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EXAMINATION OF THE SIALYL LEWIS X - CALCIUM COMPLEX BY ELECTROSPRAY MASS SPECTROMETRY

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Abstract: The Ca^{2+} binding properties of the cell-surface carbohydrate sialyl Lewis x, SLe^x, and its 2-, 3-, and 4- deoxygenated-fucose analogs, were examined by pneumatically-assisted electrospray (ion spray) mass spectrometry. Computer-assisted modeling and mass spectral evidence suggest that Ca^{2+} is coordinated primarily to the GalGlcNAc moiety of SLe^x.

Considerable interest has been generated[1-10] in the role of cell-surface carbohydrates in cell-adhesion events. These events have been shown to occur through calcium mediated carbohydrate-carbohydrate or carbohydrate-protein interactions. Specifically, Kojima and Hakomori[7] and studies[11] using pneumatically-assisted electrospray (ion spray) mass spectrometry have demonstrated calcium mediated self-association with the carbohydrate Lewis x (Le^x).

Our investigation has further utilized electrospray mass analysis to study the interaction between calcium and sialyl Lewis x (SLe^x). SLe^x is a carbohydrate that exists on the surface of leukocytes (white blood cells) and is recognized as a ligand in the calcium mediated adhesion between leukocytes and endothelial (blood vessel) cells.

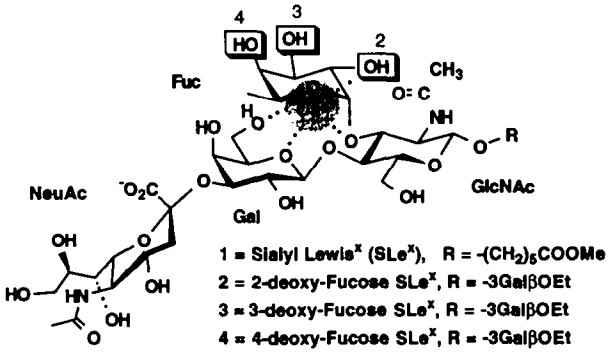

Figure 1. SLe^x, and its deoxygenated fucose analogs bound to Ca^{2+}.

The mass spectra of SLe^x and the SLe^x fucose deoxy analogs (Fig. 1)[12] were acquired from aqueous solutions. SLe^x (20 μM) was typically analyzed in solutions containing 200 μM of Ca^{2+}, Na^+, or Mn^{2+} cations. The presented data were acquired on a Perkin-Elmer SCIEX API III mass analyzer, using the pneumatically-assisted electrospray interface for sample introduction because of its utility in observing native noncovalent complexes.[11,13]

SLe^x, {NeuAcα(2→3)Galβ(1→4)[Fucα(1→3)]GlcNAcβ-O-$(CH_2)_5$COOMe, $C_{38}H_{64}O_{25}N_2$, monoisotopic mass (M_m) = 948.4} was initially analyzed with equivalent concentrations of Ca^{2+} and Mn^{2+} (100 μM). Similar ion intensities were observed for both metal complexes at low declustering potentials. However, as the declustering potential was increased, a dramatic increase (x 15) in the ratio of the Ca^{2+} complex, $[SLe^x + Ca - H]^+$, relative to the Mn^{2+} complex, $[SLe^x + Mn - H]^+$, was observed. It is likely that weak complexes between the SLe^x carboxylate and Ca^{2+} or Mn^{2+} were observed at the low declustering potentials. The carboxylate complex readily dissociated at the higher declustering potentials leaving the more tightly bound Ca^{2+}/Le^x complex (Fig. 1). The nature of the $[SLe^x + Ca - H]^+$ complex was further investigated using computer-aided modeling and tandem mass spectrometry (MS^2 and MS^3).

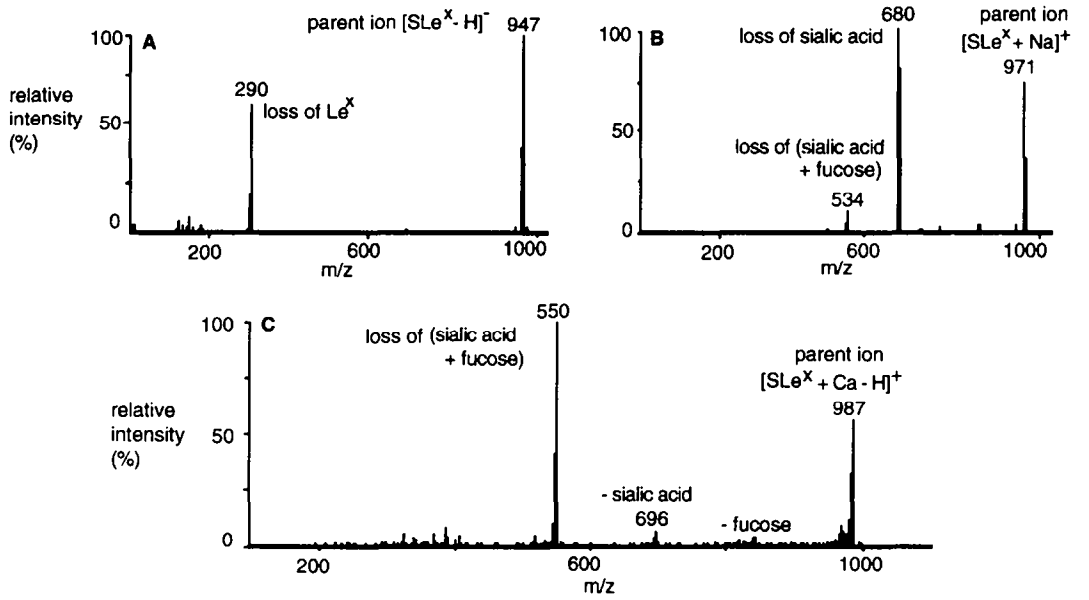

Figure 2. Tandem mass analysis (MS^2) of SLe^x, **A** - negative ion analysis of $[SLe^x - H]^-$, **B** - positive ion analysis of $[SLe^x + Na]^+$, and **C** - positive ion analysis of $[SLe^x + Ca - H]^+$

The $[SLe^x + Ca - H]^+$, $[SLe^x + Na]^+$, and $[SLe^x - H]^-$ complexes were analyzed using the MS^2 collision-induced dissociation (CID) capabilities of the electrospray ionization triple quadrupole mass spectrometer (Fig. 2). The

CID process is accomplished by selecting an ion of interest with the mass analyzer and introducing that ion into a collision cell. The selected ion will collide with a collision gas such as argon, resulting in fragmentation. The fragments can then be analyzed to obtain a daughter ion spectrum. The CID experiments were performed under identical[11] conditions to allow for qualitative comparison of the CID spectra. The collisional activation of the precursor anion, $[SLe^x - H]^-$, resulted in loss of Le^x, leaving only the sialic acid. This was revealed by the tandem mass spectrometric daughter-ion scan displayed in Fig. 2.A. Notably, no other anionic sites besides the sialic carboxylate were revealed on SLe^x in this analysis. CID analysis with the monovalent cation complex, $[SLe^x + Na]^+$, resulted in fragmentation primarily through loss of sialic acid (Fig. 2.B) with some additional loss of fucose. Collisional activation of the Ca^{2+} complex, $[SLe^x + Ca - H]^+$, resulted in fragmentation with a characteristic loss of both fucose and sialic acid to yield the most abundant fragment ion (Fig. 2.C).

CID data on the deoxy-fucose SLe^x analogs, {NeuAcα(2→3)Galβ(1→4)[Fucα(1→3)]GlcNAcβ(1→3)-Galβ-O-Et, $C_{39}H_{66}O_{27}N_2$, M_m = 994.4}, was also used to obtain information on cation coordination. Each of the analogs (2-deoxy, 3-deoxy, and 4-deoxy fucose SLe^x, Fig. 1) were analyzed under the same conditions that were maintained for SLe^x. The primary difference in the spectra was observed for the positive ions. In the positive ion CID experiments, an increase in the lability of fucose was revealed from the Ca^{2+}/2-deoxy-fucose SLe^x complex when compared to the negative ion spectra of the free acid and the positive ion analysis of the Na^+ complex. No significant difference was observed between the fucose lability of SLe^x and that of the 3- or 4- deoxy-fucose SLe^x analogs. This increase in fucose lability from the Ca^{2+} complex of the 2-deoxy analog may result from the missing 2-hydroxy-fucose/Ca^{2+} coordination site (Fig. 1). Additional experiments on the fragment ions generated from orifice collisions also allowed the MS^3 experiments to be performed on the SLe^x and SLe^x analogs.

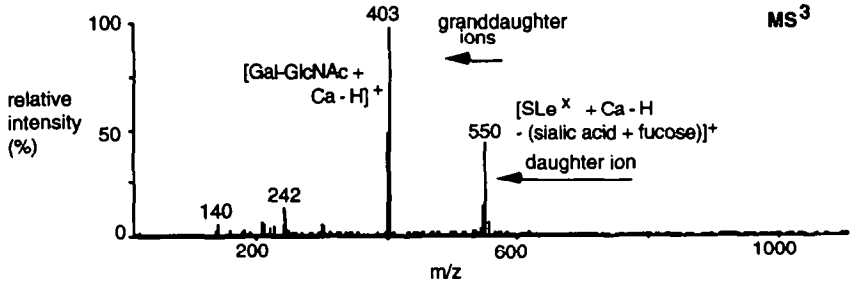

Figure 3. MS^3 of the parent ion, $[SLe^x + Ca - H]^+$, m/z = 987. The daughter ion, m/z = 550, was generated at the electrospray orifice and introduced into a collision cell to produce granddaughter ions.

It is possible to generate fragment ions at the interface (or orifice) between the electrospray ionization source and the mass analyzer. The MS^3 experiments were performed by using the orifice collisions to generate fragment ions from the $[SLe^x + Ca - H]^+$ complex. The resultant daughter ions could then be mass selected with the first quadrupole, fragmented in the second quadrupole collision cell, and the resultant granddaughter ions analyzed with the third quadrupole. The MS^3 experiments further demonstrate that Ca^{2+} remained bound to the Gal-

GlcNAc moiety of SLe^x (Fig. 3) . These results, in conjunction with computer-assisted modeling[14] of SLe^x, suggest that Ca^{2+} coordinates oxygen atoms from the Gal-GlcNAc moiety and a hydroxyl group of fucose (Fig. 1), analogous to previously performed modeling and mass analysis of Le^x.[11] It should be noted that MS^3 experiments on the deoxy analogs produced analogous results to those observed for SLe^x and that it is also possible (from the modeling data) for Ca^{2+} to be coordinating the carbonyl oxygen on the amide.

The presented results generated for the SLe^x-Ca^{2+} complex is in general agreement with a report[1] that speculates on a model for SLe^x-Ca^{2+}-E-selectin binding. The results from our experiments agree that Ca^{2+} binds at least one of the fucose hydroxyl groups and Ca^{2+} does not bind SLe^x through sialic acid. However our work further suggests that Ca^{2+} may also coordinate sites on Gal-GlcNAc (Fig. 1).

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