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Analysis of Glycans and Glycoconjugates by Matrix-assisted Laser Desorption/Ionization Mass Spectrometry (MALDI-MS)

Introduced in 1986 Matrix-assisted Laser Desorption/Ionization Mass Spectrometry (MALDI-MS) has become a rapidly expanding method. A matrix of highly absorbing organic molecules isolates the analytes and enables the desorption and ionization of intact biomolecules up to a MW of 500,000 Da (1, 2). The ions generated by the irradiation of pulsed (3-200 ns) UV- or IR-lasers are separated in a time of flight system. For proteins the limit of detection is in the low femtomole range.
In this contribution, the following examples for glycan/glycoconjugates analysis by MALDI-MS will be presented:

- native non-derivatized α-1,4-glucans
- fructans
- native and permethylated glycosphingolipids
- glycoproteins before and after enzymatic deglycosylation
- glycopeptides with a varying degree of polymerization
- characterization of an enzymatic glucan polymerization
- characterization of a non-enzymatic hydrolysis of a heteropolysaccharide (3)

The data presented here clearly show that MALDI-MS is a new and attractive alternative to standard biochemical and biophysical methods. MALDI-MS has distinct advantages such as high sensitivity, high accuracy and fast sample preparation. The time required for complete analysis is typically 10 - 15 minutes.

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Structural characterization of gangliosides from B cell derived cell lines

Activated murine T and B cells as well as tumour cell lines of T and B cell origin express different sets of glycosphingolipids (GSLs)[1]. Gangliosides of the G_{M1b}-pathway were characterized as T cell specific antigens[2] and are also expressed by the T lymphoma YAC-1[3]. B cell derived cell lines (myelomas, hybridomas) show simple ganglioside patterns in contrast to T cell descendants [1], but no detailed data of GSL-structures from B cells and related tumours are available.
In this study the gangliosides from various mouse myelomas and hybridomas (mouse-mouse, mouse-rat, human-mouse) and a human Epstein-Barr-virus (EBV) transformed B lymphocyte cell line have been characterized by immunological methods (overlay technique) and fast atom bombardment mass spectrometry (FAB-MS)[4]. Sialic acid profiles were obtained by HPLC according to Hara et al.[5]. Exclusively G_{M3} ($C_{24:1}$ and $C_{16:0}$ fatty acid) was expressed by all cell lines. Using highly specific polyclonal chicken antibodies directed to Siaα2-3Gal, all mouse-derived lines showed characteristic high G_{M3}(NeuGc) and low G_{M3}(NeuAc) content whereas the human EBV transformed B lymphocyte was characterized by G_{M3}(NeuAc), completely lacking G_{M3}(NeuGc).
In gangliosides from normal human cells NeuGc is not expressed[6]. We found that the analysed EBV-transformed human B lymphocyte retained this sialylation status by expressing exclusively G_{M3}(NeuAc) (100%). The fusion of a human B lymphocyte with a mouse myeloma, however, led to a heterohybridoma with high G_{M3}(NeuGc) content (94%). This synthesis shift may be caused by transfer of the hydroxylase gene coding for CMP-NeuAc hydroxylase activity.

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Characterisation of Glycosyl-Inositolphospholipids in *Toxoplasma gondii*

Toxoplasma gondii, a sporozoan parasite, is the causative agent of toxoplasmosis. Using human sera previous studies have described a lipophilic low molecular weight antigen. We have produced monoclonal antibodies specific for this antigen for further characterisation. Parasites were metabolically labeled with tritiated precursors and extracted with $CHCl_3$/MeOH (2:1) followed by $CHCl_3$/MeOH/H_2O (CMW 10:10:3). CMW extracts were dried and radioactivity partitioned between water and butanol and the butanol phase was analysed by TLC. Using a Berthold radioactivity scanner six [^{3}H]-glucosamine-labeled peaks were consistently detected. These glycolipids designated peak I to VI were also labeled with [^{3}H]-mannose and several [^{3}H]-fatty acids. Incorporation of [^{3}H]-amino acids was not observed for any of these peaks, whereas three glycolipids (I-III) were labeled with [^{3}H]-ethanolamine. Bands stained immunologically by mAbs and patient sera coincided with radioactive peaks. Human sera (IgM) stained all glycolipids with exception of peak III, mAbs recognized peak I-III. Glycolipids