Utilization of Antibody Beads and MALDI-TOF Mass Spectrometry to Isolate and Determine the Amount of Amyloid-β Derivatives Present in Blood Plasma

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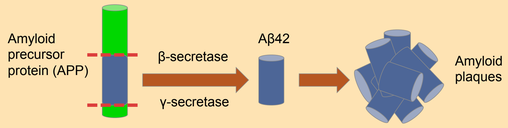
Background

Alzheimer's disease (AD) is a condition characterized by degenerative neurological function. While AD is classified as a very common disease, especially among individuals 65 and older, there are no known cures or treatments to slow the rate of AD progression. Accumulation of the protein amyloid-β peptide 42 (Aβ42) in the brain, creating extracellular deposits known as amyloid plaques, is one of the molecular hallmarks of AD. Current strategies used to evaluate the levels of Aβ42 deposits in the brain, such as positron-emission tomography (PET) scans and cerebrospinal fluid (CSF) evaluations, are invasive and costly. Furthermore, most patients are diagnosed with AD only once behavioral symptoms are present or post-mortem. However, recent evidence supports measurement of Aβ42 in the blood as a potential means to evaluate its levels in the brain for earlier detection and prevention of AD.



The objective of this research is to develop a method for measurement of Aβ in the blood through isolation with immunoprecipitation (IP) and quantification with mass spectroscopy (MS).

Methodology

Sample Preparation

Solutions of human Aβ40 peptides (AnaSpec) were prepared using 1:1 (v/v) methanol:water in order to determine the sensitivity of a MALDI-TOF MS instrument (SCIEX 5800) for detecting these Aβ peptides. The Aβ40 peptide derivative was chosen over Aβ42 because of its lower susceptibility to aggregate and precipitate out of solution. Solutions were spiked with standard isotope-labeled (SIL)-Aβ38 to function as an internal standard. The matrix solution consisted of 3 mg CHCA dissolved in 1 mL of 1:1 (v/v) acetonitrile:water + 0.1% TFA. The ratio of sample to the MS matrix solution (e.g., 1:9, 1:5, 1:1 etc.) was also optimized. Lyophilized and re-suspended peptide samples were kept at 4°C, and a new matrix solution was prepared before each spot test.

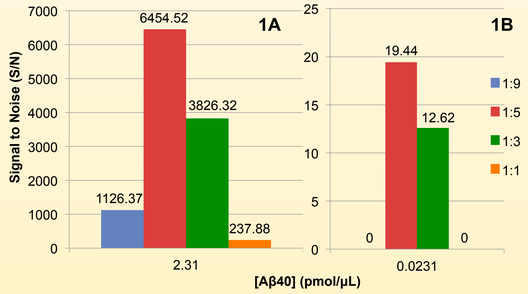
Data Collection

Samples were spotted in 1 µL aliquots to a stainless-steel plate and analyzed using MALDI-TOF MS, reflector positive ionization mode. All spot sets were calibrated with a standard peptide solution prior to data collection. To calculate the relative intensity of the Aβ40 peak, the background intensity of blank Aβ38 was subtracted from the spectra obtained of the Aβ38 spiked Aβ40 samples. The ratio of signal intensities of Aβ40 to Aβ38 was determined and used to assess relative protein quantitation.

Results

Sample to Matrix Ratio Optimization

The ideal ratio of analyte to matrix in MALDI-MS may vary according to the nature of the sample, which is why optimization is often a necessary first step. Keeping the concentration of Aβ40 constant, the v/v ratio of peptide to matrix solution was altered, and the signal to noise (S/N) ratio was recorded.



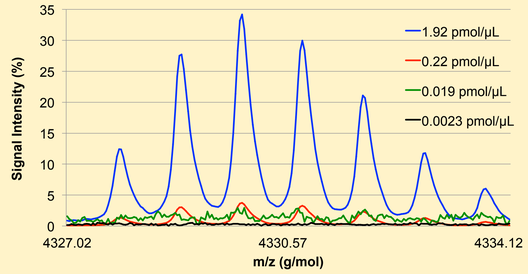
Figures 1A and 1B. Graphs of S/N ratio and specific concentrations (pmol/µL) of Aβ40 applied to spots for different peptide:matrix ratios. Ratios tested under these conditions included 1:9 (blue), 1:5 (red), 1:3 (green) and 1:1 (orange). At both 2.31 and 0.0231 pmol/µL, the S/N ratio of the 1:5 peptide:matrix ratio was highest. Signals were unable to be detected for the 1:9 and 1:1 ratios at 0.0231 pmol/µL of Aβ40.

From these results, the optimum peptide to matrix ratio was determined to be 1:5. This ratio had the highest S/N ratio at the highest peptide concentration (Figure 1A) as well as the second highest peptide concentration tested (Figure 1B).

Signal Intensity Decrease to LOD

Spectra from samples without spiked SIL-Aβ38 were first collected in order to assess the absolute limit of detection (LOD) of Aβ40 with the MALDI-MS instrument. The absence of an internal standard decreased the amount of baseline noise from fractionation or side interactions. Samples were ionized with a +1 charge, therefore the mass-to-charge ratio (m/z) was equivalent to the molecular weight.

Figure 2. Image of overlaid spectra from the MS measurements showing the Aβ40 signal intensity (%) decrease to baseline noise. The peak cluster is due to the presence of various stable heavy isotopes of the Aβ40 peptide. At the highest peak within the cluster (~4329.8 g/mol), the signal intensity decreases from 34.18% (blue) to 3.75% (red) to 2.93% (green) to immeasurable noise (black line). The signal decrease corresponds to different concentrations of Aβ40, decreasing from 1.92 to 0.22 to 0.019 to 0.0023 pmol/uL, respectively.



According to these results, the MS instrument indicated a detection limit of Aβ40 between 0.019 and 0.0023 pmol/µL utilizing standard parameters. The LOD was established as an S/N ratio of 3:1.

Within-day and Between-day Variability

The within-day and between-day variability of the MS method was evaluated by measuring the same concentration of Aβ40 spiked with internal standard Aβ38 twice within one day (n=2) and twice across two days (n=2). All samples were derived from the same stock peptide solution. Sample spots (n=10) were taken from the same peptide:matrix solutions with a new calibrant spot within-day, and with new peptide:matrix solutions and calibrant spot between-days. Blank spectra (n=3) of Aβ38 were also measured for each test and subtracted from the Aβ40 peak intensity.

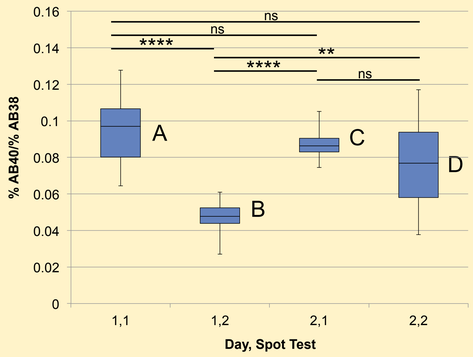


Figure 3. Boxplots showing the within-day and between-day variability in the ratio between Aβ40 and Aβ38 signal intensities (%). Mean±SD %Aβ40/%Aβ38 of spot tests A, B, C and D are 0.094±0.021, 0.047±0.009, 0.087±0.008 and 0.075±0.026, respectively. Within-day %CV of spot tests A, B, C and D are 21.97%, 19.71%, 9.38% and 34.13%, respectively. The results of a t-test are also depicted, where ns = $p > 0.05$, ** = $p \leq 0.01$ and **** = $p \leq 0.0001$.

Instrument Sensitivity with Internal Standard

A serial dilution series of Aβ40 spiked with Aβ38 was performed with each of the following concentrations spotted three times (n=3). The mean±SD signal intensity of blank Aβ38 (1.05±0.12%) was subtracted from each Aβ40 signal intensity.

Spot	[Aβ40] (pmol/µL)	Aβ38 Intensity (%)	Aβ40 Intensity - blank (%)	Aβ40 Intensity/ Aβ38 Intensity	Aβ40 Intensity/ Aβ38 Intensity Mean±SD	%CV of Aβ40 Intensity/ Aβ38 Intensity	S/N ratio
F1	2.1384	99.14	26.455	0.267	0.251±0.014	5.64	692.0
F2		98.15	24.355	0.248			177.7
F3		99.21	23.715	0.239			157.3
F4	1.7820	98.90	7.495	0.076	0.074±0.002	2.15	90.7
F5		98.94	7.265	0.073			82.7
F6		98.34	7.155	0.073			78.1
F7	1.0692	98.83	2.585	0.026	0.021±0.006	28.33	10.7
F8		97.59	1.425	0.015			12.8
F9		98.93	2.345	0.024			10.1
F10	0.3564	98.86	1.895	0.019	0.015±0.006	38.37	14.5
F11		99.18	1.815	0.018			10.2
F12		97.50	0.835	0.009			13.3
F13	0.2138	96.92	1.565	0.016	0.015±0.002	13.19	18.3
F14		95.44	1.195	0.013			22.5
F15		96.14	1.495	0.016			21.2
F16	0.0214	97.40	0.515	0.005	0.004±0.003	75.56	7.3
F17		98.23	0.505	0.005			8.5
F18		96.93	0.109	0.0005			5.6
F19	0.0021	96.72	0.255	0.003	0.004±0.001	29.50	3.1
F20		98.21	0.465	0.005			3.1
F21		98.06	0.335	0.003			3.6

Table 1. Data from an Aβ40 dilution series with Aβ38 internal standard. Calculated S/N ratios of Aβ40 show that the LOD appears to be ~0.0021 pmol/µL, which is in agreement with results found earlier without internal standard.

Conclusion

We determined the sensitivity of a MALDI-TOF MS instrument in detecting amyloid peptide standards (~0.002 pmol/µL) as well as the optimum ratio of sample to matrix solution (1:5). In the future, we will utilize this information in order to explore other experimental parameters (e.g., different matrix additives or types of sample plates) to achieve a lower sensitivity. The variability seen in within-day and between-day spot tests will also need to be addressed. This methodology will then be coupled with IP using antibody coupled beads for detecting the small amounts of Aβ typically present in blood plasma (~1.0×10^{-5} pmol/µL).

Acknowledgements

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