



Cell-free MALDI-TOF assay providing information-rich amyloid- β peptide profiles as a new tool in Alzheimer research

Simple and fast in-depth profiling of immunoprecipitated secreted amyloid-beta peptides based on label-free MALDI-TOF MS provides a promising new assay tool for Alzheimer research.

Abstract

Alzheimer's disease (AD) is the most common cause of dementia, and the number of cases is rapidly rising [1]. One hallmark of AD is the deposition of amyloid- β peptides (A β) in patient brains as amyloid plaques. Traditionally, amyloid plaques have been studied extensively using immunochemistry, which has led to a strong focus on a few well-studied A β species such as A β ₃₈, A β ₄₀ and A β ₄₂ and collective staining of multiple A β isoforms [2], respectively.

Keywords:
MALDI-TOF, rapifleX,
Alzheimer's disease,
MALDI bioassay,
amyloid-beta peptides

We describe here development and application of a Matrix-Assisted Laser Desorption/Ionization (MALDI) time-of-flight (TOF) mass spectrometry (MS) based assay for in-depth A β peptide profiling that overcomes known limitations of traditional immunochemistry based methods by enabling untargeted, label-free assay readout and providing comprehensive, information-rich A β peptide fingerprints at unprecedented analysis speed and level of robustness [3-5].

Introduction

A β peptide profiles generated from AD-causing mutations in amyloid precursor protein (APP) or presenilin 1 (PSEN1) determine the pathology of the mutations and can be used to predict the age of disease onset [5]. A β peptides are generated by sequential cleavage of APP by β - and γ -secretases. Initial cleavage of APP by β -secretase sheds a 99 amino acid long fragment (C99), which is further processed by γ -secretase in a stepwise successive manner. It has been proposed that both AD-causing mutations and environmental influences lead to a shift in the

A β -profiles by destabilizing of the γ -secretase/C99 enzyme-substrate complex [3]. Previous immunochemistry-based research has focused on only few major A β species (particularly A β 1-40, A β 1-42) 2, leaving other N- or C-terminally truncated as well as post-translationally modified A β -species understudied. Furthermore, antibodies are not available for most of these A β isoforms.

With these well-known limitations of immunochemistry-based methods in mind, we have developed a simple and fast label-free MALDI-TOF MS based assay allowing for comprehensive, untargeted A β peptide profiling at unprecedented analysis speed and robustness. The MALDI-TOF assay described here, therefore, has the potential to serve as a new essential tool in mechanistic AD research [3].

Methods

A workflow scheme of the MALDI-TOF MS based assay developed for label-free A β peptide profiling is given in Figure 1.

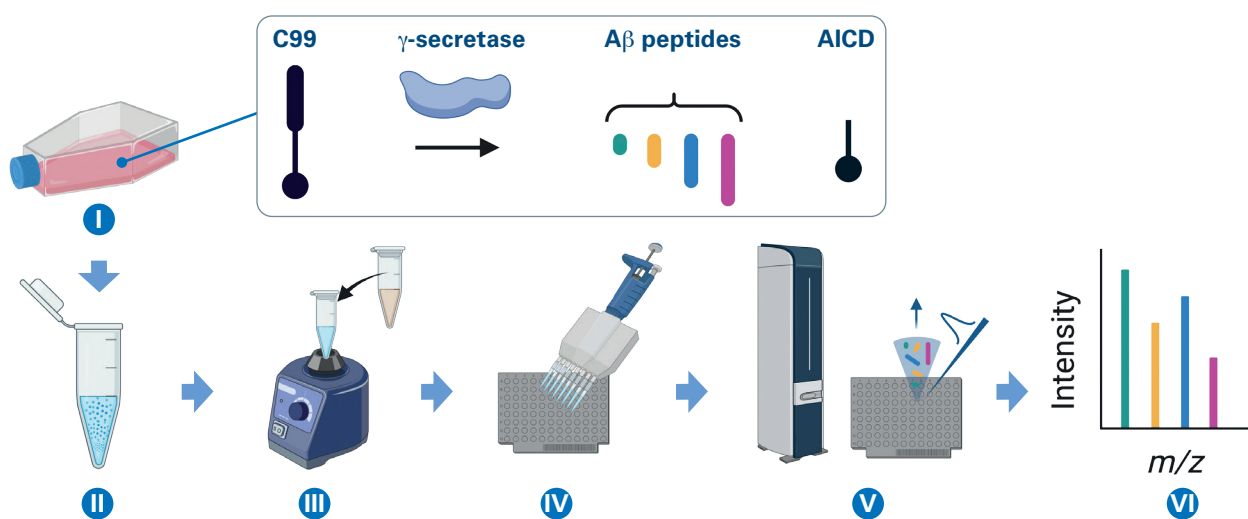


Figure 1
Schematic overview of the amyloid- β (A β) MALDI TOF MS assay.

① HEK293T cells transfected with defined point mutant amyloid precursor protein (APP) constructs are cultured, and cellular γ -secretase activity leads to secretion of various A β peptides that differ in chain length. ② A β peptide mixtures are immunoprecipitated from cell culture medium with anti-A β antibodies immobilized on agarose beads. ③ Bound A β -isoforms are released by mixing beads with MALDI matrix. ④ After centrifugation, the supernatant is spotted onto a MALDI target plate. ⑤ MALDI TOF measurement in linear positive mode is performed, and ⑥ spectra are annotated for quantitative evaluation of the data. Figure was created with BioRender.com.

Immunoprecipitation of A β peptides from conditioned media

HEK293T cells transfected with wild type (WT) or mutant APP-C99 expressing constructs were cultured as previously described [4]. One day post-transfection, the cell medium was replaced with low serum medium (DMEM/F-12, 2% FBS), and either DMSO or the γ -secretase inhibitor L 685,458 at a final concentration of 2.5 μ M was added to the medium. 24 h after medium replacement, conditioned media (CM) was collected.

To improve MS peak intensity for hydrophobic A β peptides, Tween 20 at a final concentration of 0.025% (w/v) was added to CM as described previously [6]. A β peptides were immunoprecipitated using the 4G8 antibody (4 μ g/10 mL of CM) overnight at 4°C on rotation. The 4G8 antibody targets the 17-24 aa A β epitope and, therefore, enables broadband specificity for A β peptides including N- and C-terminally truncated species. 40 μ L Protein G agarose beads (pre blocked in PBS/0.5% BSA/0.05% Tween 20 pH 7.4) were added and incubated for 3 h. Beads were washed in PBS/0.05% Tween 20 pH 7.4 two times and additionally in PBS/0.01% Tween 20 pH 7.4 to reduce the detergent concentration. Dry beads were frozen at -20 °C and subsequently subjected to MS analysis.

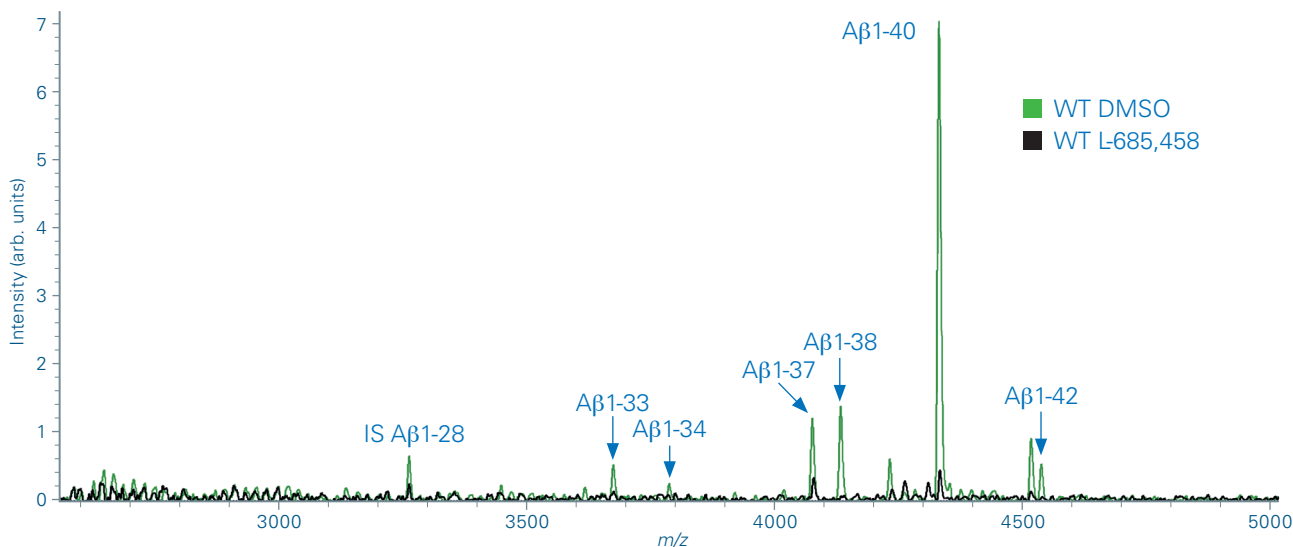


Figure 2

Spectra of A β -peptides immunoprecipitated from cell culture medium of HEK293T cells expressing wild-type (WT) APP-C99.

Black: 24-hour treatment with 2.5 μ M L-685,458 (γ -secretase inhibitor); **Green:** Vehicle (DMSO) control.

MALDI-MS sample preparation and analysis of A β peptides

Beads were resuspended in 15 μ L of sinapinic acid matrix solution (38 mg/mL in water/ACN/TFA 20/77.5/2.5 (v/v/v)) and 30 nM A β 1-28 was added as internal standard. The sample was vortexed (1 min) and centrifuged (5 min at \sim 1000 g). The supernatant was collected, and 1 μ L (9 replicates) was applied on a MALDI AnchorChip Target (Bruker Daltonics) using the dried droplet preparation method. Mass spectra were acquired on a rapiflex MALDI-TOF mass spectrometer (Bruker Daltonics GmbH & Co. KG, Bremen, Germany) equipped with a 10 kHz Smartbeam 3D laser using the AutoXecute function of the FlexControl 4.2 acquisition software.

The acquisition method was precalibrated using a 1/1/1 (v/v/v) mix of protein calibration standard I, peptide calibration standard II (both from Bruker Daltonics), and A β calibration standard using the quadratic calibration polynomial. Each spectrum was acquired in linear positive mode within the mass range of m/z 2,000 to 20,000. 25,000 laser shots were accumulated for each sample using the random walk. Subsequently, mass spectra were smoothed (Savitzky–Golay), baseline-subtracted (TopHat) and internally single-point recalibrated (using the A β 1-28 peak). Average MALDI-MS profiles were generated from individual spectra acquired from 9 replicate spots, and peaks were detected from the resulting average spectra with a S/N > 3.

Results

MALDI mass spectra obtained from immunoprecipitated secreted A β peptides from cell culture medium showed A β 40 as the major product generated from WT APP as substrate, and lower amounts of the peptide isoforms A β 33, A β 37, A β 38, A β 39, and A β 42 (Figure 2).

The fact that MALDI yields singly charged peptide ions as the vastly dominating ion species adds to the straightforwardness of the MALDI based A β peptide profiling approach. Resulting MALDI A β peptide fingerprints do not require a charge deconvolution step, are, therefore, particularly easy to interpret and, thus, provide instant access to the desired information, i.e. the relative A β -peptide intensity distribution.

Treatment with L-685,458 (a known potent γ -secretase inhibitor) completely abolished A β production (Figure 2), indicating that the detected signals were γ -secretase-dependent.

MALDI-TOF results also revealed A β s varying from 29 to 40 amino acids (aa) and from 20 aa to 38 aa for the E22F and D23F mutant substrates, respectively. Moreover, A β 33 and minor amounts of A β 34, A β 29, and A β 28 were detected for the K28F mutant substrate (Figure 3 B and C).

As an outlook to further potential applications, the MALDI-TOF MS based profiling workflow, when integrated in an immunoprecipitation-free in-vitro assay format, may enable fully agnostic broadband monitoring of A β -peptides including further γ -secretase cleavage products such as AICD's [3].

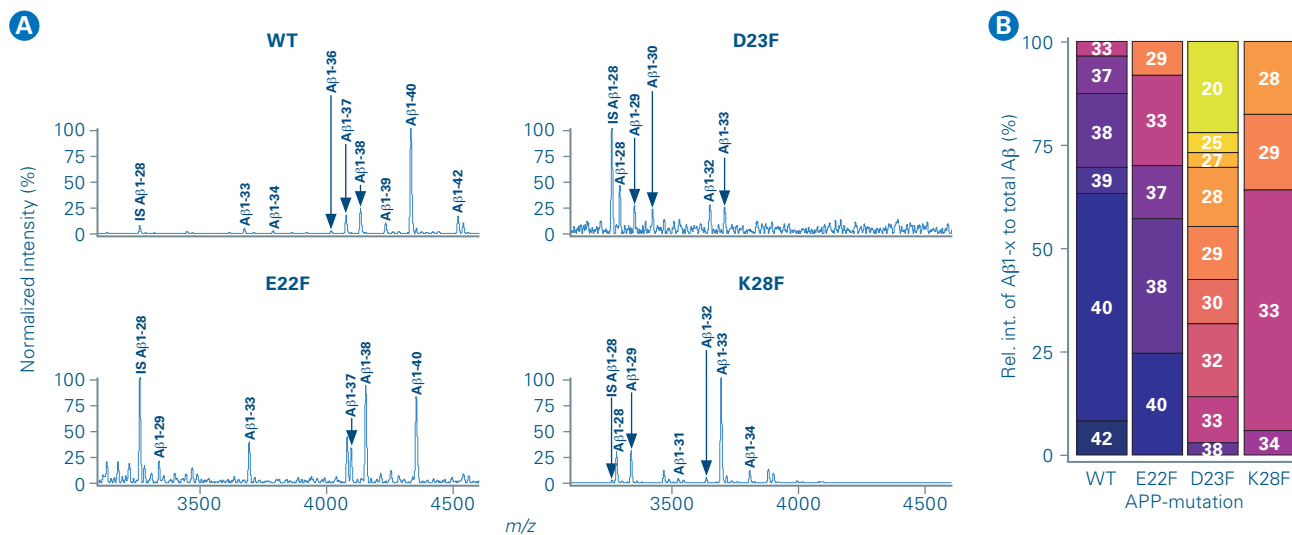


Figure 3
(A) Rapiflex MALDI-TOF MS spectra of A β -peptides immunoprecipitated from conditioned media of HEK293T cells expressing WT or mutant (E22F, D23F, and K28F) APP-C99. Synthetic A β 1-28 peptide was used as internal standard (IS). **(B)** A β peptide profiles derived from MALDI-TOF MS spectra. A β peptides that appeared in at least two independent biological replicates are shown. Numbers and color temperature indicate N-terminus of A β 1-x isoforms. Percent values represent mean A β -proportion of total A β from N \geq 3 independent experiments.

Conclusion

- MALDI-TOF MS enables label-free readout of bioassays at unprecedented speed and level of robustness.
- In contrast to traditional immunochemistry based methods, MALDI-TOF MS is capable of untargeted in-depth A β -peptide profiling providing comprehensive, information-rich A β -peptide fingerprints from in-vitro γ -secretase reactions.
- MALDI-TOF based in-vitro A β -peptide profiles offer promising potential for improved prediction of the onset of Alzheimer's disease [5].
- Its unique ability to monitor the complete range of A β -peptides makes the MALDI-TOF MS based assay a new, easy-to-use tool aiming for better understanding of the mechanisms of Alzheimer's disease [3,4].

References

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