DNA Damage

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Selective Excision of C5 from D-Ribose in the Gas Phase by Low-Energy Electrons (0–1 eV): Implications for the Mechanism of DNA Damage**

*Ilko Bald, Janina Kopyra, and Eugen Illenberger**

Sugar is the central unit within a nucleotide connecting the DNA base with the phosphate group, which itself couples to the neighboring nucleotides within single-stranded DNA. The study of the excitation, ionization, and fragmentation of biomolecular systems is essential for the understanding of many problems in the area of life sciences such as the mechanism of radiation damage in cellular systems or the action of radiosensitisers used in tumor therapy.

The passage of high-energy radiation through dense media such as water or a living cell leaves a trace of free electrons. These secondary electrons are created in numbers (5×10^4 per MeV of deposited energy[1]) that makes them the most abundant radiolytic species. In the course of thermalization they can induce further ionization or excitation processes, but they can also efficiently attach at specific energies (resonances) and sites to DNA, forming transient negative ions that subsequently dissociate (dissociative electron attachment, DEA).[2]

Ample evidence exists that DEA with its unique features plays an important role in the nascent states of cellular DNA radiolysis.[2] To date, these phenomena have been investigated at two extremes of DNA complexity, namely, plasmid DNA and isolated nucleobases 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 these experiments it became apparent 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.

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Studies on isolate nucleobases (NBs) in the gas phase[5–11] have demonstrated that they undergo DEA in the range of roughly 6–9 eV and also at much lower energies (< 3 eV) where SSBs are observed.[5] While the high-energy feature leads to loss of H^- and further fragment ions associated with the rupture of the NB ring structure,[5–7] the low-energy resonance exclusively leads to the loss of neutral hydrogen with the excess charge remaining on the nucleobase.

In a recent theoretical study[12] modeling a section of DNA composed of cytosine, sugar, and the phosphate group, an interesting mechanism for electron-initiated strand breaks was proposed. The calculations predict a low-lying anionic potential energy surface that connects the initial π^* anion state of the base to a σ^* state in the backbone. An electron 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. On the other hand, very recent experiments on thymidine (thymine coupled to sugar)[13] indicate that such an electron transfer is not operative; instead it appears that sugar moiety itself has a pronounced ability to capture low-energy electrons with subsequent fragmentation. For the detailed investigation of the response of sugar following electron attachment we use D-ribose ($C_5H_{10}O_5$) and some isotopically labeled analogues (1-^{13}C, 5-^{13}C, *C*,1-D). For simplicity we will use the term ribose for D-ribose throughout this manuscript.

A previous study by the Innsbruck Laboratory on deoxyribose ($C_5H_{10}O_4$) revealed that electron capture at energies already close to 0 eV induces a variety of fragmentation reactions.[14] As we shall demonstrate, isotopic labeling enables us to identify the underlying decomposition process and to specify the site of the target molecule involved. This provides essential information for the molecular process of DNA damage by low-energy electrons.

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).[15] The components were housed in a ultrahigh-vacuum chamber at a base pressure of 10^{-8} mbar. A well-defined electron beam generated from a trochoidal electron monochromator[16] (resolution 90–120 meV fwhm) intersected orthogonally with an effusive molecular beam consisting of ribose molecules. They emanated from a resistively heated oven directly connected to the reaction chamber by a capillary. At a temperature of about 370 K (measured by a platinum resistance) the density of intact ribose molecules was high enough to yield a reasonable negative-ion signal. The generated anions were extracted by a small electric field towards the entrance of the QMA where they were 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. Ribose and the 5-^{13}C analogue were obtained from Sigma Aldrich (stated purity 98 and 99%, respectively), [1-^{13}C]ribose and [*C*,1-D]ribose were obtained from Cambridge Isotope Laboratories, Inc. (stated purity 99 and 98%, respectively). All samples were used as delivered.

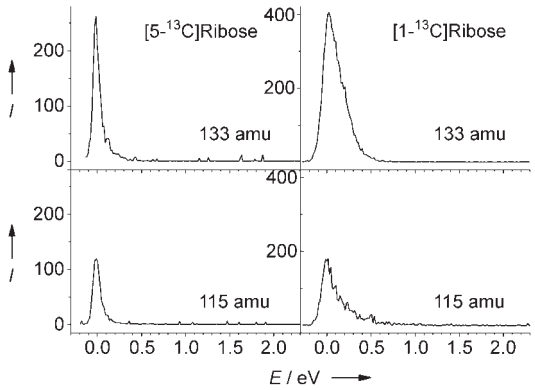


Figure 1. Ion yields obtained from [5-^{13}C]ribose and [1-^{13}C]ribose at 133 amu ($C_5H_8O_4^-$) and 115 amu ($C_5H_6O_3^-$) due to the loss of one and two water molecules, respectively.

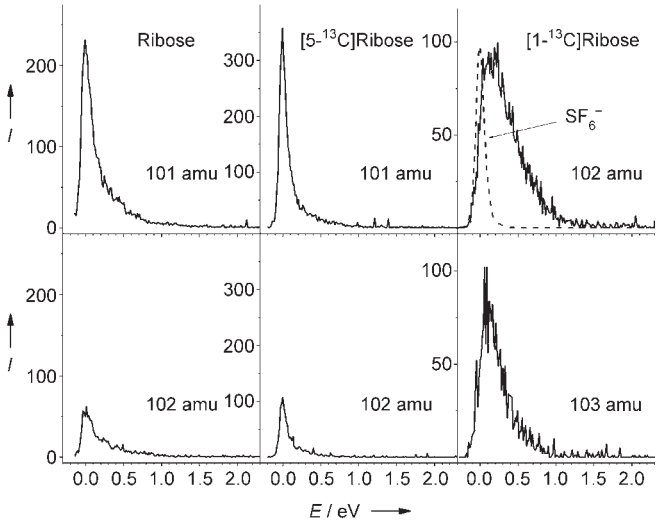


Figure 2. Ion yields from ribose (left) at 101 amu ($C_4H_5O_3^-$) and 102 amu ($C_4H_6O_3^-$) arising from the excision of a carbon-containing unit. In [1-^{13}C]ribose (right) the signal is shifted by one mass unit, while in [5-^{13}C]ribose (middle) this is not the case.

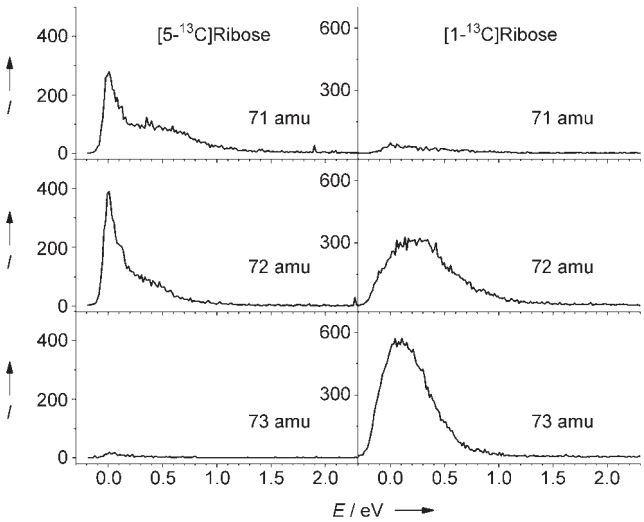


Figure 3. Ion yields at 71 amu ($C_3H_3O_2^-$) and 72 amu ($C_3H_4O_2^-$) arising from the excision of two C atoms. In [5-^{13}C]ribose (left) the situation corresponds to that in nonlabeled ribose (not shown), while in [1-^{13}C]ribose (right) and [*C*,1-D]ribose (not shown) the signal is shifted by one mass unit.

Before considering the present results, we note that in DNA the deoxyribose unit is present as a furanose five-membered-ring structure with the C1 position connected to the DNA base through the glycosidic C–N bond and the C5 position to the phosphate group through the C–O bond. The nucleotide is then formed from its building blocks (base–sugar–phosphate) by a condensation reaction releasing two H_2O molecules. In gas-phase experiments it was demonstrated that ribose and deoxyribose exist in the six-membered ring form (pyranose form).[17] It was shown that the crystalline structure of pentose sugars (the pyranose form) is in fact preserved in the course of thermal evaporation, and we hence assume that in the present experiments the ribose molecule is present as a six-membered ring. Owing to the availability of isotopic labeling we use ribose instead of deoxyribose. This should not affect the general conclusions since ribose, deoxyribose,[14] and fructose[18] all show a rather similar fragmentation behavior in the way that electrons at very low-energies decompose the respective molecule by the loss of one or more water molecules as well as C-containing neutral units.

From the selected DEA spectra shown in Figure 1, Figure 2, and Figure 3 it is immediately obvious that ribose ($C_5H_{10}O_5$, 150 amu) and its isotopically labeled analogues show a unique and surprisingly rich fragmentation behavior in the course of electron interaction at very low energies (< 1 eV). In a tentative reaction scheme [Eqs. (1)–(6)] we list the dominant negative fragment ions as observed from nonlabeled ribose in the order of decreasing mass numbers.

$$e^- + C_5H_{10}O_5 \rightarrow C_5H_{10}O_5^{-\#} \rightarrow \mathbf{C_5H_8O_4^-} + H_2O \quad (132\ \text{amu}) \quad (1)$$

$$C_5H_{10}O_5^{-\#} \rightarrow \mathbf{C_5H_6O_3^-} + 2H_2O \quad (114\ \text{amu}) \quad (2)$$

$$C_5H_{10}O_5^{-\#} \rightarrow \mathbf{C_4H_5O_3^-} + 2H_2O + CH \quad (101\ \text{amu}) \quad (3a)$$

$$\rightarrow \mathbf{C_4H_5O_3^-} + CO_2 + 2H_2 + H \quad (3b)$$

$$C_5H_{10}O_5^{-\#} \rightarrow \mathbf{C_3H_4O_2^-} + CO_2 + CH_3OH + H_2 \quad (72\ \text{amu}) \quad (4)$$

$$C_5H_{10}O_5^{-\#} \rightarrow \mathbf{C_2H_3O_2^-} + CO_2 + C_2H_6 + OH \quad (59\ \text{amu}) \quad (5)$$

$$C_5H_{10}O_5^{-\#} \rightarrow \mathbf{HCOO^-} + \text{neutral fragments} \quad (45\ \text{amu}) \quad (6)$$

$C_5H_{10}O_5^{-\#}$ corresponds to the transient anion formed upon electron capture. The reactions result from the loss of one or more neutral water units as well as the excision of one and more C-containing units from the target molecule. Since we do not observe a measurable change of the relative intensity between light and heavy fragment anions when the

temperature is increased to 410 K we can assume that the gaseous sample consists completely of intact ribose molecules.

Since the present experiment yields only information on the ionic products, the neutral decomposition channels assigned in reactions (3)–(5) are tentative and refer to reasonable and energetically favorable dissociation limits. Also, the stoichiometric assignment of the ions can be ambiguous; for example, the 101-amu anion ($C_4H_5O_3^-$) could also be assigned as $C_5H_9O_2^-$. As we shall substantiate below, however, experiments using the isotopically labeled analogues immediately show that only $C_4H_5O_3^-$ is generated. More importantly, isotopic labeling enables us to specify the site of the target molecule involved in the reaction under consideration.

The observation that free electrons with essentially no (or little) energy can trigger such reactions is a remarkable result. Water loss, for example, is associated with multiple bond cleavages and formation of new bonds. On the other hand, from the energetic point of view it has to be remembered that sugar molecules are (thermodynamically) rather unstable species with respect to the loss of water units; this can be rationalized easily by considering the corresponding thermodynamic values[19] (Table 1). Of course in the neutral system

Table 1: Heats of formation ($\Delta H_f°$) for some compounds relevant to the present reactions.[a]

Compound	$\Delta H_f°$ [kJ mol^{-1}]
$C_5H_{10}O_5$ (D-ribose, solid)	−1050
$C_5H_8O_4$ (pentanedioic acid, solid)	−960
H_2O (liquid)	−242
H_2O (gas)	−286
C (gas)	717
CH (gas)	594
CO_2 (gas)	−394
CH_3OH (liquid)	−239
CH_3OH (gas)	−201
H	218

[a] Taken from reference [19].

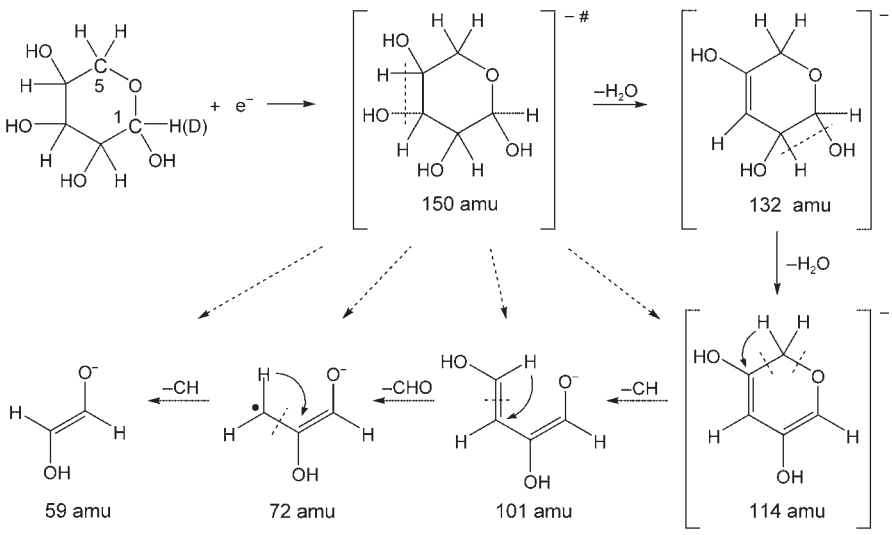
such a reaction possesses large activation barriers, and hence sugar molecules can be considered as stable compounds also on a macroscopic time scale. Obviously, the presence of an excess electron changes the situation completely, as the transient anion decomposes into all the negatively charged fragments indicated above on a microscopic time scale. In this case, the electron affinity (which is not known for the larger of the observed anions) will further energetically drive the reaction. It must be emphasized that all the anionic fragments are simultaneously detected within the time window of the present experiment extending from 0 (formation of the transient ion) to 8–20 μs corresponding to the (mass-dependent) traveling time of an ion from the reaction zone to the entrance of the mass spectrometer.[15] Ions decomposing within the quadrupole will strike the rods and will not be detected.

We do not have direct information on the neutral products or the question to what degree the underlying reactions are sequential or concerted. A further point concerns the mechanism of electron attachment. In the usual picture of resonances, electron attachment is pictured as accommodation of the extra electron into virtual MOs. In ribose these are σ* MOs which, however, are expected to be located at considerably higher energies within in the Franck–Condon region. It is therefore likely that a description by the usual resonance mechanism may no longer apply for the present system (see below).

In ribose the loss of one and two neutral water units is observed on the ion signals appearing at 132 and 114 amu, respectively (not shown here). With [5-^{13}C]ribose and [1-^{13}C]ribose (Figure 1) the ion signals are completely shifted to 133 and 115 amu as expected. Figure 1 shows, however, a noticeable difference in the width of the corresponding ion yield curve between the two isotopes which is probably related to the mechanism of anion formation (see below). More interestingly, by taking the *C*,1-D isotope (not shown here) the signals are also shifted to 133 and 115 amu, respectively, indicating that the *C*,1-D site is not involved in the abstraction of the neutral water units. We hence propose a sequential and/or concerted abstraction of the two water units according to Scheme 1 with the excess electron finally residing in a π*-type orbital of the corresponding cyclic structure.

Figure 2 shows the ion yields in the vicinity of 101 amu [Eq. (3)] for ribose and the ^{13}C-labeled isotopes. Obviously the nonlabeled molecule (left) also generates a comparatively smaller signal (≈25%) at 102 amu which is to some extent due to the natural ^{13}C isotopes (4%) of $C_4H_5O_3^-$. The majority of the intensity, however, has to be assigned to the ion $C_4H_6O_3^-$ associated either with the neutral channel $2H_2O + C$ or $CO_2 + 2H_2$. With the [5-^{13}C]ribose (middle) the relative intensity between 101 and 102 amu remains unchanged. This shows that 1) the reaction exclusively proceeds by the loss of 5-^{13}C and 2) the alternate stoichiometric composition mentioned above ($C_5H_9O_2^-$) can be excluded. Accordingly, by using [1-^{13}C]ribose (right), the signal at 101 amu completely disappears (within the detection limit) in favor of the signal at 102 amu (and 103 amu) which complements the above conclusion that only the C5 atom is excised while the 1-^{13}C atom remains on the negative-ion fragment. There are two more noticeable effects, namely, 1) the considerably broader resonance feature in the signal from the 1-^{13}C isotopomer (similar to Figure 1) and 2) the different relative intensities between the neighboring masses. The broader ion yield cannot be attributed to the slightly different energy resolution (120 meV in [1-^{13}C]ribose compared to 90 meV in [5-^{13}C]ribose, see also the SF_6^- calibration curve) and its origin is not yet clear. With [*C*,1-D]ribose the signals appear at 102 and 103 amu (at essentially the same intensity ratio as the nonlabeled compound and the 5-^{13}C-labeled ribose) while there is no detectable signal at 101 amu.

We can hence conclude that the ion $C_4H_5O_3^-$ appears from a reaction in which the C5 atom of the original molecule is excised while the C1 atom and the hydrogen (deuterium) at the C1 position remain on the negative ion. Within the detection limit of the present experiment, this decomposition is completely selective. For the deuterated compound this selectivity is remarkable as one could expect some kind of



Scheme 1. Sequential decomposition of the ribose transient negative ion (and its labeled analogues) formed by low-energy electron attachment. Alternate concerted reaction pathways are indicated by the dotted arrows (see text).

hydrogen scrambling as often observed in mass spectrometry. On the other hand, it has to be noted that the transient anion of formic acid generated at low energy is not subject to hydrogen scrambling as previously shown in DEA to the isotopomers HCOOD and DCOOH.[20]

From the thermodynamic point of view a sequential reaction creating the $C_4H_5O_3^-$ fragment at 101 amu (with the tentative structure shown in Scheme 1) and with the neutral channel consisting of $2H_2O + CH$ is rather unfavourable, while a concerted reaction associated with $CO_2 + 2H_2 + H$ is appreciably lower in energy (Table 1). For the fragment at 102 amu, the neutral channel becomes $CO_2 + 2H_2$, which is several hundred kJ below the alternate channel $2H_2O + C$. It remains to be explored what kind of isotope effect is responsible for intensity ratio between 102 and 103 amu arising from the decomposition of the 1-^{13}C isotopomer.

Figure 3 shows a selection of ion yields around 71 and 72 amu [Eq. (4)], which arise from the excision of two carbon atoms. The [5-^{13}C]ribose (left) shows the same behavior as nonlabeled ribose (not shown here), namely signals at 71 and 72 amu at approximately the same level of intensity, while in [1-^{13}C]ribose (Figure 3 right) and also in [*C*,1-D]ribose (not shown here) the signals are nearly completely shifted to 72 and 73 amu. We can therefore conclude that the final fragments $C_3H_3O_2^-$ and $C_3H_4O_2^-$ must contain the initial 1-^{13}C atom or the D atom from the original C1 position, respectively. In addition, the particular behavior concerning the broadening of the resonance curve when it originates from the ribose 1-^{13}C isotope is also preserved on this ion signal. As can be seen from Figure 3 there is now a small signal at 71 amu from the 1-^{13}C isotopomer. This indicates that the selectivity is no longer complete, but a small percentage of the reaction also proceeds by excision of the 1-^{13}C atom.

If one continues the line along decreasing masses we always find analogous behavior, namely, a remarkable selectivity in the way that both the 1-^{13}C isotope and the D atom originally attached to the C1 site are found on the final ionic product. The initial complete selectivity, however, is slightly degraded towards lower mass units.

Scheme 1 presents a reaction scheme (ending at the 59-amu ion) based on a sequential reaction mechanism. As considered above, the decomposition processes yielding the lighter fragments may rather proceed along energetically more favorable concerted reaction pathways. Apart from the structure of the 59-amu fragment indicated in Scheme 1, the acetate anion CH_3COO^- is also known as a stable negative ion.[21]

We finally note that low-energy electron attachment to ribose generates a number of more ionic fragments at low electron energies, namely at 107 amu ($C_3H_7O_4^-/C_2H_3O_5^-$), 46 amu ($CH_3CH_2OH^-/CH_2O_2^-$), and 17 amu (OH^-). O^- (16 amu) is also observed but only via resonant structures in the energy range above 6.5 eV. Ions at 46 amu were also observed in acetic acid[21] and propanoic acid,[22] but the geometrical and electronic structure of this compound has not been identified so far. It is interesting to note that ribose exhibits an additional resonance in the energy range around 7 eV which (apart from O^-) also decomposes into some of the larger fragment ions. It appears, however, that the decomposition of this excited transient anion is not as selective as that observed for the transient anion generated below 1 eV, as will be described and discussed in a forthcoming publication.[23]

The low-energy process discussed here must be associated with shape resonances, that is, accommodation of the extra electron into a virtual MO, leaving the initial electronic configuration unchanged. The energy of the relevant σ* MOs, on the other hand, are expected at higher energies and may thus not directly be accessed by Franck–Condon transitions. It is not known whether vibrational Feshbach resonances (VFR), acting as doorways for DEA or other mechanisms, are responsible for the mechanism of electron attachment to sugars. For the DNA/RNA bases thymine and uracil a VFR (supported by the large dipole of the molecule) that couples to σ* valence states was proposed as the mechanism for DEA at 1 eV.[11] In any case, the electronic structure of the precursor ion seems to have a strong tendency to localize the excess charge in the initial molecule towards the site around the C1 atom.

In conclusion from the data presented here it can be seen that ribose is appreciably sensitive towards the attack of very low energy electrons as it decomposes by the loss of water molecules and also by the excision of C5 (and more C-containing units) associated with the degradation of the cyclic structure. The decomposition is remarkably site selective in the way that C5 is excised while the excess charge is localized on the C1 site. Under the assumption that the gas pressure measured at the ionization gauge at one of the flanges behaves similarly for SF_6 and the gas-phase ribose molecules (with respect to the pressure in the reaction zone), we can

estimate the absolute cross section for a particular DEA reaction by taking the known absolute electron attachment cross section for thermal electron attachment to SF_6 generating SF_6^- (2.5×10^{-18} m^2[24,25]). This procedure results in absolute DEA cross sections for the above processes in the range of 10^{-21}–10^{-20} m^2, which is close to the geometrical cross section of a ribose molecule.

For the problem of the molecular mechanism of DNA damage by electrons it appears that the sugar itself presumably plays the most active role as a scavenger for low-energy electrons. Both the isolated DNA bases and also the phosphate group[23] are active electron scavengers, however, at energies appreciably above 0 eV. It remains to be investigated to what degree the presently studied DEA reactions are preserved when sugar is coupled to the phosphate group and to what degree the C5–O bond is involved. Rupture of the C5–O sugar–phosphate bond would represent a single-strand break, and as demonstrated here the C5 atom is selectively excised from isolated ribose.

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Communications

DNA Damage

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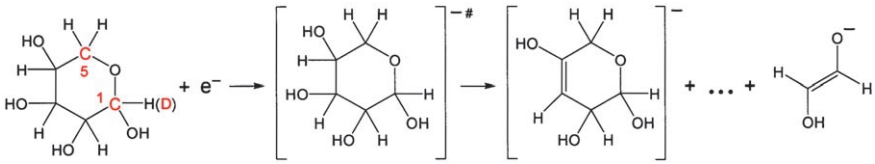
Selective Excision of C5 from D-Ribose in the Gas Phase by Low-Energy Electrons (0–1 eV): Implications for the Mechanism of DNA Damage



Attachment of low-energy electrons to D-ribose triggers a series of complex decomposition reactions associated with the loss of neutral water molecules as well as the excision of C-containing units leading to the degradation of the cyclic structure of the sugar. This excision of C-containing neutral fragments involves C5 exclusively. The sugar unit is thought to play a key role in the mechanism of DNA damage by low-energy electrons.