

Derivatization of Peptides for Analysis by High-Throughput LC-MALDI-MS/MS

Anna Pashkova; Eugene Moskovets; Xin Zhang; Roger Giese; Barry L. Karger*

Barnett Institute and Department of Chemistry, Northeastern University, Boston, MA 02115

The goal of this work was to develop peptide tags suitable for high-throughput LC-MALDI-MS/MS (TOF/TOF), with both unimolecular and high-energy CID MS/MS fragmentation. Important requirements for the tag to be used for MALDI analysis include: (1) increase in signal intensity in the MS mode; (2) facilitate generation of MS/MS (PSD and high-energy CID) spectra of improved quality (i.e. more complete ion series, higher S/N) compared to unlabeled peptides.

Initially, N-terminal positive charge (permanent cation) derivatization was suggested for increase in sensitivity in MALDI and directed MS/MS fragmentation under high-energy conditions [1,2]. We demonstrate that permanent cation tags, while significantly improving signal intensity in the MS mode, lead to severe suppression of MS/MS fragmentation, making these tags unsuitable for high-throughput MALDI MS/MS analysis. On the other side, N-terminal tagging with negative-charged sulfonic acid derivatives significantly improves unimolecular fragmentation of peptides, leading to the formation of high-intensity γ -ion series [3]. The presence of a strong negative charge, however, can lead to decrease of positive MALDI MS signals for labeled peptides.

Hydrophobicity is a second factor in the order of importance (after basicity) that influences MALDI MS signals of peptides [4]. In addition, it is known that peptides that contain aromatic amino acids, such as Phe, Tyr and Trp, usually produce MALDI MS signals of higher intensity than those lacking aromatic residues [5]. The reason is believed to be improved incorporation of these peptides in MALDI matrices (CHCA).

We found that coumarin derivatives, used as N-terminal tags, enhance intensities of MALDI MS signals of peptides. The effect is peptide-dependent and is the most pronounced for hydrophilic peptides and phosphopeptides (up to 50-fold enhancement in CHCA). The enhancement factor also depends on the MALDI matrix, being the largest for 2,5-DHAP (among CHCA, 2,5-DHAP and 2,5-DHB matrices studied). For 2,5-DHB, decrease of MALDI MS signal has been observed for coumarin-labeled peptides, compared to the unlabeled peptides. These observations also support the idea that labeling with coumarin tags improves their incorporation into hydrophobic MALDI matrices, such as CHCA and 2,5-DHAP.

Increase in MALDI MS signal intensity was the reason of higher ion current in the TOF/TOF MS/MS spectra of labeled peptides. On average, signal intensity in the MS/MS mode was 3 times higher for labeled peptides. In addition, coumarin-directed fragmentation pathway resulted in formation of b_1 - and a_1 -ions in the MS/MS spectra (these ions are not observed in MS/MS spectra of native peptides), and, in many cases, higher intensity of b_2 - and b_3 -ions. All these factors led to the increase in MASCOT scores for labeled tryptic peptides compared to unlabeled digests.

Since size, hydrophobicity and basicity of the tags are comparable with those of amino acids (e.g. tryptophan), it is expected that derivatization will not impair chromatographic behavior of peptides. This is important for the potential use of the tags for high-throughput LC-MALDI-TOF/TOF.

Both standard and tryptic digest peptides were labeled with a group of coumarin tags. The samples were deposited on a MALDI target in the dried-droplet mode. PSD and high-energy CID TOF-TOF spectra were acquired on an ABI 4700 MALDI TOF-TOF spectrometer (Applied Biosystems). The spectra of peptides before and after labeling were examined and submitted to a MASCOT search for score comparison.

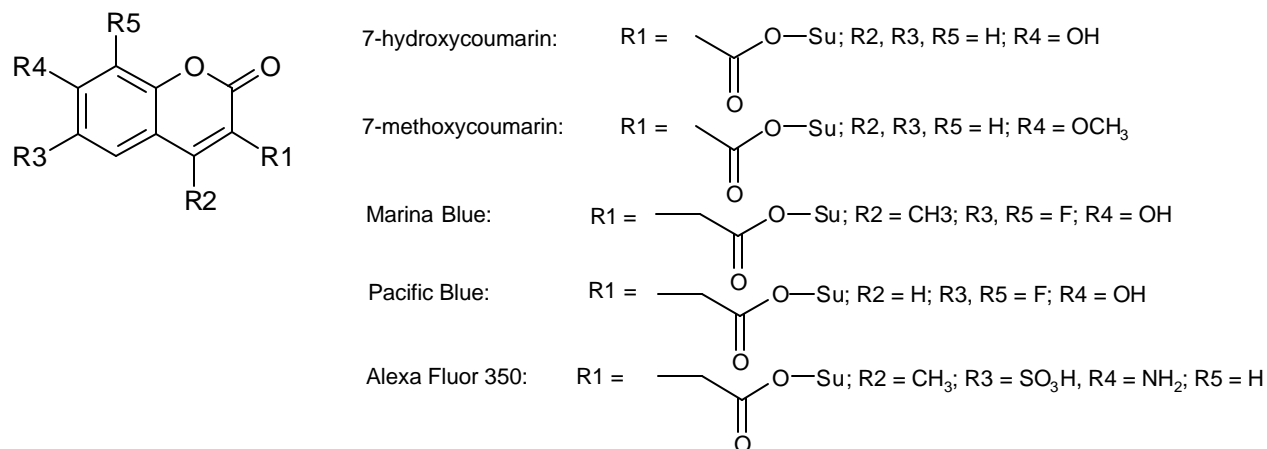


Fig. 1. Coumarin tags used for N-terminal derivatization of peptides (Su = N-oxy succinimide)

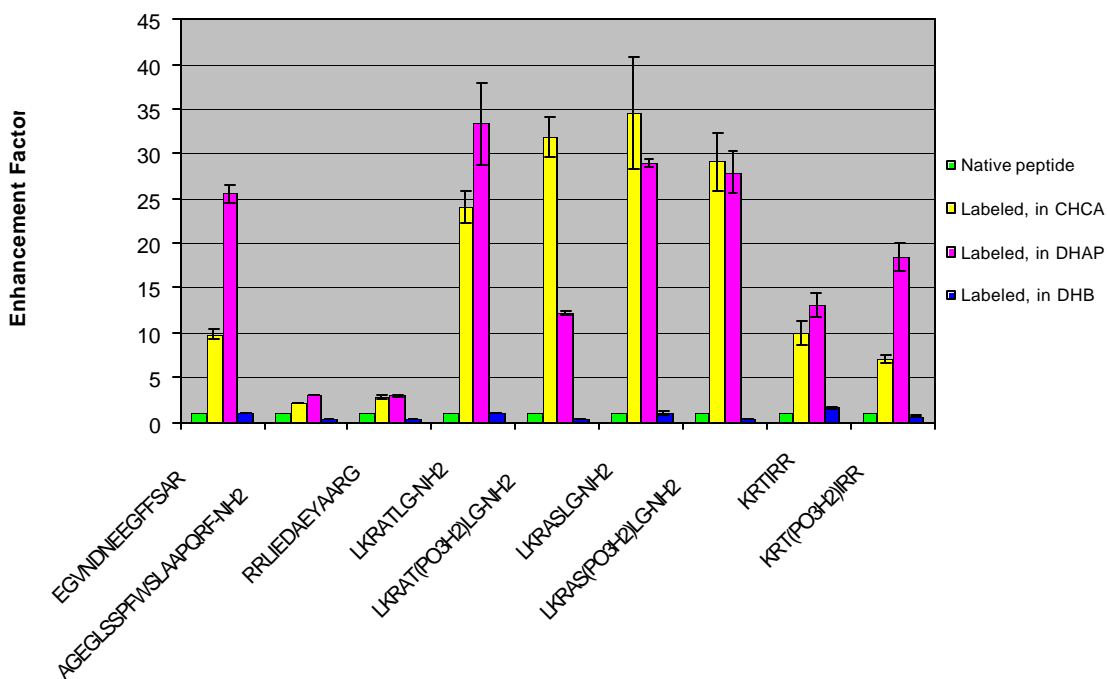


Fig. 2. MALDI MS Signal Enhancement with Pacific Blue (Coumarin tag) as a function of matrix (CHCA, DHAP and DHB)

1. Zaia, J.; Bieman, K.; *J. Am. Soc. Mass Spectrom.* 1995, 6, 428-436
2. Sadagopan N., Watson J.Th. *J. Am. Soc. Mass Spectrom.* 2001, 12, 399-409
3. Keogh, T.; Youngquist, S.; Lacey, M. *Anal. Chem.* 2003, 75, 156A-165A
4. Amado, F.; Domingues, P.; Santana-Margues, M.; Ferrer-Correia, A., Tomer, K.B. *Rapid Comm. In Mass Spectrom.* 1997, 11, 1347-1352
5. Valero, M.L., Giralt, E.; Andreau, D., in *Peptides 1996*, Ramage R., Epton, R., Eds.; Mayflower Scientific, LTD.; Kingswinford, U.K., 1998; pp.855-856