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S9.16

## Determination of Linkage Positions by FAB-Mass Spectrometry

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Recently, FAB was used for linkage position determination of intact oligosaccharides, using the negative ion mode for underivatized samples and the positive ion mode (collision-activated decompositions) for derivatized samples. We report linkage analysis of (methyl) peracetylated xylo-oligosaccharides by FAB, using the positive ion mode and unimolecular decompositions in the 3rd FFR of a four sector instrument (JMS-SX/SX102A; BEBE).

The MS/MS spectra of the *m/z* 475 oxonium ions of the disaccharides (1→2, 1→3 and 1→4) are very different. Distinction of the linkage type can be based on the presence or absence of certain fragment ions (see table). All spectra show peaks at *m/z* 415 and *m/z* 259 (loss of acetic acid and cleavage of the glycosidic bond, resp.). The spectra of the 1→2 compounds show an additional peak at *m/z* 355 (loss of a second molecule of acetic acid), which is absent in the other spectra, and a low intensity peak at *m/z* 199 (formed by loss of acetic acid from the *m/z* 259 ion). The spectra of the 1→3 compounds show a peak at *m/z* 373 (loss of $C_4H_6O_3$) and an abundant peak at *m/z* 199, formed mainly by elimination of a complete saccharide, consisting of the ring at the non-reducing end, the oxygen of the glycosidic bond and a hydrogen atom, transferred from the other ring. The MS/MS spectra of *m/z* 475 of the peracetylated tri-, tetra- and pentasaccharides (Xyl(1→n)Xyl(1→4)Xyl (n: 2, 3 or 4), $Xyl_4$ and $Xyl_5$ only 1→4) show identical fragment ions as their corresponding disaccharides.

The MS/MS spectra of oxonium ions with higher masses (three, four and five ring systems, resp.), all show only loss of one molecule of acetic acid and cleavage of the glycosidic bonds, confirming a 1→4 linkage between the two rings at the reducing end.

Diagnostic peaks in the MS/MS spectra of the oxonium ions at *m/z* 475.

| linkage | *m/z* 415 (−HOAc) | *m/z* 373 (−AcOAc) | *m/z* 355 (−2HOAc) | *m/z* 259 | *m/z* 199 |
|---|---|---|---|---|---|
| 1→2 | + | − | + | + | − |
| 1→3 | + | + | − | + | + |
| 1→4 | + | − | − | + | − |

+ indicates presence at a 5% level or more, relative to the *m/z* 259 peak

− indicates less than 5%

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## Structural Studies on the Glycosylation of the Gastric $H^+$, $K^+$-ATPase

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The $H^+$, $K^+$-ATPase is the membrane protein which is responsible for the acidity of gastric secretions. The protein consists of α and β subunits of which only the b subunits are glycosylated. According to the primary peptide sequence the β subunit contains seven potential *N*-glycosylation sites. Both *N*- and *O*-linked oligosaccharides have been found (Weitzhandler *et al.*, (1993) *J. Biol. Chem.* in press) and the *N*-linked chains are apparently novel asialo-structures, based on chromatographic studies.

We have begun studies to detemine (i) which peptide loci are glycosylated (ii) the molecular weight profile of oligosaccharides at each glycosylation site and (iii) the presence of unique structural features, all using pmol quantities of the $H^+$, $K^+$-ATPase. From a single HPLC electrospray mass spectrometric analysis, (which utilized 6–8 pmols of a tryptic digest injected into the mass spectrometer), we observed a series of ions suggesting that at least three of the potential *N*-glycosylation sites are occupied by asialo-complex structures some of which are elongated by lactosamine repeats and capped with terminal non-reducing Hex residues (possibly a-linked Gal). Ions arising from a glycopeptide containing Asn-222 were consistent with a biantennary series with up to two lactosamine repeats. A tri- or tetra-antennary series with up to five or six lactosamine repeats, respectively, some of which may be capped with Hex residues was consistent with the observed ions from the Asn-161-containing glycopeptide. The tryptic fragment containing Asn-146 produced ions suggesting tri- or tetra-antennary structures with two or three lactosamine repeats, respectively, all capped with Hex residues.

Studies are under way to ascertain which other potential *N*-linked sequons are glycosylated and the locations of *O*-linked chains. Also, released oligosaccharides from the b subunit are being further characterized using high-pH anion-exchange chromatography with pulsed amperometric detection and matrix assisted laser desorption mass spectrometry.

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S9.18

## Structural Studies on the Chondroitinase ABC-Resistant Sulfated Tetrasaccharides Isolated from Chondroitin Sulfate A, C, D and E

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Previously we made observations [1,2] that the commercial protease-free preparation (Prep. 1) of *Proteus vulgaris* chondroitinase ABC, which has been purified from the conventional, commercial preparation (Prep. 2), cannot degrade certain sulfated oligosaccharides unlike the latter. In the present study various commercial chondroitin sulfate preparations including an A isomer from whale cartilage, C and D isomers from shark cartilage, and an E isomer from squid cartilage, were exhaustively digested with Prep. 1 (Seikagaku Corp.). Gel chromatography of each digest yielded a disaccharide fraction and an oligosaccharide fraction which