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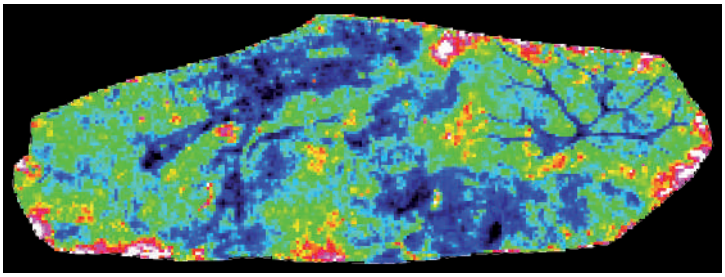
Application Note #MSI-13

Distribution of GABA with label free MALDI Mass Spectrometry Imaging



Objectives

Based on litterature, we developed a protocol to investigate distribution and quantification of GABA. We used the 4-hydroxy-3-methoxycinnamaldehyde (CA) as a derivatisation reagent to specifically identify GABA on brain tissue and enhance its sensitivity. Thanks to this derivatization strategy, we are able to detect and precisely locate GABA on brain tissue using MALDI-FTICR analysis.

For this purpose, it is important to differentiate GABA from endogenous isoforms that have the same exact mass in the literature. Therefore, specific identification of GABA was developed at the same time as the derivatisation strategy. Indeed, out of 6 isoforms, 2 of them could be confused with GABA after derivatisation: the 3-aminobutyric acid (BABA) and the 3-amino-isobutyric acid (BAIBA).

Context

GABA (γ-aminobutyric acid) is one of the major inhibitory neurotransmitter in the Central Nervous System and is involved in neural and mood disorders such as bipolar disorder, schizophrenia, Huntington, and Parkinson diseases. The ability to achieve precise distribution and concentration of this endogenous molecule on tissue may allow a better understanding of disease and drug efficacy. However, to know the localisation of the compounds, it depends on their specificity and sensibility: 2 challenging tasks of Mass Spectrometry Imaging (MSI) based studies.

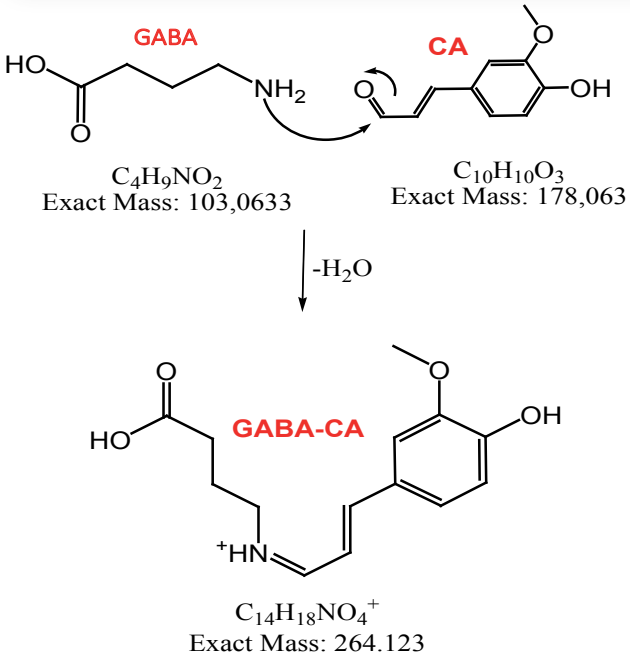


Figure 1: Derivatisation reaction between GABA and 4-hydroxy-3-methoxycinnamaldehyde (CA).

MSI developments are subsequently steered towards enhancement of detection and spatial resolution to enable the analysis of more and more compounds. Endogenous molecule such as neurotransmitters, amino acids, and metabolites are known to be difficult to detect by MALDI-MSI; and new approaches are being investigated. Therefore, ImaBiotech has decided to embark on developing strategies for GABA analysis.

Solution

Firstly, derivatisation analyses were performed to allow specific identification and localisation of GABA in brain tissues. Indeed, isoforms were detected in LC-MS/MS analyses and their differentiation needed to be performed in MSI. Except for BABA, isoforms were described in the literature. BAIBA needed to be confirmed due to a small difference with GABA fragmentation. In order to discriminate specific fragments considered as being specific to CA reagent (*m/z* 161.0597) and to derivatisated compounds, the study of fragmentation pathway of derivatisated GABA, BAIBA, and BABA was performed with MALDI-FTICR on CASI-CID mode after overnight incubation.

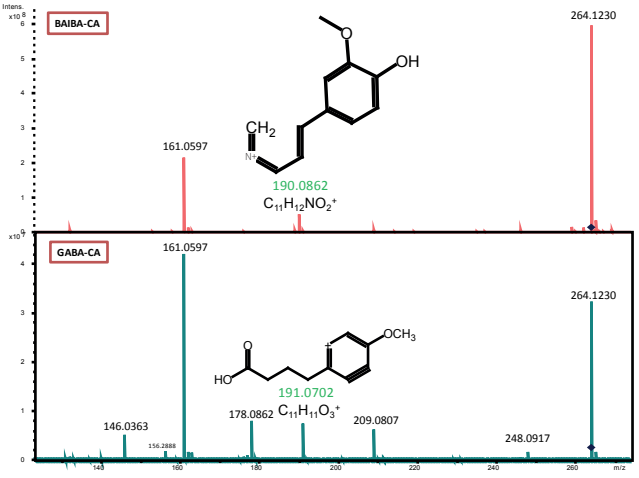


Figure 2: Fragmentation pathway of BAIBA and GABA for on tissue differentiation.

After fragmentation of the same parent ion at *m/z* 264.1235, MS^2 spectra analyses indicated a completely different fragmentation pathway between BABA and GABA. In addition, the differentiation of GABA against BAIBA could be performed with the GABA daughter ion at *m/z* 191.0702 (Figure 2). Indeed, BAIBA could be differed from GABA due to a single fragment change between both fragmentations pathways.

After the confirmation of the specific identification of GABA, the derivatisation strategy was also applied on a brain tissue. With usual MSI analysis, GABA wasn't detected on brain tissue but the derivatisation strategy developed with CA enhances the GABA sensitivity. Therefore, derivatisation was performed on tissue with the SunCollect™ system, and after incubation, GABA distribution was achieved with MALDI-FTICR/CASI-CID analysis. The CASI mode is used to improve the sensitivity by trapping more ions in the Quadrupole. Thanks to this derivatisation strategy, distribution of GABA could be obtained based on MS^2 imaging. Indeed, all derivatisated isoforms own the same parent ion at *m/z* 264.1230 (Figure 3, A) so

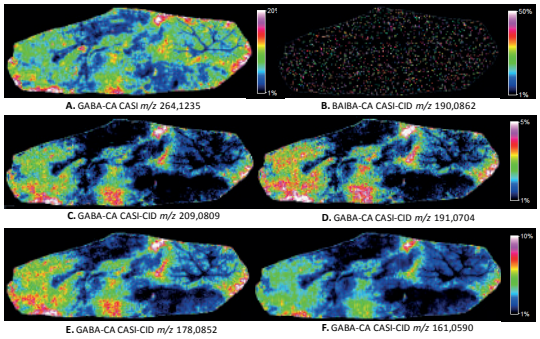


Figure 3: MS^2 imaging of GABA ions

fragmentation is necessary to distinguish them, and more precisely between GABA and BAIBA. Ions at *m/z* 209.0807, 178.0862 and 161.0597 (Figure 3, C, E and F) are common to GABA and BAIBA and don't allow their differentiation on MS^2 imaging. That's why, ImaBiotech based GABA distribution one the m/z 191.0704 (Figure 3, D). Furthermore, BAIBA distribution doesn't represent specific localisation on brain tissue contrary to GABA. Actually, GABA is especially concentrated in neurons, and with this method, the white matter is clearly distinguish from grey matter. On this MS^2 images, the GABA distribution highlights the arbor vitae in the cerebellum.

Summary

To detect GABA on brain tissue, the derivatisation strategy has been used. The reaction between GABA and the derivatisation reagent has increased the signal due to additional charge. Moreover, the differentiation between isoforms and GABA was, therefore, made though specific fragmentation analysis of derivatisated GABA with the 4-hydroxy-3-methoxycinnamaldehyde. Images of GABA distribution were successfully obtained with MALDI-FTICR-MS^2 analyses to performed localisation of GABA on the grey matter. Moreover, distinction between grey and white matter allow visualising the arbor vitae in cerebellum with only slight delocalisation induce by the derivatization method. This strategy allow the localisation on brain tissue of the major inhibitory neurotransmitter in the Central Nervous System and could provide a better understanding of its involvement in neural and mood disorders as bipolar disorder, schizophrenia, Huntington and Parkinson diseases.

References

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Keywords

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