BrdU, generally have very large cross sections for DA to near 0-eV electrons.[19–21] For the reactions of electrons with XUs in water, Rivera and Schuler[17] successfully observed the formation of anionic XU^- states that have a transient absorption peak at 330 nm in their nanosecond pulse radiolysis experiments. The XU^- absorption at approximately 330 nm slightly red-shifts from the absorption at 280–290 nm of their parent neutral XUs. In gas-phase experiments, Abdoul-Carime et al.[20] showed that the efficiency for the uracil-5-yl radical formation by DA of nearly 0-eV electrons is in the order of BrU > ClU > IU, whereas the efficiency for the halogen atomic radical is ClU > BrU > IU. They thus concluded that ClU should be the most effective radiosensitizer among halouracils. This conclusion seems inconsistent with the observations of the biological and therapeutic effects of XdUs, which have so far shown effective radiosensitization by IdU and BrdU only[11–16] and with the theoretical calculations by Li et al.[22] that have showed the DA efficiency of BrU = ClU ≫ FU to low-energy electrons.

By using time-resolved femtosecond (fs) laser spectroscopy, we have recently revealed that it is the UET reaction of BrdU with a short-lived, weakly bound precursor electron (e_{pre}^-) rather than with the deeply trapped (well-bound) hydrated electron (e_{hyd}^-), that is responsible for the radical formation from BrdU in the aqueous environment.[23] A schematic diagram to illustrate this UET process is shown in Figure 1. It turns out that the nanosecond-resolved radiolysis experiments correctly showed the relevance of the reactive radical formation to the reactions of XUs with electrons generated in radiolysis and the absorption of anionic states reaching a peak at around 330 nm.[17] However, they actually showed the transient absorption spectra of long-lived (nanosecond), relaxed XU^-, rather than the ultrashort-lived (picosecond) dissociative vibrationally excited XU^{*-} that is the true transition state of the UET reaction.[23] Herein, we extend this study to all four potential radiosensitizers XdUs (X = F, Cl, Br, and I; see Figure 1). Strikingly, we find that the efficiency of DA of e_{pre}^- to XdUs is in the order of IdU ≫ BrdU > CldU ≫ FdU (essentially no DA occurs for FdU). These results can be well related to our direct, real-time

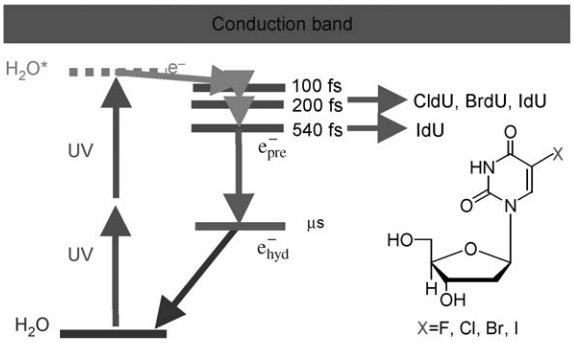

Figure 1. Schematic diagram for the production of the precursor electron (e_{pre}^-) and the hydrated electron (e_{hyd}^-) through two-UV-photon excitation in water and the reactions of e_{pre}^- with halopyrimidines (XdUs), whose structures are also shown.

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observations of the reaction transition states, which show that the DA to IdU involves two e_{pre}^- states of approximately 170- and approximately 540-fs lifetimes, respectively, whereas only one e_{pre}^- state is involved in the DAs to CldU and BrdU and no e_{pre}^- states are available for DA to FdU. The results indicate that IdU should be explored as the most effective radiosensitizer among XdUs.

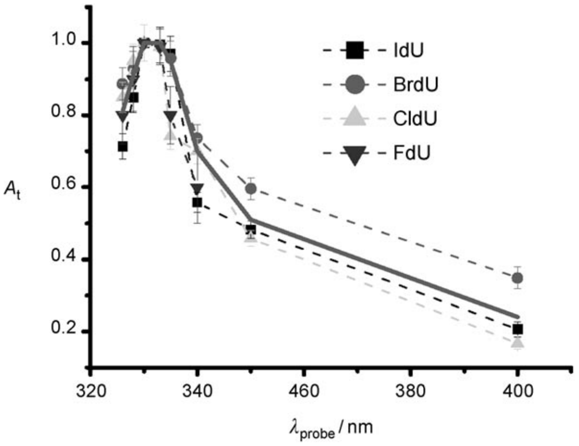

Figure 3. Transient absorption spectra of IdU, BrdU, CldU, and FdU detected at either the peak(s) or any delays after the peak(s) from Figure 2 versus probe wavelength in the 325–400-nm range with the pump wavelength fixed at 320 nm. The absorption intensities are normalized to those at the probe wavelength of 330 nm. The solid line in red is an aid for the eye. A_t = normalized transient absorbance.

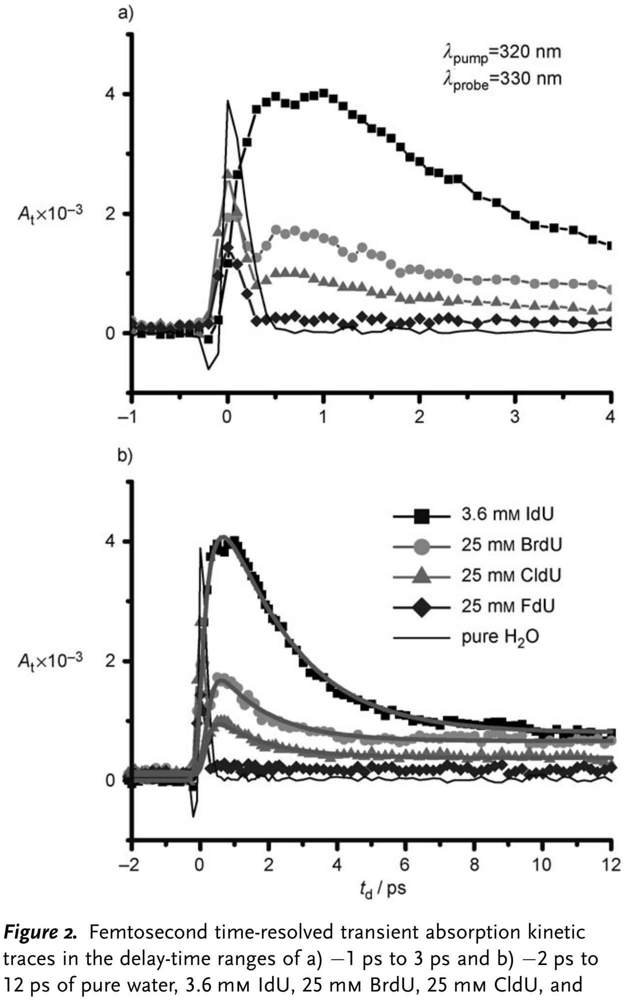

Figure 2. Femtosecond time-resolved transient absorption kinetic traces in the delay-time ranges of a) −1 ps to 3 ps and b) −2 ps to 12 ps of pure water, 3.6 mM IdU, 25 mM BrdU, 25 mM CldU, and 25 mM FdU with the pump and probe wavelengths indicated. The sharp peak at time zero is the coherence "spike". The solid lines in red are the best fits to the experimental data (see text). A_t = transient absorbance, t_d = probe–pump delay time.

In the described experiments, a pump wavelength greater than or equal to 320 nm was selected to avoid the direct absorption of the pump pulse by XdUs and to produce excess electrons by two-photon excitation of water.[23,24] The probe wavelengths around 330 nm were selected to search the transition states (XdU^{*-}) of the reactions of e_{pre}^- with XdUs.[23] Femtosecond transient-absorption kinetic traces of XdUs probed with $\lambda_{probe} = 330$ nm are shown in Figure 2 a and b together with the absorption for the pure water. The transient-absorption signal intensity detected at the peak(s) or any delays after the peak(s) in Figure 2 is shown as a function of probe wavelength with the same pump wavelength in Figure 3. Indeed, the absorption spectra of XdU^{*-} transition states exhibit a peak around 330 nm for all XdUs, which is consistent with our earlier observation for BrdU only[23] and with the observation by nanosecond radiolysis experiments.[17] The latter fact indicates that the ultrashort-lived (picosecond) dissociative vibrationally excited XdU^{*-} states have an absorption profile that is nearly identical to their relaxed, nondissociative XdU^- states. We have previously shown that the transient absorption signal for BrdU have a linear dependence on BrdU concentration in the range of 0.1–30 mM;[23] this linear dependence has now been reconfirmed for other XdUs, as shown in Figure 4 for IdU. Thus, higher XdU (X = Br, Cl, and F) concentrations were used to obtain the signal intensities that are comparable with that for IdU in Figure 2. The transient absorption trace for FdU exhibits no considerable peak in the first picosecond after the pump excitation, indicating that essentially no DA of any weakly bound e_{pre}^- to FdU could occur. Instead, only an extremely weak, flat (long-lived) signal was observed for FdU, which can be attributed to the nondissociative anion FdU^-.[23,25] In contrast, CldU shows a transient absorption kinetics similar to that for BrdU: it exhibits a single peak at the delay time of approximately 0.55 ps with one fast decay time of 1–2 ps superposed on a long-lived (nanosecond), slow decay tail; the transient absorption intensity for CldU is significantly weaker than that for BrdU. The fitted transient absorption kinetic traces, also shown in Figure 2, give a rise time, τ_1, of approximately 150 fs, a fast decay time of 1.5 ps, and a slow decay time in nanoseconds for both BrdU and CldU. As the absorptions of XdU^{*-} states are identical to their relaxed XdU^- states, the decay kinetics of the transient absorption can be solely attributed to the dissociation of XdU^{*-}. This is also confirmed by the above-described results with FdU (no DA leads to no decay in the signal). Thus, the rise time and picosecond decay and the nanosecond decay times of the transient absorption are due to the formation and the dissociation of $BrdU^{*-}$ ($CldU^{*-}$) and the lifetime of the nondissociative $BrdU^-$ ($CldU^-$), respectively.[23] These results

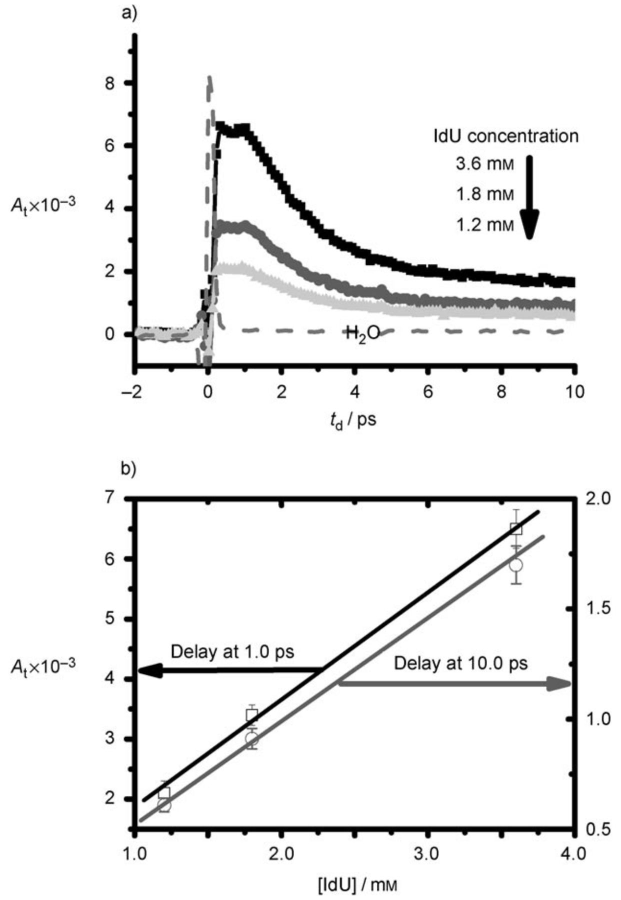

Figure 4. a) Femtosecond time-resolved transient absorption kinetic trace of IdU with various concentrations, probed at 330 nm; the dashed line is the kinetic trace for pure water; b) transient absorption intensities at 1.0 ps and 10.0 ps versus IdU concentration. A_t = transient absorbance, t_d = probe–pump delay time.

indicate that the same excited-state precursor contributes to the formation of both BrdU*⁻ and CldU*⁻. Most strikingly, the transient absorption signal for IdU is much stronger than for BrdU and CldU, even when the concentration of IdU is about one order of magnitude lower. For IdU, the transient absorption spectrum exhibits a second bump just visible at approximately 1.0 ps located just beside the first peak at approximately 0.55 ps. This indicates that there are two precursor (e_{pre}^-) states contributing to the formation of IdU*⁻. Assuming two consecutive e_{pre}^- states in water,[26–30] the best fit to the transient absorption kinetic trace of IdU, shown in Figure 2, gives two rise times, a fast decay time in picoseconds and a long-lived tail in nanoseconds. They are $\tau_1 = 170 \pm 40$ fs, $\tau_2 = 540 \pm 40$ fs, $\tau_3 = 1.80 \pm 0.20$ ps, and τ_4 fixed at 10 ns, respectively. The relative amplitude of the two rise times is $A(\tau_1){:}A(\tau_2) \approx 1{:}0.5$. Similar to the case for BrdU[23] and CldU, the τ_1 rise time and τ_3, and τ_4 decay times correspond to the lifetime of a precursor e_{pre}^-, the dissociative lifetime of IdU*⁻, and the lifetime of the long-lived molecular anion IdU⁻, respectively. Interestingly, the fact that IdU*⁻ has two rise times provides clear evidence of two e_{pre}^- states contributing to its formation. Thus, IdU*⁻ results from not only the first precursor e_{pre}^- of an approximate 170-fs lifetime, but a second precursor e_{pre}^- that has a lifetime of approximately 540 fs. Indeed, fs laser spectroscopic studies have reported several consecutive e_{pre}^- states with lifetimes of approximately 100 fs, approximately 200 fs, and 540–1000 fs, following two-photon excitation of H_2O by a UV (266–320 nm) pump pulse (Figure 1).[26–30] In particular, Long et al.[27] have observed a two-state model with lifetimes of 180 fs and 540 fs and proposed that the latter is the well-known "wet" electron that is a p-excited state of the hydrated electron, which is consistent with the theoretical prediction by Rossky and Schnitker.[31] Laenen et al.[29] have shown clear evidence for the existence of consecutive e_{pre}^- states with lifetimes of 110 fs, 200 fs, and 540 fs, which have absorption peaks at 2900 nm, 1600 nm, and approximately 900 nm, respectively, following twooton excitation of H_2O. Moreover, a number of previous studies[32–34] have obtained interesting results about the ET reactions of e_{pre}^- with conventional electron scavengers, mainly by studying the formation and decay kinetics of the hydrated electron and the e_{hyd}^- yield. However, real-time observation of the transition state of an e_{pre}^--scavenger reaction was only obtained recently.[23]

As mentioned above, gas-phase experiments have shown that halouracils (IU, BrU and ClU) and halouridines (BrdU) have very large cross sections for DA resonances to electron energies that are nearly 0 eV.[20,21] The excess energy, ΔE, for 0-eV electron-induced C–X (X = I, Br, Cl, and F) bond breakage in XUs in the gas phase is given by $\Delta E \approx E_A(X) - E_{BD} \equiv \Delta E^0$, where $E_A(X)$ is the electron affinity of the halogen atom X and E_{BD} is the C–X bond-dissociation energy. In the condensed phase, the excess energy of reaction is increased by the polarization energy (E_P) of the medium: $\Delta E = \Delta E^0 + E_P$. With the gaseous ΔE^0 values (−0.9 to −1, 0.01, 0.24, and 0.42 eV for FU, ClU, BrU, and IU, respectively)[20] and $E_p \approx 1.0$ eV for water,[35] 0-eV electron-induced C–X bond breakage for CldU, BrdU, and IdU in water are exothermic by 1.01, 1.24, and 1.42 eV, respectively, whereas the C–F bond breakage for FdU in water is nearly thermoneutral ($\Delta E \approx 0$ eV). This means that the lowest nergy limits of the DA resonance states CldU*⁻, BrdU*⁻, and IdU*⁻ lie at −1.01, −1.24, and −1.42 eV, respectively, whereas the lowest FdU*⁻ state lies around the vacuum level (0 eV). DA resonances are therefore expected to occur for CldU and BrdU with the first weakly bound e_{pre}^- state that has an energy slightly higher than the p-like excited state of the hydrated electron. In bulk water, the absorption of e_{hyd}^- peaks at 1.7 eV (≈720 nm) is believed to be a transition from the s-like ground state at approximately −3.2 eV to a p-like excited state at approximately −1.5 eV below the vacuum level.[36,37] Thus, a second DA channel can additionally occur for IdU with the p-like excited state, which is very close to the lowest nergy IdU*⁻ state at approximately −1.42 eV when energy-level broadening is taken into account. In contrast, no precursor states are available for DA to FdU. Thus, UET from excited-state e_{pre}^- to XdUs is expected to occur efficiently, leading to DA to IdU, BrdU, and CldU with the expected efficiency: IdU ≫ BrdU > CldU, but no DA to FdU. This prediction is in good agreement with the observed results.

We have obtained real-time observations of the reactions of XdUs with e_{pre}^- generated by ionizing/UV radiation in water. This study has multiplefold significance. First, the present results prove that it is the e_{pre}^- states, rather than e_{hyd}^-, that are responsible for DAs to XdUs. This reaction leads to the formation of the reactive radical, which is a key step in the mechanism of action of these prodrugs in radiotherapy of cancer. Our results have challenged a long-accepted mechanism that long-lived e_{hyd}^- is responsible for the radical formation from halogenated molecules,[17,18,38] which predicts a formation time of XdU^{*-} in the time scale beyond nanoseconds and corresponds to the e_{hyd}^- lifetime. Second, we have clearly revealed that the DA reaction efficiency is $IdU \gg BrdU > CldU$, whereas no DA of e_{pre}^- to FdU occurs. This is due to the availability of two e_{pre}^- states for DA to IdU, of one e_{pre}^- state for DAs to BrdU and CldU, and no e_{pre}^- for DA to FdU. Thus, IdU should be explored as the most effective radiosensitizer. As a more general conclusion, our results demonstrate that despite their ultrashort lifetimes in subpicoseconds, nonequilibrium e_{pre}^- can play a crucial role in many ET reactions occurring in aqueous environments, especially for chlorine-, bromine-, and iodine-containing molecules. Thus, this study can have clear significance for understanding of the role of water in electron-initiated reactions and radical chemistry in many chemical, biological and environmental systems.

Experimental Section

The standard methodology for pump–probe femtosecond transient absorption measurements was described previously.[23,24] 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 NIR. The polarization of pump and probe pulses was set at the magic angle (54.7°) to avoid contribution from polarization anisotropy owing to orientation motions of molecules. To avoid the direct absorption of the pump pulse by XdUs, a pump wavelength greater than or equal to 320 nm was used in these experiments and excess electrons were produced by two-photon excitation of water.[23,24,26–30] The probe wavelengths of around 330 nm were selected to search the transition states (XdU^{*-}) of the reactions of e_{pre}^- with XdUs.[23] A small pump pulse energy (≤ 100 nJ) was used to make the solvated electron signal negligible when detected at (probe) wavelengths around 330 nm and to avoid any nonlinear effects. A coherence "spike" unavoidably appears at delay time zero, which is clearly seen in the kinetic trace for the pure water. This "unwanted" spike, however, offers not only a visible and reliable reference in situ for the pump–probe delay time zero, but also the instrument-response function. In our experiments, an instrument-response function of 300 fs was directly given by the full width at half maximum (FWHM) of the coherence spike. Transient absorption kinetics of XdU^{*-} were corrected by the subtraction of the kinetic trace for the pure water; this also significantly reduces or completely removes the coherence spike.

The best fits to the experimental data were obtained by using a least-squares fitting program. The time-dependent transient absorption signal was given by a number of exponential functions. These exponential terms were convoluted with the instrument-response function represented by a Gaussian function. In our fits, the time zero and the FWHM ($= 300$ fs) of the pump–probe cross-correlation function are not adjustable fitting parameters, but are fixed at the values determined by the coherence spike appearing in the pure-water sample. This procedure should give rise to more-reliable fitted results.

All measurements were conducted at room temperature. The sample was held in a 5-mm cell with a stirring bar to avoid photoproduct accumulation. Ultrapure water with a resistivity of greater than $18\,\mathrm{M\Omega\,cm^{-1}}$ was obtained from an ultrapure water system. XdUs from Sigma-Aldrich were used as supplied and their concentrations were calibrated by taking static absorption spectra from a UV/Vis/NIR spectrophotometer. Except for IdU, which has a saturation concentration of approximately 4.0 mM at 300 K, BrdU, CldU, and FdU have excellent solubility in water.

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[1] R. A. Marcus, *Angew. Chem.* **1993**, *105*, 1161; *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 1111.
[2] C. Wan, T. Fiebig, S. O. Kelley, C. R. Treadway, J. K. Barton, A. H. Zewail, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 6014.
[3] H. C. Becker, A. Broo, B. Norden, *J. Phys. Chem. A* **1997**, *101*, 8853.
[4] S. J. Dixon-Warren, E. T. Jensen, J. C. Polanyi, *Phys. Rev. Lett.* **1991**, *67*, 2395.
[5] H. Hotop, M.-W. Ruf, M. Allan, I. I. Fabrikant, *Adv. At. Mol. Phys.* **2003**, *49*, 85.
[6] Q.-B. Lu, L. Sanche, *Phys. Rev. Lett.* **2001**, *87*, 078501; Q.-B. Lu, L. Sanche, *Phys. Rev. B* **2001**, *63*, 153403; Q.-B. Lu, L. Sanche, *J. Chem. Phys.* **2004**, *120*, 2434.
[7] J. C. Polanyi, A. H. Zewail, *Acc. Chem. Res.* **1995**, *28*, 119.
[8] A. H. Zewail, *Angew. Chem.* **2000**, *112*, 2688; *Angew. Chem. Int. Ed.* **2000**, *39*, 2586.
[9] M. A. O'Neill, H. C. Becker, C. Z. Wan, J. K. Barton, A. H. Zewail, *Angew. Chem.* **2003**, *115*, 6076; *Angew. Chem. Int. Ed.* **2003**, *42*, 5896.
[10] T. Polivka, V. Sundstrom, *Chem. Rev.* **2004**, *104*, 2021.
[11] W. C. Dewey, B. A. Sedita, R. M. Humphrey, *Science* **1966**, *152*, 519.
[12] F. Hutchinson, *Q. Rev. Biophys.* **1973**, *6*, 201.
[13] H. Sugiyama, Y. Tsutsumi, I. Saito, *J. Am. Chem. Soc.* **1990**, *112*, 6720.
[14] T. Chen, G. P. Cook, A. T. Koppisch, M. M. Greenberg, *J. Am. Chem. Soc.* **2000**, *122*, 3861.
[15] E. E. Blatter, Y. W. Ebright, R. H. Ebright, *Nature* **1992**, *359*, 650; M. C. Willis, B. J. Hicke, O. C. Uhlenbeck, T. R. Cech, T. H. Koch, *Science* **1993**, *262*, 1255.
[16] M. D. Prados et al., *Int. J. Radiat. Oncol. Biol. Phys.* **1999**, *45*, 1109.
[17] E. Rivera, R. H. Schuler, *J. Phys. Chem.* **1983**, *87*, 3966.
[18] S. Cecchini, S. Girouard, M. A. Huels, L. Sanche, D. J. Hunting, *Biochemistry* **2005**, *44*, 1932.
[19] E. Illenberger, *Chem. Rev.* **1992**, *92*, 1589.
[20] H. Abdoul-Carime, M. A. Huels, E. Illenberger, L. Sanche, *J. Am. Chem. Soc.* **2001**, *123*, 5354.
[21] H. Abdoul-Carime et al., *Chem. Phys. Lett.* **2004**, *393*, 442; S. Denifl et al., *Eur. Phys. J. D* **2005**, *35*, 391.
[22] X. Li, L. Sanche, M. D. Sevilla, *J. Phys. Chem. A* **2002**, *106*, 11248; X. Li, M. D. Sevilla, L. Sanche, *J. Am. Chem. Soc.* **2003**, *125*, 8916.
[23] C.-R. Wang, A. Hu, Q.-B. Lu, *J. Chem. Phys.* **2006**, *124*, 241102.
[24] Q.-B. Lu, J. S. Baskin, A. H. Zewail, *J. Phys. Chem. B* **2004**, *108*, 10509.
[25] I. I. Fabrikant, *Eur. Phys. J. D* **2005**, *35*, 193.

[26] A. Migus, Y. Gauduel, J. L. Martin, A. Antonetti, *Phys. Rev. Lett.* **1987**, *58*, 1559.
[27] F. H. Long, H. Lu, K. B. Eisenthal, *Phys. Rev. Lett.* **1990**, *64*, 1469.
[28] C. Silva, P. K. Walhout, K. Yokoyama, P. F. Barbara, *Phys. Rev. Lett.* **1998**, *80*, 1086.
[29] R. Laenen, T. Roth, A. Laubereau, *Phys. Rev. Lett.* **2000**, *85*, 50.
[30] P. Kambhampati, D. H. Son, T. K. Kee, P. F. Barbara, *J. Phys. Chem. A* **2002**, *106*, 2374.
[31] P. J. Rossky, J. J. Schnitker, *Phys. Chem.* **1988**, *92*, 4277.
[32] A. C. Chernovitz, C. D. Jonah, *J. Phys. Chem.* **1988**, *92*, 5946.
[33] S. M. Pimblott and J. A. Laverne, *J. Phys. Chem. A* **1998**, *102*, 2967.
[34] T. K. Kee, D. H. Son, P. Kambhampati, P. F. Barbara, *J. Phys. Chem. A* **2001**, *105*, 8434.
[35] Q.-B. Lu, A. D. Bass, L. Sanche, *Phys. Rev. Lett.* **2002**, *88*, 147601.
[36] J. V. Coe et al., *J. Chem. Phys.* **1990**, *92*, 3980.
[37] L. Turi, D. Borgis, *J. Chem. Phys.* **2002**, *117*, 6186.
[38] B. C. Garrett et al., *Chem. Rev.* **2005**, *105*, 355.