Available online at www.sciencedirect.com
SCIENCE DIRECT®
Analytical Biochemistry 345 (2005) 122–132
ANALYTICAL BIOCHEMISTRY
www.elsevier.com/locate/yabio

Characteristic mass spectral fragments of the organophosphorus agent FP-biotin and FP-biotinylated peptides from trypsin and bovine albumin (Tyr410)

Lawrence M. Schopfer [a,*], Matthew M. Champion [b], Nate Tamblyn [c], Charles M. Thompson [c], Oksana Lockridge [a]

[a] *University of Nebraska Medical Center, Eppley Institute, Omaha, NE 68198, USA*
[b] *Applied Biosystems, Foster City, CA 94404, USA*
[c] *Department of Biomedical and Pharmaceutical Sciences, University of Montana, Missoula, MT 59812, USA*

Received 3 May 2005
Available online 2 August 2005

Abstract

A mass spectrometry-based method was developed for selective detection of FP-biotinylated peptides in complex mixtures. Mixtures of peptides, at the low-picomole level, were analyzed by liquid chromatography and positive ion, nanospray, triple quadrupole, linear ion trap mass spectrometry. Peptides were fragmented by collision-activated dissociation in the mass spectrometer. The free FP-biotin and peptides containing FP-biotinylated serine or FP-biotinylated tyrosine yielded characteristic fragment ions at 227, 312, and 329 *m*/*z*. FP-biotinylated serine yielded an additional characteristic fragment ion at 591 *m*/*z*. Chromatographic peaks containing FP-biotinylated peptides were indicated by these diagnostic ions. Data illustrating the selectivity of the approach are presented for tryptic digests of FP-biotinylated trypsin and FP-biotinylated serum albumin. A 16-residue peptide from bovine trypsin was biotinylated on the active site serine. A 3-residue peptide from bovine albumin, YTR, was biotinylated on Tyr410. This latter result confirms that the organophosphorus binding site of albumin is a tyrosine. This method can be used to search for new biomarkers of organophosphorus agent exposure.

Keywords: FP-biotin; Quadrupole mass spectrometry; Characteristic ions; Albumin; Trypsin

FP-biotin (10-fluoroethoxyphosphinyl-*N*-biotinamidopentyldecanamide)[1] is a fluorophosphinate linked to biotin via a 17-atom spacer (Fig. 1, [593]). It is an important new probe that was designed for directed, activity-based protein profiling of the serine hydrolase superfamily [1,2]. The fluoroethoxyphosphinate moiety forms a covalent adduct with the catalytic serine (e.g., Ser198 of human butyrylcholinesterase). The biotin moiety provides a convenient handle that can be used for (i) identifying the labeled protein via fluorescently labeled avidin and for (ii) purifying and enriching the labeled proteins via avidin-agarose. The linker is sufficiently long that the biotin is accessible even when the phosphinate is bound to active sites buried deep in the target protein (e.g., butyrylcholinesterase, acetylcholinesterase) [3].

To date, identification of FP-biotinylated proteins has been made by mass spectrometry using the following

* Corresponding author. Fax: +1 402 559 4651.
E-mail address: lmschopf@unmc.edu (L.M. Schopfer).

[1] *Abbreviations used:* FP-biotin, 10-fluoroethoxyphosphinyl-*N*-biotinamidopentyldecanamide; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; MALDI, matrix-assisted laser desorption ionization; LC–MS/MS, liquid chromatography–tandem mass spectrometry; CAD, collision-activated dissociation; TPCK, *N*-tosyl-L-Phe-chloromethyl ketone; HABA, 4-hydroxy azobenzene-2-carboxylic acid; TAME, *N*α-*p*-toluene-sulfonyl-L-arginine methyl ester HCl; +EMS, positive ion enhanced mass spectrum; +ER, positive ion enhanced resolution; EPI, positive ion enhanced product ion; TIC, total ion chromatogram; XIC, extracted ion chromatogram.

0003-2697/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.ab.2005.07.016

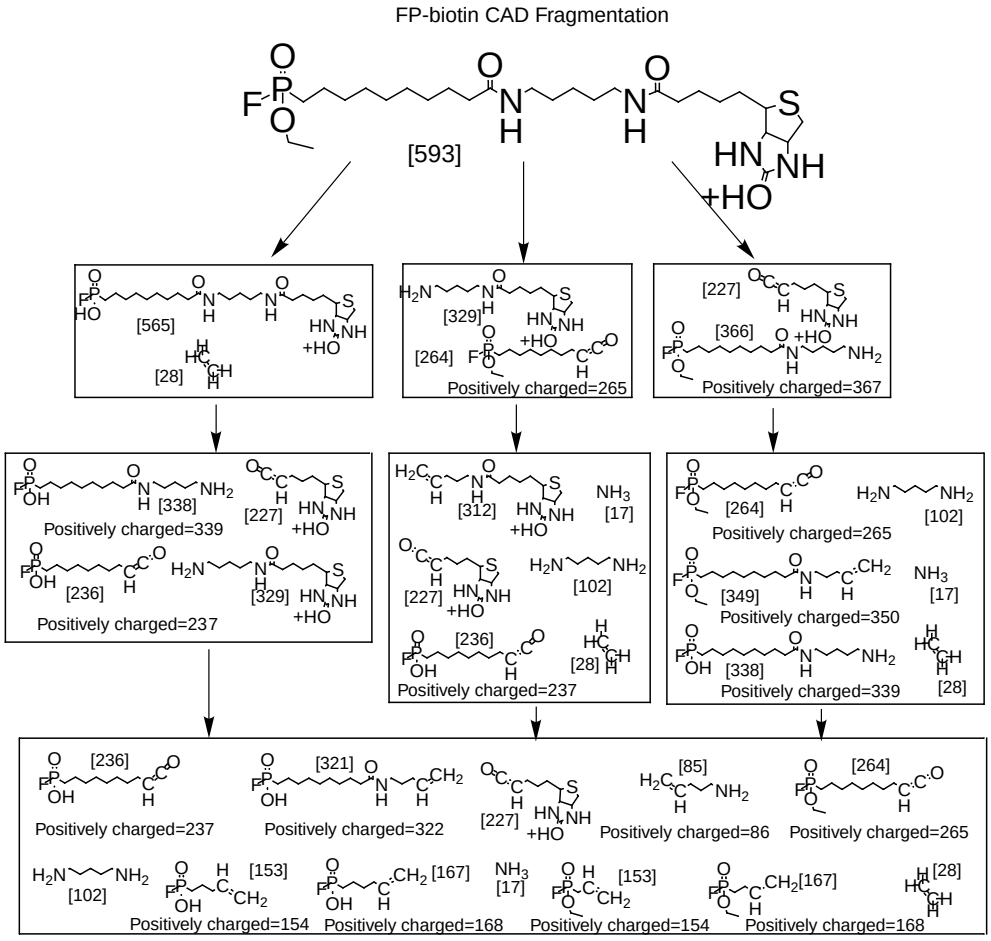

Fig. 1. Fragments of FP-biotin produced by collision-activated dissociation (CAD). The singly charged FP-biotin ion has an *m*/*z* of 593 amu. The fragments were produced by CAD. A total of 18 positively charged fragments were observed and are listed in Table 1. The structures presented are not intended to imply fragmentation mechanisms; rather, they are simply consistent with the masses of the observed fragments and serve to indicate the types of fragmentation that occurred and the sites of fragmentation. The number in square brackets next to each structure indicates the molecular weight of the piece.

protocol. A complex proteome was labeled. The labeled proteins were partially purified and enriched by avidin–agarose chromatography. Then the purified proteins were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). Proteins visible on Coomassie blue staining were digested with trypsin. The tryptic peptides were matched to proteins by mass spectrometry using either matrix-assisted laser desorption ionization (MALDI) [1,2] or liquid chromatography–tandem mass spectrometry (LC–MS/MS) quadrupole time-of-flight [4]. In no case was the FP-biotinylated peptide specifically identified. Although identification of a protein by matching it to peptides derived from a tryptic digest is the state of the art, the identification would be more compelling if it included the labeled peptide. This is especially important when the identified protein does not fall into the serine hydrolase superfamily. Peeples et al. [4] identified such a protein when they found serum albumin to be labeled after FP-biotin was injected into mice.

To address this issue, we have developed a mass spectrometry-based strategy specifically for identification of FP-biotin-labeled peptides that uses characteristic fragments of FP-biotin that are generated by collision-activated dissociation (CAD). This approach was modeled after work on phosphoproteins [5–7].

Although simple MALDI spectra can be used to identify FP-biotinylated peptides, CAD was chosen because it provides extensive sequence information for the selected peptide from which the labeled amino acid can be specifically identified. Direct identification of the labeled residue is particularly important when the FP-biotin reacts with proteins that are not part of the serine hydrolase superfamily such as serum albumin. In addition, MALDI is best suited for relatively simple samples such as the digest of a single protein. When CAD is used in conjunction with electrospray ionization and liquid chromatography on a quadrupole mass spectrometer, a convenient system suitable for more complex samples is created. The ultimate purpose of