Dissociative Electron Attachment to Phosphoric Acid Esters: The Direct Mechanism for Single Strand Breaks in DNA

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We use dibutyl phosphate to simulate the behavior of the phosphate group in DNA towards the attack of low energy electrons. We find that the compound undergoes effective dissociative electron attachment within a low energy resonant feature at 1 eV and a further resonance peaking at 8 eV. The dissociative electron attachment (DEA) reactions are associated with the direct cleavage of the C-O and the P-O bond but also the excision of the PO^-, PO_3^-, $H_2PO_3^-$ units. For the phosphate group coupled in the DNA network these reactions represent single strand breaks. We hence propose that the most direct mechanism of single strand breaks occurring in DNA at subexcitation energies (< 4 eV) is due to DEA directly to the phosphate group.

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The phosphate group is the central unit along the DNA backbone connecting the two 2-deoxyribose moieties of the two adjacent nucleosides via the P-O-C5 and P-O-C3 bonds, respectively (Fig. 1). The study of the response of the phosphate group to the interaction of low energy electrons is of particular interest since cleavage of any of the P-O-C bonds would represent a single strand break in DNA. The study of DNA damage by low energy electrons is directly relevant for the general problem of radiation damage in cellular systems and, correspondingly, for the action of radiosensitizers used in tumor therapy. Here we study dissociative electron attachment (DEA) to dibutyl phosphate (DBP) and triethyl phosphate (TEP) by means of a crossed electron-molecule beam experiment and mass spectrometric detection of the anions. DBP can directly be viewed as model system for the phosphate group coupled in the molecular network of DNA.

The passage of high-energy radiation through a living cell leaves a trace of free electrons [1]. There is ample evidence that DEA with its unique features plays a particular role in the nascent states of cellular DNA radiolysis [2]. So far these phenomena have been investigated at two extremes of DNA complexity, namely, plasmid DNA *versus* isolated DNA building blocks in the gas phase. Experiments on plasmid DNA have demonstrated that low energy electrons can efficiently induce single strand breaks (SSBs), as well as double strand breaks (DSBs) [3]. In the very low energy domain (0–3 eV), below the threshold of electronic excitation, only SSBs are observed [4]. In any of these experiments it became obvious that the efficiency of both DSBs and SSBs as a function of the primary electron energy exhibits a *resonant behavior* indicating that the formation of *negative ion resonances* is the initial step.

Concerning the single gas phase DNA building blocks, experiments so far have been performed for the different DNA nucleobases [5–11], for 2-deoxyribose [12] [and related sugar compounds [13]] and for thymidine [14], representing a thymine coupled to 2-deoxyribose via the N1-C1 glycosidic bond. These gas phase studies revealed that (i) isolated nucleobases (NBs) undergo DEA in the range $\approx$6–9 eV and also at much lower energies ($<$3 eV) where SSBs are observed [5], (ii) sugar molecules are remarkably sensitive towards low energy electrons which already at very low energies (close to zero eV) induce complex DEA reactions associated with the degradation of the ring structure.

Within the study of the intrinsic properties of the isolated gas phase DNA building blocks, the phosphate group is the missing unit. In DNA the phosphate group is negatively charged which is compensated by an appropriate counterion. The adjacent sugar units are linked by the C5-O-P and P-O-C3 bonds, respectively (Fig. 1). The use of a phosphodiester (in the present case DBP) then serves as an appropriate model to explore the behavior of the phosphate group in the DNA network.

Density functional theory (DFT) calculations on a sugar-phosphate-sugar unit [15] suggested that near zero eV electrons can induce strand breaks via rupture of the C3-O and C5-O bond, respectively. A study on the electron

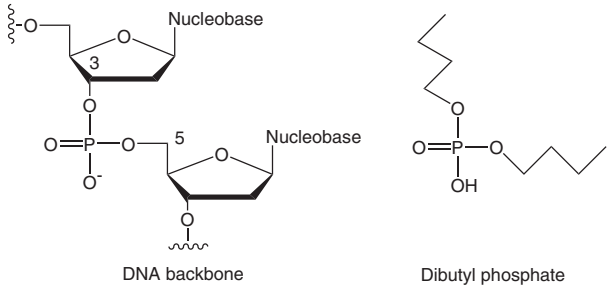

FIG. 1. Molecular structure of dibutyl phosphate (DBP) compared to the structure of the DNA backbone. Positions 3 and 5 of the sugar are labeled.

interaction (4–15 eV) with molecular films of short single strand DNA (GCAT) [16,17] indicated cleavage of the *N*-glycosidic and phosphodiester bonds via formation of DEA resonances. It appears that rather the C-O bond is involved than the P-O bond. We finally mention a recent study on electron impact to self-assembled monolayer films [18] made of single and double DNA strands prepared from 40-mer oligonucleotides investigating electron stimulated desorption (ESD) of OH^-. The authors concluded that direct DEA to the phosphate unit is operative, visible via ESD of OH^- with a maximum in the resonant yield function at 6.7 eV. This result is confirmed by an investigation of thin films of NaH_2PO_4 [19] published in the course of the revision of the present Letter. In addition to OH^- desorption of H^- and O^- was observed at 8.8 and 8.0 eV, respectively, that is ascribed to O-H and P = O bond cleavage.

To our knowledge, no DEA experiments on gas phase phosphate compounds have been reported so far. The present results on DBP hence represent the first study on the intrinsic behavior of the phosphate group in DNA towards the attack of low energy electrons (0–12 eV).

The experiments were carried out in a crossed electron-molecular beam arrangement consisting of an electron source, an oven and a quadrupole mass analyzer (QMA) [20]. The components are housed in a UHV chamber at a base pressure of 10^{-8} mbar. A well-defined electron beam generated from a trochoidal electron monochromator [21] (resolution $\approx 90 - 120$ meV FWHM) intersects orthogonally with an effusive molecular beam consisting of the phosphoric acid esters which are both liquids under normal conditions. The vapor pressure of DBP is sufficiently low that the sample could be placed directly into the oven inside the vacuum system. By heating to 370 K the vapor pressure increases to a degree that reasonable ion signals could be obtained. TEP was introduced into the chamber through a heated gas inlet line resulting in less vapor pressure in the collision zone and a correspondingly lower ion signal. The presently used vaporization temperature is appreciably lower than those previously used to sublimate the solid DNA bases [5–11], where temperatures up to 500 K were applied.

The anions generated in the collision region are extracted by a small electric field towards the entrance of the QMA where they are analyzed and detected by a single pulse counting technique. The energy scale was calibrated using the well-known resonance in SF_6 near 0 eV generating metastable SF_6^-. To prevent ion-molecule reactions involving SF_6^- ions, the flow of the calibration gas was switched off prior to each measurement.

Figures 2 and 3 show the ion yields observed from DBP ($(C_4H_9O)_2P(O)OH$, 210 amu) indicating that DEA mainly occurs at low energies but also within a resonance located near 8 eV, particularly visible on the fragment ion at 153 amu (Fig. 2), but also weakly on the PO_3^- fragment (not within the scale of Fig. 3). Before considering the underlying reactions in more detail we note that from the ion yields of Figs. 2 and 3 it is directly obvious that the dibutyl ester is subjected to a variety of DEA reactions which would directly lead to SSBs for the phosphate group connected in the DNA network. In particular, formation of the ions $H_2PO_3^-$, PO_3^-, and PO^- represents the excision of the central phosphate unit from the system.

The ion signals presented in Fig. 2 are due to the loss of a neutral hydrogen atom creating an ion at 209 amu [$(DBP\text{-}H)^-$], the loss of the hydroxyl anion OH^- (17 amu), or the loss of an entire neutral butyl unit (C_4H_9, 57 amu) leading to an ion at 153 amu. These ions can principally be formed by a single bond cleavage along the corresponding coordinate and among these formation of the 153 amu fragment would also correspond to a direct single strand break.

The dominant DEA channel is observed at 1 eV due to hydrogen loss according to

$$e^- + \mathrm{DBP} \rightarrow \mathrm{DBP}^{-\#} \rightarrow (\mathrm{DBP})^- + \mathrm{H},$$

where $DBP^{-\#}$ represents the transient negative ion (TNI). Another low energy feature appears at 3.2 eV and 2.8 eV on the $(DBP\text{-}H)^-$ and the $(DBP\text{-}C_4H_9)^-$ fragment, respectively (Fig. 2). It is reasonable to assume that loss occurs from the OH site, in analogy to hydrogen loss from formic acid as identified by isotope labeling [22]. A closer inspection of the mass range around 209 amu (obtained at higher mass resolution) reveals that also a small signal (15% of

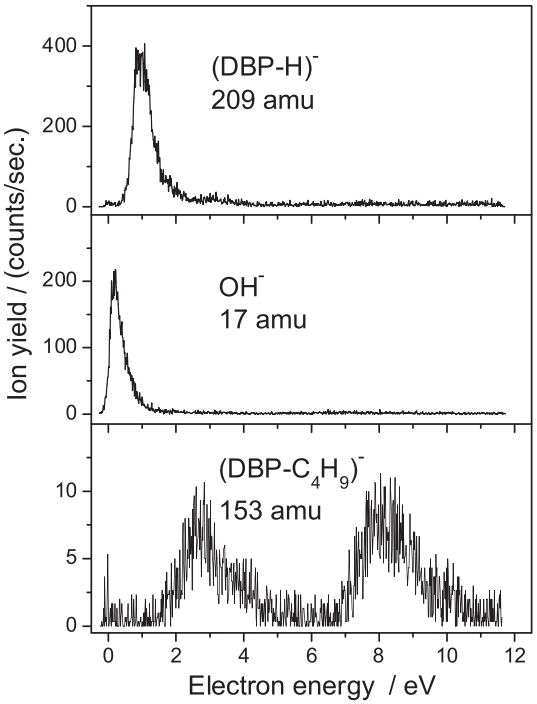

FIG. 2. Ion yields from dibutyl phosphate (DBP) at 209 amu [$(DBP\text{-}H)^-$], 17 amu (OH^-), and 153 amu [$(DBP\text{-}C_4H_9)$]. These ions can principally be formed by a single bond cleavage along the corresponding coordinate; formation of the 153 amu fragment would correspond to a direct single strand break in DNA.

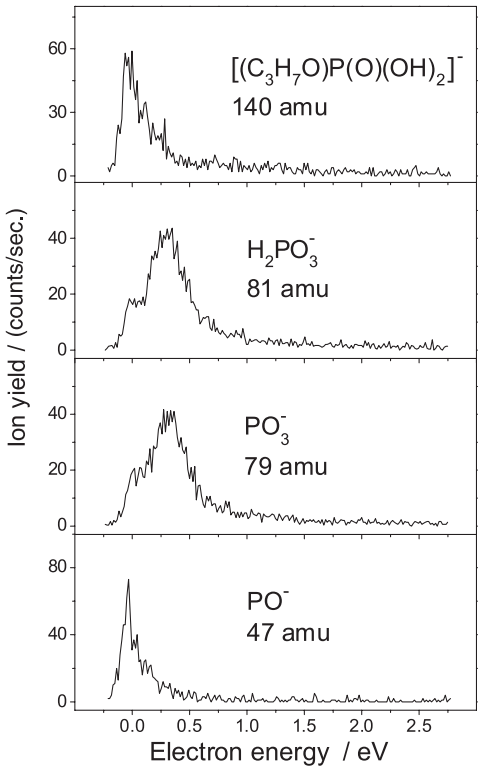

FIG. 3. Ion yields from dibutyl phosphate (DBP) at low energy.

that at 209 amu) is present at 210 amu (well separated from 209 amu) which must be due to the nondecomposed parent anion. Such metastable parent anions are sometimes observed in larger gas phase molecules [23].

The second dominant fragment is the hydroxyl anion with a peak at 0.2 eV, likely due to a direct cleavage of the P-OH bond.

Figure 3 presents some of the ion yields of P containing fragments on an expanded scale in the low energy region. It appears that the low energy feature is composed of two overlapping resonances, one close to zero eV and the other at 0.3 eV. The ion yields presented in Fig. 3 are due to complex reactions involving multiple bond cleavages. A reasonable structure for the fragment at 140 amu is $[(C_3H_7O)P(O)(OH)_2]^-$ while the ions at 81 amu, 79 amu, and 47 amu can unambiguously be assigned as $H_2PO_3^-$, PO_3^-, and PO^-, respectively. Bond breaking is likely to occur predominantly at the C-O bond and hence confirms the results from electron interaction with thin films of oligonucleotides mentioned above [16,17]. The presence of the PO^- ion, however, clearly indicates that P-O bond cleavage cannot be ruled out. The release of the central phosphate unit represents a direct route for strand breaks in DNA.

Some of the phosphate anions (PO_2^-, PO_3^-) are established as remarkably stable negative ions with respect to the binding energy of the extra electron. The other thermodynamic values like heats of formation or bond dissociation energies are so far less explored for these systems [24]. From photoelectron spectroscopy the electron affinity of PO_3 was found to be as large as 4.95 eV [25] and that of PO_2 as 3.42 eV [26]. Interestingly, this latter ion is neither observed from DBP nor from TEP while PO^- is present with its comparatively lower electron binding energy of 1.09 eV [27]. We additionally observed ions (not shown here) at 48 amu (HPO^-), 92 amu ($C_2H_5OPO^-$), and 114 amu with peaks at about 0 eV and an ion at 62 amu (CH_3OP^-) peaking at about 0.7 eV.

To investigate whether the observed reactions are restricted to possibly particular conditions which apply, when the butyl units are coupled to the phosphate, we also performed DEA experiments on TEP [$(C_2H_5O)_3PO$, 182 amu]. From this compound, the ion $(TEP\text{-}H)^-$ is missing which confirms the above suggestion that from DBP the loss of neutral H occurs from the O-H site. Like in DBP we observe PO_3^-, $H_2PO_3^-$ and additionally an ion at 137 amu $[(C_2H_5O)_2PO^-]$.

Figure 4 presents the ion yields of PO_3^- and $(C_2H_5O)_2PO^-$ which are formed by the loss of an entire C_2H_5O group from the precursor ion. Interestingly, this signal essentially appears from the high-energy resonance located near 8 eV and hence behaves similar to the 153 amu fragment from DBP which was assigned to the loss of an entire butyl group. In saturated hydrocarbons, low energy shape resonances are usually not present, while core excited resonances are often found in the region 6–9 eV [28]. From that it is likely that the low energy features can be characterized as shape resonances with the excess electron residing in a virtual MO of the *phosphate group* of π^* character. The high-energy feature particularly leads to the loss of entire neutral C_4H_9 and C_2H_5O groups from DBP and TEP, respectively, and may then be assigned to core excited resonances in the hydrocarbons. Both types of resonances are involved in bond cleavages which would represent single strand breaks in the DNA network.

In a recent theoretical study [29] modeling a section of DNA composed of cytosine, sugar, and the phosphate group, a mechanism for electron initiated strand breaks was proposed and frequently adopted. These calculations predict a low lying anionic potential energy surface which connects the initial π^* anion state of the base to a σ^* state in the backbone. An electron initially captured by a DNA base may thereby be transferred to the backbone leading to rupture of the C-O bond between the phosphate and the sugar. Direct attachment to the phosphate group is predicted to be possible at energies between 2–3 eV. This contrasts the DFT results mentioned above [15] which anticipate bond breaking at energies close to 0 eV. Our results now clearly demonstrate that DEA to the phosphodiester is in fact operative at energies appreciably below 2 eV.

Very recent experiments obtained from thymidine [14] demonstrate that a low energy electron, initially localized on thymine, is *not* transferred to the sugar leading to subsequent DEA in the sugar unit. Instead, the sugar itself dissociatively captures low energy electrons and can be

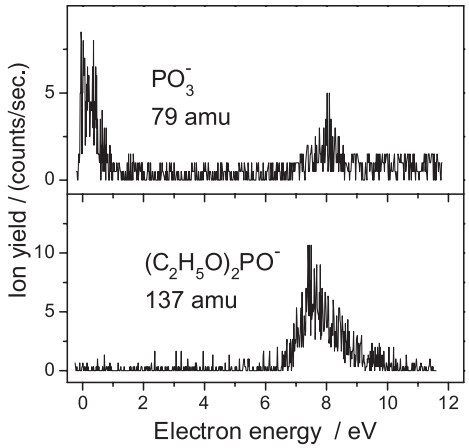

FIG. 4. Ion yields from triethyl phosphate (TEP) at 79 amu (PO_3^-) and 137 amu [$(C_2H_5O)_2PO^-$]).

considered as an active center towards strand breaks initiated by subexcitation electrons [13]. These observations may not question the electron transfer model [29] as the presence of the phosphate group may, in fact, enable the electron transfer from the base over an intact sugar to the phosphate unit.

The study of Zheng *et al.* [17] carried out at 10 eV electron energy showed a dependence of phosphodiester bond cleavage on the nucleobases and also the sequence, indicating that electron attachment to a nucleobase with subsequent transfer to the backbone may also contribute to SSBs. However, the processes observed in the present study were mainly observed at lower energies (<4 eV). In a biologically more relevant environment, i.e., within the DNA network in the condensed phase and surrounded by water molecules, the situation becomes considerably more complex. This can lead to a decrease but also to an enhancement of DEA cross sections [30,31]. Irrespective of such possible modifications, the present results clearly demonstrate the intrinsic properties of the phosphate group to directly capture an electron leading to rupture of the C-O and also the P-O bond.

While the situation in the energy range above 5 eV concerning SSBs and, in particular, DSBs, is still unclear, we arrive at a rather detailed molecular picture how low energy electrons in the subexcitation region induce SSBs. Electrons captured by the nucleobases may be transferred to the phosphate thereby cleaving a C-O bond as predicted by theory [29]. In addition, the sugar unit is also sensitive towards low energy electrons and in ribose, excision of units containing C5 is particularly selective [13]. The most direct mechanism for SSBs, however, is DEA directly to the phosphate group as shown in this study. We conclude that different molecular mechanisms contribute to SSBs. To quantify the role of these different pathways further gas phase studies on entire nucleotide moieties are necessary.

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[1] V. Cobut *et al.*, Radiat. Phys. Chem. **51**, 229 (1998).
[2] L. Sanche, Eur. Phys. J. D **35**, 367 (2005).
[3] B. Boudaiffa *et al.*, Science **287**, 1658 (2000).
[4] F. Martin *et al.*, Phys. Rev. Lett. **93**, 068101 (2004).
[5] G. Hanel *et al.*, Phys. Rev. Lett. **90**, 188104 (2003).
[6] S. Ptasinska *et al.*, Angew. Chem. Int. Ed. **44**, 1647 (2005).
[7] S. Ptasinska *et al.*, Phys. Rev. Lett. **95**, 093201 (2005).
[8] H. Abdoul-Carime, S. Gohlke, and E. Illenberger, Phys. Rev. Lett. **92**, 168103 (2004).
[9] S. Ptasinska *et al.*, Angew. Chem. Int. Ed. **44**, 6941 (2005).
[10] R. Abouaf and H. Dunet, Eur. Phys. J. D **35**, 405 (2005).
[11] A. M. Scheer, K. Aflatooni, G. A. Gallup, and P. D. Burrow, Phys. Rev. Lett. **92**, 068102 (2004).
[12] S. Ptasinska, S. Denifl, P. Scheier, and T. D. Märk, J. Chem. Phys. **120**, 8505 (2004).
[13] I. Bald, J. Kopyra, and E. Illenberger, Angew. Chem. Int. Ed. (to be published).
[14] S. Ptasinska *et al.*, Angew. Chem. Int. Ed. **45**, 1893 (2006).
[15] X. Li, M. D. Sevilla, and L. Sanche, J. Am. Chem. Soc. **125**, 13 668 (2003).
[16] Y. Zheng *et al.*, J. Chem. Phys. **124**, 064710 (2006).
[17] Y. Zheng *et al.*, J. Am. Chem. Soc. **127**, 16 592 (2005).
[18] X. Pan and L. Sanche, Phys. Rev. Lett. **94**, 198104 (2005).
[19] X. Pan and L. Sanche, Chem. Phys. Lett. **421**, 404 (2006).
[20] R. Balog *et al.*, Int. J. Mass Spectrom. **233**, 267 (2004).
[21] A. Stamatovic and G. J. Schulz, Rev. Sci. Instrum. **41**, 423 (1970).
[22] I. Martin *et al.*, Phys. Chem. Chem. Phys. **7**, 2212 (2005).
[23] T. Oster, A. Kühn, and E. Illenberger, Int. J. Mass Spectrom. Ion Proc. **89**, 1 (1989).
[24] NIST Chemistry web book: http://webbook.nist.gov/chemistry.
[25] X. B. Wang and L. S. Wang, Chem. Phys. Lett. **313**, 179 (1999).
[26] C. Xu, E. de Beer, and D. M. Neumark, J. Chem. Phys. **104**, 2749 (1996).
[27] P. F. Zittel and W. C. Lineberger, J. Chem. Phys. **65**, 1236 (1976).
[28] J. B. Hasted and D. Mathur, in *Electron-Molecule Interactions and Their Applications*, edited by L. G. Christophorou (Academic, New York, 1984).
[29] J. Berdys, I. Anusiewicz, P. Skurski, and J. Simons, J. Am. Chem. Soc. **126**, 6441 (2004).
[30] E. Illenberger, Surf. Sci. **528**, 67 (2003).
[31] I. Le Coat *et al.*, Chem. Phys. Lett. **296**, 208 (1998).