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Fragmentation of laser-desorbed 9-substituted adenines

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Abstract

In laser desorption of a series of cyclically 9-substituted adenines, followed by multiphoton ionization, we observed characteristic fragmentation. This is in marked contrast to laser desorption under identical conditions of cyclically 9-substituted guanines, for which we obtained predominantly the parent mass. By applying resonant 2 photon ionization spectroscopy (R2PI) to the laser-desorbed, jet-cooled fragments, we show that the fragmentation is induced by the desorption process and not by the excitation/ionization process. Fragmentation takes place between C′2 and C′3 and between C′1 and O6 in the sugar ring. By adding a propyl bridge between C′2 and C′3, the fragmentation pathway changes to a cleavage of the C′4–C′5 bond in the sugar ring. This allowed us to record R2PI spectra of a fragment species that can serve as a model for adenosine. (Int J Mass Spectrom 219 (2002) 133–138)

Keywords: Fragmentation; Adenines; R2PI spectrum; Laser desorption; Jet-cooling; DNA bases

1. Introduction

Techniques for bringing DNA molecules and their building blocks into the gas phase are increasingly important for two reasons. First, there is a great interest in analytical mass spectrometry for these types of compounds. Secondly, the gas phase offers ideal conditions for the study of intrinsic molecular properties, free of interactions. Common approaches include matrix-assisted laser desorption ionization (MALDI), [1] electrospray ionization (ESI) [2] and fast atom bombardment (FAB). While these techniques enable vaporization of large organic molecules with low vapor pressures, they are designed to produce ions rather than neutrals. Neutrals can be produced by thermal heating but this approach is of limited use for these compounds, because most are thermally unstable. Among purine bases adenine and adenosine are distinctly more stable than guanine and guanosine [3]. Several authors have used thermal heating in the source of a supersonic expansion to produce neutral molecules of some of the more stable bases. This technique was used by Brady et al. to study the spectroscopy of uracil and thymine [4]. Kim et al. obtained ionization potentials of adenine and thymine by thermal heating and jet-cooling [5]. More recently, Kim et al. reported vibronic spectra of adenine obtained by this approach [6].

In order to circumvent the problem of thermal degradation, we use pulsed laser desorption [7–9]. All of the DNA bases can be brought into the gas phase as neutrals by laser desorption of neat material from

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suitable substrates. We have reported the resonant 2 photon ionization (R2PI) spectra of guanine [10,11] and of adenine and a series of derivatives based on laser-desorption with jet-cooling [12]. The next step is to extend this technique to larger neutral DNA compounds, that cannot be volatilized by thermal heating. Li and Lubman have laser-desorbed adenosine, guanosine and various of their derivatives, which they entrained in a CO_2 jet and photoionized at 266 nm [13]. They obtained mass spectra that were similar to those from electron impact (EI) ionization with minor parent mass peaks and characteristic fragments [14]. We have successfully used laser desorption to volatilize several guanosines and 7-deazaadenosine [15]. For all of these we recorded R2PI spectra on the parent molecular peaks.

However, in the case of adenosine and a number of its derivatives, we obtained only a minor parent mass peak, for which we did not record a resonant R2PI spectrum. Instead we did record an R2PI spectrum on a major fragment peak at mass 177 Da. We found this fragment mass to be characteristic for an entire class of compounds, related to adenosine. As will be discussed below, we use the R2PI spectroscopy results to determine that this fragmentation takes place in the desorption rather than in the ionization step. This suggests that either adenosine is more fragile than guanosine, or its photochemistry selectively interferes with multiphoton ionization.

2. Experimental

The experimental setup has been described in detail elsewhere [7,16]. In brief, material is laser desorbed from a sample probe in front of a pulsed nozzle. All chemicals were obtained from Sigma–Aldrich and used without further purification. The desorption laser is a Nd:YAG laser operated at its fundamental wavelength of 1064 nm. At this wavelength one does not expect photochemical interaction with the compounds that we desorb while the graphite substrate absorbs effectively. Typical laser fluences are of the order of 1 mJ/cm^2 or less. The laser is focused to a spot of the order of 1 mm diameter within 2 mm in front of the nozzle. The nozzle consists of a pulsed valve with a nozzle diameter of 1 mm. We usually operate with Ar as a drive gas at a backing pressure of about 5 atm. In earlier work we optimized the geometry for effective entrainment by mapping entrained perylene with laser induced fluorescence [17].

Downstream ionization lasers intersect the beam inside the source region of a reflectron time-of-flight (TOF) mass spectrometer (R.M. Jordan Co.). A Nd:YAG-pumped dye laser, used for one color, two photon ionization, intersects the beam at right angles. By monitoring specific mass peaks while varying the two photon ionization wavelength, we obtain mass selected excitation spectra.

3. Results

Figs. 1 and 2 show the structures of the molecules in this study. Fig. 1 shows the structure of adenine and that of adenosine with the numbering scheme for their atoms. In Fig. 2 the letter A denotes the adenine moiety, substituted in the N9 position. The letter

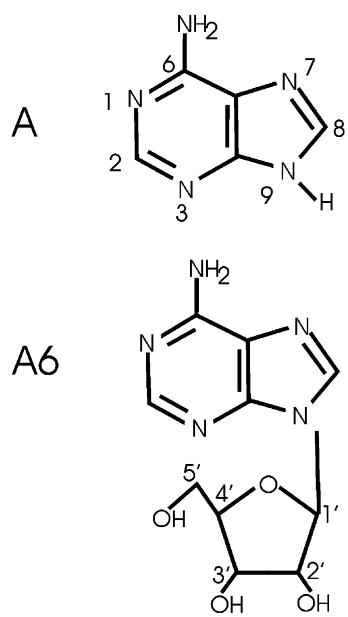

Fig. 1. Structures of adenine (A) and adenosine (A6).

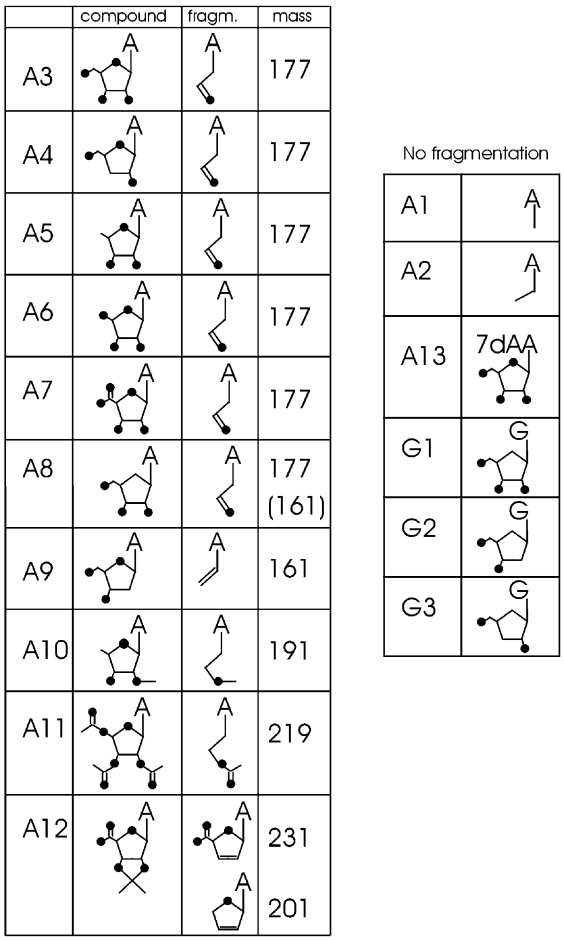

Fig. 2. Compounds used in this study. 'A' denotes the adenine moiety, substituted in the pan and 9 position. 'G' denotes the guanine moiety, substituted in the pan and 9 position. Likewise, '7dAA' denotes the 7-deazaadenine moiety, in which the N7 nitrogen of adenine is replaced by CH. Fragment structures show tentative assignments. Circles denote oxygen atoms.

G likewise denotes guanine, substituted in the N9 position as well. All measurements were performed under identical conditions of sample preparation, desorption and ionization. With laser desorption, jet cooling and photoionization, we obtained R2PI spectra on the parent molecular masses for all of the guanine-based compounds but not for all of the adenine-based compounds. In the case of adenine compounds we recorded fragment-free mass spectra for adenine itself and for 9-ethyl adenine (A2). Kim et al. [6], Luhrs et al. [18] and Kleinermanns [19] obtained R2PI spectra on the parent molecular masses as well, while thermally heating adenine in a seeded beam source. By the same technique Luhrs et al. also obtained fragment-free spectra of 9-methyl adenine (A1) [18]. However, all the compounds, A3–A11, in which adenine was substituted in the N9 position by a sugar ring exhibited distinctive fragmentation. This is in marked contrast to the corresponding guanine based compounds, G1–G3, for each of which we observed the parent molecular ion with no difficulty. Compounds A3–A11 exhibit fragmentation very similar to that of EI ionization. The abundance of the parent mass is small and we did not record an R2PI spectrum on the parent mass. Compounds A3–A8 all produced mass 177 Da as their most abundant fragment corresponding to the mass of the adenine base plus 43. This is similar to results from EI ionization, where usually a dominant fragment is reported at the mass of the base plus 44. Compounds A9, A10 and A11 lead to a major fragment at masses 161, 191 and 219 Da, respectively. Fig. 3 shows the R2PI spectra recorded on each of these masses. These R2PI spectra were recorded on the indicated fragment mass and were identical irrespective of which parent molecule produced the particular fragment.

The fact that we did not observe a major parent R2PI spectrum can be due to several factors. We should note that we cannot compare absolute abundances between different experiments. Therefore, the possibility exists that parent molecules are present but go undetected because the photochemistry is adverse to multiphoton ionization. In other words, it is possible that the fragments that we detect constitute only a minor part of the molecules that are actually present in the beam. For example, the intermediate state of the parent molecule could be very short-lived or the wavelength could be shifted outside of the range that we studied. On the other hand, it is also possible that these compounds fragment very extensively either upon desorption or upon excitation. To address these questions we wish to understand whether the observed fragments are formed in the desorption step or in the ionization step. This issue is also significant from a mechanistic

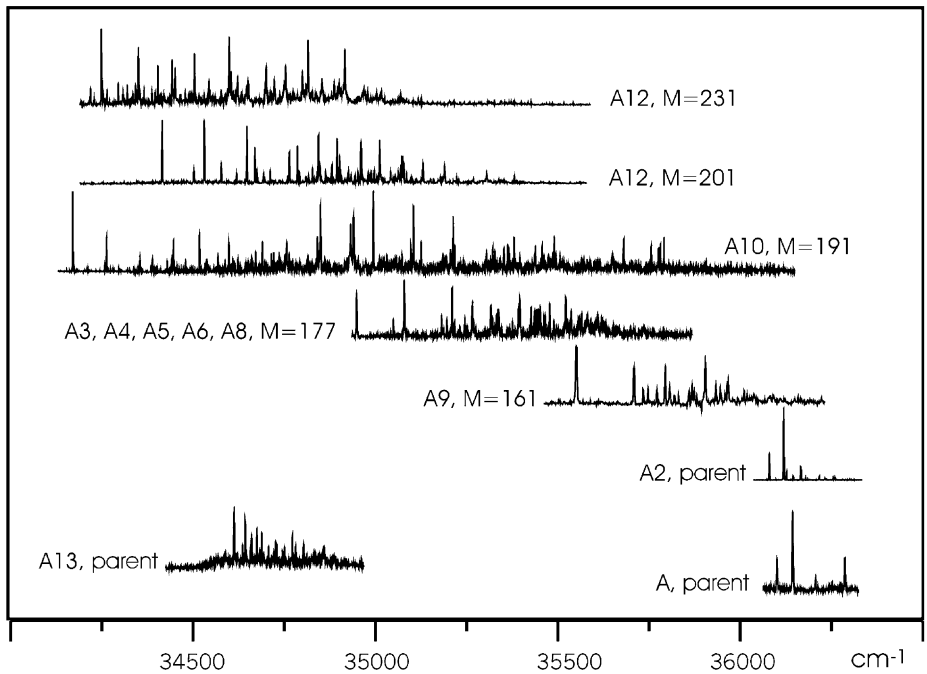

Fig. 3. R2PI spectra of the indicated compounds, and recorded at the indicated masses.

and from a practical point of view. It is important to acertain whether these molecules are fragile because of their thermochemistry, causing decomposition during desorption, or because of their photochemistry, causing a unimolecular reaction during excitation. We can address this question by examining the R2PI spectra. If fragmentation occurs in the ionization step, then the wavelength spectrum at the fragment mass reflects the vibronic transitions of $S_1 \leftarrow S_0$ excitation of the *parent*. On the other hand, if this fragmentation has already taken place in the desorption source then the wavelength spectrum is due to the excitation of the *fragment*. We can make this distinction by comparing the R2PI spectra of compounds A3–A8, recorded in each case on the fragment mass of 177 Da. Since each of these fragment wavelength spectra are identical, irrespective of which was the parent molecule, as shown in Fig. 3, this immediately excludes the possibility that the 177 Da fragments were created after photoionization. Otherwise we would have obtained different R2PI spectra for each of the different parent species.

A second conclusion can be drawn from the identical R2PI spectra for compounds A3–A8. Not only does this fragmentation occur in the desorption step, but moreover each of these compounds produces the same fragment. The fact that these compounds differ only by substitutions on C′3, C′4 or C′5 suggests the fragmentation products shown in Fig. 4, namely the f1, enol, or f2, ketene, fragment. Similar fragmentation has been observed in dissociation of ions rather than neutrals [20–23]. Shaw et al. discussed different pathways in

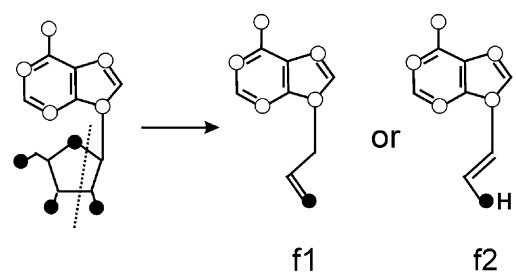

Fig. 4. Possible fragmentation pathways as discussed in the text.

EI ionization and suggested f1 as the more likely product, based on subsequent fragmentation patterns [24]. It is also possible that multiple isomers are produced simultaneously, which in principle could be observed by a spectral hole burning experiment [25]. This pathway implies a concerted cleavage in the sugar ring of bonds C′2–C′3 and C′1–O6, as will be discussed below. To test this explanation we have laser-desorbed compounds A9, A10 and A11, which differ in their substitution in the C′2 position. Their major fragments were 161, 191 and 219 Da, respectively. This is consistent with the cleavage pattern in the sugar ring postulated above. The R2PI spectra recorded on each of these most abundant fragment peaks appears in Fig. 3.

Given the proposed fragmentation pathway, it is of interest to examine compound A12, in which the C′2–C′3 bond is tethered by an isoproylidene group. Laser desorption of this compound leads to a fragments at masses 201 and 231 Da, the R2PI spectra of which appear in Fig. 3. This fragment most likely results from loss of the isoproylidene. This suggests that in this molecule the concerted fragmentation, seen in the other compounds, does not take place because the cleavage of the C′2–C′3 bond is inhibited. If we assume that in this case the fragmentation also occurs in the source, then the R2PI spectrum of Fig. 3 should be very similar to that of adenosine. This is especially likely since the spectra of the three different guanosines are shifted with respect to each other by only seven wavenumbers. Therefore, it is unlikely that the adenosine absorption is shifted strongly with respect to that of its deoxy compounds, and thus such a scenario would not explain why we failed to observe adenosine at the parent mass.

Compound A13, 7-deazaadenosine, differs from all the other compounds in this study by the fact that N7 in the adenine moiety is replaced by a CH. In this case we do observe the parent molecular peak and Fig. 3 shows the resulting R2PI spectrum. It is remarkable that a change in the adenine chromophore affects a change in the decomposition of the sugar ring. This observation can lead one to speculate about the mechanism.

4. Discussion

A possible pathway to the cleavage of the sugar ring could be initiated by a cleavage at the C′1 position, followed by cleavage at the C′2, leading to formation of f1, which could subsequently also tautomerize to f2. If this postulation is correct, the electron density at N9 could play an important role in order to stabilize the electron deficient intermediate radical at C′1. In that scenario the difference between adenosine on the one hand, and guanosine and 7-deazaadenosine on the other hand, could be in the charge distribution involving the N9 position. In our previous work on the purine bases, we have found that methyl substitutions of adenine and guanine in any position other than N9 causes only minor shifts in the electronic excitation spectrum. Substitution at the N9 position, however, appears to cause large shifts in opposite directions for adenine and guanine, respectively, suggesting a special role for the N9 electrons [12]. Ab initio calculations may be able to elucidate this issue. Another possibility to be considered is an effect due to the desorption from the bulk. For example, a different crystal structure could lead to different proximity effects for the different compounds. However, the fact that extensive substitutions do not appear to affect the observed trends, seems to suggest that the key element is the difference in nucleobase moiety. This is also strongly suggested by the fact that Wilson and McCloskey, using thermal heating and chemical ionization, observed significant abundances of adenosine parent ions [20]. Thus, it is quite possible that the fragments reported here reflect only part of the desorbed neutrals, while the parent molecules go undetected. In that case the different photochemistry between adenosines and guanosines might parallel the electronic structure differences we have observed between their respective bases [12]. We are exploring variations to our desorption technique to achieve desorption at lower temperature, to see if it will be possible to reduce this fragmentation pathway [26]. This will potentially improve control in laser desorption of these compounds for use in mass spectrometry. Improved ability to produce neutral parent molecules will also allow us to further explore the

spectroscopy of these compounds for increased understanding of their photochemistry and structure.

Acknowledgements

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