Metastable decay of deprotonated deoxynucleosides in matrix assisted laser desorption/ionization.

Flosadottir HD, Ómarsson B, Ingólfsson O
Science Institute, University of Iceland, Dunhagi 3, 107 Reykjavík, Iceland
odduring@hi.is

Abstract. Here we present a study on the metastable decay of the deprotonated deoxynucleosides dC, dT, dA and dG. We discuss the underlying mechanisms and compare the fragmentation pattern we observe to the fragment formation observed through dissociative electron attachment. The deprotonated deoxynucleosides show characteristic decay spectra which composition is mainly governed by the nature of the base, i.e., if the deoxynucleoside contains a pyrimidine or a purine base. The deoxynucleosides containing purine decompose predominantly through the simple rupture of the glycosidic bond, leading to the observation of the negatively charged, deprotonated base \([B-H]^-\). The only other fragment observed for dA and dG is \([B+C_2H_2]^-\). The deoxynucleosides containing pyrimidine (dC and dT) show a considerably richer fragmentation pattern with one of the dominating fragments being the negative ion of the high electron affinity fragment NCO. In the case of dT all other fragments may be traced to decomposition of the sugar moiety. The fragmentation spectra of dC on the other hand are governed by the decomposition of the base.

1. Introduction
The increasing use of radiation and radioactive substances for diagnostics and treatment in the modern health care depends on better understanding of the influence high energy radiation has on living organism. Though the physics of the interaction of electromagnetic radiation with material was already fairly well understood in the early 20th century we still have a long way to go to understand the complex chemical processes that may be induced by such radiation in a living organism. When high energy radiation passes through dense media such as water, it causes ionization and bond rupture along its track, thereby leaving a trace of reactive species such as ions, radicals or free electrons. In a living organism, these reactive species may lead to a sequence of chemical processes that in the end cause sufficient damage to destroy the organism. It has long been believed, that the radical spices formed along this radiation track play the dominating role in initiating the chemistry that causes the actual damage. However, low energy electrons (\(E_{kin} < 20\) eV) are one of the most abundant species along the track [1] with an yield of about \(5 \times 10^4\) per MeV of the incident radiation[2]. In the last decade the interaction of low energy electrons with biologically relevant molecules has been studied on a variety of systems. These studies include the attachment of electrons with an incident energy in the range from 0-20 eV to the nucleobases [3-8], ribose,[9,10] deoxyribose[11] and phosphodiesters[12]. The experiments show clearly that electrons in this energy range can cause substantial damaged to the building blocks of one of the most essential biological molecule, the DNA. This has also been clearly demonstrated by low energy electron irradiation of plasmide DNA condensed on a surface. These experiments show that low energy electrons can cause both single- and
double strand brakes of the DNA through a resonant attachment process \[13\]. This is a fairly well understood process that has been studied on a variety of molecular splices throughout the last decades. The first step is the formation of a transient negative ion (TNI) through a vertical transition from the electronic ground state of the neutral molecule to the potential energy surface of the anion formed (Frank-Condon transition).

\[
e^- + ABCD \rightarrow ABCD^- . \tag{1}
\]

The TNI is formed in an excited state which excess energy \(E^0\) comprises the incident energy of the attached electron and the molecules electron affinity. This TNI can relax by ejecting the electron again, i.e., through *autodetachment*. However, it may also relax through dissociation to form a negatively charged fragment and its radical counterpart, a process that can often be described within the framework of a diatomic dissociation along the dissociative asymptote of a repulsive state. This is in particular the case when the molecule in question is fairly simple and the incident energy of the electron low:

\[
ABCD^- \rightarrow AB + CD^- . \tag{2}
\]

However, as the molecule becomes more complex the lifetime of the TNI generally becomes longer. The excess energy can be distributed among the large number of vibrational degrees of freedom and the resulting fragments may still contain considerable amount of internal energy.

\[
ABCD^- \rightarrow AB^{(\alpha)} + CD^{(\beta)}. \tag{3}
\]

As a consequence further fragmentation may take place on a comparatively long time after the initial dissociation, hence leading to the formation of secondary fragments on the metastable time scale.

\[
CD^{(\beta)} \rightarrow C + D^- . \tag{4}
\]

In the case of reaction (4) where the dissociating fragment \(CD^{(\beta)}\) is diatomic the total excess energy is transformed into the kinetic energy of fragments C and D.

Here we present a study on the metastable decay of the deoxynucleosides dC, dT, dA and dG. We discuss the underlying mechanism and compare the fragmentation pattern we observe to the fragment formation observed through DEA.

2. Experimental

The experiments were carried out on a RFLEX IV (Bruker Daltonics, Bremen Germany), a UV-MALDI-TOF reflectron. The RFLEX IV is equipped with a 400 µJ/pulse N₂-Laser operating at 10 Hz and 337 nm. All experiments were carried out in negative ion mode by gating a selected parent ion into the field free linear flight tube and reaccelerating them with the reflectron after the linear flight. Pulsed delayed extraction with 200 ns delay time was used for the ion extraction and the gate was operated with a ±4 Da width. The total acceleration voltage through the linear region was 27.8 kV resulting in about 20 µs flight time. This flight time defines the time window within which we observe metastable decay. At the end of the linear flight tube the ions are decelerated and reaccelerated with a grid-less reflectron and detected on a double micro channel plate detector in chevron configuration which is operated at 1600 V. To assure for collection and detection of all fragment masses the reflectron voltage is stepped down in 9 segments and one part of the mass spectra is recorded at the time. Every segment is the sum of 400 shots. By attenuation the laser power was kept the same in all experiments and was set to be about 10% above the detection threshold for the analytes. During acquisition the laser spot is moved manually over the sample to try to average out sample inhomogeneities. All control of the recording of individual segments is done with the Fragmentation Analyses and Structural TOF method FAST, within the data acquisition and instrumental control software FlexControl®. The pasting of individual segments of the mass spectra to form a whole post source decay spectra and the mass calibration is carried out with the data handling
software FlexAnalyses®, provided by the instrument manufacturer. The accuracy of the mass calibration was verified by comparing the measured mass of the de-protonated molecular ions and that of the base fragment with the calculated mass.

The samples were prepared on a stainless steel sample plate using bisbenzimide as matrix. A 0.5 µL of the matrix solution (1 mg/mL) was pre-spotted on the plate and allowed to dry at air. When the matrix solution had dried, 0.5 µL of the sample solution (10 mg/mL) was added on top of the spot and let dry in open air.

3. Results and discussion
In this paper we present data on the metastable decay of the negatively charged (deprotonated) deoxynucleosides; deoxyadenosine (MW; 251 amu), deoxyguanosine (MW; 267 amu), deoxycytidine (MW; 227 amu) and deoxythymidine, (MW; 242 amu). These are the four building blocs that make up the DNA when linked by a phosphodiester bridge between the 5’ and 3’ carbon of the adjacent sugar moieties. When considering the similarities and differences between the individual deoxinucleosides we must look at the different structures of the individual bases. From their general structural properties the bases may be classified into two groups, pyrimidine bases and purine bases. Figure 1 shows the structure of the different deoxynucleosides. The purine bases adenine and guanine have the same core structure and are both linked to the sugar moiety at N-9 of the base through the glycosidic bond. From the chemical point of view however these bases differ significantly. While the only functional group on the adenine is the primary amine group at C-6 the guanine has an acetic proton at the N-1 and a thereto conjugated keto-group at C-6. The situation is similar for the pyrimidines cytosine and thymine. Though both have a keto-group at C-2 only thymine has an acetic proton at the N-3 which deprotonation may be stabilized through conjugation with the carboxylic group at C-2 or with the carboxyl group at the C-4. Cytosine on the other hand has a primary amine in this position (C-4).

![Figure 1. Structure of the deoxynucleosides; 2’-dA, 2’-dG, 2’-dC and 2’-dT.](image)

The deprotonation enthalpies of the different deprotonation sites of the isolated nucleobases and related model compounds have been determined, both by calculations and by experiments[14-24]. The general trend is that the secondary amines are found to be more acetic than the primary amines and the deprotonation enthalpies for the hydrogens bound to the carbon atoms of the rings are the highest. Calculations on the deprotonation enthalpies for the 5’- and 3’-phosphate mononucleotides that take into account stabilization of the respective deprotonation sites through intramolecular interaction show for the deoxynucleotides that the phosphate group is the most acetic functional group. The deprotonation enthalpies for the 3’-phosphate mononucleotides are, however very similar for the phosphate group and the 5’ hydroxyl group[14].

Figure 2 shows the PSD spectra of the deprotonated deoxynucleosides dC, dT, dA and dG. The predominating fragmentation observed for dT, dA and dG is the cleavage of the glycosidic bond leading to the negatively charged base. All the deoxynucleosides also show a prominent peak that corresponds to [B+26]–, i.e., the base with a C2H2 fragment from the sugar moiety attached. For the
nucleosides containing the purine bases these are the only two fragments observed. The pyrimidine containing nucleosides on the other hand show a considerably more extended fragmentation pattern. Both thymidine and cytidine show a prominent peak at 42 amu which we attribute to the formation of NCO$^-$ through decomposition of the corresponding pyrimidine bases. This peak is not observed from dA and dG which both lack the keto-group at the carbon adjacent to the nitrogen of the glycoside bond.

With the exception of the NCO$^-$ formation the fragmentation of thymidine is, like the fragmentation of the purine containing nucleosides, limited to decomposition of the deoxyribose unit. The fragments observed correspond to the loss of water, C$_2$H$_3$O and C$_3$H$_7$O$_2$ from the deprotonated thymidine. Cytosine on the other hand shows predominantly fragmentation of the base. The dominating fragment from cytosine is [C-18]$^-$ corresponding to the loss of ammonium or a neutral hydroxyl group from the deprotonated base. Other fragments from cytosine that result from the decomposition of the base are NCO$^-$ the mass 166 amu that can only be explained as the deprotonated deoxyribose with NCO attached through the glycosidic bond and the mass 67 which corresponds to the complimentary fragment to NCO, i.e., C$_3$H$_3$N$_2$.

Chipuk et al.[14] calculated the deprotonation enthalpies for the 5'- and 3'-phosphate mononucleotides. As expected, they found the phosphate group to be the most acetic functional group for all the phosphate mononucleotides. However, for the 3'-phosphate mononucleotides the deprotonation enthalpies were very similar for the phosphate group and the 5' hydroxyl group. For all 3'-phosphate mononucleotides they found the deprotonation enthalpies for the primary and secondary amines of the bases to be considerably higher than for the hydroxyl group. This is also true for the 5'-phosphate mononucleotides with the exception of 5'-dCMP where the acidity of the 3'-hydrogen and
the secondary amine of the base are found to be comparable and 5′-dGMP where both the primary and secondary amine groups of the base are considerably more acetic than the 3′-hydrogen. In all cases the deprotonation enthalpies for the 5′-hydrogen are considerably lower than for the 3′-hydrogen and comparable to the deprotonation enthalpy of the phosphate group.

These deprotonation enthalpies may not apply to the deoxynucleosides as the deprotonation enthalpies of the monophosphates are influenced by the possible stabilization through intramolecular interaction of the phosphate group with the respective deprotonation site. However, we observe the same fragmentation pattern for all the deoxynucleosides, and those are dominated by the decomposition of the sugar moiety. Furthermore the observed fragmentation of the base can in most cases be explained by the preceding decomposition of the deoxyribose. We therefore assume that the relative deprotonation enthalpies as calculated by Chipuk et al. also apply to the deoxynucleosides and that the preferred deprotonation site is the 5′-hydroxylgroup. The bulk of the fragmentation channels observed may then readily be explained by elimination of the 2′-hydrogen through the formation of a six member cyclic transition state.

Figure 3 shows the proposed mechanism leading to the [B+C \_2H \_2]^− fragment formation from the deoxynucleotides. The initial step is the stabilization of the deprotonated sugar moiety through the formation of a six member ring involving the 5′-hydroxylgroup and the 2′-hydrogen. The resulting elimination of the 2′-hydrogen then leads to the formation of a double bond between C-1 and C-2 of the sugar and a rupture of the ether bond between C-1 and the oxygen in the 4′-position. This results in the formation of [B+C \_2H \_2]^− and a neutral fragment which may stabilize through the formation of 1,3-Dihydroxy-propan-2-one by transfer of the 4′-hydrogen to the 3′-position of the sugar.

![Figure 3. Proposed mechanism leading to the [B+C \_2H \_2]^− fragment formation from the deoxynucleotides.](image)

Beside the cleavage of the glycosidic bond leading to the formation of the [B-H]^− fragment, this is the only channel observed for the purine containing deoxynucleosides.

The pyrimidine bases on the other hand, show a considerable richer fragmentation pattern indicating a destabilization of fragment (1) through connection to the formamid-like part of the pyrimidine ring, i.e., the NCO sequence. Figure 4 shows a proposed mechanism for the [NCO]^− formation for dT and dC from fragment (1).

![Figure 4. Proposed mechanism for the [NCO]^− formation for dT and dC from fragment (1).](image)

Considering the high electron affinity of NCO and the higher stability of ethyne as compared to the deprotonated ethene unit the reaction must be presumed to be energetically highly favorable. This is also apparent from the high intensity of the [NCO]^− fragment in the PSD spectra of dC and dT.
The same mechanism also explains the cleavage of the glycosidic bond to form the negatively charged base and ethylene. The negative charge can then readily be stabilized on the exo-cyclic oxygen in the case of the pyrimidines and through delocalization in the case of the purin bases. For cytosine however the deprotonated base is instable and decomposes further by the loss of NH$_3$ or a neutral hydroxy group. Judging from the fact that this channel is not at all observed for thymidine and that the NH$_2$ group is placed with a hydrogen in ortho- and meta position we favor the explanation that the mass 93 amu is due to the loss of NH$_3$ from the deprotonated cytosine. However that may be, it is puzzling that the deprotonated cytosine decomposes to this extend. This is especially true as the base should be readily stabilized by the formation of the alcohohol of the 4-amino-pyrimidin-2-ol. Instead we observe a elimination of either NH$_3$ or OH, which in both cases would leave a pyrimidin anion with two carbon centered radicals, an anion that is bound to relax by ring opening.

Free electron attachment to the nucleobases has been studied quite thoroughly in the last decade for electrons with kinetic energy in the range from few meV to about 20 eV [3-8]. Due to the thermal instability of the deoxynucleosides and nucleotides much less experimental gas phase work has been reported on these species. Nonetheless, electron attachment to thymidine has been studied experimentally[25] and theoretical calculations for both nucleosides and nucleotides have been conducted[26-31]. Electron attachment to thymidine is found to lead to hydrogen abstraction and rupture of the glycosidic bond but no further fragmentation is observed. Electron attachment to the nucleobases predominantly leads to the formation of [B-H]$^-$ through a number of low energy resonances centered around 1-2 eV but also through core excited resonances between 5-10 eV. In all cases the higher energy resonances also lead to the high electron affinity fragments CN$^-$ and NCO$^-$. Guanine behaves somewhat different from the other bases in the sense that the high electron affinity fragments dominate whereas [B-H]$^-$ only appears with comparably low intensities. We have not looked at metastable decay products below 35 amu, however, the dominating NCO$^-$ formation we observe from the pyrimidin containing nucleosides is also the most prominent decay channel we observe from the deprotonated nucleobases U, T and C (not shown here). This shows that the pyrimidine bases form NCO$^-$ on the metastable time scale independent of the formation mechanism of the parent ion [B-H]$^-$, i.e., independent of if [B-H]$^-$ is formed direct through deprotonation of the base or if it is formed through the loss of the sugar moiety from the nucleoside. This may indicate that the same mechanism is responsible for the formation of these fragments through DEA, i.e., that they are formed through metastable decay of the deprotonated parent ion rather than a direct dissociation of the initially formed TNI. We do however not observe these fragments from the purine containing deoxynucleosides, neither do we observe them from the purine bases directly. The explanation may be that the parent ions that are deprotonated in the MALDI process experience multiple collisions before they are mass selected and contain considerably less excess energy than the [B-H]$^-$ ion that results from the attachment of a 5 eV electron to a precursor that has been brought into the gas phase through heating above its sublimation temperature.

### 4. Conclusions

The deprotonated deoxynucleosides show characteristic decay spectra which composition is mainly governed by the nature of the base, i.e., if the deoxynucleoside contains a pyrimidine or a purine base. The purine containing deoxynucleosides decompose predominantly through the simple rupture of the glycosidic bond, leading to the observation of the negatively charged, deprotonated base [B-H]$^-$.

The only other fragment observed for dA and dG is [B+C$_2$H$_2$]$.^+$ The pyrimidine containing nucleosides dC and dT show a considerably richer fragmentation pattern with one of the dominating fragments being the negative ion of the high electron affinity fragment NCO. In the case of dT all other fragments may be traced to decomposition of the sugar moiety. The fragmentation spectra of dC on the other hand are governed by the decomposition of the base. The dominating NCO$^-$ formation we observe from the pyrimidin containing nucleosides is also the most prominent decay channel from the deprotonated nucleobases U, T and C (not shown here), showing that the pyrimidine bases form NCO$^-$ on the metastable time scale independent of the formation mechanism of the parent ion [B-H]$^-$. 


Acknowledgement
This work was supported by the Icelandic Centre for Research (RANNIS) and by the University of Iceland Research Fund.

References
[1] International Comission on Radiation Units and Measurements, Washington, DC, 1979.
[2] Cobut V, Frongillo Y, Patou J P, Goulet T, Fraser M J, Jay-Gerin J P, 1998 Radiation Physics and Chemistry 51 229-43.
[3] Hanel G, Gstir B, Denifl S, Scheier P, Probst M, Farizon B, Farizon M, Illenberger E, Märk T D, 2003 Physical Review Letters 90.
[4] Ptasińska S, Denifl S, Scheier P, Illenberger E, Märk T D, 2005 Angewandte Chemie-International Edition 44 6941-3.
[5] Ptasińska S, Denifl S, Mroz B, Probst M, Grill V, Illenberger E, Scheier P, Märk T D, 2005 Journal of Chemical Physics 123.
[6] Abdoul-Carime H, Langer J, Huels M A, Illenberger E, 2005 European Physical Journal D 35 399-404.
[7] Abouaf R, Dunet H, 2005 European Physical Journal D 35 405-10.
[8] Scheer A M, Aflatooni K, Gallup G A, Burrow P D, 2004 Physical Review Letters 92.
[9] Bacarelli I, Gianturco F A, Grandi A, Sanna N, Lucchese R R, Bald I, Kopyra J, Illenberger E, 2007 Journal of the American Chemical Society 129 6269-77.
[10] Bald I, Kopyra J, Illenberger E, 2006 Angewandte Chemie-International Edition 45 4851-5.
[11] Ptasińska S, Denifl S, Scheier P, Märk T D, 2004 Journal of Chemical Physics 120 8505-11.
[12] König C, Kopyra J, Bald I, Illenberger E, 2006 Physical Review Letters 97.
[13] Boudaiffa B, Cloutier P, Hunting D, Huels M A, Sanche L, 2000 Science 287 1658-60.
[14] Chipuk J E, Brodbelt J S, 2007 Journal of the American Society for Mass Spectrometry 18 724-32.
[15] Chen E C M, Herder C, Chen E S, 2006 Journal of Molecular Structure 798 126-33.
[16] Chen E C M, Chen E S, 2000 Journal of Physical Chemistry B 104 7835-44.
[17] Chen E S D, Chen E C M, Sane N, 1998 Biochemical and Biophysical Research Communications 246 228-30.
[18] Chandra A K, Nguyen M T, Uchimaru T, Zeegers-Huyskens T, 1999 Journal of Physical Chemistry A 103 8853-60.
[19] Freitas M A, Shi S D H, Hendrickson C L, Marshall A G, 1998 Journal of the American Chemical Society 120 10187-93.
[20] Freitas M A, Marshall A G, 2001 Journal of the American Society for Mass Spectrometry 12 780-5.
[21] Huang Y Q, Kenttamaa H, 2003 Journal of Physical Chemistry A 107 4893-7.
[22] Huang Y Q, Kenttamaa H, 2004 Journal of Physical Chemistry A 108 4485-90.
[23] Lee J K, 2005 International Journal of Mass Spectrometry 240 261-72.
[24] Robinson J M, Greig M J, Griffey R H, Mohan V, Laude D A, 1998 Analytical Chemistry 70 3566-71.
[25] Ptasińska S, Denifl S, Gohlke S, Scheier P, Illenberger E, Märk T D, 2006 Angewandte Chemie-International Edition 45 1893-6.
[26] Winstead C, McKoy V, Sanchez S D, 2007 Journal of Chemical Physics 127.
[27] Kumar A, Sevilla M D, 2007 Journal of Physical Chemistry B 111 5464-74.
[28] Winstead C, McKoy V, 2006 Journal of Chemical Physics 125.
[29] Gu J D, Wang J, Leszczynski J, 2006 Journal of the American Chemical Society 128 9322-3.
[30] Li X F, Sanche L, Sevilla M D, 2006 Radiation Research 165 721-9.
[31] Gu J D, Xie Y M, Schaefer H F, 2006 Journal of the American Chemical Society 128 1250-2.