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MASS SPECTROMETRY OF OLIGOSACCHARIDES BY CHLORIDE-ATTACHMENT REACTIONS: THE ORIGIN OF FRAGMENT LOSS

DANIELLE PROMÉ*, JEAN-CLAUDE PROMÉ, GERMAIN PUZO, AND HÉLÈNE AURELLE
Centre de Recherche de Biochimie et Génétique Cellulaires C.N.R.S., 118 Route de Narbonne, 31062 Toulouse Cédex (France)

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ABSTRACT

The direct exposure, negative chemical ionisation, chloride-attachment mass spectrometry of trehalose and sucrose gave abundant chloride-attached molecular ions. The same feature was observed when these sugars were subjected to fast-atom bombardment (f.a.b.) in a glycerol matrix containing ammonium chloride. No characteristic fragment ion was found when trehalose was analysed by either method. In contrast, sucrose gave intense chloride-containing fragments, arising by glycosidic cleavage, when analysed by the first method, whereas such cleavage was not detectable by f.a.b.–ammonium chloride analysis. However, the mass-analysed ion kinetic energy (m.i.k.e.) spectra of the $(M + Cl)^-$ ions from either trehalose and sucrose, generated under f.a.b.–ammonium chloride conditions, showed glycosidic cleavage reactions in addition to a large loss of HCl. These cleavage reactions might be attributed to S_N2-like reactions on the acetal carbon atom and to base-induced eliminations, and they were enhanced by collision-induced dissociations. However, the relative abundance of such glycosidic cleavages from the ionic state would be too weak to explain the presence of the large chloride-containing fragments in the direct exposure spectra of sucrose. Thus, these ions were mainly produced by a thermal cleavage followed by chloride-attachment reactions.

INTRODUCTION

Recent developments in ionisation techniques in mass spectrometry have allowed the direct analysis of underivatised saccharides and glycosides. Most of the reports concerned proton- and cation-attachment reactions, and little attention has been paid to anion-attachment reactions.

Under negative chemical ionisation (c.i.) conditions, using Freon 12 as reactant gas and a direct exposure probe, Ganguly *et al.*[1] showed that some glycosides produced chloride-attached molecular ions. In addition, abundant chloride-con-

*To whom correspondence should be addressed.

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taining fragment ions arising from glycosidic cleavages were observed, which made possible the sequencing of the oligosaccharidic moiety.

By field-desorption negative mass spectrometry, using LiCl in a poly(ethylene oxide) matrix, oligosaccharides gave[2] only two peaks due to the $(M + Cl)^-$ and Cl^- ions. The negative ion f.a.b. spectra of oligosaccharides[3], using a glycerol matrix containing inorganic chloride salts, showed only unfragmented molecular ions, mainly $(M + Cl)^-$ and $(M - H)^-$.

The fragment ions which appeared in the direct exposure spectra might be attributed to either thermal cleavage followed by ionisation of the fragments by chloride attachment, or decomposition in the gas phase of the $(M + Cl)^-$ ions. We now report on the fragmentation pathways of such cluster anions.

RESULTS

Sucrose and α,α-trehalose were selected as model compounds for the chloride-attachment studies.

Negative c.i., direct exposure spectra in the presence of chloride ions[4]. — The chloride reactant ions were generated by electron bombardment of either dichloromethane or dichlorodifluoromethane (Freon 12) in a high-pressure source at 200°, and the samples were desorbed from a direct exposure probe (Nermag). Although a high source-temperature was used in order to reduce the intensity of the cluster ions due to the reactant gas[5], a computer background-subtraction was needed in order to lower the relative height of such cluster peaks.

The spectra of trehalose and sucrose were measured at the beginning of the desorption process. The spectra from trehalose (Fig. 1) contained only unfragmented ions, mainly the chloride-attached molecular ion at *m/z* 377 and the chloride-attached dimer at 719. Under similar conditions, sucrose gave the chloride-attached molecular ion at *m/z* 377, but intense fragment ions were also formed, each of which contained a chlorine atom. These ions resulted from the loss of water, namely (sucrose $- H_2O + Cl)^-$ at *m/z* 359, or from glycosidic cleavage, mainly (hexose $+ Cl)^-$ at *m/z* 215, (hexose $- H_2O + Cl)^-$ at *m/z* 197, and (hexose $- 2\,H_2O + Cl)^-$ at *m/z* 179. The abundance of the ion $(M - H)^-$ at *m/z* 341 was low.

When the probe temperature was increased, some fragments began to appear in the spectra from trehalose, whereas the intensity of the above-mentioned fragments in the spectra of sucrose increased relative to the intensity of the $(M + Cl)^-$ ion. Moreover, these spectra were modified when very small amounts of sample were deposited on the probe. As shown in Fig. 2, the spectra of sucrose were very different depending on whether 50 or 10 ng were deposited. In particular, the abundance of the fragments was enhanced and new types of chlorine-containing fragments were seen, such as that at *m/z* 207 the origin of which remains to be elucidated. These decompositions might be due to stronger interactions in the solid state, involving the sample and the metallic probe on to which it was deposited as a thin layer.

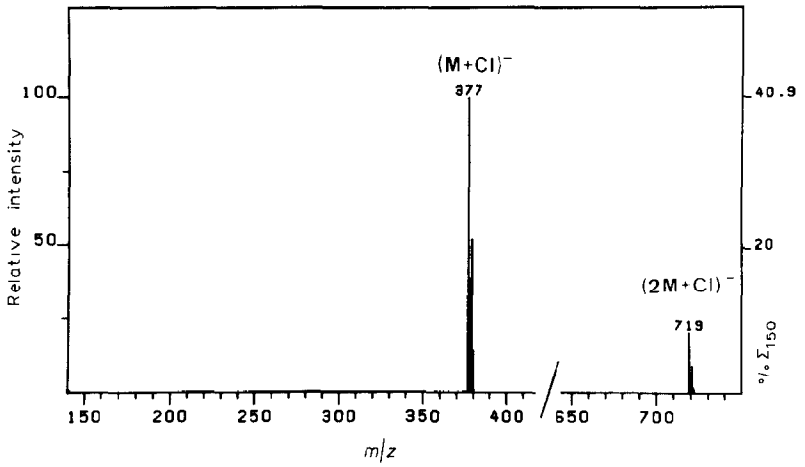


Fig. 1. Direct exposure, negative c.i. mass spectra of trehalose in the presence of CF_2Cl_2.

Negative ion, f.a.b. in the presence of chloride salts. — The spectra of trehalose and sucrose were produced by focusing a molecular beam of xenon atoms on a solution of the sample in glycerol containing ammonium chloride. The peaks of the ions from the matrix were subtracted by a computer. These spectra remained unchanged for long periods of time. As observed in Fig. 3, the main ions were the chloride-attached molecular ion at *m/z* 377 and the ion $(M - H)^-$ at *m/z* 341. The only detectable fragment ion corresponded to the loss of H_2 from $(M + Cl)^-$. All other fragment ions, if any, had a low intensity and were masked by the remaining chemical background. No cleavage of the glycosidic bond could be detected.

M.i.k.e. spectra of the chloride-attached molecular ions. — As noted above, the absence from f.a.b. spectra of ions associated with glycosidic cleavage did not prove that such reactions did not occur. One of the advantages of m.i.k.e. spectrometry is the possibility of detecting ions that arise from decomposition reactions of very low probability, because of the absence of chemical background. Thus, if fragmentation of the $(M + Cl)^-$ ions occurred, even with a very low abundance, they should be detected by measuring the corresponding m.i.k.e. spectra. The $(M + Cl)^-$ precursor ions were generated by f.a.b., and the distinction between fragments containing or not containing a chlorine atom was made by parallel experiments on the isotopic $(M + {}^{35}Cl)^-$ and $(M + {}^{37}Cl)^-$ ions.

In such spectra from trehalose and sucrose, the main decomposition process corresponded to the loss of hydrogen chloride (Table I). However, other decomposition pathways were observed, involving cleavage of the glycosidic bonds which produced mainly three types of ions, namely, $(\text{hexose} + Cl)^-$, $(\text{hexose} - H_2O + Cl)^-$, and $(\text{hexose} - H)^-$. The isomeric disaccharides could be differentiated by the relative abundances of these fragments; the ratio of intensities of the ions $(\text{hexose} + Cl)^-$ and $(\text{hexose} - H)^-$ was 0.5 for trehalose and 10 for sucrose.

Collision-induced dissociation (c.i.d.) spectra of the $(M + Cl)^-$ ions. — These

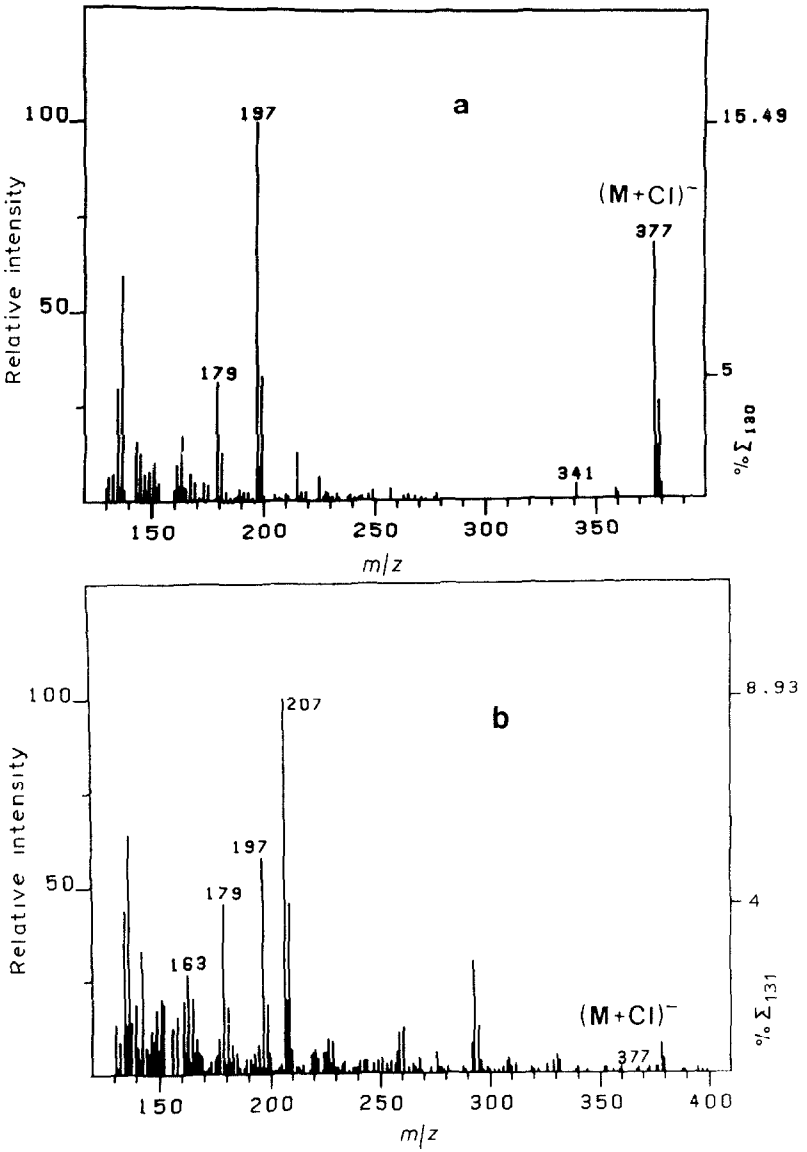


Fig. 2. Direct exposure, negative c.i. mass spectra of sucrose in the presence of CF_2Cl_2: (*a*) 50 ng, (*b*) 10 ng.

spectra were produced by the introduction of helium as a collision gas into the gas cell placed in the second field-free region in order to reduce the ion beam of the $(M + Cl)^-$ ions to 50% of its original value. The c.i.d.–m.i.k.e. spectra measured under these conditions reflect complex disruption of the sugar backbone. Since the width of the peaks was increased, the assignments of the corresponding *m/z* values were less accurate and some peaks were clearly due to the superposition of ions of different *m/z* values. The abundances of some characteristic ions are presented in Table II.

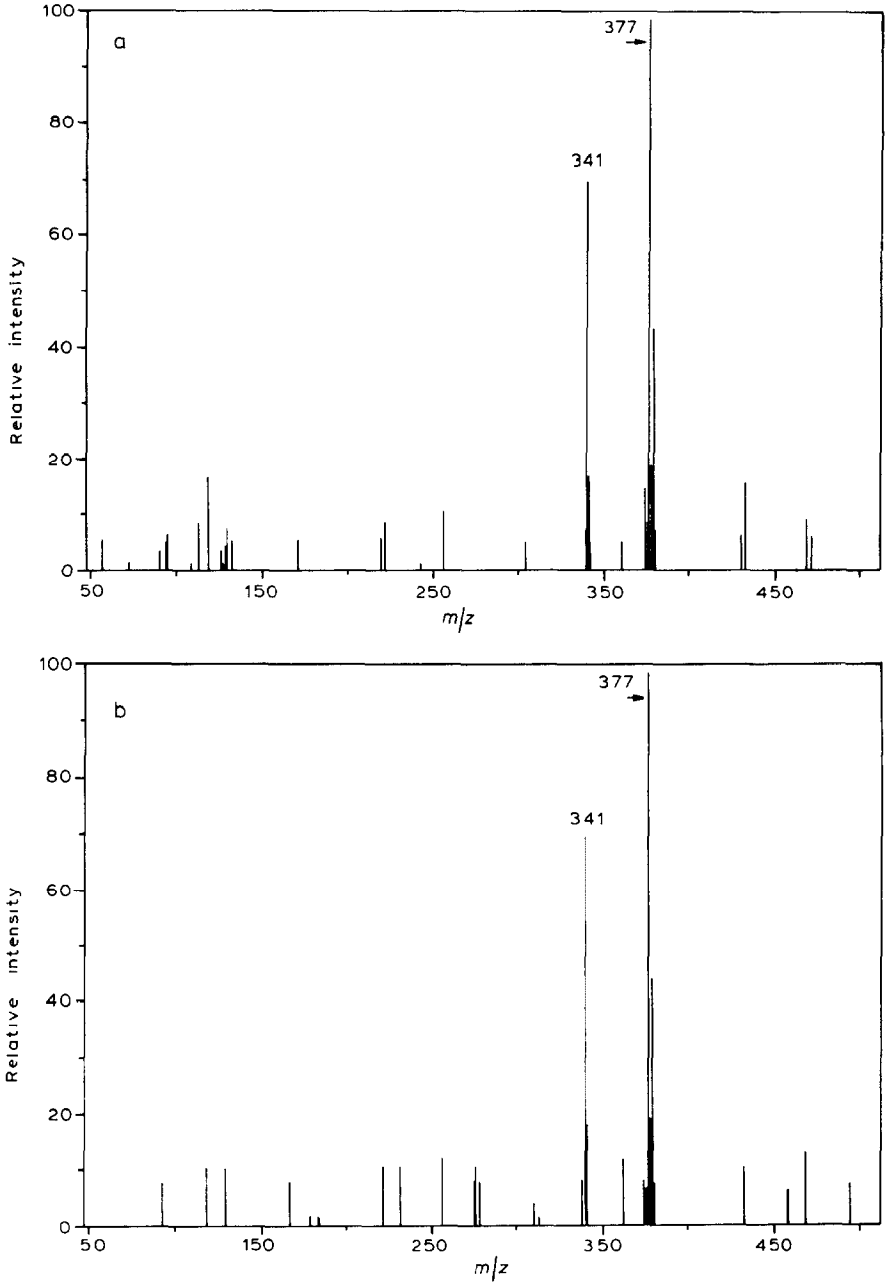


Fig. 3. F.a.b. mass spectra in a glycerol matrix containing ammonium chloride: (*a*) trehalose, (*b*) sucrose.

TABLE I

M.I.K.E. SPECTRA OF THE $(M + {}^{35}Cl)^-$ IONS FROM TREHALOSE AND SUCROSE

Calc. m/z value of the fragments	*Relative height of the fragment peaks (%)*		*Assignment*
	Trehalose	*Sucrose*	
359	3.7	2.3	loss of H_2O
341	100.0	100.0	loss of HCl
323	2.0	1.2	loss of $(H_2O + HCl)$
215	3.0	6.9	$(hexose + Cl)^-$
197	1.7	1.3	$(hexose - H_2O + Cl)^-$
179	5.5	0.7	$(hexose - H)^-$
35	1.7	0.1	Cl^-

TABLE II

MAIN FRAGMENT IONS[a] IN THE C.I.D.–M.I.K.E. SPECTRA FROM TREHALOSE AND SUCROSE

Calc. m/z value at the top of the peak	*Relative height of the fragment peaks (%)*		*Assignment*
	Trehalose	*Sucrose*	
359.0	10	5	loss of H_2O
345.5	17	5	loss of MeOH
341.0	100	100	loss of HCl
215.0	40	25	$(hexose + Cl)^-$
213.5	50	8	$(hexose - H_2 + Cl)^-$
196.7	16	13	$(hexose - H_2O + Cl)^-$
182.8	30	13	$(hexose - CH_3OH + Cl)^-$
179.0	38	20	$(hexose - H)^-$
34.9	15	11	Cl^-

[a]The complex distribution in the range *m/z* 150–50 is not shown.

Besides the intense formation of the $(M - H)^-$ ion, which remained the major decomposition process of the $(M + Cl)^-$ ion, the main fragmentations corresponded to the loss of methanol and to glycosidic cleavages, yielding $(hexose + Cl)^-$, $(hexose - H_2 + Cl)^-$, $(hexose - H_2O + Cl)^-$, $(hexose - MeOH + Cl)^-$, and $(hexose - H)^-$ ions. Although the c.i.d.–m.i.k.e. spectra of trehalose and sucrose were not identical, the differences were less pronounced than in the m.i.k.e. spectra.

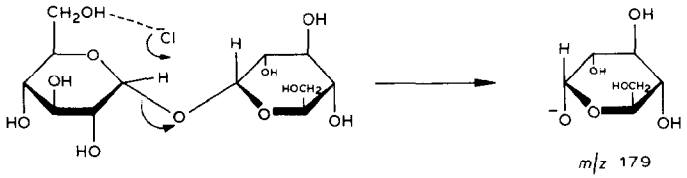
DISCUSSION

The m.i.k.e., c.i.d.–m.i.k.e. experiments described above showed that the chloride-attached molecular ions of either trehalose or sucrose were dissociated through cleavage of the glycosidic bonds. The low-energy processes shown in the m.i.k.e. spectra corresponded to two main pathways. In the first pathway, the

chlorine atom was lost together with an anhydrohexose moiety, leading to the ion (hexose − H)$^-$. In the second pathway, the elimination of a hexose or an anhydrohexose molecule was promoted, leading to the ions (hexose − H_2O + Cl)$^-$ or (hexose + Cl)$^-$, respectively. However, the main decomposition process of the (M + Cl)$^-$ ions remained the loss of HCl.

It is generally assumed that chloride-attached molecular ions of proton-donor molecules possess solvation-complex structures in which the anion is linked through a hydrogen bond to the proton donor site[6]. The main pathway of decomposition for such complexes would be the loss of hydrogen chloride or the formation of chloride ion by cleavage of hydrogen bonds. This process corresponds to the main pathway of decomposition of the (M + Cl)$^-$ ions from trehalose and sucrose.

The cleavage of the glycosidic bond in the chloride-attached molecular ion of disaccharides is surprising from a chemical point of view. In sugar molecules, the glycosidic bonds are usually resistant to attack by anions and are stable in basic media[7]. However, acetal groups can be cleaved by strong bases, such as Grignard reagents, under drastic conditions[8]. In the gas phase, although the existence of stable, pentacoordinate-carbon, negative atoms, such as an S_N2 transition complex generated by the attack of a nucleophile on a saturated carbon atom, has been the subject of debate[9], nucleophilic substitutions were frequently observed[6] and their rates can be many orders of magnitude larger than for reactions occurring in solution[10]. The S_N2 reaction corresponding to cleavage of the glycosidic bond by attack by the chloride ion is shown in **1→2**. This pathway generates the (hexose − H)$^-$ ion. It is likely that the chloride ions remain partially solvated by hydroxyl groups and that this reaction is the result of intramolecular attack allowed by favorable conformations.



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The second pathway (chlorine atom remaining on the negatively charged fragment) can be explained on the assumption that the approach of the chloride ion near to the acetal function enhances the negative charge on the oxygen atom of the interglycosidic bond, thus promoting a base-induced elimination of a sugar moiety by means of proton transfer associated with cleavage of the carbon–oxygen bond.

Although these fragment ions were clearly observed in the m.i.k.e. and c.i.d.–m.i.k.e. spectra, their intensity was too low to be detected in the "normal" f.a.b. spectra. The reason could be that this fragmentation process was probably endothermic and that most of the (M + Cl)$^-$ ions formed in the ion source did not possess sufficient internal energy to undergo fragmentation within the allowed timescale.

In contrast, the direct exposure, negative c.i. spectra of sucrose in the presence of chloride ions gave intense chloride-containing fragment ions. This difference in behaviour could be due to a larger amount of internal energy in the $(M + Cl)^-$ ions or to a thermal process prior to ionisation. However, since the main fragmentation pathway of the $(M + Cl)^-$ ions involved loss of HCl, either at low (m.i.k.e.) or high (c.i.d.–m.i.k.e.) energy, the very low abundance of the $(M - H)^-$ ions in the direct exposure spectra could account only for some of the minor fragment peaks formed from the ionic gaseous state. Thus, the intense fragment ions arose mainly from thermal cleavage rather than from ionic decompositions.

EXPERIMENTAL

The direct exposure, chemical ionisation experiments were made on a Nermag R-10 10 quadrupole instrument working in the negative mode. The ion-source temperature was 200° and the emission current was 50 μA. The ion-source pressure of reactant gas (CH_2Cl_2 or CF_2Cl_2) was adjusted so as to produce maximum intensity of the chloride-ion beam (at ~1 Torr). The samples were deposited on the probe from aqueous solutions and the probe temperature was increased linearly. The spectra were recorded by computer, using a background-subtraction programme.

The f.a.b. spectra were produced by a VG-MM ZAB 2F instrument. The accelerating voltage was 8 keV and the molecular beam of xenon atoms was accelerated at 8 keV. The samples were deposited on the f.a.b. probe as a solution in glycerol (2 μL) and M ammonium chloride (1 μL). The spectra were recorded by a computer system and a subtraction was made from spectra obtained without any sugar in the matrix. The c.i.d.–m.i.k.e. experiments involved ions accelerated at 8 keV and recorded on u.v.-sensitive paper.

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