Site- and bond-selective H\(^{-}\) formation in methylated pyrimidine bases driven by potassium-molecule collisions

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Abstract: Electron transfer in alkali-molecule collisions to gas phase thymine and uracil yielding H\(^{-}\) formation is selectively controlled in the energy range between 5 and 65 eV. By tuning the collision energy, electron transfer from the alkali to methylated thymine (at the N\(_{1}\) position), methylated uracil (at the N\(_{3}\) position) and partly deuterated thymine, enables H\(^{-}\) formation. Such process proceeds not only through the breaking of the (C-H) against (N-H) bonds but also through N\(_{1}\) against N\(_{3}\) sites. Such selectivity, as far as bond and site are concerned, is here reported for the first time in collision induced dissociation experiments by alkali-molecule interactions.
1. Introduction

The control of chemical reactions has drawn the attention of the physical chemistry community over the years, where several studies have been pointed out to the mechanisms and processes that allow controlling the pathways in chemical reactions to be controlled. Several experimental techniques have been used, where mode-selective excitation, stereodynamic and orbital alignment control [1-4] have been reported in molecular collisions. Another method has included ultra-short chirped laser pulses to control unimolecular reactions [5-6]. Moreover, it has recently been shown that electrons provide an even simple method of specific bond cleavage, particularly through the use of scanning tunnelling microscopes, where not only selective bonds in molecules are broken within a surface but also control of molecular motion has been made possible [7-13]. Soft x-rays have also been used in order to control chemical reactions in thin films of organic polymers [15-16]. Prabhudesai et al. [14] by means of low dissociative electron attachment (DEA) experiments have shown functional group dependent site specific fragmentation in molecules as small carboxylic acids, alcohols and amine.

As far as biomolecules are concerned, several DEA experiments have been performed mainly in the Innsbruck and Berlin laboratories. These studies have shown that at sub-excitation energies (< 3eV) electrons can efficiently induce structural damage to biologically relevant molecules such as DNA bases. By isotopic labelling, it was possible to describe the different anionic fragmentation pathways with identification of proper sites within the molecule [17-20]. These studies have shown that one of the pathways leading to $\text{H}^-$ formation in thymine upon DEA has remarkable site and bond selectivity by proper tuning of the electron energy [17-19]. $\text{H}^-$ loss proceeds not only through the breaking of the (C-H) against (N-H) bonds but also through $\text{N}_1$ against $\text{N}_3$ sites. Bald et al. have performed DEA studies on D-ribose and have shown that this molecule is very sensitive to the attack of very low energy electrons, i.e. the decomposition upon low energy electron attack is remarkably site-selective in the C$_5$ position. [20]

In atom-molecule collisions, the electron donor projectile interacts with a given molecule and electron transfer may take place. In the collision energy regime where the formation of a transient negative anion (TNI) occurs, several pathways including fragmentation into stable anionic species can be achieved.

In the present studies, electron transfer leading to site- and bond-selective excision triggered by potassium molecule interactions is reported in an energy region ranging from ~ 5 to 65 eV. Figure 1 schematically shows the three molecular targets used in these experiments. These studies were carried out by proper tuning of the kinetic energy of the neutral potassium beam in the lab frame from 14 eV up to 100 eV. Negative ion mass spectra were obtained at several collision energies in order to investigate the $\text{H}^-$ yield.

![Chemical structures of molecules used: a) 1-methylthymine, b) thymine-methyl-D3-6-D, c) 3-methyluracil. Atoms labelling colour: blue (N); grey (C); red (O); white (H) and green (D).](image-url)
2. Experimental Set-up

Negative ion time-of-flight (TOF) mass spectra were obtained in potassium atom collisions with 1-methylthymine, thymine-methyl-D3-6-D and 3-methyluracil in a crossed beam set-up described elsewhere [21, 22]. Briefly, an effusive molecular beam crosses a primary beam of fast neutral potassium (K) atoms. \( K^+ \) ions produced in a potassium ion source were accelerated in the lab frame from 14 to 100 eV, before passing through an oven where they resonantly charge exchange with neutral potassium atoms, yielding a beam of fast (hyperthermal) atoms. Residual ions from the primary beam were removed by electrostatic deflecting plates outside the oven. The intensity of the neutral potassium beam was monitored using a Langmuir-Taylor ionisation detector.

The target effusive beam passing through a 1 mm diameter slit crosses the neutral hyperthermal potassium beam. The anions produced between the two parallel plates with a mutual distance of 1.2 cm, were then extracted by a 250 V cm\(^{-1}\) pulsed electrostatic field. The typical base pressure in the collision chamber was \( 4 \times 10^{-5} \) Pa and the working pressure did not rise upon heating the powder samples. Calibration was carried out on the basis of the well-known anionic species formed after potassium collisions with the nitromethane molecule [22].

The solid samples of 1-methylthymine, thymine-methyl-D3-6-D and 3-methyluracil used in the presented experiment were purchased from Sigma-Aldrich with a minimum purity \( \geq 99\% \). The samples were heated to 473K and the temperature was controlled using a PID unit. In order to test no thermal decomposing of the molecular beam, several TOF mass spectra were recorded at different heating temperatures. No differences were observed in the relative peak intensities for the different temperatures at a fixed collision energy. The extraction region and the TOF system were heated to approximately 393 K throughout measurements in order to prevent any sample condensation and thence charge accumulation on the electrodes.

3. Results and discussion

In the present studies, mass spectra for the different molecules were recorded as a function of the collision energy where we have looked at \( \text{H}^- \) formation. Spectra were recorded at 17, 19 and 100 eV collision energies for 1-methylthymine, at 17, 30 and 70 eV for deuterated thymine and at 14, 17 and 100 eV for 3-methyluracil. Thus, from the analysis of the mass spectra was possible to infer from which site and bonds in the pyrimidine ring the \( \text{H}^- \) loss preceded. The collision energy of the potassium projectile is directly related to the available energy in the centre-of-mass system leading to fragmentation. Thus this energy is given by:

\[
E_f = \frac{m_t}{m_t + m_K} \times E_{iab} \times 0.9 - E_{iK}
\]

Where \( E_f \) is the available energy, \( m_t \) is the target mass, \( m_K \) is the potassium mass, \( E_{iab} \) is the collision energy in the laboratory frame, \( E_{iK} \) is the potassium ionisation energy and 0.9 a scaling factor due to geometry and space charge effects in the ion source.

Figure 2 shows the TOF mass spectra for the three studied molecules where in the upper panel we present the results for 1-methylthymine, in the middle for 3-methyluracil and in the lower for thymine-methyl-D3-6-D. Site position (i.e. \( \text{N}_1 \) or \( \text{N}_3 \)) can be discriminated by using thymine and uracil methylated molecules. In the case of 1-methylthymine, the H atom from the \( \text{N}_1 \) position is blocked by adding a methyl group whereas in 3-methyluracil the \( \text{N}_3 \) position is blocked. By comparing 1-methylthymine at 7.5, 9.0 and 64.5 eV with 3-methyluracil at 5.5, 7.5 and 64.5 eV, a clear distinction of \( \text{H}^- \) formation through \( \text{N}_1 \) and \( \text{N}_3 \) positions is observed. For 1-methylthymine, when the available energy is tuned to energies below 8 eV no \( \text{H}^- \) formation is detected. However, when the energy is increased from 8 eV up to 64.5 eV, \( \text{H}^- \) formation is observed. The profile resonances obtained by dissociative electron attachment experiments show broad structures at around 9 and 10 eV. These two resonances correspond to \( \text{H}^- \) formation from \( \text{C}_6 \) and \( \text{CH}_3 \) positions, respectively [17].
Looking at 3-methyluracil mass spectra where the N₃ position is blocked by a methyl group, H⁻ formation is observed for all collision energies, since the resonance for H⁻ formation from the N₁ is below the available energy in the current experiments. In the 3-methyluracil DEA resonance profiles, the main peak is observed at around 5 eV, corresponding to H⁻ formation from the N₁ position. This evidence allows us to conclude that H⁻ formation at low energies, i.e. below 8 eV, only stems from the N₁ position. A close inspection to the 1-methylthymine mass scans at 9.0 eV shows that it is possible to assign H⁻ formation due to even a weak contribution. Gas phase studies of Ptasinska et al. [16] showed that the resonance centred at ~7 eV, corresponding to N₃ position abstraction, is about one third of the resonance corresponding to N₁ abstraction. Due to the sensitivity of the present TOF and technical characteristics in intensity of the potassium beam, H⁻ formation may be attributed to the broad plateau. This plateau is due to the rate of formation of such anions and is due to an experimental artefact based on the pulsing shape of the extraction voltage (for more details see ref. [22]). Though we can conclude that H⁻ abstraction from the N₁ position is exclusively attributed to energies below ~8 eV, whereas for energies between 8 and 9 eV, H⁻ abstraction from the N₃ position becomes visible and for higher energies, in the mass spectra at 64.5 eV, the corresponding H⁻ signal is achieved through all possible positions in the thymine ring.

As far as bond selectivity is concerned, a close inspection to the deuterated thymine TOF mass spectra (lower panel in Figure 2) will reveal such a process. The D⁻ loss DEA resonance for this molecule has two peaks centred at around 8.5 and 10 eV [17], since the methyl group and the C₆ positions are isotopically labelled with deuterium. While H⁻ resonances are centred at around 5.5 eV and 7 eV, D⁻ formation is suppressed at low energy (~7.5 eV) but at ~15.5 and ~44 eV, its formation becomes evident. Therefore, for collision energies below 8 eV, H⁻ formation proceeds exclusively through the N-H bonds in contrast to energies above 8 eV where D⁻ abstraction proceeds from the C-D bonds. Although this is not particularly evident at 30 eV in the lab frame, a close inspection reveals a small contribution that lies slightly above the background level, almost identical to the H⁻ signal.

![Figure 2](image-url)
4. **Conclusions**  
We have studied electron transfer induced processes in collisions between potassium atoms and methylated pyrimidine molecules. Negative ion TOF mass spectra were obtained at different collision energies. In an unprecedented study we have shown that by tuning the collision energy, electron transfer from the alkali to methylated thymine (at the N1 position) and methylated uracil (at the N3 position), yields H formation not only through the breaking of the (C-H) against (N-H) bonds but also through N1 against N3 sites. Making use of partly deuterated thymine, bond selectivity (N-H against C-H) has been demonstrated. These findings may have particular relevance for the investigation of early molecular processes in the nascent stages of DNA damage by secondary electrons, especially those related to strand breaks.

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