

Structure determination of MHC class I peptides of renal carcinoma cells on an AP-MALDI / ESI ion trap and a MALDI-PSD-TOF instrument

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MHC (Major Histocompatibility Complex) class I peptides play a prominent role in immune response. These peptides have a chain length between eight and ten amino acids and originate from intracellular proteins. The processing and presentation pathway of these peptides is aimed at detecting foreign and endogenous tissues, cancer or viral infections in cells. For that purpose these oligopeptides are presented on the cell surface as part of the major histocompatibility complex I molecules (MHC I), and the differentiation between foreign and endogenous is based on recognition by T lymphocytes (via the T cell receptor). [1]

MHC class I peptides of renal carcinoma cells have been concentrated and isolated in a series of preparation steps with a final reversed phase HPLC separation. These HPLC fractions represent highly complex peptide mixtures with peptide material less than a picomol often distributed over up to 100 different peptides. Identification of these peptides by mass spectrometry is quite challenging and tedious work. For primary structure determination of MHC peptides it is essential to find the optimal ionization technique. Nano-electrospray ionization (NSI), atmospheric pressure MALDI (AP-MALDI) and standard MALDI-TOF were compared in this study. MALDI at atmospheric pressure and NSI experiments were carried out on a Finnigan LCQ Deca XP ion trap mass spectrometer with MSⁿ option. MALDI spectra were recorded on a Bruker Ultraflex TOF/TOF time-of-flight mass spectrometer (MS²).

Often NSI ion trap and MALDI-TOF mass spectra of the same MHC-HPLC fractions strongly differ from each other. There are cases where nice ion signals are observed in a MALDI-TOF instrument but only noise signals are obtained in the NSI ion trap instrument, for the same sample investigated. Using the information from the MALDI-TOF spectrum it is nevertheless sometimes possible to obtain MS/MS spectra from the noisy NSI ion trap spectrum by "blindly" selecting the mass of the invisible precursor (fig.1).

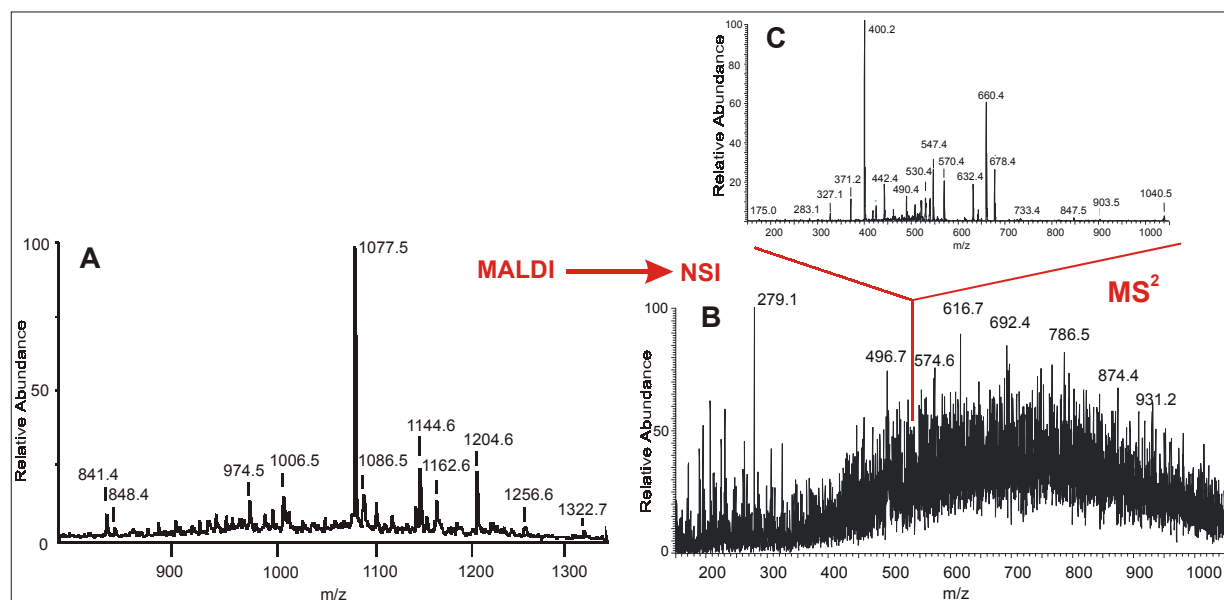


Figure 1: MALDI spectrum (A), NSI spectrum (B), and NSI-MS² spectrum (C) of the same MHC-HPLC fraction.

Automated identification of MHC peptides using MS/MS data and automated database-aided search algorithms (e.g. Sequest, Mascot, ...) mostly fails, because important information is missing such as that on the host protein and on the proteolytic specificity. Therefore *de novo* sequencing is mandatory for getting structural information on these peptides.

MS/MS and MSⁿ spectra of singly charged precursor ions have been recorded on the same ion trap instrument but using different ionization techniques and fragmentation parameters. A comparison of resulting mass spectra shows that the fragmentation patterns are very similar and that the spectra are rather complex (fig.2). In contrast to that MS/MS spectra of double charged precursor ions are less complicated, but precursor ion signals are generally much less intense. MALDI-PSD spectra are easier to interpret because of the accessibility of the complete mass range down to low mass immonium ions. In conclusion, MALDI-TOF and ion trap mass spectrometry were found to be complementary tools in the analysis of MHC peptides.

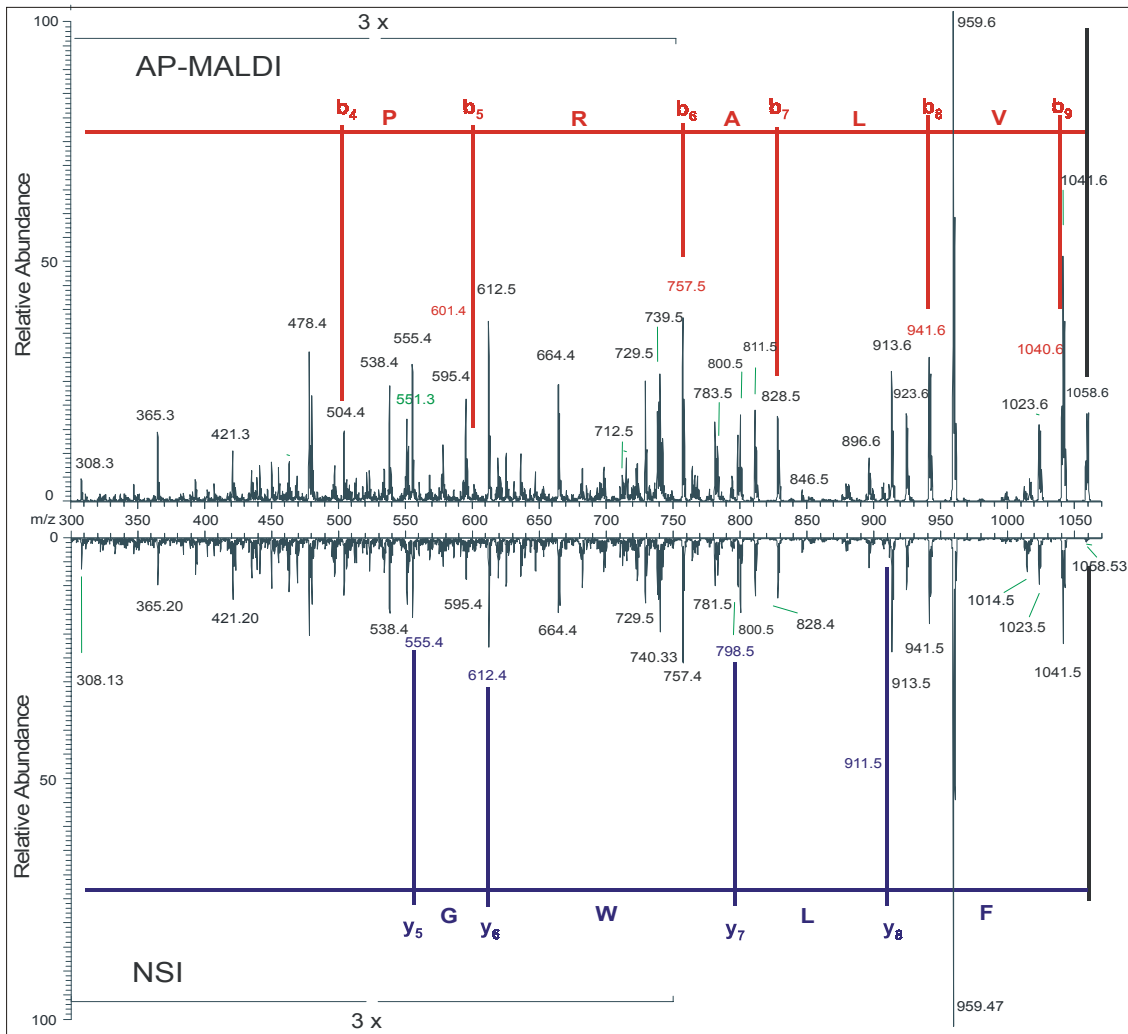


Figure 2: Comparison of the fragmentation patterns using different ionization techniques. MS/MS spectra of the single charged ion of FLWGPRALV (synthetic peptide derived from MAGE 3) are shown.

[1] Th. Flad, B. Spengler, H. Kalbacher, P. Brossart, D. Baier, R. Kaufmann, P. Bold, S. Metzger, H. Meyer, B. Kurz, C.A. Müller, *CANCER RESEARCH*, 58 (1998) 5803-5811.

Acknowledgement: Financial support through BMBF grant 0312834A and DFG grant Sp314/3-2 is gratefully acknowledged.