Analyte Derivatization for Enhancement of Mass Spectrometry Response for Quantitation of β-amyloid Peptides

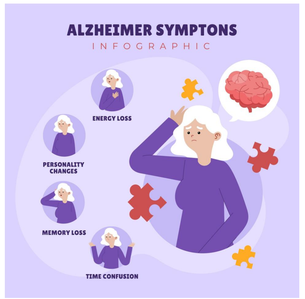
Fangjun Chen[1,2], Priscilla S.-W. Yeung[1,2], Morgan W. Mann[1,2], Run-Zhang Shi[1,2], Ruben Y. Luo[1,2]

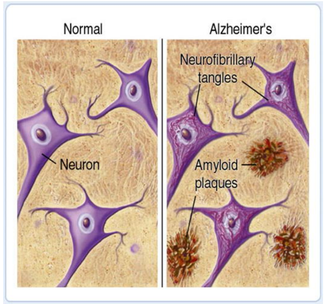
[1]Department of Pathology, Stanford University, Stanford, CA, USA; [2]Clinical Laboratories, Stanford Health Care, Palo Alto, CA, USA

This study employed a diverse range of derivatization reagents to amplify the mass spectrometry signal of the Aβ peptides and determine optimal derivatization conditions.

1. Introduction

Alzheimer's Disease (AD) is a debilitating neurodegenerative condition characterized by progressive cognitive decline and memory impairment. Early and precise diagnosis of AD is pivotal for effective management and intervention. Key pathological features include the presence of β-amyloid (Aβ) plaques and neurofibrillary tangles composed of aggregated tau protein. Consequently, beta-amyloid 1-40 (Aβ40), beta-amyloid 1-42 (Aβ42), total tau, and phosphorylated tau (p-tau) serve as primary diagnostic markers for AD[1-2].





While immunoassays have been commonly utilized to quantify Aβ peptides, their efficacy is hindered by nonspecific binding, resulting in high intra- and inter-laboratory variability. To overcome these limitations, alternative methods such as liquid chromatography-tandem mass spectrometry (LC-MS/MS) have emerged. LC-MS/MS offers enhanced precision and selectivity, utilizing multiple reaction monitoring (MRM) to improve analyte specificity[3-4].

Nevertheless, quantifying Aβ peptides in plasma via LC-MS/MS presents challenges due to the lower concentrations compared to cerebrospinal fluid (CSF)[5]. To address this, analyte derivatization emerges as a promising solution to boost the analytical sensitivity of LC-MS/MS assays for measuring endogenous Aβ40 and Aβ42 in plasma.

In conclusion, while traditional immunoassays have been foundational in AD diagnosis, advancements in LC-MS/MS technology coupled with techniques like analyte derivatization hold potential for refining the accuracy and reliability of AD diagnosis through plasma biomarkers.

2. Materials and Methods

Aβ peptides could be derivatized by dansyl chloride (5-N,N-dimethylaminonaphthalene-1-sulfonyl chloride) and also derivatized by EZ-Link™ NHS-PEG4-Biotin（A39259, Thermo Fisher Scientific ）

To implement the derivatization reaction, the derivatization reagent was directly added into Aβ peptide samples, The mixture will be incubated at different temperatures for different periods of time to find the most suitable derivatization conditions. Ammonium hydroxide in water was added to quench the reagent.

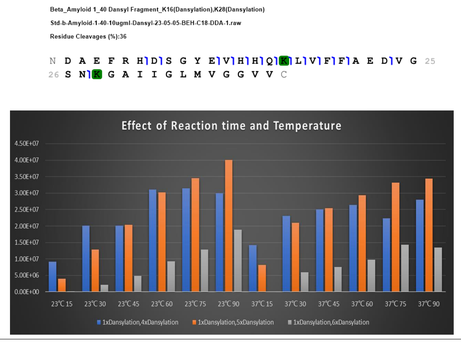
LC-MS/MS analysis was implemented in a Vanquish Flex-Orbitrap Q-Exactive system (Thermo Fisher Scientific). The LC separation was carried out using a C18 column, and a gradient elution of mobile phase A (0.1% ammonium hydroxide in water) and mobile phase B (ACN). The MS analysis was carried out in MRM mode.

3. Dansyl derivatization effect and optimal reaction conditions

Derivatized Aβ peptides were observed in mass spectra, and each peptide contained multiple derivatization groups on lysine side chain, tyrosine side chain, phenylalanine side chain, and peptide N-terminus. Although the derivatization method did not significantly increase the ionization efficiency of Aβ peptides, it significantly enhanced the MRM response by providing higher-abundance signature fragments resulted from the derivatization groups. For example, dansyl chloride derivatization resulted in a signature fragment at 317 m/z.

Top: Dansylation first took place on K16 and K28 in the β-amyloid 1-40 peptide.

Bottom: Our work have tried a variety of incubation temperatures and durations time, and found that room temperature and 90 minutes were the best derivatization conditions, and the derivatization products were mainly six dansylation groups.

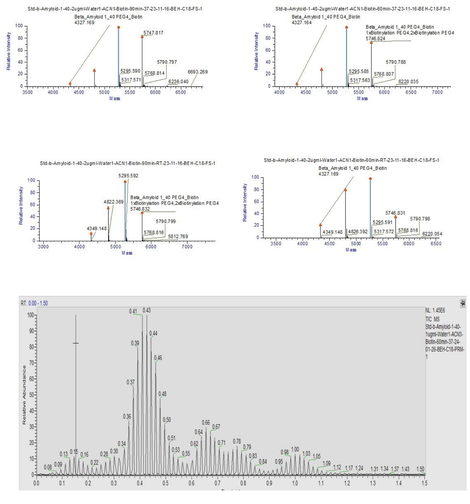


4. Biotin derivatization effect and optimal reaction conditions

Derivatization of EZ-Link™ NHS-PEG4-Biotin did not increase the ionization efficiency of β-Amyloid 1-40. However, it significantly enhanced the MRM response by providing a high-abundance fragment at 270 m/z.

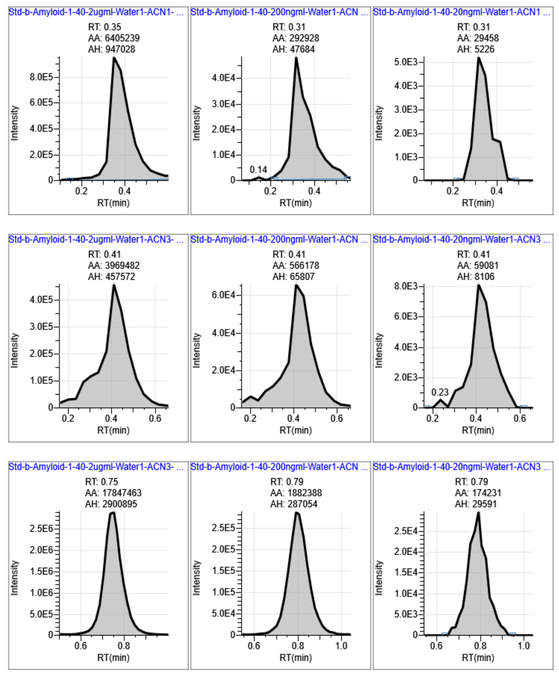
Top: There are three main forms of biotin derivatization, which are the combination of one, two and three biotin groups. And the derivatization efficiency is different under different solvents, incubation temperatures, and incubation durations.

Bottom: Our work have tried a variety of incubation temperatures and durations time, and found that 37 degrees and 60 minutes were the best derivatization conditions, and the derivatization products were mainly two biotin groups.



5. Mass spectrometry response amplification for derivatization

Under the most suitable derivatization conditions, compared to Aβ samples without derivatization. The MS response was amplified for both dansylation and biotinylation. The samples were freshly prepared to avoid adsorption effect.



LC-MS/MS extracted ion chromatograms of Aβ40 (upper), dansylated Aβ40 (middle), and biotinylated Aβ40 (lower). The sample concentrations were 2 μg/ml (left), 0.2 μg/ml (middle), and 20 ng/ml (right).

References

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