Photoionization of Organic Phosphates by 193 nm Laser Light in Aqueous Solution: Rapid Intramolecular H-Transfer to the Primarily Formed Phosphate Radical. A Model for Ionization-Induced Chain-Breakage in DNA?

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Received December 29, 1997

Abstract: In aqueous solution, 193 nm (6.4 eV) photolysis of inorganic and organic phosphates such as ribose-5-phosphate leads to ionization with formation of the corresponding oxygen-centered phosphate radicals, $O_3PO^{\bullet}$. These (oxidizing) radicals function as traps with respect to hydrogens attached to α-, β-, or, possibly, γ-carbons, whereby in the case of the β-hydrogens a six-membered transition state for transfer of the hydrogen to the phosphate oxygen is possible, leading to high rate constants (up to $>5 \times 10^7$ s^{-1}) for H-transfer in these unimolecular reactions. In the case of (deoxy)ribosephosphates the six-membered transition state is possible for transfer of the hydrogen at C4 to the phosphate group at C5 as well as at C3. In DNA, the resulting C4′-radical will undergo a rapid β-elimination of the phosphate-ester group, this step representing the DNA chain break. The apparently easy H-transfer from a carbon to a phosphate radical, by which these radicals are "repaired", is why phosphate radicals are not observed in irradiated DNA. Insofar as hereby the C4′-radical is formed, the mechanism of DNA chain breakage is the same for the "direct" and the "indirect" effect.

Introduction

Despite more than a generation of radiation chemists' and biologists' efforts,[1] the understanding of the mechanistic details of the radiation or radical-induced chain breakage of DNA is still incomplete. This is particularly true with respect to the "direct effect",[2] which appears to be at least as important as the "indirect effect", which involves the mediation by the $^{\bullet}OH$ radical (produced by irradiation of the solvent, H_2O). Concerning the indirect effect, a mechanism has been proposed (the C4′-mechanism)[3] which explains well all experimental observations and which has recently been supported by experiments on DNA-model compounds (oligomers).[4] In contrast, the mechanism by which the direct effect operates, i.e., leads to the lethal chain breaks, is not at all well understood. On the basis of EPR-experiments,[1a,c,d,g] the radiation damage is localized mainly on the *bases*, the *reductive* equivalent on the pyrimidines (initially at cytosine (C), at higher temperatures at thymine (T))[1a,c,d,g,5] and the *oxidative* equivalent preferentially on guanine (G), whose (deprotonated) radical cation has been well characterized.[6] In many studies, very little evidence for sugar (deoxyribose) and no evidence for phosphate-centered radicals was obtained, although, on the basis of the relatively high mass-fraction (0.6) of deoxyribose and phosphate in DNA, these radicals are expected to be formed in appreciable yields by the unselective ionizing radiation. It has therefore been suggested that a "spin-transfer" takes place leading, finally, to a *base*-centered radical.[1,7] To study a (potential) spin-transfer from phosphate to the deoxyribose moiety and to obtain information about a chain breakage mechanism starting out from a directly ionized phosphate ester function, the present experiments were performed.

Results and Discussion

1. UV-Absorption Spectra of Phosphates. In Figure 1 are presented the absorption spectra in aqueous solution of the phosphate dianion, HPO_4^{2-}, and of the organic phosphates, *n*-butyl phosphate and triethyl phosphate, and in Figure 2 those of 2-hydroxyethyl phosphate (ethylene glycolphosphate) and ribose-5-phosphate.

With HPO_4^{2-} and the monoalkyl phosphates, the ϵ at 193 nm is between 100 and 180 M^{-1} cm^{-1}. The absorption band of the phosphate function, which extends into the region <187 nm, where measurements with our equipment were not possible, has been assigned to charge transfer to solvent (CTTS).[8]

(1) For reviews on DNA, see, e.g., (a) Bernhard, W. A. *Adv. Radiat. Biol.* **1981**, *9*, 199. (b) Steenken, S. *Chem. Rev.* **1989**, *89*, 503. (c) Close, D. M. *Magn. Res. Rev.* **1991**, *15*, 241. (d) Hüttermann, J. In *Radical Ionic Systems: Properties in Condensed Phases*; Lund, A.; Shiotani, M. Kluwer: Dordrecht 1991; p 435. (e) Steenken, S. *Free Rad. Res. Commun.* **1992**, *16*, 349. (f) O'Neill, P.; Fielden, E. M. *Adv. Radiat. Bio.* **1993**, *17*, 53. (g) Becker, D.; Sevilla, M. D. *Adv. Radiat. Bio.* **1993**, *17*, 121. (e) Steenken, S. *Biol. Chem.* **1997**, *378*, 1293.

(2) See, e.g.; Le Sech, C.; Frohlich, H.; Saint-Marc, C.; Charlier, M. *Radiat. Res.* **1996**, *145*, 632.

(3) Dizdaroglu, M.; von Sonntag, C.; Schulte-Frohlinde, D. *J. Am. Chem. Soc.* **1975**, *97*, 2277. For reviews, see: Schulte-Frohlinde, D. In *Mechanisms of DNA Damage and Repair*; Simic, M. G., Grossman, L., Upton, A. C., Eds.; Plenum: New York, 1980; p 19. Schulte-Frohlinde, D. In *Radiation Protection and Anticarcinogens*; Nygaard, O. F., Simic, M. G., Eds.; Academic: New York, 1983; p 53.

(4) For a review, see: Giese, B.; Beyrich-Graf, X.; Erdmann, P.; Petretta, M.; Schwitter, U. *Chem. Biol.* **1995**, *2*, 367.

(5) Barnes, J. P.; Bernhard, W. A. *J. Phys. Chem.* **1995**, *99*, 11248.

(6) (a) In aqueous solution: Candeias, L. P.; Steenken, S. *J. Am. Chem. Soc.* **1989**, *111*, 1094. Steenken, S.; Jovanovic, S. V. *J. Am. Chem. Soc.* **1997**, *119*, 617. (b) In the solid state: Hole, E. O.; Nelson, W. H.; Sagstuen, E.; Close, D. M. *Radiation Res.* **1992**, *129*, 119.

(7) In the case of thymidine-6-yl radical, the feasibility of the *reverse* reaction has recently been studied with the result that its rate is low (<2 s^{-1}): Barvian, M. R.; Barkley, R. M.; Greenberg, M. M. *J. Am. Chem. Soc.* **1995**, *117*, 4894.

(8) Halman, M. *Top. Phosph. Chem.* **1967**, *4*, 49.

S0002-7863(97)04393-X CCC: $15.00
Published on Web 04/11/1998

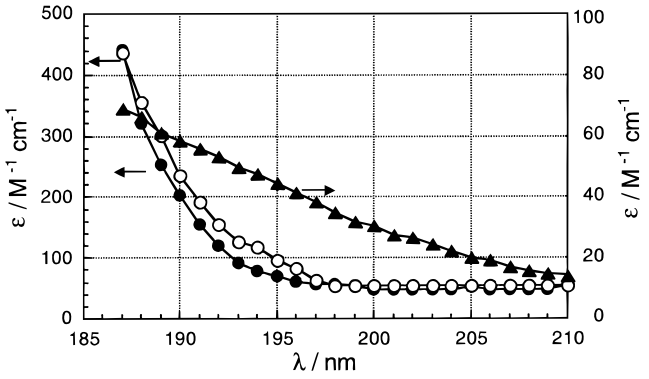


Figure 1. UV spectra of HPO_4^{2-} (filled circles), *n*-$BuOPO_3^{2-}$ (open circles) and $(EtO)_3PO$ (triangles) in aqueous solution pH 7−8.

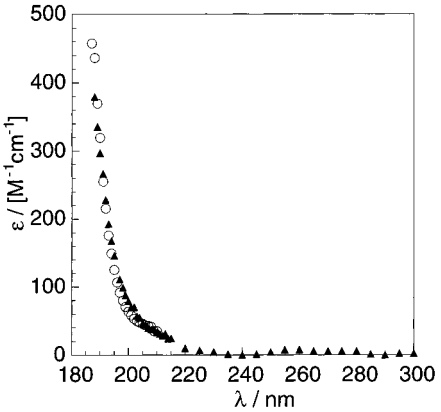


Figure 2. Absorption spectra of of $HOCH_2CH_2OPO_3^{2-}$ (circles) and of ribose-5-phosphate (triangles) in water, pH 8.

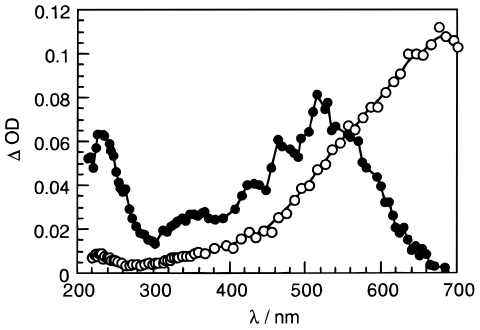


Figure 3. Absorption spectra recorded at 0.1 μs after 193 nm photolysis of 20 mM Na_2HPO_4 in deoxygenated (open circles) and at 0.8 μs in oxygenated (filled circles) aqueous solution. The latter spectrum has been magnified by the factor 5.

2. Laser Flash Photolysis (LFP) of Phosphates. Inorganic Phosphate and Simple Alkyl Phosphates. On photolysis of the phosphates with 20 ns pulses of 193 nm laser light from a ArF-excimer laser, the hydrated electron, e^-_{aq}, was formed, as evidenced by its characteristic[9] absorption spectrum with λ_{max} at ≈700 nm and its reactivity with e^-_{aq}-scavengers.[10] E.g., in Figure 3 is shown the spectrum observed on 193 nm photolysis of a 20 mM solution of HPO_4^{2-} in the absence (open circles) and presence of O_2 (filled circles). In the absence of O_2, the broad band extending from ca. 400 nm to ≥700 nm is that of e^-_{aq}, whereas the weaker band at ≈225 nm is due to the phosphate radical, $HPO_4^{\bullet-}$, whose main absorption band (at 515 nm with $\epsilon = 1550\ M^{-1}cm^{-1}$)[11] becomes visible (Figure 3, filled circles) on removal of e^-_{aq} by O_2, whereby $O_2^{\bullet-}$ is formed whose absorption ($\lambda_{max} = 241$ nm)[12] adds on top of that of $HPO_4^{\bullet-}$ (Figure 3, filled circles).

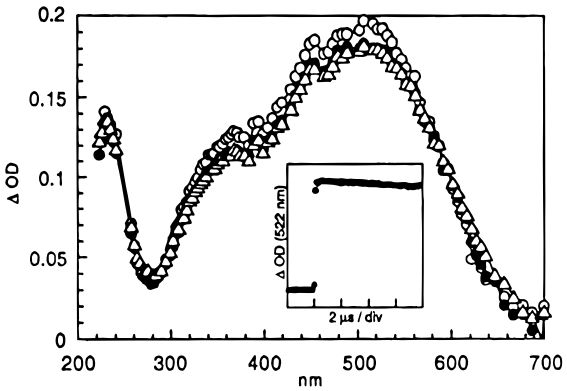
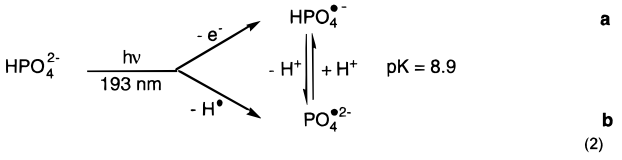


Figure 4. Spectra recorded at 125 ns (open circles), 0.8 μs (closed circles), and 8 μs (triangles) on 248 nm photolysis of an oxygenated 25 mM solution of $Li_4P_2O_8$ in water, pH 9.2.

The formation of $HPO_4^{\bullet-}$ was checked by producing it independently (see Figure 4), i.e., by 248 nm photolysis of the peroxide, $^-HO_3P-O-O-PO_3H^-$, reaction 1:

$$^-HO_3P-O-O-PO_3H^- \xrightarrow[248\ nm]{h\nu} 2HPO_4^{\bullet-}\ \Phi = 0.8\ ^{13} \quad (1)$$

From a comparison of Figure 3, filled circles, with Figure 4 it is apparent that the phosphate radical, $HPO_4^{\bullet-}$, is the product of the ionization of HPO_4^{2-}. From a quantitative inspection of Figure 3 it is also clear that photoionization cannot be the only photoreaction of HPO_4^{2-} induced by the 193 nm light. On the basis of the optical density (filled circles) at 515 nm, measured using an oxygenated (to scavenge e^-_{aq}) aqueous solution of HPO_4^{2-}, compared with the optical density at 700 nm (open circles), due to e^-_{aq}, and taking account of the extinction coefficients of $HPO_4^{\bullet-}$ at 515 and of e^-_{aq} at 720 nm (19000 $M^{-1}cm^{-1}$),[9] the photochemical yield of $HPO_4^{\bullet-}$ compared to that of e^-_{aq} turned out to be 224%. Therefore, there must be an additional source of $HPO_4^{\bullet-}$, other than ionization (eq 2a) of HPO_4^{2-}. A reasonable path to $HPO_4^{\bullet-}$ would be homolysis of the O−H bond, eq 2b:



The photon energy of 193 nm light (6.4 eV = 148 kcal/mol) is sufficient to break an O−H bond (110−120 kcal/mol). The formation of the phosphate radicals via 2a amounts to 45% and to 55% via 2b. The pK_a-value of $HPO_4^{\bullet-}$ has been determined[11] to be 8.9.

Similar experiments were performed with a series of simple *organic* phosphates. In Figure 5, a and b, are shown the spectra obtained on 193 nm photolysis of an aqueous solution of methyl

(9) Hug, G. E. *Nat. Stand. Ref. Data Ser. Nat. Bur. Stand.* **1981**, *69*, 6.
(10) Buxton, G. V.; Greenstock. C. L.; Helman, W. P. *J. Phys. Chem. Ref. Data* **1988**, *17*, 513.
(11) Maruthamuthu, P.; Neta, P. *J. Phys. Chem.* **1978**, *82*, 710.
(12) Czapski, G.; Dorfman, L. *J. Phys. Chem.* **1964**, *68*, 1169.
(13) Quantum yield for decomposition of the peroxide at pH 9.2, measured by comparison with that (0.9, cf. Faria, J. L.; Steenken, S. *J. Phys. Chem.* **1992**, *96*, 10869) for decomposition of $S_2O_8^{2-}$ and taking (from ref 11) $\epsilon(HPO_4^{\bullet-})_{515\ nm} = 1550\ M^{-1}\ cm^{-1}$. The value 0.8 was also obtained if the photoionization in water of I^- with 248 nm light ($\Phi = 0.29$; Jortner, J.; Levine, R.; Ottolenghi, M. Stein, G. *J. Phys. Chem.* **1961**, *65*, 1232, see also ref 14b) was used for actinometry.

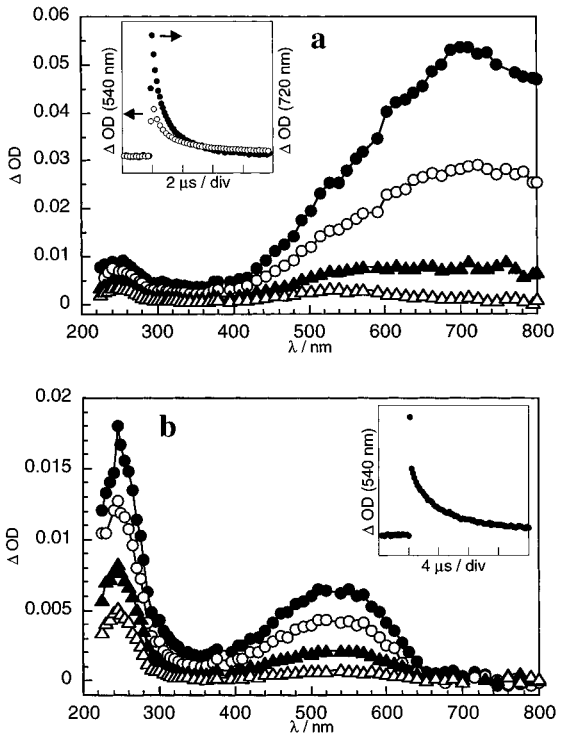


Figure 5. (a) Time-resolved spectra recorded on 193 nm photolysis of a deoxygenated aqueous solution of 12 mM $MeOPO_3^{2-}$ at pH 8. Filled circles: 275 ns, open circles: 775 ns, filled triangles: 2.75 μs, open triangles: 7.75 μs after the flash. Inset: Time-dependence of the absorption at 540 nm and at 720 nm. (b) Same solution as in (a) but containing 1 mM O_2 to scavenge e^-_{aq}. Filled circles: 350 ns, open circles: 1.55 μs, filled triangles: 5.5 μs, open triangles: 15.5 μs after the flash.

phosphate, $CH_3OPO_3^{2-}$, in the absence (a) and in the presence of oxygen (b). In Figure 5a, the presence of e^-_{aq} is clearly seen, as is a peak at ≈240 nm, indicative of a phosphate-centered radical. If the electron is removed by reaction with O_2, the presence of a species absorbing at ≈520 nm is evident (Figure 5b).

On the basis of the similarity of its spectrum with that of $HPO_4^{\bullet-}$, the species is identified as the methyl phosphate radical, $CH_3OPO_3^{\bullet-}$. As seen in the inset in Figure 5b, the species decays with a first half-life of ca. 2 μs (which is much shorter than that of the inorganic phosphate radical, see inset in Figure 4) in a reaction which is predominantly second order, but which has a first-order component, the rate of which turns out to be smaller than 10^5 s^{-1}.

On photolysis of *ethyl* phosphate under the same conditions, there also was a band at ca. 520 nm, which is assigned to the phosphate radical, $EtOPO_3^{\bullet-}$, but its intensity at 350 ns after the pulse was only about half that in the case of *methyl* phosphate (see Figure 6). It was found that the radical $EtOPO_3^{\bullet-}$ had a much shorter lifetime than $MeOPO_3^{\bullet-}$ or $HPO_4^{\bullet-}$, decaying with first-order kinetics (see inset in Figure 6) with $k = 7.9 \times 10^5$ s^{-1} as measured at 455−560 nm.

When *n-butyl* phosphate was photolyzed, the 520 nm band was hardly discernible. A similar observation was made in the case of diisopropyl phosphate (see Figure 6).

In the 193 nm ionization of the phosphates, the yields of e^-_{aq} were in all cases proportional to the laser light intensity (see, e.g., inset in Figure 7), so the process leading to the production of e^-_{aq} (ionization) is monophotonic. From the linear e^-_{aq}-yield vs laser-power plots, taking the photoionization of Cl^- in aqueous solution as an actinometer with $\phi(e^-_{aq}) = 0.46$,[14] the quantum yields for photoionization as listed in Table 1 were obtained.

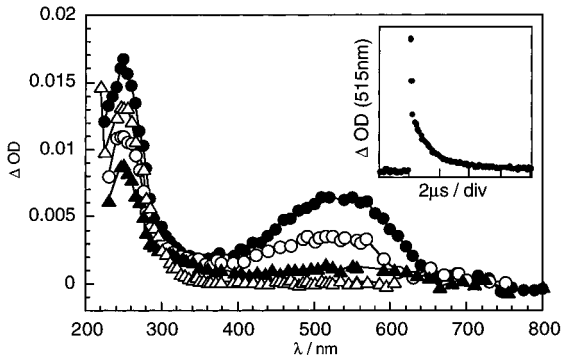


Figure 6. Absorption spectra recorded at 350 ns after the 193 nm laser pulse on photolyzing oxygenated aqueous solutions at pH 8 of methyl phosphate (filled circles), ethyl phosphate (open circles), *n*-butyl phosphate (open triangles), and diisopropyl phosphate (filled triangles). In the inset is shown the (first-order) decay of $EtOPO_3^{\bullet-}$, produced by photolysis of an oxygenated 8 mM solution of ethyl phosphate at pH 8.

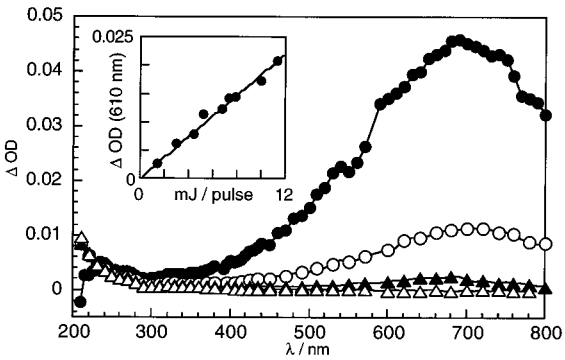


Figure 7. Time-dependent absorption spectra observed on 193 nm photolysis of a deoxygenated 10 mM aqueous solution of ribose-5-phosphate, recorded at 150 ns (filled circles), 775 ns (open circles), 1.5 μs (filled triangles), and 7.75 μs (open triangles) after the pulse (≈30 mJ). Inset: yield of e^-_{aq} as a function of laser power/mJ.

Table 1. Quantum Yields for the 193 nm Photoionization of Phosphates in Aqueous Solution

phosphate	$\phi(e^-_{aq})^a$
HPO_4^{2-}	0.53[b]
monomethyl phosphate[c]	0.61
monoethyl phosphate[c]	0.65
mono-*n*-butyl phosphate[c]	0.58
ethyleneglycolphosphate[c]	0.43
glycerol-1-phosphate[c]	0.61
glycerol-2-phosphate[c]	0.55
ribose-5-phosphate[c]	0.46 (0.47)[b]
2-deoxyribose-5-phosphate[c]	0.42
$H_2PO_4^-$	0.33[b]
ribose-5-phosphate monoanion	0.24[b]
diethyl phosphate	0.11 (pH 6)
di*iso*propyl phosphate	0.35
diethyleneglycolphosphate	0.29
triethyl phosphate	0.085

[a] Error ±10%. [b] From ref 15. [c] Dianion. [d] pH ≈ 8.

As is evident from Table 1, for the *mono*-alkyl phosphates the quantum yields are in the range 0.42−0.65, which is similar

(14) (a) Dainton, F. S.; Fowles, P. *Proc. R. Soc.* **1965**, *287*, 312. See also: (b) Jortner, J.; Ottolenghi, M.; Stein, G. *J. Phys. Chem.* **1964**, *68*, 247. However, there is also a more recent value for photoionization of Cl^- ($\Phi = 0.41$): Iwata, A.; Nakashima, N.; Kusaba, M.; Izawa, Y.; Yamanaka, C. *Chem. Phys. Lett.* **1993**, *207*, 137.

Table 2. Products and Their Quantum Yields[a] from the 193 nm Photolysis of $HOCH_2CH_2OPO_3^{2-}$ at pH 8[b]

product	ϕ/Ar	ϕ/O_2
e^-_{aq}	0.43	
H_2	0.09	$\leq 0.00_5$
inorganic phosphate	0.69	0.46
acetaldehyde	0.70	0.02
glycolaldehyde	0.00_8	0.13
formaldehyde	0.00_5	0.04
succinaldehyde	≈0.02	traces

[a] Error limits ±10%. [b] The power per pulse was 15−16 mJ.

to that (0.53) for the phosphate dianion, HPO_4^{2-}. There is obviously only a very small influence of the nature of the organic group on the quantum yield, which indicates that the photogenerated e^-_{aq} stems from the phosphate and not from the alkyl function. For the *di*-alkyl phosphates, the ionization quantum yields appear to be somewhat smaller (average = 0.25 ± 0.12), and an even smaller number is observed for *tri*-ethyl phosphate ($\phi = 0.085$). Since alkylation of the phosphate anion corresponds to protonation and since protonation reduces the electron density of an anion, the observed decrease of $\phi(e^-_{aq})$ with increasing alkylation reflects the corresponding decrease[16] in the ionization potential. Concerning DNA, in this molecule are, of course, present *di*alkyl phosphate groups (= *mono*anions) which should have *higher* ionization potentials than the *mono*alkyl phosphate *di*anions discussed in this study.

(Deoxy)ribose-5-phosphate and 2-Hydroxyethyl Phosphate. In Figure 2 is contained the absorption spectrum of ribose-5-phosphate in aqueous solution at pH 8 (natural pH). Visible is the phosphate band with $\lambda_{max} \leq 187$ nm. On irradiation into this band with 193 nm light, a strong signal due to e^-_{aq} was seen (Figure 7), whose half-life is ca. 0.3 μs. As shown in the inset, the formation of e^-_{aq} is monophotonic. From the slope of this dependence, compared with that from Cl^-, $\phi(e^-_{aq})_{ribose-5-phosphate} = 0.46$. Based on the ΔOD at ≈ 515 nm in oxygenated solution, there was no evidence for the presence of a phosphate radical, which means that its lifetime is ≤ 20 ns (= pulse length of laser).

With 2-hydroxyethyl phosphate, 193 nm photoionization occurs with a quantum yield of 0.43, which is similar to that (0.46) for ribose-5-phosphate. Despite this relatively high quantum yield as reflected by a strong signal of e^-_{aq}, there was no evidence, based on the signal at ≈515 nm, for the presence of a phosphate radical. This means that the phosphate radical necessarily produced in the ionization step must have a lifetime of <20 ns.

3. Product Analysis Results. $HOCH_2CH_2OPO_3^{2-}$. For $HOCH_2CH_2OPO_3^{2-}$, it was possible to perform a quantitative product analysis study, the results of which are presented in Table 2 and Figure 8.

As seen from Table 2, in the absence of O_2, the main products—other than e^-_{aq} and H_2—are inorganic phosphate and acetaldehyde, whose yields are equal. The small yield of glycolaldehyde probably indicates traces of oxygen in the solution. This is concluded from the fact that the yield of glycolaldehyde increases by the factor 16 in going from an Ar- to an O_2-saturated solution. The effect of varying the dose/pulse was also investigated, the results of which are shown in Figure 8. From Figure 8 it is evident that the yield of inorganic phosphate and of acetaldehyde depends on the intensity per pulse of the 193 nm light, *in*creasing with *de*creasing dose/pulse (in the experiments described in Figure 8, the *total* dose was kept constant at 1200 mJ).

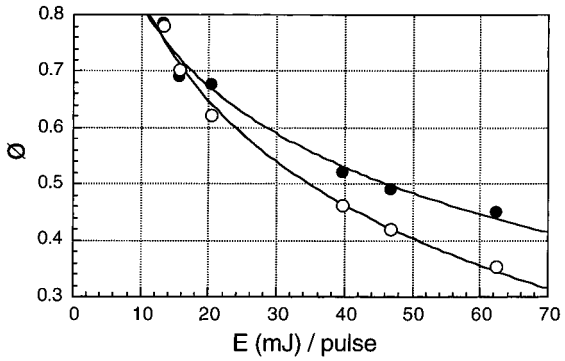


Figure 8. Dependence of the quantum yields for formation in deoxygenated solution of inorganic phosphate (filled circles) and acetaldehyde (open circles) on the light intensity per pulse, measured as mJ/pulse.

This dose-rate dependence is indicative of a chain reaction occurring. On this basis, the results so far presented are explained in terms of the following reactions:

$$HOCH_2CH_2OPO_3^{2-} \xrightarrow[193\ nm]{h\nu} HOCH_2CH_2OPO_3^{\bullet -} + e^-_{aq}, \quad \phi = 0.43 \quad (3)$$

$$HOCH_2CH_2OPO_3^{\bullet -} \rightarrow HOC^{\bullet}HCH_2OPO_3^{2-} + H^+ \quad (4)$$

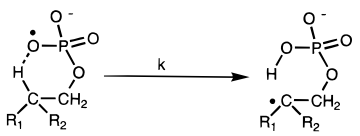
$$k \geq 5 \times 10^7\ s^{-1}$$

$$HOC^{\bullet}HCH_2OPO_3^{2-} \rightarrow OCHC^{\bullet}H_2 + HPO_4^{2-} \quad (5)$$

$$k \geq 2.3 \times 10^6\ s^{-1}$$

$$OCHC^{\bullet}H_2 + HOCH_2CH_2OPO_3^{2-} \xrightarrow{slow} CH_3CHO + HOC^{\bullet}HCH_2OPO_3^{2-} \quad (6)$$

The first step (eq 3) is the photoionization of the phosphate function in EGP, leading to the phosphate radical where the unpaired spin is probably strongly localized on an oxygen atom.[17] This oxyl-radical must be very short-lived, since, even immediately after the 20 ns pulse, spectroscopic evidence for it (expected at ≈ 520 nm) was not found. As the reason for this, we suggest a very rapid intramolecular H-atom shift, from the β-carbon to the phosphato oxygen (eqs 4 and 7). On the basis of the nonobservability of the $-OPO_3^{\bullet -}$ signal at 520 nm, the rate constant for this reaction must be ≥1/20 ns = 5×10^7 s^{-1}. As the product of this 1,5 H-shift, which can proceed through a six-membered ring, a carbon-centered α-hydroxy-β-



(7)

$R_1 = R_2 = H \quad k = 7.9 * 10^5\ s^{-1}$

$R_1 = H, R_2 = OH \quad k \geq 5 * 10^7\ s^{-1}$

phosphato radical is formed (eq 4). Radicals of this type have

(15) Candeias, L. P.; Steenken, S. *J. Am. Chem. Soc.* **1992**, *114*, 699.
(16) See Tasaki, K. Yang, X.; Urano, S.; Fetzer, S.; LeBreton, P. R. *J. Am. Chem. Soc.* **1990**, *112*, 538.
(17) For ESR spectra of phosphate radicals see, e.g.: Subramanian, S.; Symons, M. C. R.; Wardale, H. W. *J. Chem. Soc. (A)* **1970**, 1239.

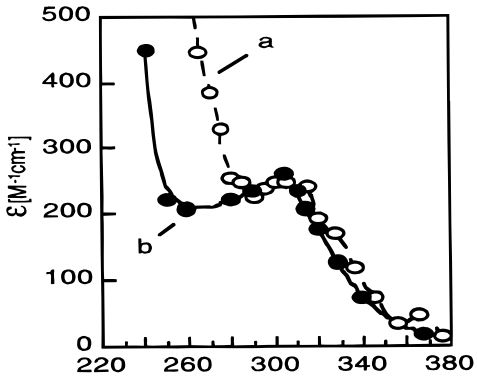


Figure 9. Absorption spectrum measured on reaction of •OH with 1 mM EGP at pH 8 (a). Comparison with absorption spectrum of $^{\bullet}CH_2CHO$ (b; from ref 20).

been shown[18] to undergo a rapid, heterolytic elimination of inorganic phosphate to yield β-oxoalkyl radicals which are oxidizing in character.[19] In this reaction (eq 5), the formyl methyl radical is produced which has been shown[20] to be able to abstract an H-atom from α-hydroxyalkanes. If this happens (eq 6), a chain reaction is started, the chain carrier being $^{\bullet}CH_2CHO$, the products of the chain reaction are acetaldehyde and inorganic phosphate, in equal yields, as experimentally observed (see Table 2).

In the presence of oxygen, the chain carrier, $^{\bullet}CH_2CHO$, is scavenged, as is visible from the dramatic decrease in the yield of CH_3CHO and the increase in the yield of glycolaldehyde. Under this condition, the yield of inorganic phosphate should be equal to that of the primarily produced (by the ionization) phosphate radicals, and this is in fact the case, as seen from Table 2 ($\phi(\text{phosphate})_{\text{oxygen}} = 0.46$ as compared to $\phi(e^-_{aq}) = 0.43$).

To check the formation of the formylmethyl radical from ethyleneglycolphosphate, pulse *radiolysis* experiments were performed with an N_2O-saturated aqueous solution of 1 mM $HOCH_2CH_2OPO_3^{2-}$. Under this condition, the OH-radicals produced by the pulse are expected to abstract an H-atom from a carbon atom of the substrate, preferably from the β-carbon relative to the phosphate group. This carbon is activated by the OH-group it carries. The resulting α-hydroxy-β-phosphato radical is the same as that from the intramolecular H-atom shift to the phosphato radical (eq 4/7) and is expected to rapidly undergo the heterolytic β-phosphate elimination reaction 5 yielding the formylmethyl radical. In Figure 9 the measured spectrum is compared with that of the authentic[21] formylmethyl radical: The similarities between the two spectra with respect to band position and epsilon are satisfactory.[22] The rate constant measured for the reaction of $OH^{\bullet}$ with $HOCH_2CH_2OPO_3^{2-}$ is 2.3×10^9 $M^{-1}s^{-1}$, similar to that (2.4×10^9 $M^{-1}s^{-1}$)[21] for reaction with ethyleneglycol. From the fact that there was no delayed formation of absorption due to $^{\bullet}CH_2CHO$ in a 1 mM solution of $HOCH_2CH_2OPO_3^{2-}$ at pH 8 it is concluded that the rate constant for (the intramolecular) elimination of phosphate from the β-phosphato radical (eq 5) is $\geq 2.3 \times 10^9$ M^{-1} s^{-1} $\times$ 10^{-3} M $= 2.3 \times 10^6$ s^{-1}, a value in agreement with analogous cases.[23]

Concerning the possibility of *intra*molecular H-transfer from a C to a phosphate oxyl radical, it may be relevant that the analogous *inter*molecular reaction, i.e., that between $HPO_4^{\bullet -}$/$PO_4^{\bullet 2-}$ and ribose or deoxyribose, does in fact exist, the rate constants measured at pH 9[24] being 9×10^7 and 7.5×10^7 M^{-1} s^{-1}.[25]

Support for the *intra*molecular transfer of an $H^{\bullet}$ from a C to a phosphate oxygen (eq 4/7) comes from ESR-measurements of γ-irradiated silver diethyl phosphate, where the $^{\bullet}CH_2CH_2O(CH_3CH_2O)PO_2^-$ radical was seen and interpreted as resulting from H-transfer to the initially formed radical oxidation product $(EtO)_2PO_2^{\bullet}$.[26] Intramolecular H-transfers have been found also in other alkyl phosphates.[27] Of relevance is also the fact[28] that in the mass spectra of trialkyl phosphates, the positive molecular ion decomposes very rapidly such that its contribution to the recorded spectrum is almost zero. In the case of tributyl phosphate, this has been interpreted as resulting from an intramolecular H-transfer from a carbon to the oxygen of the P-oxyl group.[29]

As evident, the H-transfer mechanism eq 7 involves a six-membered transition state, which is a way possible only for alkyl phosphates with R ≥ Et. For R = CH_3, the transition state necessarily involves the less favorable five-membered ring, and this, together with the higher C−H bond energy of a *primary* vs a *secondary* alkyl group, is probably the reason why the rate constant for H-transfer in *methyl* phosphate ($\leq 10^5$ s^{-1}) is lower than that with ethyl- (7.9×10^5 s^{-1}) or the higher phospates ($>5 \times 10^7$ s^{-1}). In the case of 2-hydroxyethyl phosphate, H-abstraction from the 2-position should be strongly enhanced by the activating effect of the OH-group. In agreement with this is the fact that k(abstraction) is in fact larger than 5×10^7 s^{-1}.

Ribose-5-phosphate. Product analysis experiments were also performed after 193 nm photolysis of ribose-5′-phosphate at pH 8. It was found that in aqueous deoxygenated solution (Ar or N_2O-saturated) the quantum yield of inorganic phosphate was 0.2 which dropped to 0.08 when O_2 (1 mM) was admitted. Of organic compounds in deoxygenated solution, the main photolysis products were 5-deoxypentos-4-ulose, pentos-4-ulose, and pentodialdose. In the presence of O_2 (1 mM), the yield of 5-deoxypentos-4-ulose dropped to below the detection limit, whereas those of pentos-4-ulose and pentodialdose remained approximately the same.

These results can be explained (Schemes 1 and 2) by photoionization of the phosphate group ($\phi = 0.46$) followed by intramolecular H-abstraction by the ionization-produced phosphato-radical from C4 (main reaction), and from C5. H-abstraction from C4 is via a *six*-membered transition state, as shown in Scheme 1.

The resulting C4-radical then eliminates a phosphate anion in a heterolytic fashion. This is the famous C4′-mechanism[3,4]

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(22) The fact that in the $HOCH_2CH_2OPO_3^{2-}$ case the absorption at <280 nm is stronger than that of the formylmethyl radical may be due to the additional formation of the α-phosphatoalkyl radical from the reaction of $HOCH_2CH_2OPO_3^{2-}$ with $OH^{\bullet}$.

(23) See, e.g., ref 18 and Behrens, G.; Koltzenburg, G.; Schulte-Frohlinde, D. *Z. Naturforsch.* **1982**, *37c*, 1205.

(24) Due to $pK_a(HPO_4^{\bullet -}) = 8.9$,[10] the rate constant refers mainly to the less reactive form, i.e., $PO_4^{\bullet 2-}$.

(25) Nakashima, M.; Hayon, E. *J. Phys. Chem.* **1970**, *74*, 3290.

(26) Bernhard, W. A.; Ezra, F. S. *J. Phys. Chem.* **1974**, *78*, 958.

(27) Haase, K. D.; Schulte-Frohlinde, D.; Kourim, P.; Vacek, K. *Int. J. Radiat. Phys. Chem.* **1973**, *5*, 351.

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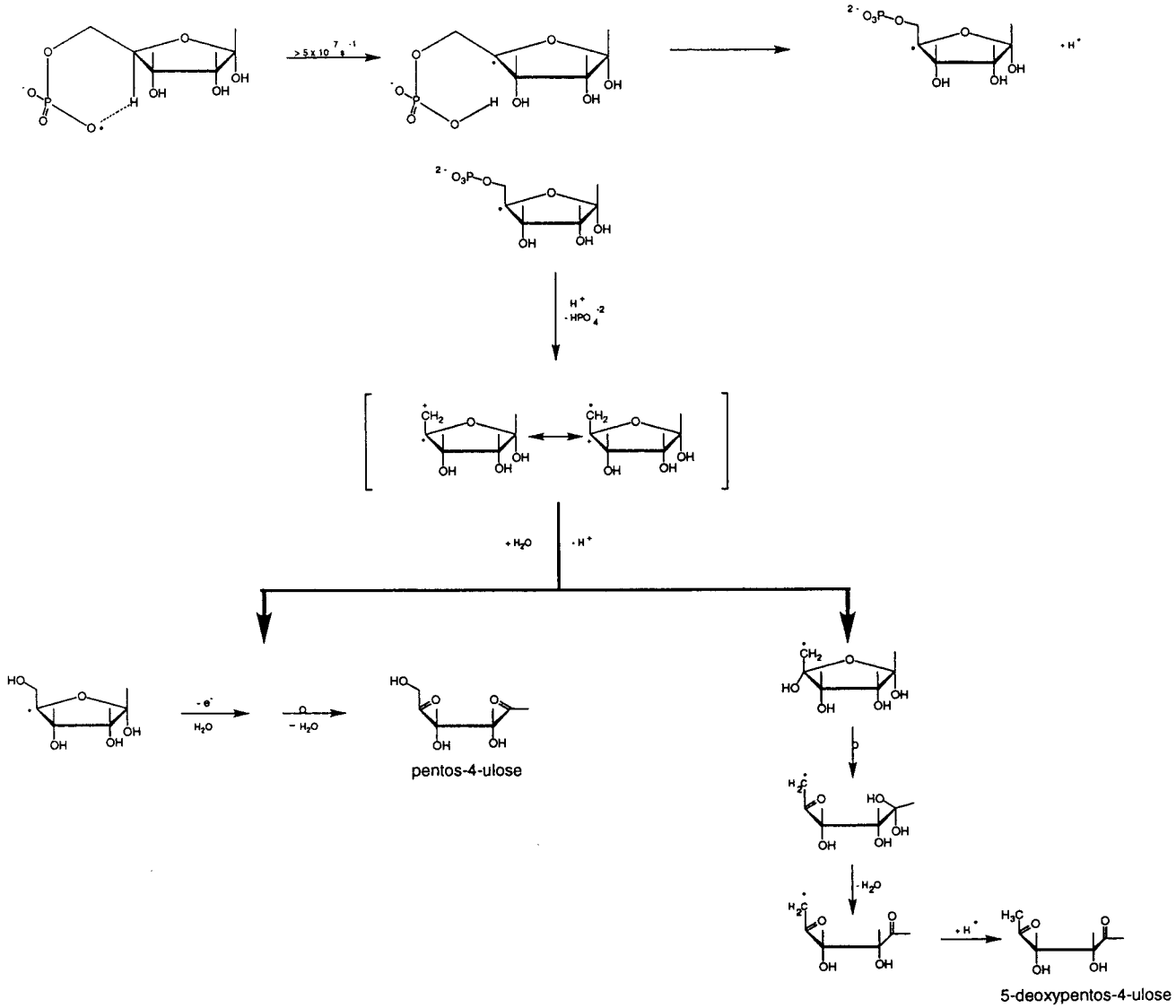
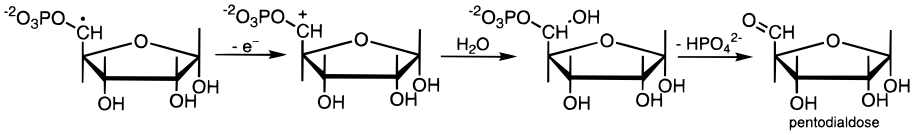
(29) .Wilkinson, R. W.; Williams, T. F. *J. Chem. Soc.* **1961**, 4098.

Scheme 1



Scheme 2



of DNA-chain breakage. The C4′-radical can be produced also by H-abstraction via a six-membered transition state if the phosphate group *in the 3′-position* is ionized. On this basis the C4′-radical is quite a likely radical to be formed (and therefore a chain break induced) on ionization of a phosphate group in DNA.[30] Obviously, by this reaction the phosphate radical is "repaired". In this connection it is relevant that phosphate radicals, in contrast to sugar radicals, have never been seen in γ-irradiated nucleotides.[6b,31] If irradiated with densely ionizing particles, evidence has been found also in DNA for *sugar* but not for phosphate radicals.[32] Furthermore, Scheme 1 (and 2) provides a mechanistic basis for the observation[33] that the frank strand breaks induced by <200 nm UV-irradiation of plasmid DNA result from excitation of the sugar−phosphate backbone. The strand breaks induced in model DNA compounds by monochromatic soft X-rays[34] can be explained on the same basis.[35]

To explain the formation of the product pentodialdose, it is necessary to assume abstraction by the phosphate radical of the H at C5, a reaction which involves a five-membered ring, followed by oxidation, addition, and elimination, as shown in Scheme 2.

(30) To which extent these concepts, which are based on simple, low-molecular weight model compounds, whose intramolecular motions are not restricted, can be applied to the rather stiff DNA polymer remains to be seen. It is obvious that model calculations on H-transfer to phosphate radicals at C3′ or C5′ from "adjacent" deoxyribose C−H groups would be very helpful.

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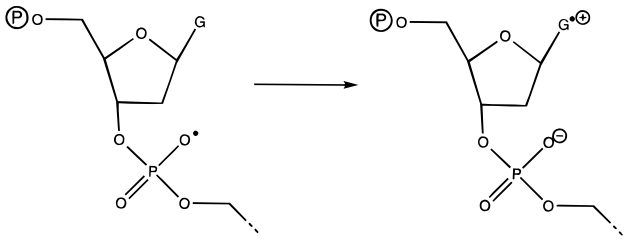
(32) Becker, D.; Razskazovskii, Y.; Sevilla, M. D. *Radiat. Res.* **1996**, *146*, 361.

(33) Gut, I. G.; Farmer, R.; Huang, R. C.; Kochevar, I. E. *Photochem. Photobiol.* **1993**, *58*, 313.

(34) Ito, T.; Saito, M.; Kobayashi, K. *Int. J. Radiat. Biol.* **1992**, *62*, 129.

(35) The authors, however, propose a different mechanism.

In principle, it is also possible that a phosphate radical is repaired intramolecularly by *electron transfer* from a base, e.g., G, as indicated:



To test this idea, applied, however, to a *bi*molecular reaction, the photochemically produced (via eq 1) phosphate radical was reacted with 2′-deoxyguanosine, monitoring the absorption at 525 nm. At pH 7, the rate constant for this reaction, in which the one-electron oxidized guanine radical[6a] was formed, was measured to be 8.7×10^8 M^{-1} s^{-1}, which is considerably higher than that ($\approx 1 \times 10^8$ M^{-1} s^{-1}, see above) for reaction with d-ribose, which indicates that the reaction mechanism of $HPO_4^{\bullet -}$ with the "aromatic" nucleoside may be different from that with the aliphatic ribose-unit. However, the *yield* of the G-centered radical from the reaction of $HPO_4^{\bullet -}$ was 2.4-times *lower* than that (=100%)[6a] from the reaction of $SO_4^{\bullet -}$. This is indicative of a higher tendency of $HPO_4^{\bullet -}$, as compared to $SO_4^{\bullet -}$, for H− relative to electron transfer.

Summary and Conclusions

In aqueous solution, 193 nm photolysis of inorganic and organic phosphates such as ribose-5-phosphate leads to ionization with formation of the corresponding oxygen-centered phosphate radicals, $O_3PO^{\bullet}$.[36] These radicals abstract hydrogens attached to α-, *β*-, or, possibly, *γ*-carbons, whereby in the case of the *β*-hydrogens a six-membered transition state for transfer of the hydrogen from the carbon to the phosphate oxygen is possible, leading to high rate constants (up to $> 5 \times 10^7$ s^{-1}) for H-transfer in these unimolecular reactions. In the case of (deoxy)ribosephosphates the six-membered transition state is possible for H-transfer from C4 to the phosphate group at C5 as well as at C3. In DNA, the resulting C4′-radical will undergo a rapid *β*-elimination of the phosphate-ester group, this step representing the DNA chain break. The apparently easy H-transfer from a carbon to a phosphate radical, by which these radicals are repaired, is probably why phosphate radicals are not observed in irradiated DNA. A consequence of the involvement of the C4′-radical is that the mechanism of DNA chain breakage is the same for the direct and the indirect effect.

(36) In the case of DNA, *neutral* phosphato radicals are expected from the DNA *mono*anionic phosphate ester function. These neutral radicals should be considerably *more oxidizing* (i.e., even better H-abstractors) than the phosphato radical *anions* discussed in this paper.

Experimental Section

Inorganic phosphate (analytical grade) was from Merck. Ribose- and 2-deoxy-5-ribosephosphate were from Fluka or Sigma. Dialkyl phosphates were prepared from the corresponding trialkyl phosphates (from Fluka) by partial hydrolysis with aqueous NaOH, following a procedure as described for diethyl phosphate.[37] Ethyleneglycolphosphate was synthesized from 2-chloroethanol and polyphosphoric acid according to ref. 38. The water was purified with a Millipore-Milli-Q system. The optical densities of the solutions at 193 nm were ca. 0.4−1/cm. 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 ca. 3 mL/min, using pulse rates of 0.4 Hz. Unless otherwise indicated, the pH was 8, and the temperature was 20 ± 1 °C. The experiments were carried out with an argon fluoride excimer laser (Lambda Physik EMG103MSC) that delivered unfocused 20 ns pulses with a power of 3−50 mJ (measured at the position of the cell with a Gentech ED-200 power meter). The optical signals were digitized with Tektronix 7612 and 7912 transient recorders interfaced with a DEC LSI11/73$^+$ computer, which also controlled the other functions of the apparatus and on-line preanalyzed the data. Final data analysis was done with a Microvax II connected to the LSI.

Quantum yield measurements of 193 nm photoionization are based on e^-_{aq} yields from argon-saturated aqueous solutions of NaCl (Merck) of the same OD at 193 nm as those of the phosphate solutions. The quantum yield for the production of e^-_{aq} from Cl^- is assumed to be the same as that with 185 nm light, reported as 0.46.[14] For the product analysis measurements, the Farkas-actinometer (5 M ethanol in water, formation of H_2 (measured by GC)) was used, taking $\Phi(H_2) = 0.4$.[39]

JA974393M

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