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Two sodium acetate gradients were employed to obtain separation of sialic acid-containing oligosaccharides released from various gangliosides via endo ceramidase treatment or ozonolysis. Addition of a terminal neutral monosaccharide to the linkage decreases the retention time of oligosaccharides from mono- and disialogangliosides which run in following order: OS-GM1, OS-GM2, OS-GM3, OS-GD2, OS-GD3. However, addition of an internal Gal-GlcNAc sequence to monosialogangliosides with terminal sialic acid (sialosyl-nLc$_4$ versus sialosyl-nLC$_6$) does not significantly effect the retention time. Oligosaccharide structural isomers generated from ganglioside series "b" are eluted earlier then from series "a" (OS-GD1b before OS-GD1a and OS-GT1b before OS-GT1a). Substitution of *N*-acetylneuraminic acid by *N*-glycolylneuraminic acid in the linkage drastically increases the retention time (OS-NANA GM3 before OS-NGNA GM3). Mapping of separated oligosaccharides from gangliosides by HPAE chromatography is a useful tool for studying impurities of gangliosides as well as an alternative to the TLC method for checking minor gangliosides in mixtures. This procedure is being used in our laboratory for the analysis of gangliosides in disorders of myelination.

S19.26

A New Source of GD2 and Several Other Gangliosides

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GD2 ganglioside is a minor component of normal neural tissue, but the concentration is greatly enhanced in tumors of neuroectodermal origin: neuroblastoma, retinoblastoma, brain tumors, small cell carcinoma of the lung and some melanomas. Therefore, CD2 is of great interest as a target molecule for immunotherapy of all of these tumors, particularly of neuroblastoma.

Recently, the preparation of *O*-Acetyl-GD3, GM3, GD1a gangliosides and several neutral glycosphingolipids from the liver of Rainbow Trout was reported by the Hakomori group (Ostrander, G. K. *et al.*, *Arch. Biochem. Biophys.*, **284**, 1991, 413 – 421). We have used this source and also the liver tissues from other fishes for extraction and isolation of gangliosides. We confirmed the presence of substantial amounts of 9-*O*-Acetyl-GD3 in the liver of Rainbow Trout.

In addition, however, we have also found the GD2 ganglioside. These gangliosides have been characterized chemically with the aid of HPTLC, HPLC, NMR and FAB-MS. For the immunological characterization by HPTLC-immunostaining and ELISA-test monoclonal antibodies ME-361, ME-311, R 24 and Mab 126 were used. The activities thus measured were comparable with those observed for GD2, obtained by β-galactosidase treatment of GD1b.

S19.27

Characterization of Ceramides by Fast Atom Bombardment and Tandem Mass Spectrometry of Cationized Species at High and Low Collision Energy

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The molecular characterization of native, underivatized ceramides can be accomplished by fragment ion analysis, of protonated, deprotonated and cationized species desorbed by FAB[1]. The latter species, in particular, undergoes collision-induced decomposition at kilovolt energies, which results, among other structurally valuable fragmentation pathways, in the charge-remote electrocyclic fission of the hydrocarbon tail, mainly of the fatty acid residue, so that modifications such as double bonds and methyl branching can be located. According to the same principles the hydrocarbon chain of the sphingosine can be mapped by fragment ion analysis of the (Sph-H_2O + H)$^+$ ion/s present in the source spectrum[2,3].

Selective detection of the ceramides in mixture can be obtained with a quadrupole-based precursor scan of the (Sph-2H_2O + H)$^+$ ion, which yields the m/z values of the (MH-H_2O)$^+$ ceramide molecular species[3].

Low-energy collision-induced decomposition of lithiated ceramides triggers a beta-fission of the fatty acid hydrocarbon chain, promoted by polarization of the amide carbonyl induced by the metal cation. The thus generated lithiated *N*-acriloyl-sphingosine fragment undergoes further charge-remote electrocyclic fission of the long-chain base hydrocarbon tail. The complementarity of fragmentation pathways under the two collision regimes allows to separately characterize the two hydrocarbon tails in the ceramide molecule.

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S19.28

Negative Ion Electrospray Mass Spectrometry (ESIMS) of Glycolipids — Comparison with (-)FABMS

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FABMS is still a method of choice for structural analyses of glycolipids, provided that the sample is ionized by this method and that the molecular weight is within the mass range of the instrument. However, glycoconjugates having larger sugar-chains or multiple number of acidic functional groups are better ionized by ESIMS. We have found that ESIMS is an excellent ionization method not only for proteins but also for large oligosaccharides. Now we have compared ESIMS with FABMS, and found that the negative ion mode for neutral glycolipids produces different types of ions in the two ionization methods. In FABMS, the molecule-related ions for both neutral and acidic compounds are $[M-H]^-$. On the other hand, (-)ESIMS for neutral glycolipids such as CMH (GalCer) and CTH (Gb3Cer) produce $[M+Cl]^-$ (1) and $[M+89]^-$ or $[M-H+90]^-$ in addition to the deprotonated molecule if the solvent is MeOH/chloroform. Acidic glycolipids such as a ganglioside GD2, where dissociative hydrogens are available, showed $[M-H]^-$ and $[M-2H]^{2-}$ as the molecule-related negative ions in ESIMS. The chemical species of the adduct (MW 90) was identified by the use of deuterated and ^{13}C-isotopic solvents as 1,2-dimethoxyethane ($C_4H_{10}O_2$) derived from methanol.

The authentic CTH sample was a kind gift from Dr. S. Ando of Tokyo Metropolitan Institute of Gerontology. We