

Derivatization of Peptides with Alexa Fluor 350 for Analysis of Proteomic Samples by High-Throughput LC-MALDI-TOF/TOF MS

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It has previously been demonstrated that coumarin derivatives, used as N-terminal tags, enhance intensities of MALDI MS signals of peptides¹, due to specific analyte-matrix interactions that facilitate incorporation of the peptides into hydrophobic MALDI matrices, such as CHCA and 2,5-DHAP, during co-crystallization². Labeling with Alexa Fluor 350, a coumarin tag containing a sulfo group, was found to improve unimolecular fragmentation of peptides and formation of high-intensity y-ion series, while the peptide intensities in the MS mode were not severely affected. This N-terminal tag was used for LC-MALDI-TOF/TOF MS analysis of complex peptide mixtures, such as tryptic digests of standard proteins and SCX fractions of *E.coli* lysate.

MS/MS sequencing of peptides labeled with Alexa Fluor 350 was performed using an AB4700 MALDI TOF/TOF mass spectrometer in both the PSD (unimolecular fragmentation) and CID modes. Analysis of the distribution of MS/MS fragment ions revealed changes in the fragmentation pattern caused by the tag when compared to the native peptide spectra. In particular, labeling with Alexa Fluor 350 caused a dramatic increase of the y-ion fraction, while the fractions of the rest of the ion fragments, such as a-, b-, immonium and internal ions, decreased. Enrichment of the y-ion series, in terms of both the number of y-ions and their intensity, and simultaneous decrease of the less informative internal and neutral ion series, was found to be very beneficial for MASCOT scores of the Alexa Fluor 350 labeled peptides.

To evaluate advantages of the use of Alexa Fluor 350 labeling for the analysis of proteomic samples, an SCX LC fraction of a tryptic digest of *E. coli* lysate was split in two parts, one of which was maintained native and the other modified by guanidation of lysines, followed by N-terminal labeling with Alexa Fluor 350. Both fractions were analysed in parallel by RP (C18) nanoLC-MALDI-TOF/TOF MS using a home-built LC-MALDI deposition device³, and the data were submitted to MASCOT for database searching. The results showed both improved peptide scores and an increased number of identified peptides due to labeling. Surprisingly, many more unique (non-overlapping) peptides were identified in either the native or labeled samples than peptides identified in both samples. In particular, longer peptides with C-terminal arginine prevailed among the peptides identified in the native sample, whereas shorter peptides with C-terminal lysine (guanidated) were more common for the labeled sample. As a result, confidence in correct identification was increased for proteins found in both samples, as many proteins were identified by different peptides. These results suggest that Alexa Fluor 350 labeling could be run in parallel with the native sample analysis, similar to the complementary use of the MALDI and ESI MS methods.

It is important to note that derivatization did not impair chromatographic behavior of peptides, as narrow (30-40 s) chromatographic peaks were obtained for the labeled peptides, with relatively small changes in their retention times, compared to the unlabeled peptides. Labeling conditions were relatively mild and allowed analysis of some post-translational modifications, such as phosphorylation. Tags used in this study are commercially available (Molecular Probes) and relatively inexpensive. All these factors suggest that labeling with Alexa Fluor 350 has a real potential for the use for high-throughput LC-MALDI-TOF MS/MS analysis of proteomic samples.

Fig. 1. Coumarin and CAF⁴ tags used for N-terminal derivatization of peptides (Su = N-oxysuccinimide)

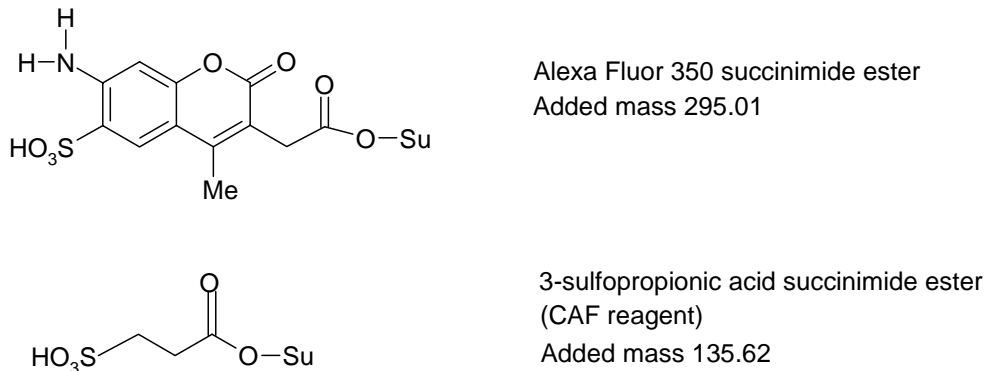
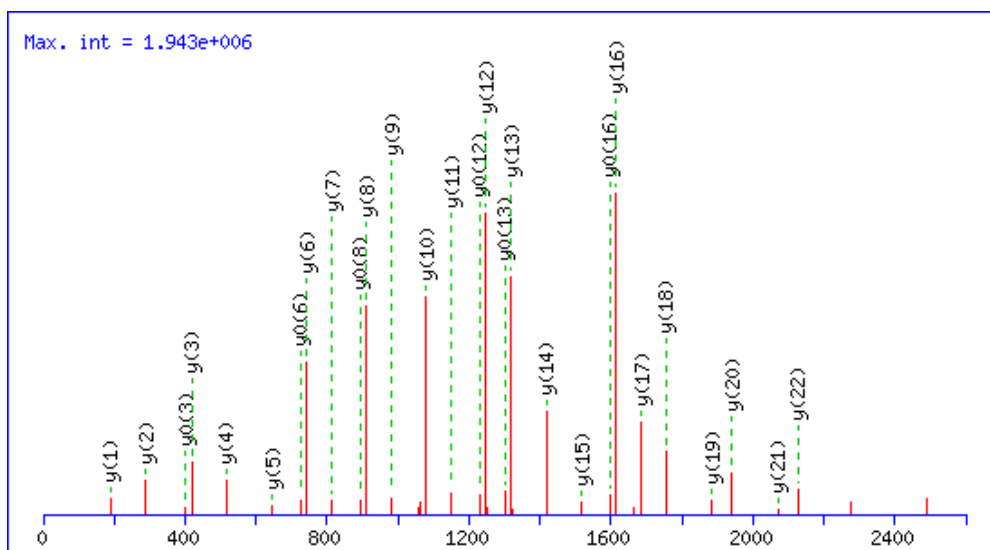


Fig. 2. An example of MS/MS spectrum of the Alexa Fluor 350 labeled peptide FGQGEAAPVVAPAPAPEVQTK (found in OMPA_ECOLI, (P02934) Outer membrane protein A precursor), m/z 2568.2 with complete y-series, which resulted in MASCOT score 252.



References:

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