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A theoretical and mass spectrometry study of the novel mechanism of N-glycosidic bond cleavage in nucleoside

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ABSTRACT

The fragmentation pathways of ribonucleosides, deoxynucleosides and isopropylidenenucleosides were investigated by electrospray ionization tandem mass spectrometry (ESI-MS/MS) in both positive and negative mode. Novel fragmentation pathways investigated using deuterium-label experiment provided important insight on the nature of N-glycosidic bond cleavage. The deuterium of 5′-hydroxyl group on ribose moiety was deprived by the nucleobase, and a novel five-member ring through 5′-oxygen and 1′-carbon was formed in the ribose residue as the lost neutral molecule, instead of a new double bond generating between 1′-C and 2′-C as the literature reported. The novel fragmentation pathway was supported by density functional theory calculations.

1. Introduction

Electrospray ionization tandem mass spectrometry (ESI-MS^n), a very powerful tool for structural determination, has played an important role in the characterization of nucleosides, nucleotides and the sequence analysis of oligonucleotides, RNA or DNA [1–3]. It is well known that ribonucleosides (adenosine (A), guanosine (G), cytidine (C), uridine (U)) and deoxynucleosides (deoxyadenosine (dA), deoxyguanosine (dG), deoxycytidine (dC), thymidine (dT)) are the building blocks of RNA and DNA. Therefore, knowledge of detailed dissociation routes of the compounds is essential for successful pattern recognition and fragment ion prediction of the oligonucleotides, RNA and DNA. In the past years, nucleobases and nucleosides were studied in details using mass spectrometry mostly in positive mode [4–10]. However, the study in negative mode was seldom reported, and especially the negative ESI mass spectra of cytidine were not reported previously. In this paper, ribonucleosides, deoxynucleosides and isopropylidenenucleosides (structure showed in Scheme 1) were studied by ESI-MS/MS both in positive and negative mode.

Both in positive and negative ESI-MS/MS, the cleavage of N-glycosidic bond was not only a dominant progress for all nucleosides [7–11], but also a common fragmentation pathway in nucleotides, dinucleotides, trinucleotides and oligonucleotides [12–19]. Therefore, it was important to investigate the mechanism clearly. For nucleosides, all the studies still threw their light on the fragmentation pathways of cationized nucleobases [7–10], but neglected to study the mechanism of N-glycosidic bond cleavage. For oligonucleotides, it was firstly reported that the N-glycosidic bond cleavage was a 1, 2-elimination reaction, involving the 2′ non-exchangeable sugar hydrogen with a new double bond generating between 1′-C and 2′-C simultaneously [13,15]. This mechanism was rejected by Wan et al. [20]. They suggested that an exchangeable hydrogen from phosphate group transfer to the nitrogen on the base initially, and then the oxygen of phosphate group deprived the 2′ non-exchangeable sugar hydrogen with the double bond generating between 1′-C and 2′-C. In our study, to elaborate the mechanism of N-glycosidic bond, the deuterium-label experiment of ribonucleosides was performed using ESI-MS/MS and the results suggested that the hydroxyl group on ribose moiety was involved in the N-glycosidic bond cleavage. To understand the mechanism in detail, deuterated deoxynucleosides and isopropylidenenucleosides were also analyzed using ESI-MS/MS.

In this paper, the tentative fragmentation pathway deduced from the mass spectral data were confirmed by quantumchemical calculations in which theoretical information on the anticipated mechanism was obtained using the density functional theory (DFT) method. To this end, equilibrium geometries were obtained starting from the tentative ion structures, and the change of Gibbs energies (ΔG) between precursor ions and fragments were determined. In addition, the intermediate products of the cleavage were localized and the corresponding activation energies were calculated. The

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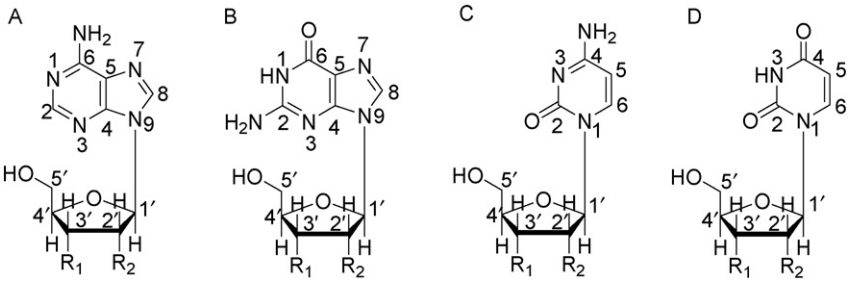


Scheme 1. The structure of studied nucleosides[$R_1 = R_2 = OH$ or $R_1 = OH$, $R_2 = H$, or $R_1 = R_2 = O_2CH(CH_3)_2$][A: adenosine (A); B: guanosine (G); C: cytidine (C); D: uridine (U)].

results illustrated that the calculation data were consistent favorably with the tentative mechanisms.

2. Experimental

2.1. Chemicals

Nucleosides and deoxynucleosides was purchased from Shanghai Sangon Company and Sigma Chemical Company (St. Louis, MO, USA). Isopropylidenenucleosides were synthesized according to the literature [21–24]. Deuterated solvents D_2O were from Beijing Chemical Factory (Beijing, China).

2.2. Mass spectrometry

Experiments were conducted on an Esquire 3000 ESI-MS with an ion trap mass spectrometer in both positive and negative ionization modes (Bruker Daltonik Gmbh, Germany). The MS^n spectra were obtained by collision-induced dissociation (CID) with helium after isolation of the appropriate precursor ions. Ionization of analysis was carried out using the following setting of the ESI: nebulizer gas flow 7 psi, dry gas 4.5 L/min, dry temperature 300 °C, spray voltage 4000 V. Scan range was 15–500 *m/z* and scan resolution was normal (13,000 *m/z*/s). Collision energies were 0.37–0.70 V for $[M+H]^+$ and $[M-H]^-$, and 0.55–1.10 V for $[M+Na]^+$.

2.3. Theoretical calculations

All calculations were performed with the B3LYP/6-31G(d, p) model [25,26], using Gaussion 03 [27] suite of programs in the gas phase. The geometries of the neutral and ionized molecules were fully optimized and the vibrational frequencies analysis indicated that all structures were minima in the potential energy surface. The electron density was analyzed by natural bond orbitals (NBO) [28,29] with Gaussian 03.

3. Result and discussion

3.1. ESI-MS/MS negative fragmentation pathways of the studied nucleosides

In positive mode, the main fragment ions of all the studied nucleosides were the cationized nucleobase resulting from the cleavage of N-glycosidic bond, consistent with the literature [7–10]. In negative mode, the fragment pathways were relatively complicated. For purine nucleosides (adenosine, guanosine, deoxyadenosine, deoxyguanosine, isopropylideneadenosine and isopropylideneguanosine), the fragment ions were deprotoned nucleobase obtained from the same way as their positive mode. However, in terms of pyrimidine nucleosides (cytidine, uridine, deoxycytidine, deoxyuridine, isopropylidenecytidine and isopropylideneuridine), the fragment ions were quite different (Data showed in Table 1). All the pyrimidine nucleosides underwent characteristic dissociation in the pyrimidine moiety analogous to the retro-Diels-Alder (RDA) reaction[30], losing HNCO (43 Da) which could be accounted for by N-3, C-2 and O-2 (Scheme 1)[6] and obtaining the ions at *m/z* 199, 200, etc. (Table 1). Another series of fragment ions at *m/z* 152, 135, 153, 136 (Table 1), resulting from the ribose cleavage, consisted of the intact base with portions of the sugar moiety containing C-1′, C-2′ and O-2′ (for cytidine and uridine)[11,31]. For cytidine, uridine and deoxycytidine, both the ribose and nucleobase cleavages were observed and the product ions at *m/z* 109, 93, 110 (Table 1) were obtained accordingly. But for thymidine, the main fragment ion was *m/z* 125 resulting from the cleavage of N-glycosidic bond. It was interesting to find that in negative mode, the N-glycosidic bond was stable enough to keep intact all along like the fragment ions of pseudouridine (except thymidine) [11]. The fragmentation pathways were the characteristic of pyrimidine nucleosides, and were also observed in the product ions of dinucleotides. For example, the main fragment ions of UpC (precursor ion *m/z* 548) was *m/z* 505 with 43 Da loss [32]. Therefore, it could be expected that the results would provide some help for the base recognition and

Table 1
Negative ESI/MS/MS data of pyrimidine nucleosides.

Name of pyrimidine nucleosides	Precursor ions (*m/z*) $[M-H]^-$	Fragment ions *m/z* (relative intensity, %)[a]			
		Base ring open $[M-H-43]^-$	Ribose ring open $[M-H-90]^-$	Both base and ribose ring open $[M-H-43-90]^-$	$[Base-H]^-$ $[M-116]^-$
Cytidine	242	199 (8)	152 (16)	109 (100)	
Deoxycytidine	226	183 (20)	136 (14)	93 (100)	
Isopropylidenecytidine	282	239 (100)			
Uridine	243	200 (100)	153 (29)	110 (59)	
Deoxyuridine	227	184 (100)	137 (69)		
Isopropylideneuridine	283	240 (100)			
Thymidine	241	198 (10)	151 (16)		125 (100)

[a] $[M-H-43]^-$: the ions lost HNCO (43 Da); $[M-H-90]^-$: the ions lost $C_3H_6O_3$ (90 Da); $[M-H-43-90]^-$: the ions lost HNCO (43 Da) and $C_3H_6O_3$ (90 Da) successively.

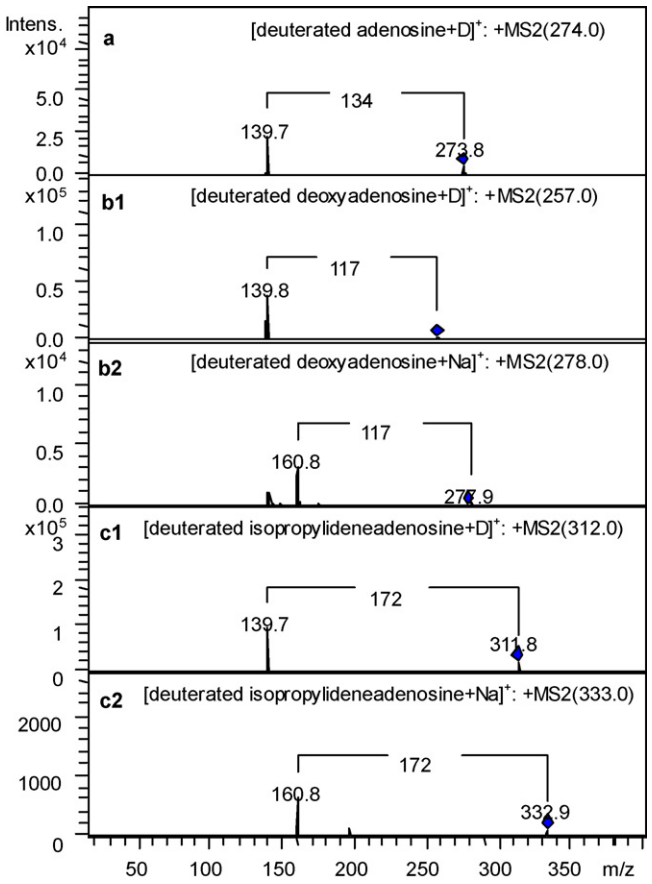


Fig. 1. ESI-MS/MS of deuterated adenosine (a), deoxyadenosine (b1, b2) and isopropylideneadenosine (c1, c2) (collision energies were 0.37–0.70 V for $[M+H]^+$ and $[M-H]^-$, and 0.55–1.10 V for $[M+Na]^+$).

sequence identification in the analysis of oligonucleotides and RNA.

3.2. Novel dissociation mechanism of N-glycosidic bond in deuterated nucleosides

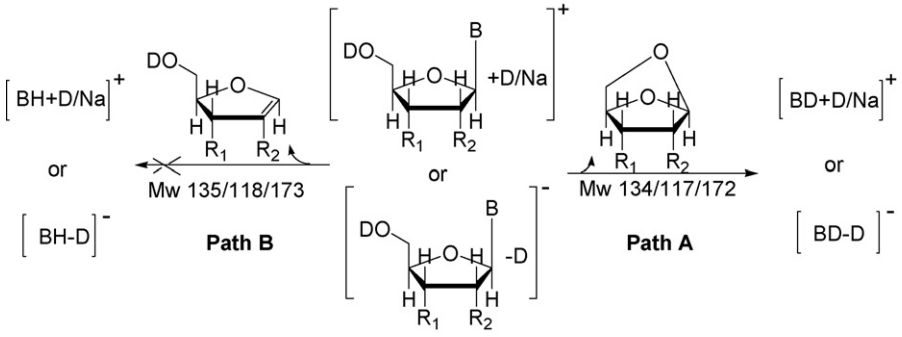
Ribonucleosides were dissolved in the D_2O and shaken for 30 min to obtain the deuterated nucleosides in which all the active hydrogens were exchanged. Then the solutions were analyzed by ESI tandem mass spectrometry both in positive and negative mode. Adducts of deuterated nucleosides with deuterium or sodium were selected to be fragmented. The MS/MS spectra of deuterated adenosine, deoxyadenosine and isopropylideneadenosine were shown as the example in Fig. 1. All the ESI-MS/MS data of deuterated ribonucleosides, deoxynucleosides and isopropylidenenucleosides were shown in Table 2.

Table 2
ESI-MS/MS data of deuterated nucleosides, deoxynucleosides and isopropylidenenucleosides.

Deuterated nucleosides[a]	Precursor ions		Fragment ions (m/z)	Mass loss (Da)
	Ion name	m/z		
Adenosine	$[M+D]^+$	273.8	139.7	134
	$[M-D]^-$	269.8	135.7	134
Guanosine	$[M+Na]^+$	311.9	177.8	134
	$[M+K]^+$	327.9	193.8	134
	$[M-D]^-$	286.7	152.6	134
Cytidine	$[M+D]^+$	249.9	115.9	134
	$[M+Na]^+$	270.9	136.8	134
Uridine	$[M+D]^+$	249.9	115.9	134
	$[M+Na]^+$	270.8	136.8	134
Deoxyadenosine	$[M+D]^+$	256.9	139.8	117
	$[M+Na]^+$	277.9	160.8	117
	$[M-D]^-$	252.7	135.7	117
Deoxyguanosine	$[M+Na]^+$	294.9	177.8	117
	$[M-D]^-$	269.7	151.7	118
Deoxycytidine	$[M+D]^+$	232.8	115.8	117
	$[M+Na]^+$	253.8	136.7	117
Deoxyuridine	$[M+Na]^+$	253.8	136.8	117
Thymidine	$[M+D]^+$	246.8	129.8	117
	$[M-D]^-$	242.7	125.7	117
Isopropylideneadenosine	$[M+D]^+$	311.8	139.7	172
	$[M+Na]^+$	332.9	160.8	172
	$[M-D]^-$	307.8	134.7	173
Isopropylideneguanosine	$[M+Na]^+$	349.9	177.8	172
	$[M-D]^-$	324.8	151.7	173
Isopropylidenecytidine	$[M+D]^+$	287.8	115.8	172
	$[M+Na]^+$	308.8	136.7	172
Isopropylideneuridine	$[M+D]^+$	287.9	115.9	172
	$[M+Na]^+$	308.9	136.8	172

[a] All the active hydrogens were deuterated.

According to the literature reported, the fragment ions should be deuterated nucleobase with a new N–H bond resulting from the cleavage of N-glycosidic bond [13,15] as the path B shown in Scheme 2. However, the ions obtained were actually 1 Da heavier than the expected ions. For example, the fragment ion of [deuterated adenosine + D]$^+$ was the ion at m/z 140, not the ion at m/z 139 as expected, and the main fragment ions of [deuterated deoxyadenosine + Na]$^+$ was the ion at m/z 161, not the expected ion at m/z 160 as shown in Fig. 1a and b2. Therefore, it was suggested that in the cleavage of N-glycosidic bond, the nitrogen (N9 of adenosine and guanosine, N1 of cytidine and uridine, Scheme 1) acquired a deuterium, not a hydrogen from the ribose ring. In the nucleosides, 2′-, 3′- and 5′-OD on the ribose moiety could be the deuterium supplier, then which deuterium was deprived by the nitrogen? To investigate the mechanism, the deoxynucleosides and isopropylidenenucleosides were analyzed by ESI-MS/MS on the same way of deuterated nucleosides (Fig. 1, Table 2). Without the deuterium on



Scheme 2. Suggested novel mechanism of N-glycosidic bond cleavage in deuterated nucleosides, deoxynucleosides and isopropylidenenucleosides (B = Base A, G, C, U, $R_1 = R_2 = OD$ or $R_1 = OD$, $R_2 = H$, or $R_1 = R_2 = O_2CH(CH_3)_2$).

Table 3
Values of atomic Fukui functions indicating the most probable sites of protonation.

Atom	N(13)	N(16)	N(19)	N(22)	O(1)	O(7)	O(28)	O(30)
f^-	0.054539	0.196836	0.049611	0.05548	0.028963	0.016247	0.037646	0.068895

2′- of the ribose moiety in deoxynucleosides and on 2′-, 3′- in isopropylidenenucleosides, as shown in Fig. 1b and c, the fragment ions were still the protonated base with a N–D bond (except the ions of deprotoned deoxyguanosine, isopropylideneadenosine and isopropylideneguanosine), so 5′-OD was suggested to be the deuterium supplier. Therefore, the mechanism was proposed as the Path A shown in Scheme 2. The nucleobase deprived the deuterium of 5′-OD at the time of the N-glycosidic bond cleavage, and the ribose residue left away with a new five-member ring formed by the attack of 5′-O to 1′-C.

From the discussion above, it could be know that in negative mode the fragment pathways of nucleosides were very complicated, so was the deuterium-label experiment. There is some exception to this rule either in deoxynucleosides or isopropylidenenucleosides. As shown in Table 2, the fragment ions of deprotoned deoxyguanosine, isopropylideneadenosine and isopropylideneguanosine were the deprotoned base with a N–H bond, not a N–D bond.

In order to further investigate the novel mechanism, the structure of neutral and ionized adenosine (using as example) were modeled using Gaussion 03 suite of programs. The geometry was optimized at the B3LYP/6-31G(d, p) level and frequency calculation was performed for the optimized structure (Fig. 2).

Firstly the protonation site of adenosine was obtained by the analysis of the electrophilic Fukui function, f^-, which is found to be great higher at the N(16) atom than at the O(30), N(22), N(13) and N(19) atoms (Fig. 2, Table 3). Then the structure of the adenosine cation protonated at N(16) was fully optimized by using of B3LYP/6-31G(d, p) model. The increases of bond length in the C(8)–N(10), O(1)–H(32) and a decrease of the distance between N(22) and H(32) were observed upon comparing the protonated and neutral adenosine molecule (Table 4). The differences of structure were the initial

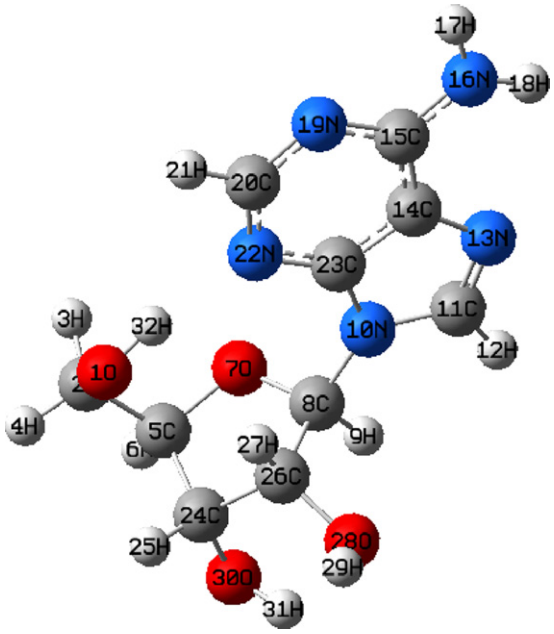
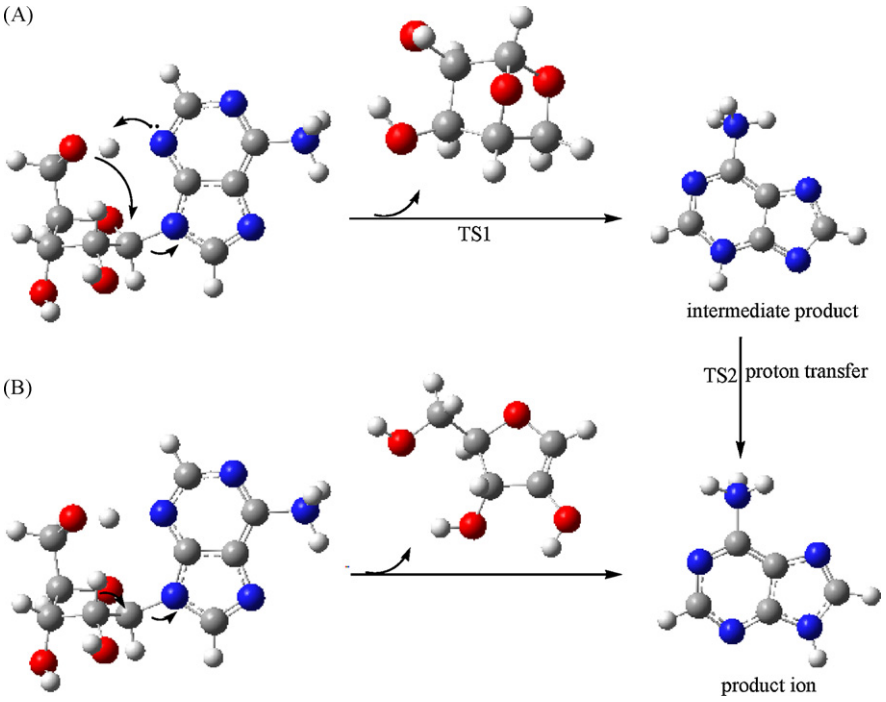


Fig. 2. The optimized geometry of adenosine and the atom labels.

Table 4
The value of bond length for neutral and protonated adenosine.

	Bond length of C(8)–N(10)	Bond length of O(1)–H(32)	Distance between N(22) and H(32)
A	1.454	0.983	1.885
AH^+N(16)	1.494	1.079	1.605



Scheme 3. The two possible cleavage pathways of adenosine (A: pathway suggested in this paper; B: pathway reported in the literature).

Table 5
The changes of Gibbs energy (ΔG, kcal/mol) in the cleavage of N-glycosidic bond.

Nucleoside name	ΔG of pathway A[a]			ΔG of pathway B[a]	ΔG of pathway B[a] − ΔG of pathway A[a]
	$\Delta G_{A\text{-}TS1}$	$\Delta G_{A\text{-}TS2}$	ΔG	ΔG	$\Delta\Delta G_{AB}$
Adenosine	24.38	−12.84	11.54	22.37	10.83
Guanosine	66.29	−51.80	14.49	24.30	9.81
Cytidine	12.23	−1.53	10.70	20.50	9.80
Uridine	27.61	−16.18	11.43	21.24	9.81

[a] The cleavage pathway in Scheme 3.

evidences for the weakening of N-glycolsidic bond and the transfer of H(32) from 5′-hydroxyl on the ribose to N(22) on nucleobase after protonation(Scheme 3).

According to the structure change discussed above, the mechanism of N-glycolsidic bond was presented in Scheme 3A. At the time N-glycolsidic bond of precursor ion was cleaved, the intermediate product ion was formed by N(22) depriving the hydrogen of 5′-OH and the ribose leaving away with a new five-member ring by the attacking of 5′-O to 1′-C. Then the product ion was obtained through the proton transfer from N(22) to N(10) (Scheme 3A). The changes of Gibbs energy (ΔG) for each progress were +24.38 and −12.84 kcal/mol. So the total change of Gibbs energies of pathway A was 11.54 kcal/mol, 10.83 kcal/mol lower than 22.37 kcal/mol-change of Gibbs energies if N-glycolsidic bond cleavage followed the pathway B as reported in the literature (Scheme 3, Table 5). On the same way, the changes of Gibbs energy (ΔG) in the cleavage of N-glycosidic bond in guanosine, cytidine and uridine were also calculated. The datas were showed in Table 5. It could be concluded that for all the nucleosides, the energy changes of pathway A were lower than that of pathway B. The cleavage of N-glycosidic bond at 5′-terminal of oligonulceotides is a common dissociation. According to our study, it is suggested that in the N-glycosidic bond cleavage, the effect of free 5′-OH should be taken into account.

4. Conclusions

Ribonucleosides, deoxynucleosides and isopropylidenenucleosides were investigated by ESI-MS/MS in both positive and negative mode. In positive mode, the main fragment ion of all the studied nucleosides was the cationized nucleobase resulting from the cleavage of N-glycosidic bond. In negative mode, the fragment pathways of purine nucleosides were different with those of pyrimidine nucleosides. For purine nucleosides, the fragment ions were deprotoned nucleobase obtained from the same way as their positive mode. For pyrimidine nucleosides, ribose and base cleavage product ions were observed, and all fragment ions contained the intact N-glycosidic bond except that of thymidine. A novel mechanism was found, in which 5′-hydroxyl group on the ribose was involved in N-glycosidic bond cleavage in positive mass spectra of deuterated ribonucleosides, deoxynucleosides and isopropylidenenucleosides and in negative mass spectra of purine nucleosides. Firstly, the mass spectra of deuterated nucleosides revealed that an active hydrogen on the ribose moiety migrated to the nitrogen atom of the nucleobase moiety at the time N-glycosidic bond cleaving, not an inactive hydrogen transfer as reported in the literature. Secondly, 5′-hydroxyl group was proved to be the active hydrogen supplier from the mass spectra of deuterated deoxynucleosides and isopropylidenenucleosides. Finally, the novel mechanism presented was elaborated by theoretical calculations. The cleavage of N-glycosidic bond at 5′-terminal of oligonulceotides is a common dissociation. According to our study, it is suggested that in the N-glycosidic bond cleavage, the effect of free 5′-OH should be taken into account. Therefore, the results will provide some help for successful mass dissociation pattern recognition and fragment ion prediction of the oligonucleotides and RNA.

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