International Journal of Mass Spectrometry 278 (2008) 1–9

Contents lists available at ScienceDirect

International Journal of Mass Spectrometry

journal homepage: www.elsevier.com/locate/ijms

Fragmentation of Lewis-type trisaccharides in the gas phase: Experimental and theoretical studies

Hiroaki Suzuki[a,b], Tohru Yamagaki[a,*], Kazuo Tachibana[a], Kazuhiko Fukui[b]

[a] Department of Chemistry, School of Science, The University of Tokyo, 7-3-1 Hongo, Bunkyo, Tokyo 113-0033, Japan
[b] Computational Biology Research Center (CBRC), National Institute of Advanced Industrial Science and Technology (AIST), 2-42 Aomi, Koto, Tokyo 135-0064, Japan

ARTICLE INFO

Article history:
Received 12 May 2008
Received in revised form 4 July 2008
Accepted 4 July 2008
Available online 29 July 2008

Keywords:
Fragmentation mechanism
Negative ion
Lewis-type trisaccharides
Hartree–Fock theory

ABSTRACT

The mechanisms of fragmentation of the chlorinated Lewis-type trisaccharides Lewis a and Lewis X were studied by using experimental and theoretical methods. In the fragment ion spectra obtained by ESI-CID-MS, C- and Z-type fragmentations corresponding to 4- and 3-linked glycosyl bond cleavages of the *N*-acetylglucosamine moiety were observed. The relationships of the relative ion intensities of C- and Z-type fragmentations were Z > C in Lewis a and C > Z in Lewis X, suggesting that the relationships of the activation energies were C > Z in Lewis a and Z > C in Lewis X. Anomeric and acetoamide groups were assumed to be included in the mechanisms of C- and Z-type fragmentation; we therefore analyzed 3-fucosyllactose and methylated Lewis-type trisaccharides, which are the derivatives of Lewis-type trisaccharides. These analyses revealed that C-type fragmentation was little influenced by anomeric group and that Z-type fragmentation was influenced by both the anomeric and the acetoamide groups. Fragmentation mechanisms were proposed on the basis of the experimental results and were calculated at the HF/3-21G(d), HF/6-31G(d) levels. From the calculations, C-type fragmentation of the 4-linkage was considered to take place by electron transfer from the acetoamide group, and Z-type fragmentation at the 3-linkage was considered to take place in two steps: (i) elimination of the 3-linked saccharide moiety, and (ii) deprotonation of an anomeric proton by a chloride anion. The activation energies for C- and Z-type fragmentations, as determined by HF calculations, were consistent with the experimentally assumed trends.

1. Introduction

Oligosaccharides have been the targets of intense study because they play essential roles in various biochemical processes: they act as media for cell–cell recognition, aid in the processes of fertilization and inflammation, and add functionality to proteins by post-translational modification [1,2]. There have recently been widespread developments in the field of glycobiology, which involves in vivo analysis of the molecular functions of saccharides [3]. In particular, the structural study of oligosaccharides has become an essential part of glycobiology, because oligosaccharide structure has a close relationship with the receptor molecular recognition, and carbohydrates structure directly affects carbohydrate function [4]. The quantity of oligosaccharides obtained from biological samples is in many cases so low that highly sensitive analytical methods are essential for structural analyses. As a sensitive analytical method for the structural analysis of oligosaccharides, mass spectrometry has widely been applied [5–8].

The advantage of applying mass spectrometry to such structural analyses is that fragment ion spectra that yield various structural informations can be obtained by decomposition via collision-induced dissociation (CID) [9] or postsource decay (PSD) [10,11]. Much effort has been exerted to obtain a variety of structural information, including information on sequence, isomeric configuration, and stereochemistry, such as anomeric configuration and linkage type. Conventionally, oligosaccharides have been studied in positive-ion mode, since they have a high affinity toward alkali metal cations such as lithium, sodium, and potassium and can thus easily be ionized [12–14]. In particular, the fragmentation of sodiated molecules has been intensively studied. Fragmentation from sodiated oligosaccharide molecules mainly takes place at glycosyl bonds of the B/Y-type (nomenclature by Domon and Costello [15]) via various fragmentation methods. These types of fragmentation offer oligosaccharide structural information.

In negative-ion analysis, which is an alternate to the typical positive-ion analysis, oligosaccharides are ionized as deprotonated,

* Corresponding author. Current address: Suntory Institute for Bioorganic Research, 1-1-1, Wakayamadai, Shimamoto-cho, Mishima-gun, Osaka 618-8503, Japan. Tel.: +81 75 962 7094; fax: +81 75 962 2115.
E-mail address: yamagaki@sunbor.or.jp (T. Yamagaki).

1387-3806/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijms.2008.07.012

halogenated ions or nitrate ion adducts; these negative ions give C/Z-type glycosyl bond cleavage, and also cross-ring cleavage ions with high intensity. Cross-ring cleavage ions reflect the anomeric configuration or linkage type [16–18], and can be remarkably useful for defining these structural features, which are usually difficult to identify with conventional positive-ion analysis. The ionization process is also preferable in the analysis of fucosyl or sialyl oligosaccharides, because fucose and sialic acid residue are so labile in positive-ion analysis [19–21] that the inevitable decomposition corresponding to the loss of sialic acid moiety predominates in the mass spectrum, making interpretation of the mass spectra difficult [22]. Negative-ion analyses of oligosaccharides by ESI-MS have been reported since the generation of deprotonated and chlorinated molecules was achieved [23–29], whereas analysis by MALDI-MS has lagged behind. Ever since a series of β-carbolines were introduced as efficient matrices for the negative ionization of neutral oligosaccharides [30–32], the use of negative-ion analysis of oligosaccharides has been increasing in structural analyses [33–36]. In particular, chlorinated oligosaccharides have received attention, because chlorinated molecules of oligosaccharide are stable ionic species and therefore easier to analyze [34,37].

We have been performing structural analyses of chlorinated oligosaccharides by using ESI-TOF-MS, MALDI-TOF, and ion-trap MS [38–40]. These studies concern the distinction of structural isomers of lacto-oligosaccharides that have a Lewis-type core structure; the structural isomers can be distinguished from one other because each isomer gives a different fragmentation pattern. Study of the fragmentation mechanism is indispensable to any discussion of the theoretical aspects of why each oligosaccharide produces a characteristic pattern. An approach that uses both experimental and theoretical methods can be expected to be useful for elucidating these fragmentation mechanisms; in particular the theoretical method is suitable for handling the chemical properties of molecules in the gas phase.

Theoretical analyses of oligosaccharides have been undertaken by the application of molecular mechanics, molecular dynamics, and molecular orbital calculations. There have been several reports on the fragmentation mechanisms of positively charged oligosaccharides [16,35,41,42]. In studies of the fragmentation of positive ions of oligosaccharides, theoretical methods such as semi-empirical and *ab initio* methods have proven useful in analyzing fragmentation mechanisms. Previous studies of fragmentation mechanisms of negative-ion saccharides were limited to deprotonated molecules and mono- or disaccharides [43,44], making it more desirable to study the fragmentation of chlorinated oligosaccharides.

In this study, Lewis a and Lewis X, which are the structural isomers of Lewis-type trisaccharides [45], were chosen as model compounds. We focused on the C- and Z-type glycosyl bond cleavage of chlorinated Lewis-type trisaccharides, and we propose fragmentation mechanisms based on the results of ESI-CID experiments. To identify which functional group influenced the fragmentation reaction, we compared these spectra with those of structural derivatives of Lewis-type trisaccharides. The proposed mechanisms were then simulated by using quantum chemical calculations at the HF/3-21G(d) and HF/6-31G(d) level of theory. We discuss the energetics and pathways of each fragmentation mechanism.

2. Experimental

2.1. Samples

All saccharides are commercially available. Lewis a (Le^a) and Lewis X (Le^X) were obtained from Sigma (St. Louis, MO). The structural features of these saccharides have been intensively studied by the use of NMR [46–48], crystallography [49], computational analysis [46,50–53], and mass spectrometry [54–56]. Lewis X methyl glycoside (Le^X-Me) and Lewis a methyl glycoside (Le^a-Me) were obtained from Toronto Research Chemicals Inc. (North York, Canada), and 3-fucosyllactose was obtained from Funakoshi (Tokyo, Japan).

2.2. Electrospray mass spectrometry

ESI-CID experiments were carried out with a Q-TOF 2 mass spectrometer (Waters-Micromass Inc., Manchester, UK). Nitrogen was used as a desolvation and nebulizer gas at flow rates of 500 and 100 L/h, respectively. Source temperature was kept at 110 °C, and desolvation temperature was 250 °C. Cone voltage was set to 40 V and capillary voltage was 3 kV. Argon was used as a collision gas at a pressure of 1 bar. The collision energy was varied between 10 and 30 eV to obtain the CID spectra at various energies. A monoisotopic precursor ion was selected in the CID experiments.

For CID analyses, all saccharides were dissolved in MeOH:H_2O = 1:1 (v/v) at a final concentration of 20 μM, containing 30 nmol of ammonium chloride. Sample solutions were infused at a flow rate of 10 μL/min by a syringe pump system. The scan rate was set to 2 s/scan for the CID experiments.

3. Theory

Theoretical calculations for the Lewis-type trisaccharides were performed to investigate the reaction mechanism. Quantum chemical calculations were carried out with the Gaussian 98 and Gaussian 03 programs [57] on a PC cluster at the CBRC and AIST Super Cluster at AIST. All calculations were performed by using the HF/3-21G(d), HF/6-31G(d) basis set. The initial geometries of the Lewis-type trisaccharides, which were obtained from the Cambridge Structural Database (CSD, version 5.25), were fully optimized. A full geometric optimization was performed for the neutral, chlorinated $[M+Cl]^-$ molecule of the Lewis-type trisaccharides, and all of the optimized geometries were checked to have positive vibrational frequencies. In an attempt to verify the reaction pathway, optimization of the transition state was carried out by using a quadratic synchronous guided transition state optimization (QST3) [58] at the HF/3-21G(d), HF/6-31G(d) level. The transition structures were checked against the frequencies with respect to the geometries having one imaginary frequency. All energy values were corrected with zero-point vibrational energy from the frequency calculations.

4. Results and discussion

4.1. Fragmentation of Le^a and Le^X

Structural isomers of the Lewis-type trisaccharides Le^a and Le^X were ionized as chlorinated molecules by ESI-Q-TOF-MS. Fig. 1 shows the ESI-CID spectra of Le^a and Le^X and the assignment of the fragment ions. The *m/z* 564 ion was the chlorinated molecule $[M+Cl]^-$ of Le^a and Le^X chosen as the precursor ion. In these spectra, fragment ions from each isomer were observed; $Z_{1\alpha}$ (*m/z* 348), $Z_{1\alpha}/^{0,4}A_2$ (*m/z* 288), and $C_{1\beta}$ (*m/z* 163) fragment ions were generated from Le^a (Fig. 1(a)), and $Z_{1\beta}$ (*m/z* 364), $Z_{1\beta}/^{0,4}A_2$ (*m/z* 304), and $C_{1\alpha}$ (*m/z* 179) fragment ions were generated from Le^X (Fig. 1(b)). In these molecules, C-type fragmentation indicates cleavage of the 4-linked glycosyl bond, whereas Z-type indicates cleavage of the 3-linked glycosyl bond of the GlcNAc moiety. These fragment ions are generated by decomposition of the deprotonated molecule (*m/z* 528, $[M-H]^-$), but the deprotonated molecule itself was not observed in the spectrum. This result is interesting, since deproto-

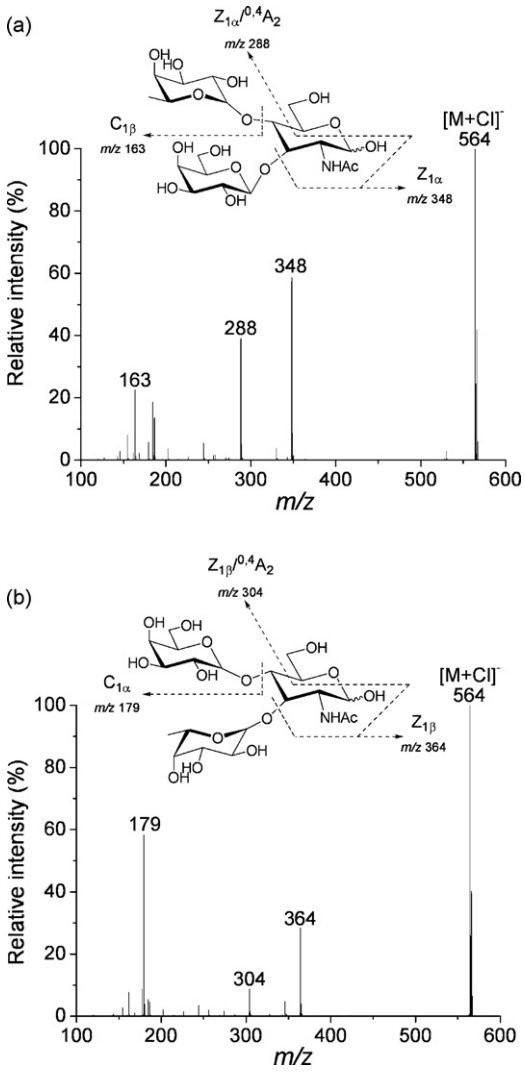


Fig. 1. ESI-CID spectra and assignment of fragment ions of Le^a (a) and Le^X (b) from the chlorinated precursors. Collision energy is 20 eV.

nation by the chloride anion is thought to take place in the first step, as suggested by a previous study [59]. We suspected that this characteristic is a key to the clarification of the mechanism of fragmentation of Le-type saccharides, as we will discuss later.

From analysis of the CID spectra of Le^a and Le^X (Fig. 1), we ordered the relative ion intensities for C-type (4-linkage) vs. Z-type (3-linkage) as C > Z in Le^X and Z > C in Le^a. When comparing Le^a and Le^X, we surmised that the C-type fragmentation reaction was energetically more favorable than the Z-type in Le^X, and that the Z-type was more favorable than the C-type in Le^a. To confirm these characteristics, we investigated the behavior of the relative intensities of fragment ions with collision energy. We plotted the relative ion abundances of the precursor ion at *m/z* 564, the C-type fragmentation ions, and the Z-type related fragment ions against collision energy (eV) (Fig. 2). Fig. 2(a) is a breakdown diagram of the precursor ions. The breakdown curves indicate that the collision energies required to decompose the precursor ions of Le^a and Le^X were similar. In the breakdown diagram of the C-type fragment ions of Le^a and Le^X (Fig. 2(b)), the relative ion abundance was Le^X > Le^a at 10–30 eV collision energy. These results indicate that the relationship of the activation energy for C-type fragmentation is Le^a > Le^X.

We constructed a breakdown diagram of Z-type fragmentation (Fig. 2(c)). In the breakdown curve of Le^a, the curves for the $Z_{1\alpha}$ ion

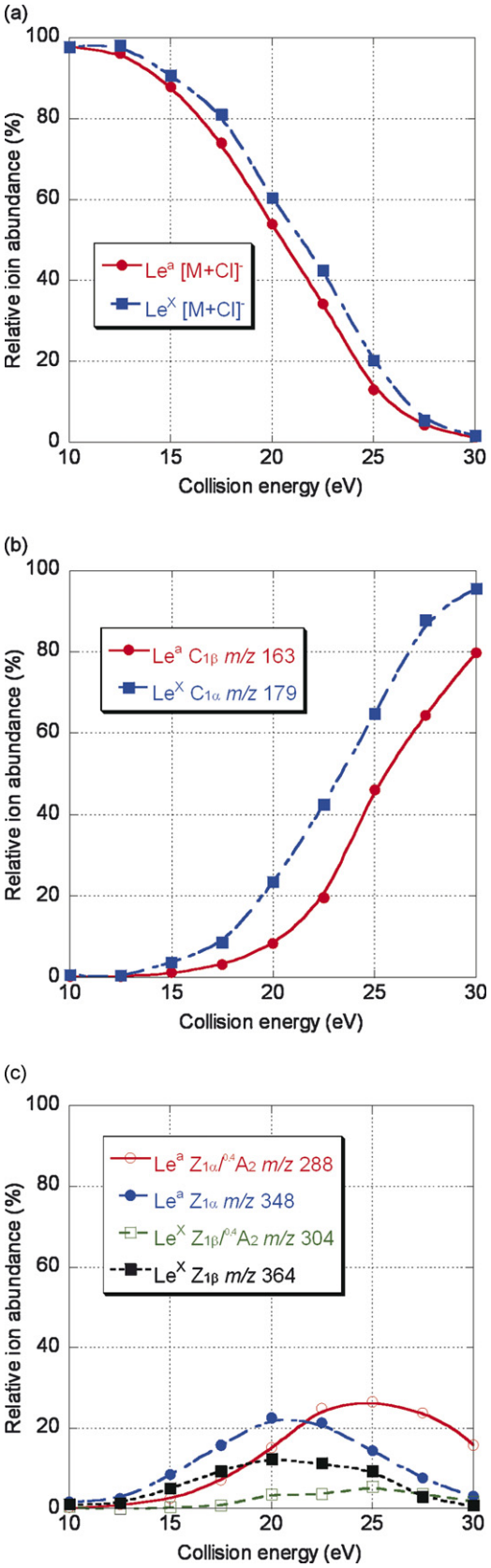


Fig. 2. Relative ion abundances vs. collisional energy (eV) of the precursor ion and product ions in the CID-MS/MS spectra of Le^a and Le^X. Breakdown diagrams for (a) precursor ions of Le^a (●) and Le^X (■); (b) C-type fragment ions of Le^a (●) and Le^X (■); (c) Z-type fragment ions of Le^a (●) and Le^X (■); and $Z_1/^{0,4}A_2$ ions of Le^a (○) and Le^X (□).

and $Z_{1\alpha}/^{0,4}A_2$ crossed at 22 eV, and the order of relative intensity was $Z_{1\alpha} > Z_{1\alpha}/^{0,4}A_2$ under 22 eV, inverting when over 22 eV. This was again different from the breakdown diagram of Le^X, where the relative intensity of the $Z_{1\beta}/^{0,4}A_2$ ion was lower than that for the $Z_{1\beta}$ ion at all collision energies. The generated $Z_{1\alpha}/^{0,4}A_2$ ion, a secondary ion of the $Z_{1\alpha}$ fragment ion, is a characteristic fragment ion for Le^a. For example, when C-type fragmentation is more favorable than Z-type fragmentation, the relative ion intensity of the $Z_1/^{0,4}A_2$ ion will decrease at high collision energy because C-type fragmentation cannot generate the $Z_1/^{0,4}A_2$ ion. In contrast, when Z-type fragmentation is more favorable than C-type fragmentation, the relative ion intensity of the $Z_1/^{0,4}A_2$ ion will increase at high collision energy because the generation of the Z_1 ion will increase, and generation of the subsequent $Z_1/^{0,4}A_2$ ion can be accelerated. From these results, we defined the activation barrier of these fragmentations as $C_{1\beta}$ (4-linkage) > $Z_{1\alpha}$ (3-linkage) in Le^a, and $Z_{1\beta}$ (3-linkage) > $C_{1\alpha}$ (4-linkage) in Le^X.

4.2. Comparison of fragmentations with derivatives of Lewis-type saccharides

Substitutional effects on fragmentation were investigated in structural derivatives to detect which functional groups influenced the C- and Z-type fragmentation reactions. Fig. 3 shows the ESI-CID spectra of Le^X methyl glycoside and 3-fucosyllactose (3-FL). 3-FL has a D-glucopyranose moiety at the reducing end, and the structures of 3-FL and Le^X differ only in the substituents at the C2 position (Glc or GlcNAc).

To investigate the influence of the anomeric hydroxyl group, Le^X methyl glycoside was analyzed. In the CID spectrum of Le^X-Me (Fig. 3(a)), the deprotonated molecule (*m/z* 542, $[M-H]^-$) was observed with high intensity, and the $C_{1\alpha}$ (*m/z* 179) fragment ion was also observed, whereas the $Z_{1\beta}$ fragment ion of the 3-linkage (*m/z* 378) was not observed. This result indicates that the anomeric group is essential for Z-type fragmentation of the 3-linkage to proceed. The influence of the *N*-acetyl group on fragmentation can be detected by comparing the CID spectra of Le^X and 3-FL. In the CID spectrum of Le^X (Fig. 1(b)), $Z_{1\beta}$ (*m/z* 364), $Z_{1\beta}/^{0,4}A$ (*m/z* 304), and $C_{1\alpha}$ (*m/z* 179) fragments were observed. In contrast, chlorinated 3-FL gave $C_{1\alpha}$ (*m/z* 179) fragmentation of the 4-linkage with high intensity and $Z_{1\beta}$ fragmentation at the 3-linkage with very low intensity (Fig. 3(b)). In positive-ion MALDI-PSD analyses of the same saccharides, Le^a and Le^X generate Z-type fragment ions; however, Z-type fragmentation is hardly observed for 3-FL [54]. The *N*-acetoamide group was involved in the mechanism of Z-type fragmentation at the 3-linkage in both positive and negative mode. These results indicated that both the *N*-acetoamide and the anomeric hydroxyl groups had an influence upon Z-type fragmentation of the 3-linkage.

4.3. Proposed fragmentation mechanism

Generally, chlorinated oligosaccharides are thought to decompose to deprotonated molecules as a result of the elimination

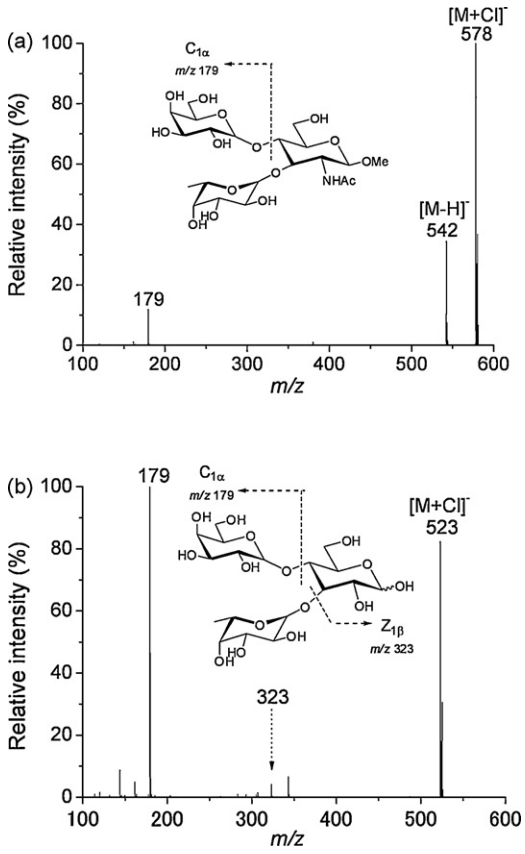
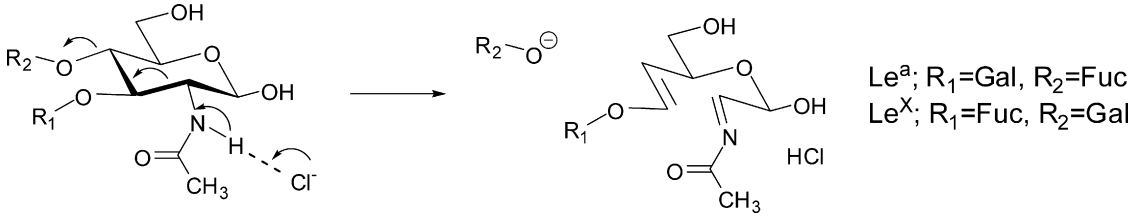


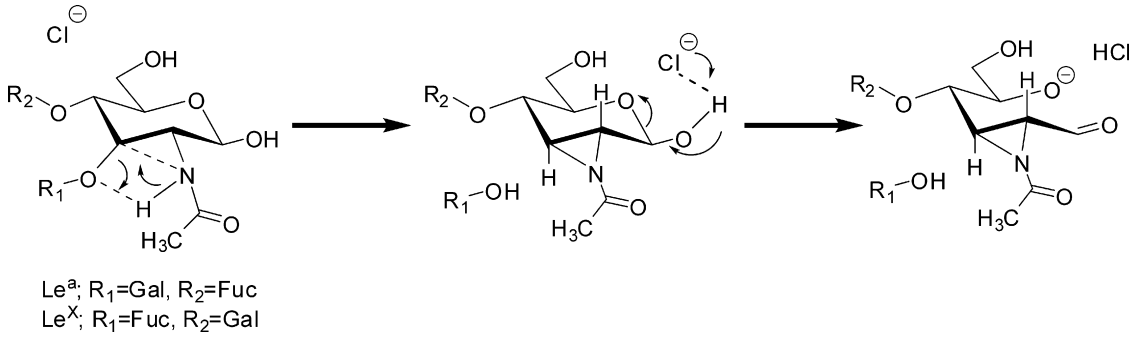
Fig. 3. ESI-CID spectra of Le^X methyl glycoside (a) and 3-fucosyllactose (b). Collision energy is 20 eV.

of hydrochloride molecules in the first step and the continuous decomposition of the deprotonated ions to each fragment ion. In such cases the deprotonated molecules can be observed in the fragment ion spectrum. However, no deprotonated molecules were observed in the CID spectra of chlorinated Le-type trisaccharides. By the computational method, deprotonation reactions on amide protons by chloride anion and all the hydroxyl groups were simulated, but all the optimized structures converged to the initial structure whereby the chloride forms a hydrogen bond with vicinal hydrogen. In the case of chlorinated Le-type trisaccharide, the existence of another reaction pathway that generates fragment ions without going through the deprotonated molecules is a reasonable explanation for the experimental results.

C-type fragmentation was commonly observed in both Le-type saccharides and methylated Le-type saccharides, although the relative intensities differed. Presumably the anomeric group itself does not strongly influence C-type fragmentation. A possible mechanism of C-type fragmentation based on this assumption is shown in Scheme 1. C-type fragmentation is assumed to take place by electron transfer from the chloride anion on the acetoamide group



Scheme 1. Proposed mechanism of C-type fragmentation at the 4-linkage in Lewis-type trisaccharides. The reaction takes place by successive electron transfer.



Scheme 2. Proposed mechanism of Z-type fragmentation at the 3-linkage in Lewis-type trisaccharides. This mechanism includes amide proton transfer to O-3 of GlcNAc and subsequent deprotonation.

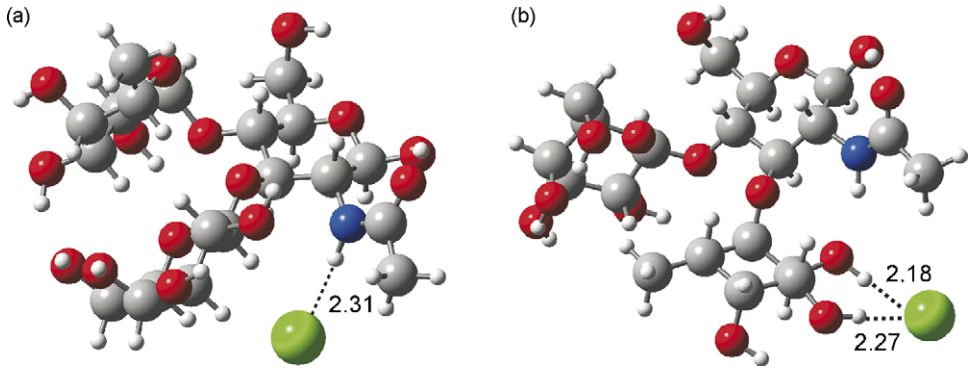


Fig. 4. Stable structures of chlorinated Le^a (a), and Le^X (b), calculated at the HF/6-31G(d) level.

to the C4 glycosyl bond of GlcNAc, and consequently the C-type fragment ion is generated. This mechanism can generate C-type fragment ion at the 4-linkage in one step. A similar mechanism has been proposed by Pfenninger et al. for deprotonated oligosaccharides containing GlcNAc [25], although the mechanism has not yet been confirmed by theoretical methods.

We proposed a possible Z-type fragmentation mechanism in light of the fact that the *N*-acetoamide and anomeric groups play important roles in Z-type fragmentation. The Z-type fragmentation pathway was divided into two steps: elimination of the 3-substituted moiety takes place first, followed by the deprotonation reaction in the second step (Scheme 2). The amide proton rearrangement produces aziridine saccharide, which has a strained 3-membered ring. The aziridine structure is suspected to be energetically labile owing to this highly distorted structure. After elimination of the 3-substituted moiety, deprotonation of the anomeric proton by chloride anion gives a Z-type fragment ion. We suspect that the deprotonation reaction from aziridine saccharide can take place owing to the distorted structure of the 3-membered ring; as a result of deprotonation the Z-type fragment ion may form an open-ring structure because of the presence of the aziridine ring.

4.4. Stabilization energy of chlorinated saccharide

Theoretical calculations were begun with the chlorinated molecule. To investigate the stable structures of chlorinated Lewis-type trisaccharides, the chloride ion was located at arbitrary positions around the saccharide molecules, and these initial geometries were optimized at both the HF/3-21G(d) and HF/6-31G(d) levels. In the optimized structures there are several positions at which the chloride anion can stably form a hydrogen bond. To evaluate the stabilization energy of chlorinated Lewis-type trisaccharides, the most stable saccharide-anion structure was investigated. The initial geometries of Le^a and Le^X were obtained from the CSD, and the initial geometries of the chlorinated complexes, $[Le^a, Le^X + Cl]^-$, were fully optimized at the HF/6-31G(d) level. Fig. 4 shows the stable structures of isomeric Lewis-type trisaccharides. Chloride anion formed hydrogen bonds between the amide proton and C1–H of Gal in Le^a, whereas binary hydrogen bonds were formed between 2-OH and 3-OH of Fuc in Le^X. Table 1 lists the total energies of the neutral molecules, complexes, and chloride anion. Both isomers were stabilized by forming complexes with the chloride anion. The stabilization energy values are 39.7 kcal/mol for Le^a and 32.1 kcal/mol for Le^X. These values are consistent with those of a density functional theory study of a glucose and chloride anion complex by Cai and Cole [37].

4.5. C-type fragmentation at the 4-linkage

The transition state structure was calculated for the proposed mechanism in Scheme 1. Fig. 5 shows the optimized structures of the initial, transition state, and product ions of $C_{1\alpha}$ fragmen-

Table 1
Thermochemical data for isomeric Lewis-type trisaccharides, calculated at the HF/6-31G(d) level

	Le^a	$Le^a + Cl^-$	Le^X	$Le^X + Cl^-$	Cl^-
Total energy	−1955.0771	−2414.6684	−1955.0856	−2414.6631	−459.5260
ΔH_{298} (kcal/mol)		−39.7		−32.1	

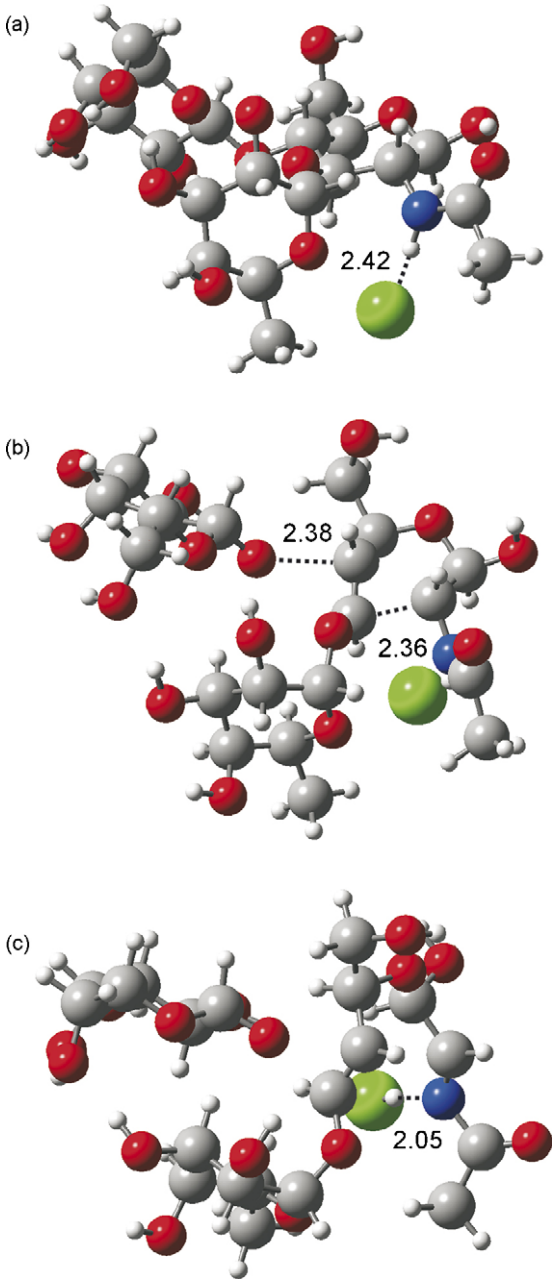


Fig. 5. Optimized structures of the chlorinated Le^X molecule (a), transition state (b), and intermediate product ion (c) of the reaction in Scheme 1. All geometries were optimized by using the HF/6-31G(d) basis set.

tation of Le^X. In the geometry of the transition state, the distance between C2–C3 and C4–O(Fuc) is elongated, resulting in 4-linked glycosyl bond cleavage. Table 2 summarizes the activation energies of Le^a and Le^X in the fragmentation mechanism. In both Le^a and Le^X, the difference in calculated activation energies in the HF/3-21G(d) and 6-31G(d) basis sets was about 10 kcal/mol, which was attributed to variations in basis size. The transition state has one imaginary frequency, and the vibrational motion of the imaginary frequency represents the stretching motions of the C2–C3 and C4–O(Fuc) bonds, which lie along the decomposition reaction coordinate. The transition state structure is more similar to that of the product than to that of the initial structure. This characteristic is rational, since fragmentation in mass spectrometry is generally an endothermic reaction; thus the transition structure tends to resemble the structure of the product ion rather than that of the initial ion, in agreement with Hammond's postulation [60].

In the reaction mechanism of Le^a, it is significant that the activation energy was about 10 kcal/mol higher than that of Le^X. This is interesting, because a similar reaction mechanism also gave a prominent difference in activation energy between Le^a and Le^X. This difference is attributed to the ranking of relative stability of the $[Gal-H]^-$ and $[Fuc-H]^-$ ions in the gas phase. The fucose moiety is comparatively negative relative to the galactose moiety, owing to the presence of the electron-abundant C6 methyl group, whereas the galactose moiety is more positive than the fucose moiety owing to the presence of the methoxy group. The product ion of C-type fragmentation has a negative charge on the anomeric oxygen; thus the fucose anion is expected to be more energetically labile than the galactose anion because the electron-rich methyl group is located near the negative charge on the anomeric oxygen. This factor may affect the activation energy of C-type reactions. In the experimental results, these chemical properties were directly reflected in the relative ion intensities of Le^a and Le^X.

4.6. Z-type fragmentation at the 3-linkage

In the mechanism shown in Scheme 2, Z-type fragmentation is suspected to take place by amide proton transfer, followed by deprotonation of the anomeric proton by chloride anion to give the aziridine product ion. First, the transition state of the elimination reaction of the 3-substituted moiety was calculated. The initial, transition state, and product-ion structures involved in the Z-type fragmentation of Le^a are shown in Fig. 6. The transition state structure, in which the 3-substituted galactose moiety has already been eliminated, is similar in structure to the product ion, in agreement with Hammond's postulation. The transition state structure has geometry in which the nitrogen atom and neighboring C2 and C3 atoms on the GlcNAc form a 3-membered intermediate, and its

Table 2
Activation energies[a] of Lewis-type trisaccharides undergoing C-type fragmentation

		Le^a		Le^X	
		Initial	TS	Initial	TS
HF/6-31G(d)	Total energy	−2414.6684	−2414.5082	−2414.6552	−2414.5103
	ZPVE[b]	0.644898	0.637834	0.644084	0.638403
	E + ZPVE	−2414.0235	−2413.8704	−2414.0111	−2413.8719
	ΔE_a (kcal/mol)		96.1		87.4
HF/3-21G(d)	Total energy	−2401.8137	−2401.6289	−2401.8022	−2401.6491
	ZPVE[b]	0.638162	0.631775	0.638588	0.632757
	E + ZPVE	−2401.1755	−2400.9971	−2401.1637	−2401.0163
	ΔE_a (kcal/mol)		111.9		92.5

[a] Energy values are in Hartree unless noted otherwise.
[b] ZPVE represents zero-point vibrational energy.

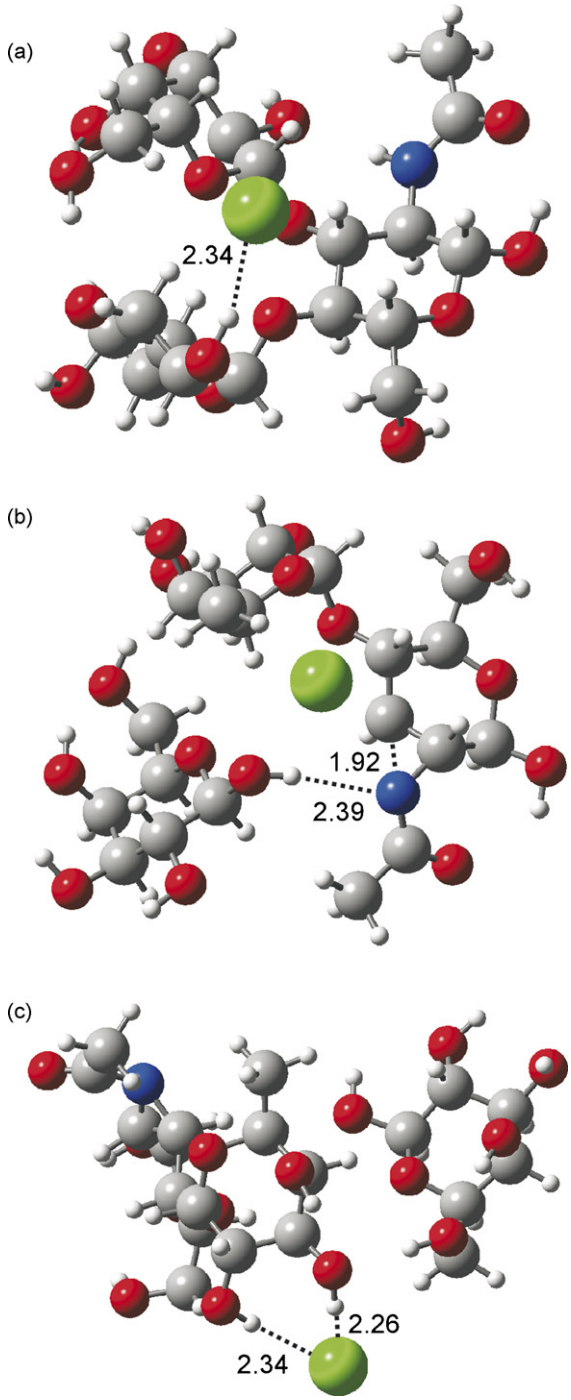


Fig. 6. Optimized structures of the chlorinated Le^a (a), transition state (b), and intermediate product ion (c) of the reaction in Scheme 2. All geometries were optimized by using the HF/6-31G(d) basis set.

vibrational motion corresponds to that of the transition state. The activation energies are listed in Table 3.

We next calculated the deprotonation reaction by the chloride anion. In this calculation, the eliminated neutral saccharide was omitted and the aziridine disaccharide was selected as the initial geometry. Fig. 7 shows the optimized structures of Le^a, along with the reaction pathway. The activation energies of the deprotonation reaction are listed in Table 4. The activation energies calculated at HF/6-31G(d) are 25.7 kcal/mol (Le^a) and 17.8 kcal/mol (Le^X)—much lower than the activation energies of Z-type fragmentations at the

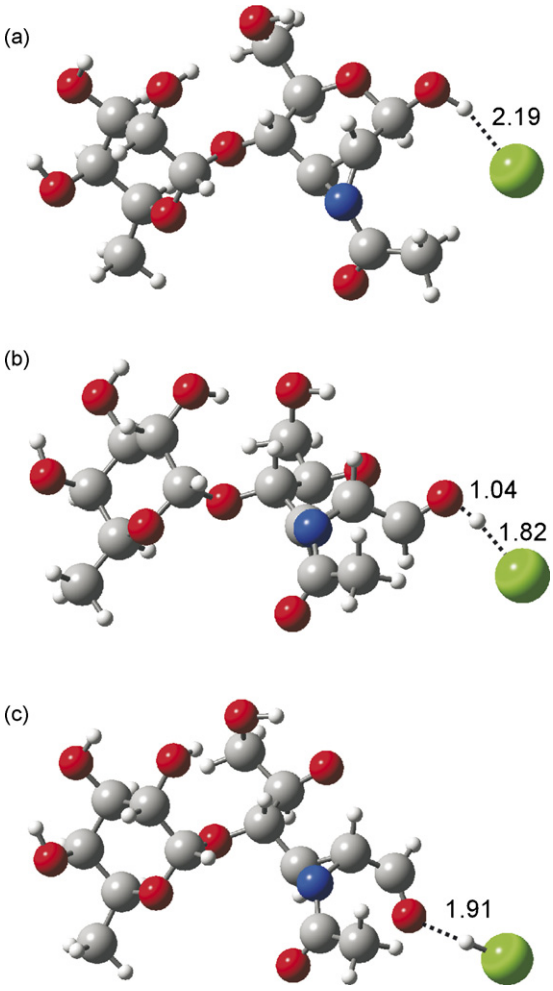


Fig. 7. Optimized structures of Le^a along with deprotonation reaction initial (a), transition state (b), and product (c) ion structures.

3-linkage (Table 3). The aziridine group is a highly distorted structure, since the conformation around the C2–C3 bond is fixed by the bridged amide bond. This distortion makes the deprotonation reaction at the anomeric hydroxyl proton by chloride anion easier, and at the same time, the ring-opening reaction takes place on the GlcNAc moiety by a deprotonation reaction. The ring-opened GlcNAc structure is more stable than retention of the 6-membered ring because of the presence of the strained aziridine group.

Comparison of the activation energies of C- and Z-type fragmentation calculated by HF theory revealed that the relationships of the calculated activation energies were $C_{1\beta}$ (4-linkage) > $Z_{1\alpha}$ (3-linkage) in Le^a, and $Z_{1\beta}$ (3-linkage) > $C_{1\alpha}$ (4-linkage) in Le^X. These results are consistent with the experimentally deduced trends in the activation energies of C- and Z-type fragmentations, as previously discussed in Section 4.1.

We also considered fragmentation mechanisms from the deprotonated molecule, and performed quantum chemical calculations from the deprotonated molecule. There are some possible deprotonation sites in Le-type saccharides, we calculated the optimized structures of various deprotonated molecules and found that the deprotonated molecule generated by amide proton withdrawal was the most stable. Calculations for both C-type (Scheme 3) and Z-type (Scheme 4) fragmentations were performed from the deprotonated molecule based on the schema. From the calculations, the activation energies were determined; C-type 78.5 kcal/mol, Z-type 71.0 kcal/mol in Le^a, C-type 76.9 kcal/mol, Z-type 72.0 kcal/mol in

Table 3
Activation energies[a] of Lewis-type trisaccharides undergoing Z-type fragmentation

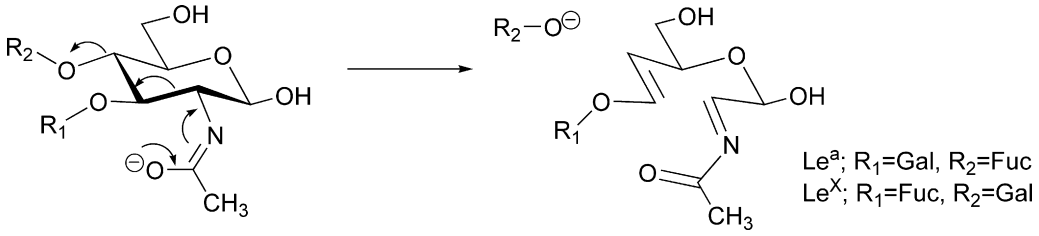
		Le^a		Le^X	
		Initial	TS	Initial	TS
HF/6-31G(d)	Total energy	−2414.6557	−2414.5011	−2414.6597	−2414.5060
	ZPVE[b]	0.646059	0.638479	0.645843	0.638751
	E + ZPVE	−2414.0096	−2413.8626	−2414.0139	−2413.8672
	ΔE_a (kcal/mol)		92.3		92.0
HF/3-21G(d)	Total energy	−2401.8137	−2401.6289	−2401.8022	−2401.6491
	ZPVE[b]	0.638162	0.631775	0.638588	0.632757
	E + ZPVE	−2401.1755	−2400.9971	−2401.1637	−2401.0163
	ΔE_a (kcal/mol)		105.4		104.6

[a] Energy values are in Hartree unless noted otherwise.
[b] ZPVE represents zero-point vibrational energy.

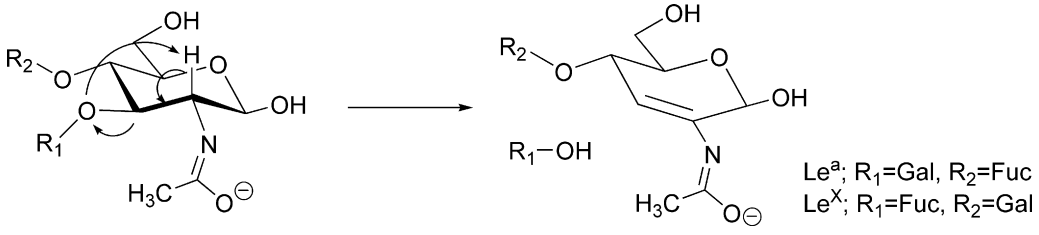
Table 4
Activation energies[a] for the deprotonation reaction of aziridine disaccharides

		Le^a		Le^X	
		Initial	TS	Initial	TS
HF/6-31G(d)	Total energy	−1731.1900	−1731.1468	−1806.0340	−1805.9903
	ZPVE[b]	0.422806	0.417228	0.428147	0.421275
	E + ZPVE	−1730.7672	−1730.7296	−1805.6059	−1805.5690
	ΔE_a (kcal/mol)		23.6		23.1
HF/3-21G(d)	Total energy	−1722.0612	−1722.0132	−1796.4811	−1796.4449
	ZPVE[b]	0.417776	0.410646	0.422316	0.41454
	E + ZPVE	−1721.6435	−1721.6025	−1796.0588	−1796.0304
	ΔE_a (kcal/mol)		25.7		17.8

[a] Energy values are in Hartree unless noted otherwise.
[b] ZPVE represents zero-point vibrational energy.



Scheme 3. Proposed mechanism of C-type fragmentation at the 4-linkage in deprotonated Lewis-type trisaccharides. The reaction takes place by successive electron transfer.



Scheme 4. Proposed mechanism of Z-type fragmentation at the 3-linkage in deprotonated Lewis-type trisaccharides. This mechanism takes place by C2 proton transfer to O-3 of GlcNAc and subsequent elimination.

Le^X. The relationship of the calculated activation energy was C-type (3-linkage) > Z-type (4-linkage), and experimental trend of deprotonated molecules of Le-type saccharides was that Z-type fragmentation took place easier than C-type fragmentation [61]. The calculated results agreed with trends of CID experiments for deprotonated molecules of Le^a and Le^X, whereas the calculated trends for the deprotonated molecule could not account for experimentally deduced tendencies of CID spectra from chlorinated Le-type saccharides.

From these discussions, the calculated reaction pathways of the C- and Z-type fragmentations of chlorinated Le-type saccharides could well account for the characteristic lack of observation of the deprotonated molecule in the CID spectra. The reaction mechanisms proposed from these results were in accordance with the experimentally deduced characteristics, which have been proven reasonable.

5. Conclusions

C- and Z-type fragmentations corresponding to 4- and 3-linked glycosyl bond cleavages of the *N*-acetylglucosamine moiety of Lewis-type trisaccharides were studied by both experimental and

theoretical methods. In the fragment ion spectra of chlorinated Le-type trisaccharides obtained by ESI-CID-MS, both C- and Z-type fragment ions were observed, but the deprotonated ion, which would be generated during the process of decomposition of chlorinated oligosaccharides, was not observed. The relationships of the relative ion intensities of C- and Z-type fragmentations were Z > C in Le^a and C > Z in Le^X, suggesting that the relationships of the activation energies were C > Z in Le^a and Z > C in Le^X. We surmised that anomeric and acetoamide groups were included in the mechanisms of these fragmentations; we therefore analyzed the structural derivatives of Lewis-type trisaccharides to identify the functional groups that influenced the fragmentations. In CID experiments on 3-FL and methylated Lewis-type trisaccharides, Z-type fragmentation was rarely observed, whereas C-type fragmentation was commonly observed. These results indicated that C-type fragmentation was little influenced by anomeric group, and that both anomeric and acetoamide groups enhanced Z-type fragmentation. In light of these results, fragmentation mechanisms of C- and Z-type were proposed and studied by quantum chemical calculations. The results of these calculations suggested that C-type fragmentation of the 4-linkage takes place by electron transfer from the acetoamide group, and that Z-type fragmentation at the 3-linkage takes place in two steps: (i) elimination of the 3-linked saccharide moiety, and (ii) deprotonation of the anomeric proton by a chloride anion. The interesting point is that the proposed reaction pathway did not yield deprotonated ions, which can well account for the experimental results. The activation energies of C-type fragmentations at the 4-linkages of Le^a and Le^X differed notably from each other; this was attributed to the difference in the stability of the Gal and Fuc anions in the gas phase. In contrast, the activation energies of the Z-type fragmentations for Le^a and Le^X were similar. By HF theory, the activation energy of the C-type was greater than that of the Z-type in Le^a, whereas the Z-type energy was greater than the C-type energy in Le^X, which was in accordance with experimentally deduced trends in the activation energies. Fragmentation mechanisms of the deprotonated molecules were also studied by performing quantum chemical calculations. Calculations for the deprotonated molecule were in good accordance with experimental trends of the deprotonated molecules of Le-type saccharides, however the calculated results did not agree with the trends of the fragmentations from chlorinated Le-type saccharides. From these discussions, the proposed mechanisms were thus proven reasonable. The combination of experimental and theoretical studies was an appropriate method for analyzing the reaction pathways and should be useful for analyzing the reaction pathways of other chlorinated oligosaccharides.

Acknowledgments

The experimental section of this study was financially supported by the Global COE Program for Chemistry Innovation and the Japan Society for the Promotion of Science (JSPS, 18-11463).

References

[1] A. Varki, Glycobiology 3 (1993) 97.
[2] R.A. Dwek, T.D. Butters, Chem. Rev. 102 (2002) 283.
[3] S. Roseman, J. Biol. Chem. 276 (2001) 41527.
[4] C.R. Bertozzi, L.L. Kiessling, Science 291 (2001) 2357.
[5] D.J. Harvey, Mass Spectrom. Rev. 18 (1999) 349.
[6] A. Dell, H.R. Morris, Science 291 (2001) 2351.
[7] J. Zaia, Mass Spectrom. Rev. 23 (2004) 161.
[8] D.J. Harvey, J. Chromatogr. A 720 (1996) 429.
[9] Y. Mechref, M.V. Novotny, Chem. Rev. 102 (2002) 321.
[10] R. Kaufmann, B. Spengler, F. Lutzenkirchen, Rapid Commun. Mass Spectrom. 7 (1993) 902.
[11] F.W. McLafferty, Science 214 (1981) 280.
[12] M.D. Mohr, K.O. Bornsen, H.M. Widmer, Rapid Commun. Mass Spectrom. 9 (1995) 809.
[13] M.T. Cancilla, S.G. Penn, J.A. Carroll, C.B. Lebrilla, J. Am. Chem. Soc. 118 (1996) 6736.
[14] U. Boesl, R. Weinkauf, E.W. Schlag, Int. J. Mass Spectrom. Ion Proc. 112 (1992) 121.
[15] B. Domon, C.E. Costello, Glycoconj. J. 5 (1988) 397.
[16] G.E. Hofmeister, Z. Zhou, J.A. Leary, J. Am. Chem. Soc. 113 (1991) 5964.
[17] D. Garozzo, M. Giuffrida, G. Impallomeni, A. Ballistreri, G. Montaudo, Anal. Chem. 62 (1990) 279.
[18] J.W. Dallinga, W. Heerma, Biol. Mass Spectrom. 20 (1991) 215.
[19] S.G. Penn, M.T. Cancilla, C.B. Lebrilla, Anal. Chem. 68 (1996) 2331.
[20] D.J. Harvey, T.S. Mattu, M.R. Wormald, L. Royle, R.A. Dwek, P.M. Rudd, Anal. Chem. 74 (2002) 734.
[21] P.B. O'Connor, E. Mirgorodskaya, C.E. Costello, J. Am. Soc. Mass Spectrom. 13 (2002) 402.
[22] A.K. Powell, D.J. Harvey, Rapid Commun. Mass Spectrom. 10 (1996) 1027.
[23] W.G. Chai, V. Piskarev, A.M. Lawson, Anal. Chem. 73 (2001) 651.
[24] W.G. Chai, V. Piskarev, A.M. Lawson, J. Am. Soc. Mass Spectrom. 13 (2002) 670.
[25] A. Pfenninger, M. Karas, B. Finke, B. Stahl, J. Am. Soc. Mass Spectrom. 13 (2002) 1331.
[26] A. Pfenninger, M. Karas, B. Finke, B. Stahl, J. Am. Soc. Mass Spectrom. 13 (2002) 1341.
[27] D.J. Harvey, J. Am. Soc. Mass Spectrom. 16 (2005) 647.
[28] D.J. Harvey, J. Am. Soc. Mass Spectrom. 16 (2005) 631.
[29] D.J. Harvey, J. Am. Soc. Mass Spectrom. 16 (2005) 622.
[30] H. Nonami, K. Tanaka, Y. Fukuyama, R. Erra-Balsells, Rapid Commun. Mass Spectrom. 12 (1998) 285.
[31] H. Nonami, F. Wu, R.P. Thummel, Y. Fukuyama, H. Yamaoka, R. Erra-Balsells, Rapid Commun. Mass Spectrom. 15 (2001) 2354.
[32] Y. Cai, Y.J. Jiang, R.B. Cole, Anal. Chem. 75 (2003) 1638.
[33] H. Suzuki, T. Yamagaki, K. Tachibana, Trends Glycosci. Glycotechnol. 18 (2006) 277.
[34] T. Yamagaki, H. Suzuki, K. Tachibana, Anal. Chem. 77 (2005) 1701.
[35] T. Yamagaki, K. Fukui, K. Tachibana, Anal. Chem. 78 (2006) 1015.
[36] T.T. Fang, J. Zirrolli, B. Bendiak, Carbohydr. Res. 342 (2007) 217.
[37] Y. Cai, R.B. Cole, Anal. Chem. 74 (2002) 985.
[38] T. Yamagaki, H. Suzuki, K. Tachibana, J. Mass Spectrom. 41 (2006) 454.
[39] T. Yamagaki, H. Suzuki, K. Tachibana, J. Am. Soc. Mass Spectrom. 17 (2006) 67.
[40] T. Yamagaki, H. Suzuki, K. Tachibana, J. Mass Spectrom. Soc. Jpn. 54 (2006) 144.
[41] M.T. Cancilla, S.G. Penn, C.B. Lebrilla, Anal. Chem. 70 (1998) 663.
[42] K. Fukui, A. Kameyama, Y. Mukai, K. Takahashi, N. Ikeda, Y. Akiyama, H. Narimatsu, Carbohydr. Res. 341 (2006) 624.
[43] J.A. Carroll, D. Willard, C.B. Lebrilla, Anal. Chim. Acta 307 (1995) 431.
[44] B. Mulroney, J.B. Peel, J.C. Traeger, J. Mass Spectrom. 34 (1999) 856.
[45] A. Imberty, S. Perez, Chem. Rev. 100 (2000) 4567.
[46] K.E. Miller, C. Mukhopadhyay, P. Cagas, C.A. Bush, Biochemistry 31 (1992) 6703.
[47] H.F. Azurmendi, M. Martin-Pastor, C.A. Bush, Biopolymers 63 (2002) 89.
[48] H.F. Azurmendi, C.A. Bush, Carbohydr. Res. 337 (2002) 905.
[49] S. Perez, N. MouhousRiou, N.E. Nifantev, Y.E. Tsvetkov, B. Bachet, A. Imberty, Glycobiology 6 (1996) 537.
[50] G.I. Csonka, C.P. Sosa, J. Phys. Chem. A 104 (2000) 7113.
[51] G.I. Csonka, C.P. Sosa, I.G. Csizmadia, J. Phys. Chem. A 104 (2000) 3381.
[52] F.I. Auzanneau, E. Sourial, J.M. Schmidt, M. Feher, Can. J. Chem. 80 (2002) 1088.
[53] A. Imberty, E. Mikros, J. Koca, R. Mollicone, R. Oriol, S. Perez, Glycoconj. J. 12 (1995) 331.
[54] T. Yamagaki, H. Nakanishi, J. Mass Spectrom. 35 (2000) 1300.
[55] T. Ii, Y. Ohashi, Y. Nagai, Carbohydr Res. 273 (1995) 27.
[56] T. Ii, Y. Ohashi, T. Ogawa, Y. Nagai, Glycoconj. J. 13 (1996) 273.
[57] M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, J.A. Montgomery Jr., T. Vreven, K.N. Kudin, J.C. Burant, J.M. Millam, S.S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G.A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J.E. Knox, H.P. Hratchian, J.B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R.E. Stratmann, O. Yazyev, A.J. Austin, R. Cammi, C. Pomelli, J.W. Ochterski, P.Y. Ayala, K. Morokuma, G.A. Voth, P. Salvador, J.J. Dannenberg, V.G. Zakrzewski, S. Dapprich, A.D. Daniels, M.C. Strain, O. Farkas, D.K. Malick, A.D. Rabuck, K. Raghavachari, J.B. Foresman, J.V. Ortiz, Q. Cui, A.G. Baboul, S. Clifford, J. Cioslowski, B.B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R.L. Martin, D.J. Fox, T. Keith, M.A. Al-Laham, C.Y. Peng, A. Nanayakkara, M. Challacombe, P.M.W. Gill, B. Johnson, W. Chen, M.W. Wong, C. Gonzalez, J.A. Pople, R.C. Gaussian 03, Gaussian, Inc., Wallingford, CT, 2004.
[58] C.Y. Peng, P.Y. Ayala, H.B. Schlegel, M.J. Frisch, J. Comp. Chem. 17 (1996) 49.
[59] Y.J. Jiang, R.B. Cole, J. Am. Soc. Mass Spectrom. 16 (2005) 60.
[60] G. Hammond, J. Am. Chem. Soc. 77 (1955) 334.
[61] J.L. Seymour, C.E. Costello, J. Zaia, J. Am. Soc. Mass Spectrom. 17 (2006) 844.