

WeP-6

OVERCOMING PROBLEMATIC FRAGMENTATION OF CARBOXYLATED PEPTIDES BY MALDI TOF MS

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MALDI TOF MS is a popular analytical technique used to determine molecular weight of biomolecules. In principle, an analyte is mixed with a suitable "matrix", allowed to air dry, and irradiated with a laser to desorb ions. The analysis of fragile biomolecules such as sialated glycopeptides and glycoproteins can result in an incorrect mass assignment which is lower than expected due to the loss of sialic acid¹. Likewise, the analysis of fragile carboxylated peptides can result in lower mass assignments if care is not taken to ensure that the molecule does not fragment during the ionization process.

In practice, it is important to establish the threshold irradiance, the laser power, which begins to generate ions. Molecular ions are generally observed at slightly higher irradiances, whereas laser power which is too high can result in extensive fragmentation of the molecule as with post source decay and in source fragmentation.

A 55Kda protein extracted from Bovine blood was purified and digested with chymotrypsin. The desired peptide (Bovine Factor 10 5229.6 Da) was isolated and purified. This fragile peptide exists naturally with all 12 Glutamic acid (Glu) residues carboxylated to gamma carboxy glutamic acid, and serves as an ideal candidate to study the effects of fragmentation with fragile materials.

It will be shown that the difficulties associated with the analysis of fragile molecules by MALDI TOF MS can be overcome when care is exercised. Of importance is taking care not to impart excessive laser energy into the analyte of interest, selecting a suitable matrix which does not facilitate fragmentation, and employing gridless ion optics when possible. Since it is possible to successfully determine the molecular weight of intact fragile carboxylated peptides by MALDI, judgement can be made about the activity of the molecule, its structure, and the extent of peptide modification.

1. J. Blackledge, A. Alexander, D. Dodsworth, Proceedings of the 46th ASMS Conference on Mass Spectrometry and Allied Topics, p. 239 (1998).