# Disentangling molecular mechanisms leading to mutagenesis via ion-induced mass spectrometry

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Keywords: radiation damage, mutagenesis, radiosensitisers, halo-uracil, tautomerisation, mass spectrometry

The bi-helical complementary base pairing structure of DNA [1] is widely considered one of the most important scientific discoveries of the 20th century. In the original formulation of mechanisms of replica of DNA, the possibility of base mispairing due to a base occasionally occurring in one of its less likely tautomeric forms was suggested as the main cause for spontaneous mutations. Indeed Topal and Fresco [2], proposed a new general base-pairing hypothesis based on chemical features and isomeric equilibria of nucleotides and nucleobases with a set of complementary base-paring wider than the two canonical, adenine-thymine (A-T) and guanine-cytosine (G-C) pairs. These non-Watson-Crick complementary bases [2] constitute a mispair due to keto-enol and amino-imino tautomerisations and give rise to mutations in DNA.

Spontaneous tautomerisation is unlikely, but it can be stimulated by external factors, like the presence of base analogues, as 5-bromouracil (5BrU), or of the water environment itself [3,4]. The large interest in 5BrU is justified by the fact that halosubstitute bases have found application as radiosensitisers in cancer therapy. The mutagenic action of 5BrU is widely accepted [5], but the basic mechanisms of such effect are still much debated. Theoretical calculations propose two leading mechanisms to explain the recognition between BrU and G, i.e. the basic mutagenesis induced by 5BrU. Both models emphasize the crucial role played by the water environment. The first mechanism is the deprotonation on the N atoms, which leads to an increase of the acidity of 5BrU (compared to U) and favors the formation of complexes with guanidine. The second one is the 5BrU keto → enol tautomerisation (figure 1). In our experiments we show that mass spectrometry performed on free biomolecules and small clusters in condition of nanosolvation, provides valuable insights into tautomerisation mechanisms.

In this work we report a study of the fragmentation of hydrated clusters of 5BrU induced by $^{12}C^{4+}$ ions. The 5BrU hydrated clusters have been produced by using a gas aggregation cluster source [6] and the fragment products have been analyzed via a time of flight mass spectrometer. The experiments have been performed at the low energy beamline, ARIBE, of the GANIL facility in Caen (France). The use of a $^{12}C^{4+}$ beam at 36 keV is motivated by the fact that multiply charged ion beams provide nowadays a very valid approach for cancer therapy, alternative to radiotherapy. The knowledge of the direct and secondary processes triggered at the nanoscale in the biological system by an energetic ion beam is of vital importance for finding the most suitable therapeutic approach and drugs. The measured mass spectra have shown the presence of several series of hydrated fragments, i.e. molecular fragments bound to an increasing number of water molecules (figures 2 and 3). Such phenomenon is barely present in the unsubstituted uracil case. This suggests that the substitution of the Br atom in the uracil molecule enhances ultrafast and intense radiation damage mechanisms, as expected for a radiosensitiser. Furthermore, the existence of hydrated series has allowed to observe that a relatively small number of water molecules in specific sites promotes the H migration from the N to the O atom (see figure 4) producing the keto → enol tautomerisation, which is the main cause of the mutagenic effect observed in 5BrU (see figure 1).

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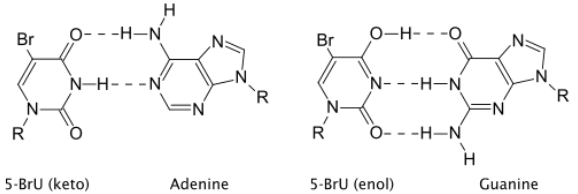


Figure 1. The keto form of 5BrU (on the left) is complementary to adenine, while the enol form (on the right) is complementary to guanine.

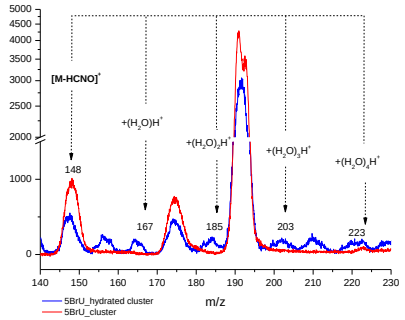


Figure 2. The series of the $[M\text{-}HCNO](H_2O)_nH^+$ hydrated fragments in the 5BrU hydrated cluster mass spectrum.

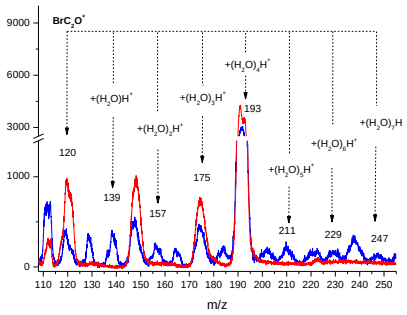


Figure 3. The series of the $(BrC_2O)(H_2O)_nH^+$ hydrated fragments in the 5BrU hydrated cluster mass spectrum.

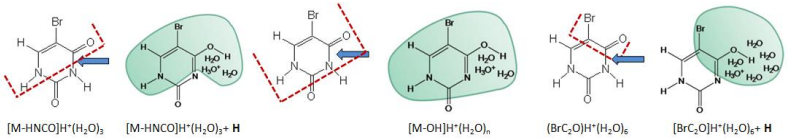


Figure 4. Schematic of the main fragments whose hydrated series are observed and assigned in the mass spectrum of hydrated clusters of 5BrU. The blue arrows indicate the suggested sites of hydration and the red dashed lines surround the charged (detected) fragment; M indicates the parent ion. The proposed tautomerisation processes mediated through the presence of a sufficient number of water molecules is also shown.

**Acknowledgements**. Progetto Galileo2012 'New Ligth on Radiosensitisers' and MIUR FIRB project 2010 (RBFR10SQZI).