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ScienceDirect
Analytical Biochemistry 361 (2007) 263–272
ANALYTICAL BIOCHEMISTRY
www.elsevier.com/locate/yabio

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry assay for organophosphorus toxicants bound to human albumin at Tyr411

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Received 3 October 2006
Available online 4 December 2006

Abstract

Our goal was to determine whether chlorpyrifos oxon, dichlorvos, diisopropylfluorophosphate (DFP), and sarin covalently bind to human albumin. Human albumin or plasma was treated with organophosphorus (OP) agent at alkaline pH, digested with pepsin at pH 2.3, and analyzed by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry. Two singly charged peaks *m*/*z* 1718 and 1831, corresponding to the unlabeled peptide fragments containing the active site Tyr411 residue, were detected in all samples. The sequences of the two peptides were VRYTKKVPQVSTPTL and LVRYTKKVPQVSTPTL. The peptide–OP adducts of these peptides were also found. They had masses of 1854 and 1967 for chlorpyrifos oxon, 1825 and 1938 for dichlorvos, 1881 and 1994 for DFP, and 1838 and 1938 for sarin; these masses fit a mechanism whereby OP bound covalently to Tyr411. The binding of DFP to Tyr411 of human albumin was confirmed by electrospray tandem mass spectrometry and analysis of product ions. None of the OP–albumin adducts lost an alkoxy group, leading to the conclusion that aging did not occur. Our results show that OP pesticides and nerve agents bind covalently to human albumin at Tyr411. The presence of Tyr411 on an exposed surface of albumin suggests that an antibody response could be generated against OP–albumin adducts.
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Keywords: Biomarker organophosphate exposure; Pepsin; Sarin; Soman; Dichlorvos; Diisopropylfluorophosphate; Chlorpyrifos oxon; Nerve agents; Pesticides

The acute toxicity of organophosphorus (OP)[1] toxicants is known to be due to inhibition of acetylcholinesterase. However, other proteins also bind OP, although their role in toxicity is less defined [1]. Albumin is a potential new biomarker of OP exposure. Mice treated with a nontoxic dose of a biotinylated nerve agent analog, FP-biotin (10-fluoroethoxyphosphinyl-*N*-biotinamidopentyldecanamide), had 1000 times more FP-biotinylated albumin than FP-biotinylated butyrylcholinesterase in their blood [2].

Albumin has been shown to covalently bind radiolabeled diisopropylfluorophosphate (DFP). Human albumin incorporated 1 mol DFP per mole of albumin when 20 to 70 μM albumin was incubated with a sevenfold molar excess of DFP at pH 8.0 for 2 h at 23 °C [3,4]. Bovine albumin also incorporated 1 mol DFP per mole of albumin [5]. The site of covalent binding of DFP to human albumin was identified by amino acid sequencing. The labeled peptide

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[1] *Abbreviations used:* OP, organophosphorus; FP-biotin, 10-fluoroethoxyphosphinyl-*N*-biotinamidopentyldecanamide; DFP, diisopropylfluorophosphate; MS, mass spectrometry; MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight; HPLC, high-performance liquid chromatography; TFA, trifluoroacetic acid; DHBA, 2,5-dihydroxybenzoic acid; CHCA, α-cyano 4-hydoxycinnamic acid; ACTH, adrenocorticotropic hormone; MS/MS, tandem mass spectra; LC–MS, liquid chromatography–mass spectrometry; GC–MS, gas chromatography–mass spectrometry.

0003-2697/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.ab.2006.11.018

had the sequence ArgTyrThrLys with DFP bound to Tyr [6]. Later, when the complete amino acid sequence of human albumin was known, the active site tyrosine was identified as Tyr411 (Tyr435 when residue 1 is Met of the signal peptide). Mass spectrometry (MS) identified Tyr410 of bovine albumin (equivalent to Tyr411 of human albumin) as the covalent binding site for FP-biotin [7]. The nerve agents soman and sarin were shown to bind covalently to human albumin on tyrosine [8,9] and to be released by treatment with potassium fluoride [9].

Albumin has also been demonstrated to be an OP hydrolase, hydrolyzing chlorpyrifos oxon, *O*-hexyl *O*-2,5-dichlorophenylphosphoramidate, and paraoxon at measurable rates [10–13]. The apparent K_m of bovine albumin is 0.41 mM for chlorpyrifos oxon and 1.85 mM for paraoxon [12], and the apparent K_m of human albumin is 3.6 mM for DFP [3]. Despite this seemingly consistent body of results, some issues have been raised regarding the reaction of OP with albumin. It has been questioned whether the observed OP hydrolase activity was associated with the albumin molecule itself or with minor phosphotriesterase contaminants in the albumin preparation [10]. In addition, the possibility has been raised that DFP binds to one site in albumin but that other OP toxicants bind to a different site [12,14].

Our goal was to determine whether Tyr411 of human albumin was the site for covalent attachment of a variety of OP toxicants. For this purpose we developed a matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS assay applicable to purified human albumin and to human plasma.

Materials and methods

Materials

Purified human serum albumin, essentially fatty acid free, was obtained from Fluka via Sigma (Cat. No. 05418, St. Louis, MO, USA). Pepsin from porcine gastric mucosa was obtained from Sigma (Cat. No. P6887). Modified trypsin, sequencing grade, was purchased from Promega (Cat. No. V5113, Madison, WI, USA). DFP was obtained from Sigma (Cat. No. D0879). Dichlorvos and chlorpyrifos oxon were purchased from Chem Services (Cat Nos. PS-89 and MET-674B, West Chester, PA, USA). Sarin-treated human plasma was a gift from Patrick Masson. Acetonitrile high-performance liquid chromatography (HPLC)-grade 99% ACROS, was purchased from Fisher Scientific (Cat. No. 61001-0040, Pittsburgh, PA, USA). Trifluoroacetic acid (TFA), sequencing grade, was purchased from Beckman Instruments (Cat. No. 290203, Palo Alto, CA, USA). 2,5-Dihydroxybenzoic acid (DHBA) matrix was purchased from Applied Biosystems (Foster City, CA, USA). α-Cyano 4-hydroxycinnamic acid (CHCA, Cat. No. 70990, Sigma) was recrystallized before use. Calibration standards for MALDI-TOF were obtained from New England Biolabs (Cat. No. P7720S, Beverly, MA, USA) and included angiotensin 1 (1297.51 amu), adrenocorticotropic hormone (ACTH) (7–38) (3660.19 amu), and ACTH (18–39) (2466.73 amu). Double distilled water was prepared in-house and was autoclaved.

Sample preparation for DFP-, dichlorvos-, and chlorpyrifos oxon-treated samples

Fatty acid-free human albumin at a concentration of 10 mg/ml, which is 150 μM, was dissolved in 25 mM ammonium bicarbonate (pH 8.6) and treated with an equimolar concentration of OP for 24 h at 37 °C. The pH of 1000 μl reaction mixture was reduced to 2.3 by the addition of 500 μl of 1% TFA. Pepsin was dissolved in 10 mM HCl to make 1 mg/ml and was stored at −80 °C. The albumin was digested with pepsin (1:250 ratio) for 2 h at 37 °C and was diluted to 1 pmol/μl with 0.1% TFA.

A 200-μl aliquot of human plasma was treated with 6.85 μl of 20 mM OP (660 μM final OP concentration) for 24 h at 37 °C. The pH was adjusted to 2.3 to 2.5 by the addition of 200-μl of 1% TFA. Proteins were digested with 50 μl of 1 mg/ml pepsin for 2 h at 37 °C. Before spotting the digest on the target plate, a 10-μl aliquot of the digest was diluted with 390 μl of 0.1% TFA so that the final plasma dilution was 1000-fold.

MALDI-TOF

A 1-μl aliquot of diluted peptic digest was applied to a stainless-steel target plate, air-dried, and overlaid with 1 μl of 2,5-dihydroxybenzoic acid matrix. The CHCA matrix gave similar results. Mass spectra were acquired with the Voyager DE-PRO MALDI-TOF mass spectrometer (Applied Biosystems/MDS Sciex) in linear positive ion mode, 20,000 V accelerating voltage, 94% grid voltage, 0.1% guide wire, 350 ns extraction delay time, and automated laser intensity adjustment from 1000 to 1600. The instrument was calibrated with a peptide calibration mixture from New England Biolabs. Mass accuracy for each standard was within 0.05% of the corresponding average molecular weight. Spectra were acquired in automatic mode by examining signals from random spots on a target. The signals from the first 10 spots that met the acceptance criteria were summed into one final profile mass spectrum The acceptance criteria were signal intensities between 1000 and 55,000 counts with signal/noise ratios of 10 or greater and minimum resolution of 50. The final spectrum was the average of 1000 shots.

The MS-Digest program from the UCSF Mass Spectrometry Facility was used to calculate the masses of the peptic peptides expected from digests of human serum albumin.

Quadrupole mass spectrometer

Tandem mass spectra (MS/MS) were acquired on a Q-Trap 2000 triple quadrupole linear ion trap mass spectrometer (Applied Biosystems/MDS Sciex) with a nano electro-

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