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Ionization and fragmentation of DNA, RNA bases induced by proton impact

A Le Padellec, P Moretto-Capelle, M Richard-Viard, JP Champeaux and P Cafarelli

Laboratoire Collisions, Agrégats, Réactivité, UMR 5589, CNRS – Université de Toulouse, Bât IIIR1 b4, 118 route de Narbonne, 31062 Toulouse Cedex 9, France

arnaud.lepadellec@irsamc.ups-tlse.fr

Abstract. We present recent results obtained in the Toulouse's group that deal with proton to base and nucleoside interactions. We stress the weakness of the sugar part in the nucleoside, i.e. the uridine molecule under scrutiny. Since some parts of the fragmentation spectrum correspond to the fragmentation of a 'pure' uracil molecule, i.e. the RNA base, an 'additivity rule' seems to prevail for the nucleoside, something that still has to be confirmed. Moreover, some results that deal with the secondary electronic emission from uracil are also displayed.

1. Introduction

Radiotherapy is a non ablative tool used to sterilize tumoral cells while irradiating them with customized radiation beams. Subsequently, sterilization or irreversible DNA damage is the consequence of a complex chain of physical, chemical and biochemical events triggered by the energy deposition along the radiation track. A simulation of the irradiation is therefore essential to define and / or refine the best radiotherapy strategy, and at the present time, several simulation codes are indeed available. Some of them are based on the simulation in a particular physical, chemical or biochemical stage with an ever-increased complexity [1] . Some others attempt to describe a sufficiently large time domain in order to bridge the gap between physical, chemical and biological stages [2] . In any case, the simulation transport of a moving particle within a given medium is managed through a Monte-Carlo scheme built on the following pivotal points: type of interaction (target nature, ionization or excitation), kinematics of the interaction (energy loss, scattering angle) and mean free path value, "reaction products" (secondary electrons, recoil cluster of atoms). It is obvious, specifically from the latter point, that there is a clear need for data that deal with elementary processes (cross sections, branching ratios) and among them, those that concern molecular targets.

This communication presents recent results we have gained on the subject of ionization and fragmentation of isolated DNA, RNA bases and uridine nucleoside induced by 25 – 100 keV proton impact. This is organized as follows. We first comment on the Bragg peak and subsequent DNA damages. The experimental set-up is then briefly presented, whereas the results are described and discussed in some details. Some conclusion and perspectives are finally given.

2. Proton transport in matter: the Bragg peak

In proton therapy, the incident energy ranges from 10 to 250 MeV, the upper choice allowing irradiation of the deepest point in a human body, i.e. 30 cm. Let us first consider the ballistic point of

view, with the transport of an incident 100 MeV proton beam into liquid water, the prototype of human tissue. This beam would experience a severe deceleration within the target medium, and would deposit a large dose at a given depth, in a well-defined volume, i.e. the so-called the Bragg peak. This can be seen in figure 1, which displays the proton stopping power in liquid water as a function of the proton energy. The curve displays a maximum for proton energies of ≈100 keV [3] that corresponds to maximum energy deposition (maximum of the ionization cross section curve). Let us now consider the biological point of view. The effects of radiation on biological systems can be directly related to alteration of the DNA molecule and single and/or double strand breaks might lead to a cell's death or generate mutation. In order to investigate the damages caused by the proton all over its track, we have performed simulations using the Monte Carlo Damage Simulation (MCDS) algorithm described in [4, 5] . The results are displayed in figure 2 and show that the number of DNA damages, such as single strand breaks –SSBs- or double strand breaks –DSBs-, increases with decreasing proton energies. Those damages are maximized around 100 keV, with a Linear Energy Transfer –LET- of about 80 keV per micron. The Relative Biological Effectiveness –RBE- is meanwhile maximised with this latter value of the LET as was shown also by Rodriguez et al [6] .

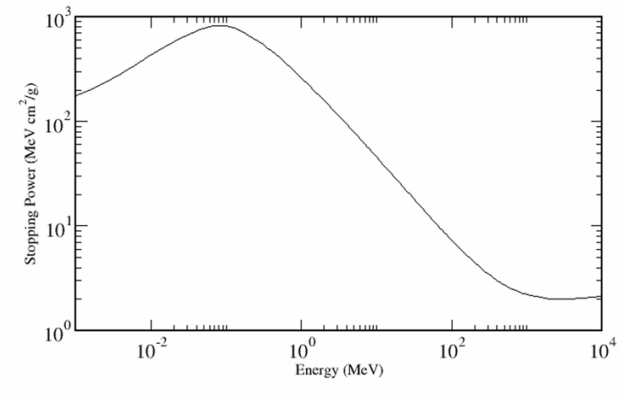

Figure 1. Stopping power in liquid water as a function of the proton energy.

3. Experimental setup and procedure

A pulsed 25-100 keV proton beam impinges at right angle on an effusive jet containing DNA or RNA bases. The jet is produced by sublimation of commercial powders placed in an oven heated to about 120°C.

Charged products, i.e. parent molecules and their fragments, are mass over charge analyzed within a time-of-flight (TOF) cell operating in second order space focusing [7] . A pulsed extraction field (1.5 kV with 10 ns rise time) is triggered once the bunch of projectile ions has reached the collision zone to ensure the measurement being independent of the proton beam width. This results in a substantial increase of the time-of-flight resolution, and consequently in the mass resolution. After the drift zone, the positive fragments are post-accelerated in order to improve their detection efficiency on a multichannel plate assembly (MCP), in front of which a negatively biased grid repels the secondary electrons towards the assembly. The detection efficiency is therefore greatly enhanced from 60 % (open area ratio of the MCP) up to 95 % [8] . Ionic TOF spectra are recorded by means of a Fast Comtec 7885 multistop module, with 5 ns resolution.

4. Results and discussion

4.1. Base fragmentation

As it can be seen in figure 2, base damage in proton-DNA interaction is the dominant process, although not necessary the most dangerous for living organisms. It can also produce mutation by simple tautomerization [9] . Some work was previously done on the subject of base fragmentation using proton impact [10] and multicharged ion impact [11, 12] . Within the energy range under consideration, the single ionization is an important process, directly accessible in single stop time-of-

flight spectra, i.e. singly charged fragments correlated to –unobserved- neutral ones. Such spectra are displayed on figure 3 for the three pyrimidine targets: cytosine, thymine and uracil. One might note the production of the C^+, CH^+, N^+, NH^+ and OH^+ fragments, though not being dominant. The production of mass 28, i.e. the CNH_2^+ or CO^+ fragments, appears efficient from the three base targets. A common feature to the three displayed bases is the emission of the HNCO group [13] . The other fragments, particularly noticeable for the uracil and cytosine molecules in the 67 to 69 mass ranges, may themselves experience the emission of a carbon monoxide molecule. This gives rise to the sizeable ionic yield in the 39 to 43 mass ranges. The very same mechanism was indeed shown by Impotra *et al* [13] in the thymine case, with a resulting 55 amu charged fragment, also visible in our spectrum.
All these fragmentation features can be rationalized from the chemical composition and initial molecular arrangement of the target bases. Although not visible on figure 3, we also studied the correlation between charged fragments, the subject of a forthcoming communication. In brief, it is noticeable from the two-charged fragment correlation spectra that 'stable' doubly charged parent targets are rather scarce, showing the intrinsic fragility of the dicationic structures.

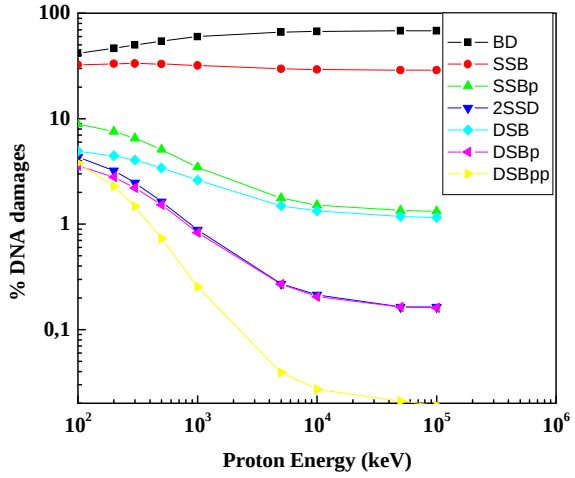

Figure 2. DNA damages induced by proton at different energies, evaluated by MCDS codes [4, 5] . Classification of damages according to Nikjoo's definition: BD bases damages, $nSSB^{(+)}$ single strand break, $DSB^{(+,++)}$ double strand break.

4.2. Nucleoside fragmentation

The next step towards complexity concerns the study of larger molecules producible in the gas phase. From that respect, a nucleoside constitutes an obvious choice that contains a base plus a sugar -an element of the DNA strand-. For sublimation's sake, we chose to study the uridine molecule, a 244 amu structure, being aware of the risk of molecular decomposition while heating the target sample. We can not exclude that our data were affected by this experimental artifact all the more because Ptasinska et al [14] observed it while studying the temperature effect on the molecular dissociation of uridine and thymidine molecules. Nonetheless, these authors observed stable structures within a certain temperature range, a range that included our oven settings. A similar finding was made by the KVI group on the collision induced fragmentation of deoxyribose [15] . Our spectrum is displayed in figure 4 and shows unfragmented uridine cations, though in quite small amount. It is divided into two mass over charge regions, that corresponding to larger masses being magnified vertically by a factor of forty. In this spectral region, the peak at 244 amu is also weak for the ionized parent uridine molecule (see points above). Other peaks at 218 and 226 amu are associated with the ablation of a C_2H_2 and H_2O molecule, respectively, whereas stronger structures at 133 and 175 amu are connected to the sugar subunit of the uridine and (tentatively) to the fragmentation within the uracil subunit, respectively. The lower part of the spectrum with mass to charge ratios comprised between 0 and 120 is –by far- the most intense one. The one stop pure uracil spectrum is superimposed in shaded area for comparison purposes as a subunit of the uridine after normalization of the two spectra on mass 112. A large part of the uridine fragmentation spectrum can be attributed to the uracil compound. Some extra peaks result in molecular rearrangement like that at 19 amu, with the formation of H_3O^+. The strongest one at 31 amu, i.e. the $[HO\text{-}CH2]^+$ fragment, is due to the fragmentation of the sugar subunit.

Comparing the respective magnitudes of the lower and larger parts of the spectrum, it follows that the emission of an unaltered sugar is unlikely, and therefore that the sugar unit, i.e. the DNA strand compound, is radiosensitive.

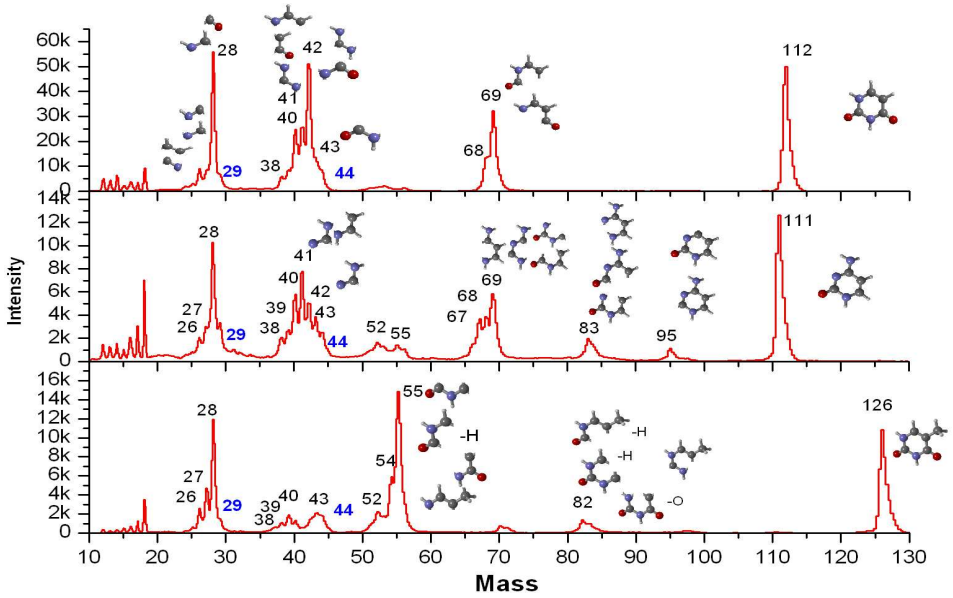

Figure 3. Mass spectra associated to fragmentation of uracil (top view), cytosine (middle view) and thymine (bottom view) induced by 100 keV proton impact.

Alvarado et al [15] showed that there is a rather complete destruction of the deoxyribose molecule under ion impact in the phase gas. Since several characteristics of the dissociation of the uracil molecule can be found in that of the uridine, we anticipate that the collision induced dissociation of the nucleoside would reflect that of the two parts (base + sugar), according to an 'additivity' rule. Using this terminology, we make the following point: the weakness of the glycosidic bond is such that any other fragmentation of the nucleoside is preceded by that of the base-sugar bond. This point will have– of course- to be strengthened by studying the sugar alone, a study under progress.

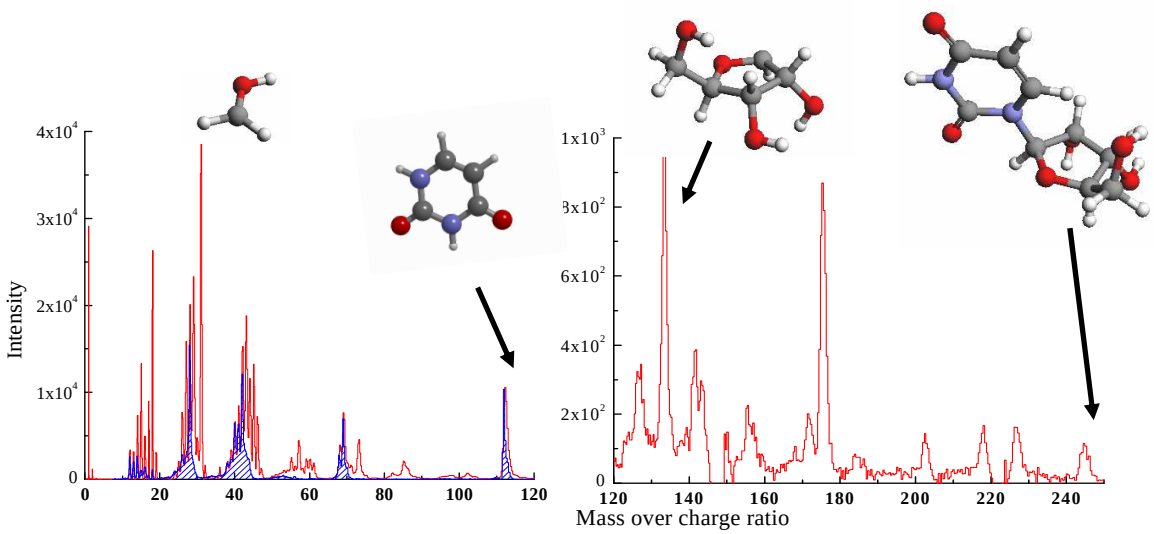

Figure 4. Mass spectrum of collision induced dissociation of uridine.

4.3. Electron emission

It is now firmly established that the interaction of energetic projectiles with molecular targets causes the emission of secondary electrons. Of special interest are the low energy electrons (< 20 eV) that are produced in a sizeable amount, well below ionization thresholds. As pointed out by Sanche and co-workers [16] , those electrons are responsible for substantial yields of single and double strand breaks in DNA, via a dissociative attachment mechanism. For larger electron energies, i.e. above ionization thresholds, direct fragmentation occurs. The knowledge of the secondary electron's energy spectrum and angular dependence in the electronic emission, is therefore of prime importance for future use as input data in track's simulation (cross section resolved in angle and energy).

Figure 5(a) displays the Double Differential Cross Sections (DDCS) for the production of secondary electrons from the uracil molecule, at the fixed angle of 35° and at proton incident energies of 25, 50 and 100 keV. No peaking is seen at low electron energy where the DDCS is found to depend weakly on proton energy. In the high energy part of the curve, the DDCS fall off exponentially due to the dipole interaction term, according to the Bethe-Born approximation [17, 18] . Also noticeable is the absence of any observable discrete structures due to Auger electrons (negligible contribution of the cross section). Moreover, we did perform a Classical Trajectory Monte Carlo (CTMC) simulation which results are displayed in figure 5(b) for 100 keV protons. For details on the simulation, one shall refer to ref [19] . The CTMC computation is in reasonable agreement with the experimental findings at 35° and while investigating the angular dependence in the electronic emission, the computation shows that electrons are emitted preferentially along the projectile direction due to the post-collision interaction.

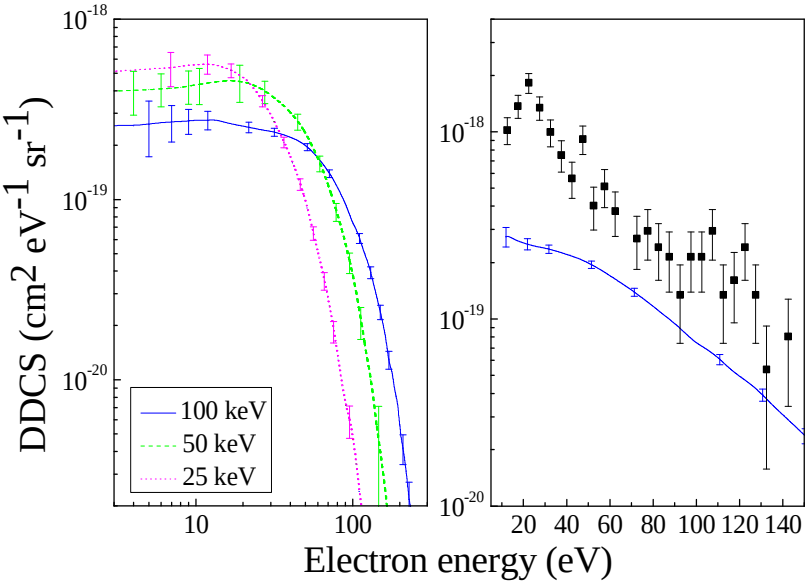

Figure 5: (a) Left: DDCS versus electron energy for the uracil molecule. Three sets of data are presented for incident proton energies of 100, 50 and 25 keV, in solid, dashed and dotted lines, respectively. (b) Right: comparison CTMC computation –in filled squares- and experimental data at 100 keV -in solid line-.

5. Conclusion and perspectives

We carried out experiments on DNA, RNA bases as well as on the uridine molecule in interaction with proton projectiles, and focused on the fragmentation and electron emission. We stress the weakness of the sugar part in the whole uridine molecule. Since some parts of the fragmentation spectrum happen to correspond to the fragmentation of a 'pure' uracil molecule, an 'additivity rule' seems to prevail for the uridine nucleoside. We plan future works on modified ribose molecules in which the missing OH radical in pure sugar could be substituted by a hydrogen atom. Indeed, the

fragmentation patterns of the complementary part of the uracil within the uridine molecule are not known. In addition, molecules used in chemotherapy that enhance the effects of radiation treatment-so-called radiosensitizers-, are also of great interest since physical mechanisms are poorly understood (see ref [20] for the action of slow electrons on the bromouridine molecule). Last, it would be very fruitful to compare -on an absolute scale- electron versus fragmentation spectra for gas phase molecules that differ by only one atom (uracil and halouracil) or those which include platinum atoms (cisplatin, carboplatin). The output might be of some relevance in proton therapy based on the 5FU-Cisplatin protocol [21] .

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