Fast Atom Bombardment Mass Spectrometry of Carbohydrate Chains Derived from Glycoproteins

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Positive as well as negative ion fast atom bombardment mass spectrometry has been applied to a series of underivatized oligosaccharides and glycopeptides derived from glycoproteins of the N-glycosidic type. The mass spectra obtained of the various compounds contain molecular weight as well as sequence information. The fragmentation of the pseudomolecular ions of the carbohydrate chains appears to take place only along a small number of specific routes. The mass spectrometric data make clear that fast atom bombardment mass spectrometry can become a valuable additional technique for the structural analysis of glycoconjugates.

INTRODUCTION

In the past 20 years mass spectrometry has become an important method in the analysis of primary structures of polysaccharides and carbohydrate moieties of glycoproteins and glycolipids. The centre of many of these investigations is methylation analysis, which provides information concerning the substitution patterns of monosaccharides in oligomers.[1-6] Mass spectrometry has also been applied to intact oligosaccharides, oligosaccharide-alditols, glycopeptides and glycolipids, mainly using their relatively volatile methylated, acetylated or trimethylsilylated derivatives.[2,6-11] For the analysis, electron impact as well as chemical ionization mass spectrometry are employed. The mass spectra of the derivatized oligomers contain sequence information as far as the type of sugar is concerned such as hexose, deoxyhexose, acetamidohexose and sialic acid residues. From the spectra of small molecules information on the positions of glycosidic linkages between the monosaccharides can sometimes be obtained.[7] Other ionization methods like field ionization, field desorption and desorption chemical ionization have mainly been used in model studies on easily available products.[7] Field desorption mass spectrometry has been employed for the characterization of carbohydrate chains of the oligomannoside type glycoproteins.[12]

Recently, fast atom bombardment (FAB) has been developed and introduced as a new ionization technique for the analysis of a wide range of compounds, including non-volatile and thermally labile, polar and non-polar species.[13-18] The possibility of obtaining mass spectra from underivatized molecules is also attractive for the structural analysis of carbohydrates as was demonstrated recently in the revision of the structure of a 6-O-methylglucose polysaccharide of Mycobacterium smegmatis.[19] This paper reports preliminary studies on FAB mass spectrometry of underivatized oligosaccharides and glycopeptides, derived from glycoproteins in which the carbohydrate chain is N-glycosidically linked to the polypeptide-backbone via a GlcNAc→Asn carbohydrate-peptide linkage.

EXPERIMENTAL

Positive and negative ion mass spectra were recorded on a VG Analytical ZAB-HF mass spectrometer, an instrument with reverse geometry and fitted with a high field magnet. The primary beam was composed of xenon atoms with a maximum energy of approximately 7.6 keV. The carbohydrate samples (0.1 mg) were dissolved or dispersed in glycerol. In some cases, small amounts of NaCl were also added. The sputtered ions were extracted and accelerated with a potential of 8 kV. A linear mass scan over 2000 daltons was made in approximately 70 s. The spectra were obtained with an ultraviolet chart recorder.

For the isolation of the carbohydrates studied, see the references in Table 1.

RESULTS

A series of oligosaccharides and glycopeptides obtained from different biological sources have been investigated; their structures are summarized in Table 1. The most important mass spectrometric data deduced from the positive and/or negative ion mass spectra of each compound are presented in Tables 2-8. Since insufficient information is currently available to decide whether discrimination between stereoisomeric monosaccharides and between type and configuration

Table 1. List of carbohydrate structures investigated[a]

No.	Oligosaccharides/glycopeptides	Reference
1	GlcNAc($\beta1 \rightarrow$ N)Asn	20
2	Fuc($\alpha1 \rightarrow$ 6)GlcNAc($\beta1 \rightarrow$ N)Asn	20
3	Man($\alpha1 \rightarrow$ 2)Man($\alpha1 \rightarrow$ 2)Man($\alpha1 \rightarrow$ 3)- Man($\beta1 \rightarrow$ 4)GlcNAc	21
4	GlcNAc($\beta1 \rightarrow$ 2)Man($\alpha1 \rightarrow$ 3)[GlcNAc($\beta1 \rightarrow$ 2)- Man($\alpha1 \rightarrow$ 6)]Man($\beta1 \rightarrow$ 4)GlcNAc	22
5	GlcNAc($\beta1 \rightarrow$ 2)Man($\alpha1 \rightarrow$ 3)[GlcNAc($\beta1 \rightarrow$ 2)- Man($\alpha1 \rightarrow$ 6)][GlcNAc($\beta1 \rightarrow$ 4)]- Man($\beta1 \rightarrow$ 4)GlcNAc	22
6	GlcNAc($\beta1 \rightarrow$ 2)[GlcNAc($\beta1 \rightarrow$ 4)]- Man($\alpha1 \rightarrow$ 3)[GlcNAc($\beta1 \rightarrow$ 2)Man($\alpha1 \rightarrow$ 6)]- [GlcNAc($\beta1 \rightarrow$ 4)]Man($\beta1 \rightarrow$ 4)GlcNAc($\beta1 \rightarrow$ 4)- GlcNAc($\beta1 \rightarrow$ N)Asn	23, 24
7	Gal($\beta1 \rightarrow$ 4)GlcNAc($\beta1 \rightarrow$ 2)Man($\alpha1 \rightarrow$ 3)- [Gal($\beta1 \rightarrow$ 4)GlcNAc($\beta1 \rightarrow$ 2)Man($\alpha1 \rightarrow$ 6)]- Man($\beta1 \rightarrow$ 4)GlcNAc($\beta1 \rightarrow$ 4)GlcNAc($\beta1 \rightarrow$ N)Asn	25, 26

[a] Abbreviations: Fuc = fucose; Man = mannose; Gal = galactose; GlcNAc = 2-acetamido-2-deoxy-glucose; Asn = asparagine. All monosaccharides occur in the pyranose ring form.

Table 2. Positive and negative ion mass spectral data of compound 1 (M = 335). Intensity $[M+H]^+ = 100$

Positive ion mode

m/z	Rel. int. (%)	Ion composition
428	9	(M + glycerol) + H
358	87	M + Na
336	100	M + H
133	45	(S_1 + H) + H

Negative ion mode

m/z	Ion composition
334	M − H

HexNAc$\underset{1}{\overset{\overset{\displaystyle\rightarrow}{\vdots\,131}}{|}}$NH—CO—CH$_2$—CH$\overset{\displaystyle COOH}{\underset{\displaystyle NH_2}{|}}$
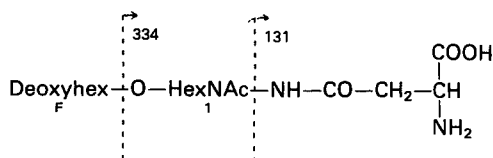
of glycosidic linkages can be derived from FAB spectra, the structures in Tables 2–8 are given in terms of hexoses (Hex), deoxyhexoses (Deoxyhex) and acetamidohexoses (HexNAc). The numbering of the monosaccharide residues in the oligosaccharides and glycopeptides corresponds with the nomenclature introduced for the analysis of this type of compound by high

Table 3. Positive and negative ion mass spectral data of compound 2 (M = 481). Intensity $[M+H]^+ = 100$; $[M-H]^- = 100$

Positive ion mode

m/z	Rel. int. (%)	Ion composition
526	9	M + 2Na − H
520	12	M + K
504	73	M + Na
482	100	M + H
336	55	(S_F + H) + H
133	88	(S_1 + H) + H

Negative ion mode

m/z	Rel. int. (%)	Ion composition
756	3	(M + glycerol$_3$) − H
664	4	(M + glycerol$_2$) − H
572	7	(M + glycerol) − H
480	100	M − H
334	28	(S_F + H) − H
131	33	(S_1 + H) − H

Deoxyhex$\underset{F}{|}$—O—HexNAc$\underset{1}{|}$—NH—CO—CH$_2$—CH$\overset{\displaystyle COOH}{\underset{\displaystyle NH_2}{|}}$

resolution ^{1}H nuclear magnetic resonance spectroscopy.[27,28] As a typical example, part of the negative ion FAB mass spectrum of compound 7 is presented in Fig. 1.

Pseudomolecular ions

In the positive ion mass spectra the molecular weight can be deduced from the relatively abundant protonated molecular ion $[M+H]^+$, and from the frequently present cationized molecular ions $[M+Na]^+$ and/or $[M+K]^+$. The abundances of the alkali-metal cationized ions were dependent on the compound under investigation, indicating different amounts of inorganic salts resulting from isolation procedures. The amount of these species can be enhanced by the addition of suitable salts to the glycerol matrix. Sometimes $[M+H+glycerol]^+$ adduct ions were also detected.

The negative ion spectra are characterized by relatively abundant $[M-H]^-$ and/or $[M+Cl]^-$ ions. Especially for larger molecules a better signal to background ratio has been observed in the negative ion
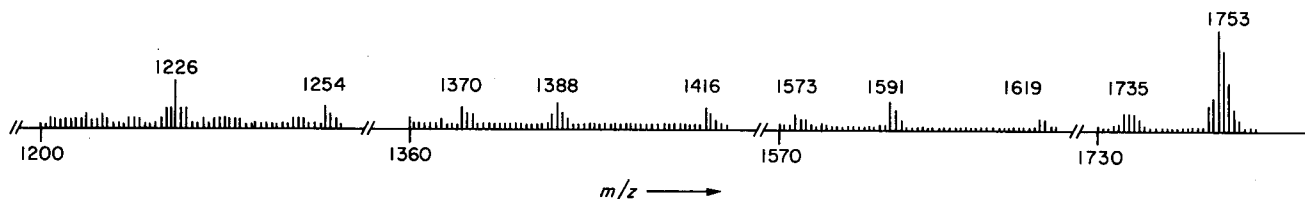
Figure 1. High mass range of the negative ion FAB mass spectrum of compound 7 (for structure and explanation, see Tables 1 and 8).

Table 4. Positive and negative ion mass spectral data of compound 3 (M = 869). Intensity [M+H]$^+$ = 100; [M−H]$^-$ = 100

Positive ion mode (with additional NaCl)

m/z	Rel. int. (%)	Ion composition
908	34	M+K
892	200	M+Na
870	100	M+H
860	58	892−CH$_3$OH
852	28	870−H$_2$O
838	32	870−CH$_3$OH
758	24	(S$_{D1}$+HCO)+Na
730	20	(S$_{D1}$+H)+Na
708	20	(S$_{D1}$+H)+H
596	34	(S$_C$+HCO)+Na
568	28	(S$_C$+H)+Na
546	34	(S$_C$+H)+H
528	30	546−H$_2$O
384	98	(S$_4$+H)+H
366	106	384−H$_2$O
222	440	(S$_3$+H)+H

Negative ion mode

m/z	Rel. int. (%)	Ion composition
904	18	M+Cl
868	100	M−H
836	36	868−CH$_3$OH
767	78	MZ−H
707	46	MZ'−H
605	31	(S$_{D1}$+H)Z−H
545	39	(S$_{D1}$+H)Z'−H

```
         →706      →544      →382      →220
Hex┼O—Hex┼O—Hex┼O—Hex┼O—HexNAc
 D1   │    C    │    4    │    3    │    2
```

Table 5. Positive ion mass spectral data of compound 4 (M = 1113). Intensity (M+H)$^+$ = 100

Positive ion mode (with additional NaCl)

m/z	Rel. int. (%)	Ion composition
1152	18	M+K
1136	80	M+Na
1114	100	M+H
1096	18	1114−H$_2$O
961	10	(S$_5$+HCO)+Na/(S$_{5'}$+HCO)+Na
933	16	(S$_5$+H)+Na/(S$_{5'}$+H)+Na
915	15	933−H$_2$O
911	25	(S$_5$+H)+H/(S$_{5'}$+H)+H
893	18	911−H$_2$O
799	21	(S$_4$+HCO)+Na/(S$_{4'}$+HCO)+Na
749	28	(S$_4$+H)+H/(S$_{4'}$+H)+H

```
          →909     →747
HexNAc┼O—Hex┼O
 5    │    4    │  ＼
      │         ＼ Hex—O—HexNAc
      │         ／   3       2
HexNAc┼O—Hex┼O
 5'   │    4'   │
          909     747
          →        →
```

Table 6. Positive and negative ion mass spectral data of compound 5 (M = 1316). Intensity [M+H]$^+$ = 100; [M−H]$^-$ = 100

Positive ion mode (with [A] and without [B] additional NaCl)

m/z	Rel. int. (%) [A]	Rel. int. (%) [B]	Ion composition
1355	38		M+K
1339	236	15	M+Na
1321	36		1339−H$_2$O
1317	100	100	M+H
1299	24	17	1317−H$_2$O
1164	50		(S$_5$+HCO)+Na/(S$_{5'}$+HCO)+Na/(S$_9$+HCO)+Na
1136	66		(S$_5$+H)+Na/(S$_{5'}$+H)+Na/(S$_9$+H)+Na
1118	54		1136−H$_2$O
1114	46	28	(S$_5$+H)+H/(S$_{5'}$+H)+H/(S$_9$+H)+H
1096	24	9	1114−H$_2$O
1002	52		(S$_4$+HCO)+Na/(S$_{4'}$+HCO)+Na
974	36		(S$_4$+H)+Na/(S$_{4'}$+H)+Na
956	28		974−H$_2$O
952	32	18	(S$_4$+H)+H/(S$_{4'}$+H)+H

Negative ion mode

m/z	Rel. int. (%)	Ion composition
1315	100	M−H
1297	17	1315−H$_2$O
1214	111	MZ−H
1196	33	1214−H$_2$O
1154	104	MZ'−H
1112	50	(S$_5$+H)−H/(S$_{5'}$+H)−H/(S$_9$+H)−H
1094	22	1112−H$_2$O
1011	61	(S$_5$+H)Z−H/(S$_{5'}$+H)Z−H/(S$_9$+H)Z−H
951	93	(S$_5$+H)Z'−H/(S$_{5'}$+H)Z'−H/(S$_9$+H)Z'−H
849	50	(S$_4$+H)Z−H/(S$_{4'}$+H)Z−H
789	102	(S$_4$+H)Z'−H/(S$_{4'}$+H)Z'−H

```
          →1112     →950
HexNAc┼O—Hex┼O
  5   │    4   │  →1112
      │         ＼
HexNAc┼O—Hex—O—HexNAc
  9   │    3            2
HexNAc┼O—Hex┼O
 5'   │    4'   │
      1112      950
      →          →
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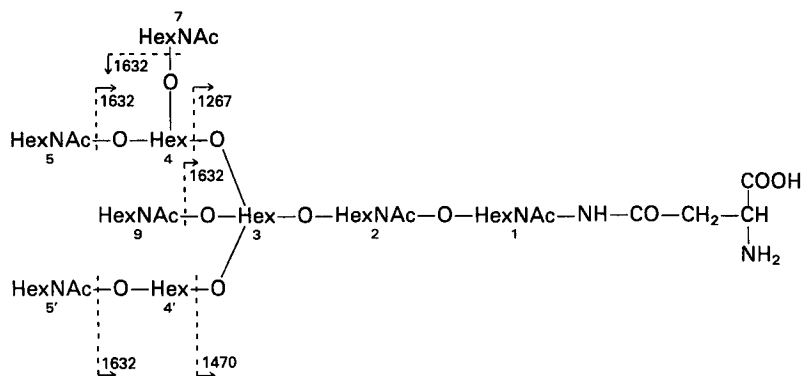spectra compared with the positive ones. Glycerol adduct ions are also observed in the negative ion mode.

Simple fragmentations of pseudomolecular ions

The positive as well as the negative ion mass spectra show peaks corresponding with the elimination of a H$_2$O molecule, e.g. [M+H−H$_2$O]$^+$ in the positive and [M−H−H$_2$O]$^-$ in the negative ion mode.

In the negative ion mass spectra of oligosaccharides with a reducing HexNAc moiety, two different fragmentations of this residue have been observed. In these reactions one or two molecules are eliminated from the even-electron [M−H]$^-$ ion, resulting in the formation

Table 7. Negative ion mass spectral data of compound 6 (M = 1836). Intensity $[M-H]^- = 100$

m/z	Rel. int. (%)	Ion composition
1835	100	$M-H$
1817	16	$1835 - H_2O$
1632	68	$(S_5+H)-H/(S_{5'}+H)-H/(S_7+H)-H/(S_9+H)-H$
1614	20	$1632 - H_2O$
1470	24	$(S_{4'}+H)-H$
1267	36	$(S_4+H)-H$

```
                    7
                 HexNAc
            ┌╌╌╌┐ │
            ↓ 1632 │
              O
         →        →
        ¦1632  │  ¦1267
HexNAc┼O—Hex┼O
   5        4   →
                ¦1632
                  \
   HexNAc┼O—Hex—O—HexNAc—O—HexNAc—NH—CO—CH₂—CH          COOH
      9       /3          2           1                  │
                                                        NH₂

HexNAc┼O—Hex┼O
   5'      4'
    ¦            ¦
    ¦1632      ¦1470
    →            →
```

of stable even-electron $[MZ-H]^-$ and $[MZ'-H]^-$ fragment ions with abundances comparable to that of the $[M-H]^-$ ion. The fragmentations are proposed to proceed as indicated in Scheme 1.
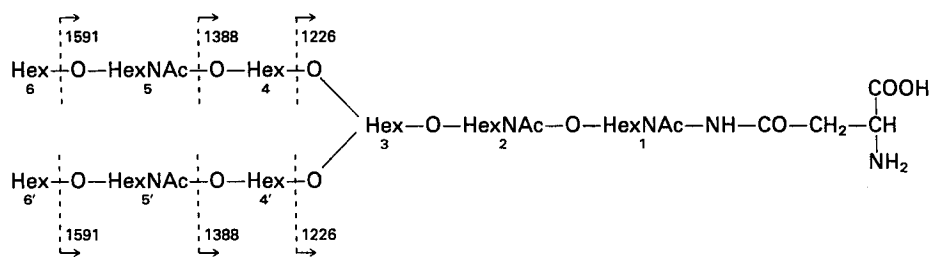
Sequence ions

The most important fragmentation reaction involves cleavage of the glycosidic bond between the anomeric carbon atom and the interglycosidic oxygen atom. This reaction occurs both in the positive and the negativ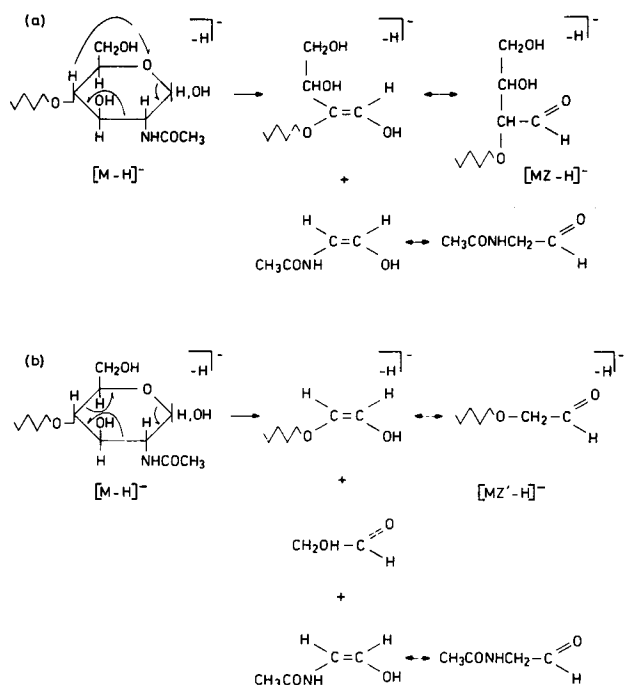e ion mode, and is always accompanied by a hydrogen migration to the interglycosidic oxygen, resulting in positive $[(S_n+H)+H]^+$ or negative $[(S_n+H)-H]^-$ even-electron fragment ions. The process is rationalized in Scheme 2. Comparable positive fragment ions have been observed in field desorption mass spectra of carbohydrate structures.[29]

Sequence ions derived from other pseudomolecular ions, like $[M+Na]^+$, have also been observed. Frequently, peaks are found in the mass spectra at m/z values 18 u less than those of the sequence ions discussed before. These ions originate from the normal sequence ions by loss of a H_2O molecule and/or from $[(M-H_2O)+H/Na]^+$ and $[(M-H_2O)-H]^-$ ions by

**Table 8. Negative ion mass spectral data of compound 7 (M = 1754). Intensity $[M-H]^- = 100$**

m/z	Rel. int. (%)	Ion composition
1753	100	$M-H$
1735	18	$1753 - H_2O$
1619	8	$(S_6+HCO)-H/(S_{6'}+HCO)-H$
1591	24	$(S_6+H)-H/(S_{6'}+H)-H$
1573	18	$1591 - H_2O$
1416	16	$(S_5+HCO)-H/(S_{5'}+HCO)-H$
1388	29	$(S_5+H)-H/(S_{5'}+H)-H$
1370	18	$1388 - H_2O$
1254	21	$(S_4+HCO)-H/(S_{4'}+HCO)-H$
1226	45	$(S_4+H)-H/(S_{4'}+H)-H$

```
  →           →           →
 ¦1591        ¦1388        ¦1226
Hex┼O—HexNAc┼O—Hex┼O
 6           5        4    \
                            Hex—O—HexNAc—O—HexNAc—NH—CO—CH₂—CH      COOH
                             3          2           1                │
                                                                    NH₂
Hex┼O—HexNAc┼O—Hex┼O
 6'          5'       4'
 ¦           ¦           ¦
 ¦1591      ¦1388      ¦1226
 →           →           →
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Scheme 1. Proposed fragmentation reactions in the reducing HexNAc moiety of [M − H]⁻ ions obtained from oligosaccharides.
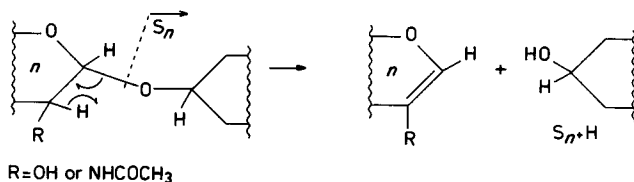
Scheme 2. Sequence ion formation by glycosidic bond cleavage. The index *n* of S_n refers to the cleaved glycosidic bond at the anomeric carbon atom of monosaccharide *n*.

cleavage of the glycosidic bonds. Cleavage on the other side of the interglycosidic oxygen atom and simultaneous hydrogen migration will also result in an ion with the same composition, but seems less plausible. A definite answer on the formation of these ions requires specific labelling.

There are indications, especially in the positive ion spectra, that the fragmentation reaction given in Scheme 2 might also lead to fragment ions in which the charge resides on the glycal part of the molecule. These ions, however, are mainly found in the lower mass region (m/z 147, Deoxyhex; m/z 163, Hex; m/z 204, Hex-NAc; m/z 325, Hex-*O*-Hex; m/z 366, HexNAc-*O*-Hex). Because the peaks are often hidden in clusters of background peaks originating from smaller ions, including glycerol adduct ions, their abundances are less reliable. For this reason they have not been included in the Tables.

The negative ion mass spectra of the oligosaccharides with a reducing HexNAc-residue show that the [MZ − H]⁻ and (MZ′ − H)⁻ ions can also undergo the cleavage of the glycosidic bond as presented in Scheme 2. In this

way new series of sequence ions are obtained, which can be presented as [(S_n + H)Z − H] and [(S_n + H)Z′ − H]⁻. In principle these ions can also originate from the sequence ions [(S_n + H) − H]⁻ by similar fragmentations as depicted in Scheme 1.

Finally, in a number of positive and negative ion spectra peaks were present which correspond with a fragmentation of monosaccharide *n*. The generation of these fragment ions, in which a formyl group is left at the interglycosidic oxygen atom, can be rationalized as in Scheme 3. Peaks are detected corresponding with [(S_n + HCO) − H]⁻ in the negative ion mode and with [(S_n + HCO) + Na]⁺ in the positive ion mode.
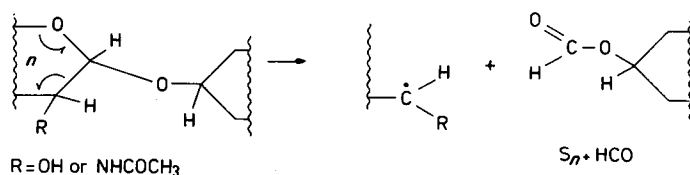
Scheme 3. Generation of fragment ions resulting from fragmentation of monosaccharide *n*.

In conclusion, the positive and negative ion mass spectra of the various underivatized carbohydrate structures contain molecular weight as well as sequence information. The fragmentation of the pseudomolecular ions appears to take place only along a small number of specific routes. Interpretation problems, especially in the lower mass region ($<m/z$ 500) of the spectra, might arise when glycerol adduct ions are interfering. However, these problems can be eliminated by applying the combined techniques of collisional activation and FAB ionization, as has been demonstrated recently for a peptide.[30] To establish the suitability of FAB in (micro)heterogeneity analysis of isolated carbohydrate materials, more investigations are necessary.

For the characterization of the primary structure of an unknown carbohydrate chain, several parameters have to be established. This requires the application of chemical and enzymatic analysis methods, chromatographic separation procedures, and instrumental techniques such as gas-liquid chromatography/mass spectrometry (up to now mainly using electron impact and chemical ionization of derivatized compounds) and high resolution nuclear magnetic resonance spectroscopy.[9] It is obvious that a combination of methods is always necessary to reach the final conclusion about the carbohydrate structure. The knowledge of the molecular weight of the carbohydrate under investigation as well as some sequence information in an early stage of the structural analysis will be of great help for the subsequent analysis strategy. From this point of view it is evident that FAB mass spectrometry of underivatized carbohydrate structures, as reported in this paper, can become a valuable additional technique in the whole arsenal of methods to be applied.

Acknowledgements

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