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**Analysis of Glycan 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 femtogram range.

In this contribution, the following examples for glycan/glycoconjugates analysis by MALDI-MS will be presented:

- native non-derivated α-1,4-glucans
- fructans
- native and permethylated glycosphingolipids
- glycoproteins before and after enzymatic deglycosylation
- glycopeptides with a varying degree of polymerization
- characterisation of an enzymatic glucan polymerisation
- characterisation of a non-enzymatic hydrolysis of a heteropolysaccharide

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 characterisation 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 Ga2p-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 (myeloma, hybridoma) show simple ganglioside patterns in contrast to T cell derivatives (4), 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) (5). Sialic acid profiles were obtained by HPLC according to Hara et al. (6).

Exclusively Ga2 (Cerpd1 and C18:1 fatty acid) was expressed by all cell lines. Using highly specific polyclonal chicken antibodies directed to Sialo-3Gal, all mouse-derived lines showed characteristic high Ga2 (NeuGc) and low Ga3 (NeuAc) content whereas the human EBV transformed B lymphocyte was characterized by Ga3 (NeuAc), completely lacking Ga2 (NeuGc).

In gangliosides from normal human cells NeuGc is not expressed (7). We found that the analysed EBV-transformed human B lymphocyte retained this sialylation status by expressing exclusively Ga3 (NeuAc) (100%). The fusion of a human B lymphocyte with a mouse myeloma, however, led to a heterobioside with high Ga2 (NeuGc) content (94%). This synthesis shift may be caused by transfer of the hydrolase gene coding for CMP-NeuAc hydroxylase activity.


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**Characterisation of Glycophospholipids 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 [3H]mannose and several [3H]-fatty acids. Incorporation of [3H]-amino acids was not observed for any of these peaks, whereas three glycolipids (I-III) were labeled with [3H]-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 and III.

Glycolipids...