137571-28-9; $3^{\bullet 3-}$, 137693-20-0; **5**, 137571-19-8; $5^{\bullet -}$, 137624-72-7; 5^{2-}, 137571-26-7; $5^{\bullet 3-}$, 137624-73-8; 5^{4-}, 137571-27-8; $5^{4-}/4Li^{+}$, 137571-18-7; **6**, 137571-20-1; $6^{\bullet -}$, 137624-74-9; **7a**, 137571-21-2; **7b**, 137571-22-3; **8**, 137571-23-4; $8^{\bullet -}$, 137624-75-0; **10**, 4284-01-9; **11**, 137594-02-6; 12^{2-}, 137571-29-0; TBABr, 1643-19-2; $TBAPF_6$, 3109-63-5; K, 7440-09-7; $CdCl_2$, 10108-64-2; $C_3H_6Br_2$, 109-64-8; I_2, 7553-56-2; MeOH, 67-56-1; diphenic dialdehyde, 1210-05-5; (3,5-di-*tert*-butylbenzyl)triphenylphosphonium bromide, 36393-44-9; lithium, 7439-93-2; anthracene, 120-12-7; stilbene, 588-59-0; lithium tetraphenylborate, 14485-20-2; sodium tetraphenylborate, 143-66-8; 15-crown-5, 33100-27-5.

Ionization of Purine Nucleosides and Nucleotides and Their Components by 193-nm Laser Photolysis in Aqueous Solution: Model Studies for Oxidative Damage of DNA[1]

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Abstract: The effect of 20-ns pulses of 193-nm laser light on aqueous solutions of purine bases, (2′-deoxy)nucleosides, and (2′-deoxy)nucleotides was investigated, and monophotonic ionization was observed. Although (deoxy)ribose and (deoxy)ribose phosphates are also ionized by 193-nm light, the photoionization of the (deoxy)nucleosides and -tides takes place predominantly (90%) at the purine moiety, due to the much higher extinction coefficients at 193 nm of the bases as compared to the (deoxy)ribose phosphates. The quantum yields of photoionization (ϕ_{PI}) of the purines are in the range 0.01 to 0.08, based on $\phi_{PI}(Cl^-)$ at 193 nm of 0.46. As shown by comparison with data obtained from pulse radiolysis, the ionized purines, i.e., the radical cations, deprotonate in neutral solution, yielding neutral radicals. The radical cation of 1-methylguanosine, produced by photoionization in oxygen-saturated aqueous solution, deprotonates with the rate constant $3.5 \times 10^5\ s^{-1}$. In the absence of oxygen, the hydrated electrons resulting from the photoionization react with the untransformed purine derivatives to yield the corresponding radical anions. As these are rapidly protonated by water (as concluded from pulse radiolysis), the photoionization in deaerated neutral solution results in two different neutral radicals: a *deprotonated* radical cation and a *protonated* radical anion.

Introduction

The chemical and biological effects of UV radiation on DNA or its constituents have been the subject of numerous studies.[2] However, these have mostly involved the use of light with wavelengths of ~250 nm. With the energies corresponding to these wavelengths, the *ionization* of purines (gas-phase ionization potentials ≳8 eV)[3] with only *one* photon is thermodynamically barely possible and is essentially not observed (with one 248-nm photon corresponding to 4.9 eV and the solvation energies of the ions formed being ≲3.5 eV,[4] ionization would at best be thermoneutral). The reported[2,5-9] ionizations of nucleic acid bases on interaction with ~250-nm light are therefore due to multiphotonic, usually biphotonic, processes, the yields of which depend, by definition, on the intensity of the exciting light.

However, with 193-nm light as emitted by argon fluoride excimer lasers, *mono*photonic ionization of purines (and pyrimidines) should be possible since ≲9.9 eV is available (the 193-nm quantum energy contributes 6.4, and there is ≲3.5 eV from the hydration of the ions produced). An advantage of 193-nm light compared to that of shorter wavelengths is that it is above the onset of light absorption by H_2O (~180 nm). This means that complications due to the formation of $H^\bullet$ and $OH^\bullet$ radicals are avoided.

UV light of wavelength 193 nm has already been used to excite DNA[10-12] or its bases.[8] With the latter, evidence for the occurrence of ionization was in fact obtained,[8] but the resulting radicals were not identified nor were any data given to judge the importance of ionization relative to other, *non*ionic photochemical processes. In the studies on the effect of ~190-nm light on DNA,[10-12] the interesting observation was made[10,11] that the type of damage produced is different from that observed[13] with low-intensity ~250-nm light but similar to that[14] with ionizing radiation. If

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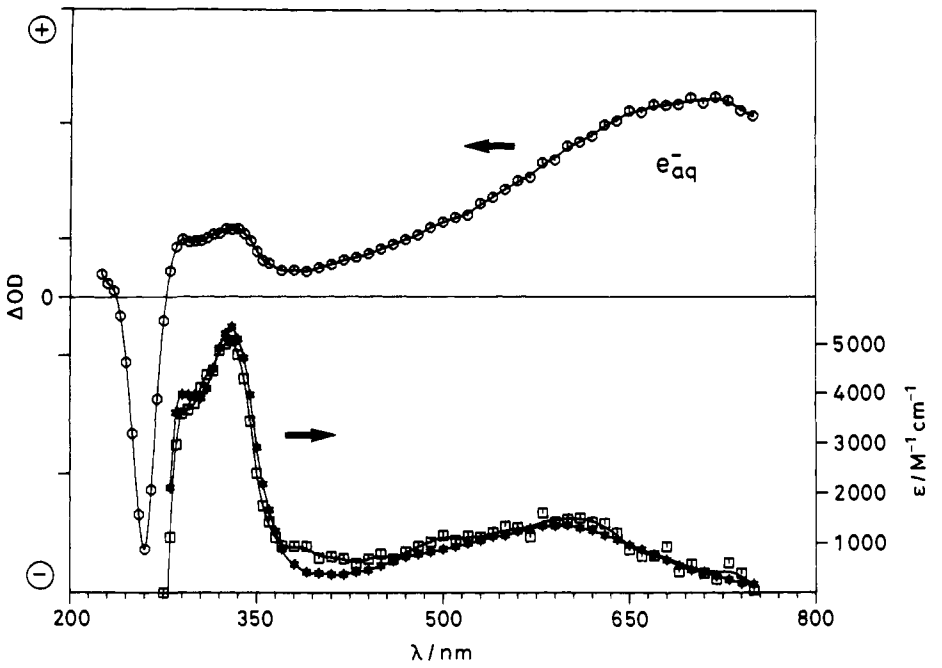

Figure 1. Absorption spectra measured on 193-nm photolysis of a neutral aqueous solution of adenosine 5′-phosphate (0.2 mM) deaerated, 0.1 μs after the pulse (circles) and saturated with oxygen 0.3 μs after the pulse and corrected for the absorption of $O_2^{\bullet-}$ (lower part, squares). The ε values were obtained by comparison with the OD values measured at 650 nm, assuming φ(adenosine 5′-phosphate radical) = φ(e^-_{aq}) and taking ε(e^-_{aq}) to be 15 900 M^{-1} cm^{-1} (from ref 18). Absorption spectrum recorded on reaction of $SO_4^{\bullet-}$ produced by pulse radiolysis with adenosine 5′-phosphate (0.2 mM) in deaerated neutral aqueous solution containing 6 mM $S_2O_8^{2-}$ and 43 mM *tert*-butyl alcohol (asterisks). To obtain the ε values, it was assumed that *G*(adenosine 5′-phosphate radical) = *G*($SO_4^{\bullet-}$) = 3.1.

this is the case, ~190-nm photolysis of the nucleic acids and their base components[15] is possibly an attractive way of simulating the direct effect of ionizing radiation. For this reason it was considered worthwhile to study this reaction in detail, using the bases and their nucleosides and nucleotides as models, and to compare the photochemically induced short-lived species with radicals generated by established techniques such as pulse radiolysis. The results are thought to contribute to a better understanding of the mechanism of DNA strand break formation and of other damages induced not only by 193-nm irradiation[10,11] and ionizing radiation[14] but also by "chemical" oxidizing agents, such as radicals produced in normal or pathological metabolic processes.

Experimental Section

The nucleic acid bases, (deoxy)nucleosides, (deoxy)nucleotides, (deoxy)ribose (phosphate), and inorganic phosphate (from Sigma, Fluka, Aldrich, or Merck) were of the highest purity available and used as received. 1,3,5,6-Tetramethyluracil was prepared by methylation of 5,6-dimethyluracil. The water was purified with a Millipore-Milli-Q system. The optical density of the solutions at 193 nm was ~1/cm, which typically corresponds to ~0.05 mM bases, nucleosides, or nucleotides.[15,16] The solutions were deaerated with argon or saturated with oxygen and flowed through the 2 mm (in the direction of the laser light) by 4 mm (in the direction of the analyzing light) Suprasil quartz cell with flow rates of ~3 mL/min, using pulse rates of 0.4 Hz. Unless otherwise indicated, the pH was 7 ± 0.5. The experiments were performed at room temperature (20 ± 1 °C).

Quantum yield measurements of photoionization are based on e^-_{aq} yields from argon-saturated aqueous solutions of NaCl (Merck) of optical density at 193 nm 1.0/cm ([NaCl] = 4 mM). The quantum yield for the production of hydrated electrons from Cl^- is assumed to be the same as that with 185-nm light, reported as 0.46.[17] The yield of e^-_{aq} was determined by measuring its absorption at 650 nm, taking ε(650 nm) = 15 900 M^{-1} cm^{-1}.[18] On photolysis of oxygen-free water, no detectable formation of e^-_{aq} was observed.

The experiments were carried out with an argon fluoride excimer laser (Lambda Physik EMG103MSC) that delivered (unfocused) ~20-ns pulses with energy of 3–45 mJ (measured at the position of the cell using a Gentec ED-200 power meter). For optical detection, a beam of analyzing light from a pulsed xenon lamp, perpendicular to the laser beam, was used. The optical signals were digitized with Tektronix 7612 and 7912 transient recorders interfaced with a DEC LSI 11/73$^+$ computer, which also controlled the various other functions of the experimental apparatus and preanalyzed the data. Final data analysis was done with a Microvax II connected to the LSI by Ethernet.

In the HPLC experiments, the mobile phase was an aqueous solution of pH 4 containing 5 mM KH_2PO_4, 20 mM $NaClO_4$, and 4% methanol, which flowed through the column (Nucleosil-5-C_{18} 4.6 × 125 mm) at a flow rate of 0.8 mL/min. UV (254 nm) or electrochemical detection (+0.7 V vs Ag/AgCl) with resorcinol (2.5 μM) as the internal standard was used.[19] At this voltage, 8-hydroxyguanosine and resorcinol have responses (peak area) per unit concentration in the ratio 1:1.2. Their retention times were 16 and 11 min, respectively.

Results and Discussion

On 193-nm pulse photolysis of deaerated aqueous solutions of purine bases, their (deoxy)nucleosides, or their (deoxy)nucleotides, relatively strong optical density (OD) changes were seen. The absorption spectra recorded at short times (50–100 ns) after the 20-ns pulses are characterized by a strong, broad band with a maximum at ~700 nm, by bands in the range 300–350 nm, and by negative signals at ~250 nm (see Figures 1 and 3). The absorption at ~700 nm is characteristic of the hydrated electron (e^-_{aq}). In agreement with this assignment, the rate of decay of this band was strongly increased by oxygen, N_2O, or the purine derivatives (see below). The negative signals at ~250 nm show that the purine bases and derivatives, which absorb in this wavelength

(15) The UV spectra of purines (and pyrimidines) are characterized by the "classical" bands at ~260 nm (ε = (1 – 1.5) × 10^4 M^{-1} cm^{-1}) and stronger ones (ε up to 3 × 10^4 M^{-1} cm^{-1}) at ~200 nm. For a description and interpretation of the spectra, see: (a) Mason, S. F. *J. Chem. Soc.* **1954**, 2071. (b) Voet, D.; Gratzer, W. B.; Cox, R. A.; Doty, P. *Biopolymers* **1963**, *1*, 193. (c) Drobnik, J.; Augenstein, L. *Photochem. Photobiol.* **1966**, *5*, 13. (d) *Ibid.*, 83. (e) Kleinwächter, V.; Drobnik, J.; Augenstein, L. *Ibid.* **1967**, *6*, 133. (f) Drobnik, J.; Kleinwächter, V.; Augenstein, L. *Ibid.*, 147. (g) Clark, L. B.; Tinoco, I. *J. Am. Chem. Soc.* **1965**, *87*, 11. (h) Ito, A.; Taniguchi, T.; Ito, T. *Photochem. Photobiol.* **1986**, *44*, 273. Ito, A.; Ito, T. *Ibid.*, 355. (i) Callis, P. R. *Ibid.*, 315.

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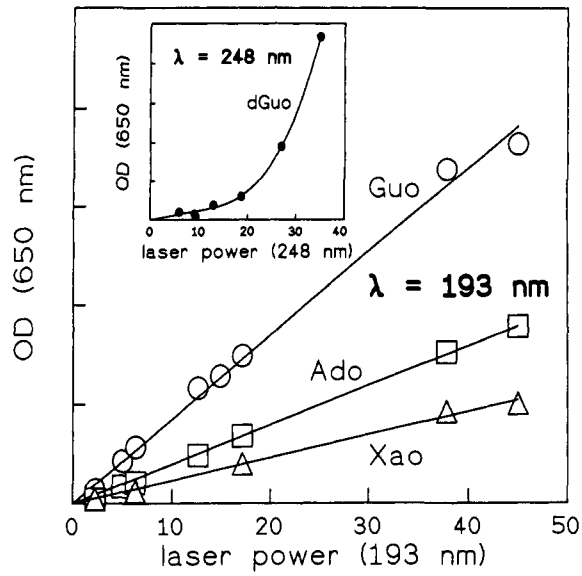

Figure 2. Dependence on laser power (mJ/pulse) of the OD at 650 nm measured immediately after the pulse on 193-nm photolysis of deaerated aqueous solutions of guanosine (Guo, circles, 0.04 mM), adenosine (Ado, squares, 0.06 mM), and xanthosine (Xao, triangles, 0.05 mM). In the inset is shown the corresponding dependence for *248-nm* photolysis of a neutral deaerated aqueous solution of 2′-deoxyguanosine (0.06 mM).

range, are depleted by the photolysis. The transients absorbing at 300–350 nm decay in the millisecond time scale and are not affected by oxygen. They are identified as the radicals resulting from the radical cations (by deprotonation, eq 1b, see below).

The formation of e^-_{aq} is direct proof for ionization of the purine compounds (≡P, see eq 1a). Since the spectral changes are completed within the laser pulse, the excited state involved in the photoionization must have a lifetime shorter than 20 ns.

$$P \xrightarrow{h\nu\ 193\ nm} P^* \xrightarrow{<20\ ns} P^{\bullet+} + e^-_{aq} \qquad (1a)$$

$$P^{\bullet+} \rightarrow P(-H)^{\bullet} + H^+ \qquad (1b)$$

With all of the purines studied, the amount of e^-_{aq} formed by photoionization, expressed by the optical density at 650 nm measured immediately after the pulse, increased linearly with the light intensity (see Figure 2). The ionization is therefore concluded to be a monophotonic process which, as pointed out in the Introduction, is thermodynamically feasible. In contrast to this, photoionization of the purines with 248-nm laser light was found to require more than one photon, i.e., to be not monophotonic (see inset of Figure 2).

From the slopes of the plots of OD at 650 nm (due to e^-_{aq}) versus laser power at 193 nm, the quantum yields of photoionization (ϕ_{PI}) were obtained, using NaCl solutions for actinometry. The values obtained are listed in Table I. The quantum yields are in all cases lower than 0.08. These low values indicate that processes other than ionization are occurring in the decay of the excited state.

The hydrated electrons formed by photoionization disappear in a deaerated solution with first-order kinetics, as shown by the exponential decay of the optical density at 700 nm. With the same rate, a further depletion of the substrates (evident from the decay of the OD at 250 nm) was observed, which resulted in the formation of new transients (buildup of OD at 300–320 nm), as shown in the insets of Figure 3 for the case of guanosine. The rates of these OD changes were found to increase linearly with the concentration of the purine derivative, and from these relations the bimolecular rate constants for reaction of e^-_{aq} with the substrates (eq 2a) were obtained. They are in the range 5×10^9 to 1×10^{10} M^{-1} s^{-1} for the nucleosides and $(2–4) \times 10^9$ M^{-1} s^{-1} for the nucleotides, and in all cases they are equal to those determined by other techniques, such as pulse radiolysis.[18,20]

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Table I. Quantum Yields of Photoionization (ϕ_{PI}) by 193-nm Light in Neutral Aqueous Solution at 20 ± 1 °C

compound	ε(193 nm), M^{-1} cm^{-1} [a]	IP, eV	ϕ_{PI} [c,d]
guanine	20 200[e]	7.77[b]	0.057
guanosine	25 400[e]		0.073
2′-deoxyguanosine	25 900[e]		0.073
2′-deoxyguanosine 5′-phosphate	19 300[e]		0.070
1-methylguanosine	24 700		0.064
adenine	15 100[e]	8.26[b]	0.032
adenosine	19 300[e]		0.034
2′-deoxyadenosine	18 600[e]		0.024
adenosine 5′-phosphate	19 700		0.040
hypoxanthine	17 600	8.44[b]	0.015
inosine	17 600		0.013
2′-deoxyinosine	19 600		0.010
xanthine (pH 5)	17 800	8.55[b]	0.048
xanthosine (pH 5)	17 000		0.020
D-ribose	80	9.7[f,g]	0.077
2′-deoxy-D-ribose	100	9.7[f,g]	0.056
D-ribose 5-phosphate (pH 5) (*mono*anion)	120		0.24
D-ribose 5-phosphate (pH 9) (*di*anion)	200		0.47
$H_2PO_4^-$ (pH 5)	<100	~5.1[g]	0.33
HPO_4^{2-} (pH 9)	150		0.52
2′-deoxycytidine	19 500[e]	8.68[b,h]	0.017
uridine	6 750	9.32[b,h]	0.070[j]
2′-deoxyuridine	6 900		0.051[j]
thymidine	5 700[e]	8.87[b,h]	0.055[j]
1,3,5,6-tetramethyluracil	6 800	8.37[i]	0.04

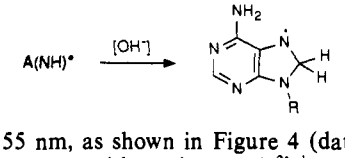
[a] Measured (error ±10%) in neutral aqueous solution unless indicated otherwise. Under these conditions, the molecules exist in their neutral forms except for the phosphates. [b] From ref 3. [c] Estimated error ±20%. [d] Based on ϕ_{PI}(NaCl) at 193 nm = 0.46. [e] From ref 15b. [f] This value was measured for tetrahydrofuran and cyclopentanol and is assumed to be the same for D-ribose. [g] From Tasaki, K.; Yang, X.; Urano, S.; Fetzer, S.; LeBreton, P. R. *J. Am. Chem. Soc.* **1990**, *112*, 538. [h] Refers to the corresponding base. [i] Measured using PES by Dr. B. Solouki, Universität Frankfurt. [j] Biphotonic photoionization; quantum yield for pulse energy of 45 mJ.

By use of pulse radiolysis with conductance detection, the radical anions of adenosine[21] and of guanosine[22] have been found to be rapidly protonated by water at a heteroatom to give neutral radicals, symbolized by PH•, as shown in eq 2b.

$$P + e^-_{aq} \xrightarrow{a} P^{\bullet-} \xrightarrow[+H_2O]{b} PH^{\bullet} + OH^- \qquad (2)$$

These neutral radicals PH• have absorption spectra with λ_{max} at 300–320 nm, and their formation explains the second component of the buildup of optical density observed at these wavelengths (as seen in inset b, Figure 3): the fast and concentration-*in*dependent component after the pulse is due to formation[20] of the (deprotonated) radical cations (see eq 1b).

In the case of adenosine, further evidence for the reaction of e^-_{aq} with the parent was obtained by photolysis in *basic* solution. In basic medium, the N-protonated electron adduct (A(NH•)) transforms in an OH^- catalyzed reaction[21] into the C8-protonated isomer[21c] (eq 3). This radical has a characteristic and strong



(3)

absorption at 355 nm, as shown in Figure 4 (data from pulse *radiolysis*), in agreement with previous work.[21a,b] When adenosine was photolyzed in a deaerated solution of pH 10.5, a buildup of optical density at 355 nm was observed, the rate of which was the same as that on reaction of adenosine with e^-_{aq} produced by

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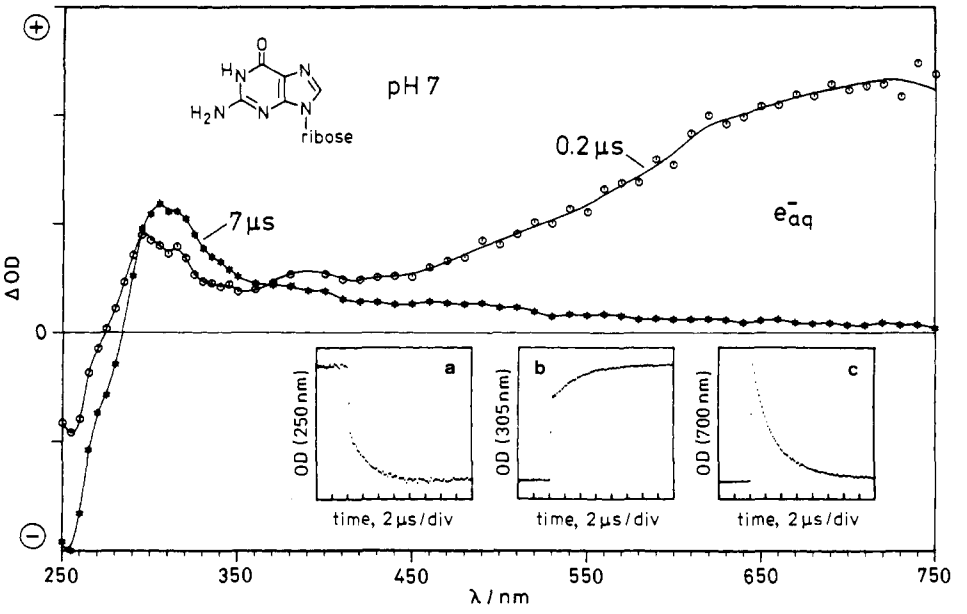

Figure 3. Absorption spectra recorded on 193-nm laser photolysis of a deaerated neutral aqueous solution of guanosine (0.05 mM), 0.2 (circles) and 7 μs (asterisks) after the pulse; insets: time dependence of the optical density at (a) 250, (b) 305, and (c) 700 nm.

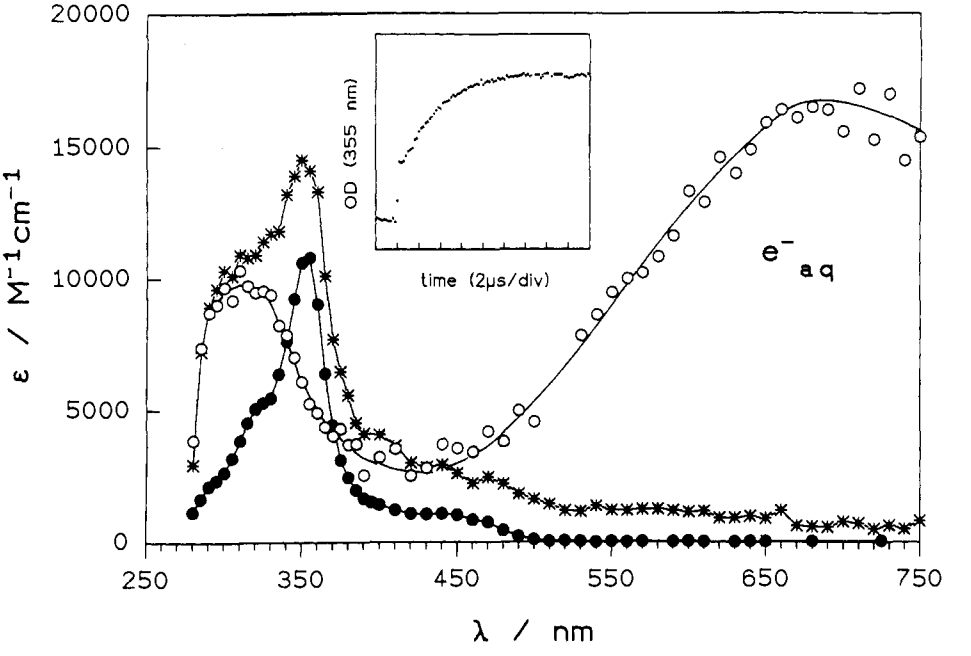

Figure 4. Absorption spectra recorded on 193-nm photolysis of a deaerated aqueous solution of adenosine (0.1 mM) at pH 10.5, 0.8 (open circles) and 14 μs (asterisks) after the pulse, and on pulse radiolysis of a deaerated aqueous solution of adenosine (0.5 mM) and 2-propanol (0.1 M) at pH 10.1, 90 μs after the pulse (full circles). Inset: Dependence on time of the optical density at 355 nm in the photochemical experiment.

pulse *radiolysis*. The absorption spectrum recorded after completion of this transformation (14 μs after the pulse) showed the characteristic absorption at 355 nm (see Figure 4), consistent with the formation (eq 3) of the C8-protonated electron adduct of adenosine, which is further support for the primary formation of e^-_{aq}, i.e., for photoionization. As is evident from Figure 4, the absorptions at 355 nm and the other wavelengths are higher in the case of the *photo*chemical experiment compared to the pulse *radiolysis* experiment. This is due to the fact that in the former not only the *reduced* but also the *oxidized* species (the deprotonated radical cation, see below) are formed.

In oxygen-saturated solution, the hydrated electrons are efficiently scavenged by O_2,[23] and their reaction with the substrate is thereby prevented. Under these conditions, the photoionization results in the $O_2^{\bullet-}$ radical, characterized by a weak absorption at ~250 nm,[24] and the neutral purine radical resulting from deprotonation of the radical cation.[25,26] The formation of the neutral radicals is deduced from the similarity of the absorption spectra recorded after complete scavenging of e^-_{aq} by O_2 (200 ns after the pulse) with those obtained on oxidation of the purines and derivatives by the sulfate ($SO_4^{\bullet-}$) or the dibromide ($Br_2^{\bullet-}$) radical. By comparison with conductance results, the organic radicals have been assigned to the deprotonated radical cations[20,25] (see Figures 1 and 5). However, from a close inspection of Figures 1 and 5, it is evident that the spectra obtained by the two methods are not *identical*. For example, in the case of adenosine 5′-phosphate (Figure 1), there is an absorption at ~400 nm on

(23) Other e^-_{aq} scavengers (e.g., aromatics) cannot easily be used due to their strong absorption at 193 nm. Even halogenated alkanes present difficulties as a result of their own photochemistry (S. Steenken, unpublished results).

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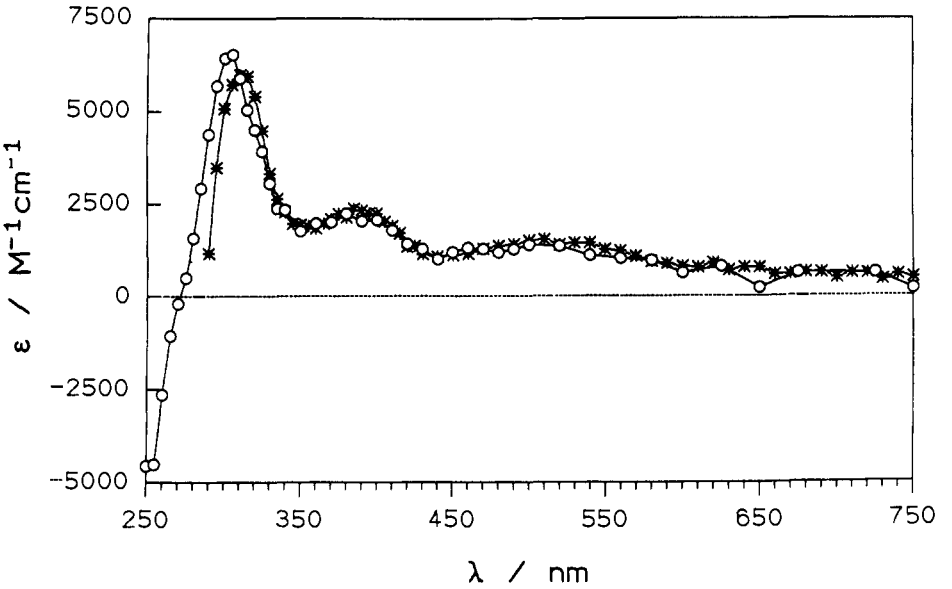

Figure 5. Absorption spectrum recorded on 193-nm laser photolysis of an oxygen-saturated neutral aqueous solution of 2′-deoxyguanosine (0.05 mM), 0.25 μs after the pulse (circles), corrected for the absorption of $O_2^{\bullet-}$. The ε calibration is as indicated in Figure 1. Comparison with that from the reaction of pulse radiolytically produced $SO_4^{\bullet-}$ with 2′-deoxyguanosine (0.1 mM) in deaerated neutral aqueous solution containing 10 mM $S_2O_8^{2-}$ and 50 mM *tert*-butyl alcohol, 10 μs after the pulse (asterisks), assuming G(2′-deoxyguanosine radical) = $G(SO_4^{\bullet-})$ = 3.1.

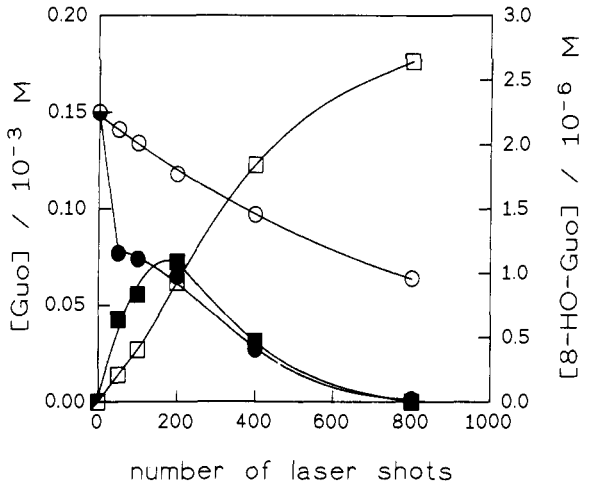

Figure 6. Results of HPLC analysis showing the formation of 8-hydroxyguanosine (squares) and the depletion of guanosine (circles) by photolysis of neutral aqueous solutions of 0.15 mM guanosine saturated with O_2 (full symbols) or deaerated with argon (open symbols) with 193-nm pulses (45 mJ/pulse).

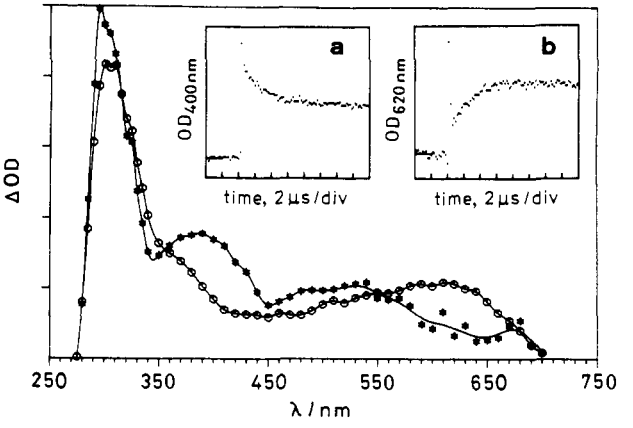

Figure 7. Absorption spectra obtained by 193-nm laser photolysis of an oxygen-saturated aqueous solution of 1-methylguanosine (0.05 mM, pH 7.1), 0.35 (asterisks) and 9 μs (circles) after the pulse; insets: time dependence of the optical density at (a) 400 and (b) 620 nm.

photolysis which is not observed on reaction with the chemical oxidant $SO_4^{\bullet-}$. With deoxyguanosine (Figure 5), there are slight differences in the absorptions at ≤330 nm.

Also, from the point of view of product analysis, there is evidence that ionization is not the only photochemically induced process. By means of product analysis by HPLC with electrochemical detection,[27] 8-hydroxyguanosine and 8-hydroxyadenosine were found in solutions of guanosine or adenosine, respectively, irradiated with 193-nm laser light. The yields of the 8-hydroxypurines were found to be much larger in the presence of oxygen than in its absence, as was the efficiency of depletion of the parent compounds (see Figure 6 for the case of guanosine). 8-Hydroxyguanosine is more sensitive to 193-nm-induced destruction than its precursor, so at higher absorbed doses its yield decreases (Figure 6). 8-Hydroxyguanosine is *not* formed, even in the presence of 0.1 mM $Fe(CN)_6^{3-}$, upon oxidation of guanosine by either $Br_2^{\bullet-}$ or Tl^{II}[28] (which gives the radical cation[25]), showing that 8-hydroxyguanosine is not derived from the radical cation. The formation of 8-hydroxy derivatives from the electronically excited purines is interesting since it shows that these compounds, which are found in γ-irradiated DNA,[29] are not necessarily indicative of the presence of the OH radical. They may be formed by the excited states resulting from the *direct effect* of the ionizing radiation.

To summarize, the overall similarity of the time-resolved spectra obtained by the two methods (193-nm photolysis in the presence of O_2 and reaction with $SO_4^{\bullet-}$; see Figures 1 and 5) leads to the conclusion that ionization is the *predominant* reaction induced by 193-nm light.

The radical cations of the purines are strong acids, with K_a values at least 5 orders of magnitude higher than those of the corresponding parent compounds.[20] Their deprotonation is therefore expected to be a fast process. For example, the radical cation of 1-methylguanosine has a pK_a of 4.7.[25] The value is such that the rate of deprotonation is expected to be measurable. In agreement with this expectation, on 193-nm laser photolysis of this compound in oxygen-saturated neutral aqueous solution, the

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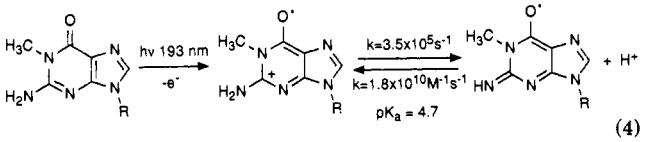
absorption spectrum measured at short times (0.35 μs) after the pulse can be identified as that of the radical cation on the basis of the similarity with that[25] observed on oxidation with $Br_2^{\bullet-}$ at pH 3. In a fast reaction, this spectrum transforms into a different one (see Figure 7), identified as the spectrum of the deprotonated radical cation as obtained[25] by oxidation by $Br_2^{\bullet-}$ at pH 7. The deprotonation reaction could be monitored by the buildup of optical density at 300 or at 620 nm (see insets in Figure 7), and its rate was determined as $k = 3.5 \times 10^5\ s^{-1}$. If this value is combined with the pK_a value of 4.7, the rate constant for protonation of the neutral radical is obtained as $1.8 \times 10^{10}\ M^{-1}\ s^{-1}$, a number that is reasonable for protonation of a heteroatom base (eq 4).[30]



(4)

When the radical cation of 1-methylguanosine was produced by photolysis at pH 4, which is *below* its pK_a, the spectrum observed was the same as the initial spectrum recorded at pH 7 but, in contrast to the situation at pH 7 (see above), no further changes occurred. This behavior is exactly as expected since net deprotonation does not take place below the pK_a.

From Table I it is obvious that among the purines, the base with the lowest ionization potential, guanine, has the highest quantum yield of photoionization. This result is in line with the well-known fact that guanine is the most easily ionizable of the DNA bases. However, there is no simple relation between (gas phase) ionization potentials and photoionization yields. Particularly, xanthine is photoionized with a higher yield than predicted on the basis of its ionization potential.

Ionization yields were also determined for some *pyrimidine* nucleosides. The data are shown in Table I. In the case of 2′-deoxycytidine, for ionization to occur, one 193-nm photon is sufficient whereas (2′-deoxy)uridine and thymidine require *two* photons. In order to see what role ionization plays as compared to other photochemical reactions in the family of pyrimidines, the model compound 1,3,5,6-tetramethyluracil (TMU) was investigated. The same spectrum was obtained as on reaction with the oxidant $SO_4^{\bullet-}$, which has been shown[31] by conductance to give the radical cation. The conclusion is thus that photolysis of TMU with 193-nm light leads (in a *mono*photonic process) predominantly to ionization, as in the case of the purines described above.

D-Ribose and D-2′-deoxyribose and their 5-phosphate derivatives, as well as inorganic phosphate, are also efficiently ionized on 193-nm laser photolysis (see Table I). It is interesting that the quantum yields for e_{aq}^- formation from ribose and deoxyribose are higher than that of the average of the free bases and the nucleosides. Even higher quantum yields are observed for inorganic phosphate, particularly if it is present as the *di*anion (as at pH 9). This is understandable since the oxidizability of the dianion is, of course, greater than that of the monoanion. Deoxyribose phosphate is almost as efficiently photoionized as is inorganic phosphate, and again the *di*anion loses the electron more readily than the *mono*anion. However, since the extinction coefficients of (deoxy)ribose and phosphate at 193 nm are about 2 orders of magnitude lower than those of the purine bases (see Table I and ref 15), the photoionization of the purine (deoxy)nucleosides is inferred to take place predominantly (90%) at the base moiety. Experimental evidence for this conclusion is the fact that the quantum yields for formation of e_{aq}^- and of the base radicals (measured at 300–330 nm) are the same (see, e.g., Figures 1 and 5). Since the radicals obtained by photoionization of (deoxy)ribose and ribose 5-phosphate show only very weak absorptions above 250 nm, such a result would not be possible if the (deoxy)ribose (phosphates) contributed significantly to the production of e_{aq}^-.

Summary and Conclusions

It has been demonstrated that 193-nm photolysis of purines and their nucleosides and nucleotides leads to their ionization in a monophotonic process. On the basis of the similarity of the absorption spectra in the range 250–350 nm of the transients observed immediately after the pulse with those[25,26] from chemically oxidized purines (with $Br_2^{\bullet-}$ or $SO_4^{\bullet-}$, produced by pulse radiolysis), it is concluded that ionization is an important chemical reaction induced by the 193-nm light. It is thus evident that 193-nm irradiation of purine nucleosides and nucleotides is a useful method to simulate the "direct" effect of ionizing radiation and to produce and study one-electron-oxidized bases as probably also formed in nonphotochemical or nonradiation chemical processes.

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Registry No. TMU, 59264-09-4; guanine, 73-40-5; guanosine, 118-00-3; 2′-deoxyguanosine, 961-07-9; 2′-deoxyguanosine 5′-phosphate, 902-04-5; 1-methylguanosine, 2140-65-0; adenine, 73-24-5; adenosine, 58-61-7; 2′-deoxyadenosine, 958-09-8; adenosine 5′-phosphate, 61-19-8; hypoxanthine, 68-94-0; inosine, 58-63-9; 2′-deoxyinosine, 890-38-0; xanthine, 69-89-6; xanthosine, 146-80-5; D-ribose, 50-69-1; 2′-deoxy-D-ribose, 533-67-5; D-ribose 5-phosphate, 4300-28-1; 2′-deoxycytidine, 951-77-9; uridine, 58-96-8; 2′-deoxyuridine, 951-78-0; thymidine, 50-89-5; oxygen, 7782-44-7.

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