Chemico-Biological Interactions 175 (2008) 68–72

Contents lists available at ScienceDirect

Chemico-Biological Interactions

journal homepage: www.elsevier.com/locate/chembioint

Fast affinity purification coupled with mass spectrometry for identifying organophosphate labeled plasma butyrylcholinesterase

He Li[a,*], Larry Tong[a], Lawrence M. Schopfer[a], Patrick Masson[b], Oksana Lockridge[a]

[a] *Eppley Institute, University of Nebraska Medical Center, Omaha, NE 68198-6805, United States*
[b] *Centre de Recherches du Service de Santé des Armées, Département de Toxicologie, Unité d'Enzymologie, BP 87, 38702 La Tronche cédex, France*

ARTICLE INFO

Article history:
Available online 2 May 2008

Keywords:
Butyrylcholinesterase
Biomarker
Organophosphate exposure
Sarin
Soman
Affinity chromatography
Mass spectrometry

ABSTRACT

Classical plasma butyrylcholinesterase (BChE) purification involves dialysis and multiple steps of chromatography. We describe a procainamide affinity gel purification scheme that takes 15–30 min to purify BChE from 1 ml plasma. The method uses a microfuge spin column to build a 0.2 ml procainamide affinity column. The eluted BChE contains 3–4 μg of 500-fold purified BChE, free from 99% of contaminating plasma proteins. The BChE was further purified by gel electrophoresis. Tryptic peptides from the BChE containing gel electrophoresis band were prepared by in-gel digestion, separated by reverse phase liquid chromatography and identified by mass spectrometry. The 29 residue active site tryptic peptide labeled with the nerve agents soman or sarin was identified.

1. Introduction

Butyrylcholinesterase (BChE) is a scavenger protein that protects the cholinergic system against anticholinesterase poisons [1,2]. Most of the U.S. population has been exposed to organophosphorus (OP) pesticides in their homes, workplaces, outdoors, or through trace contaminants in food [3]. The high reactivity of BChE with OP makes BChE an ideal biomarker of OP exposure. OP inhibits BChE by covalently binding to its active site serine. Proteolysis of OP-inhibited BChE generates an OP-peptide conjugate whose molecular mass distinguishes nerve agents from OP pesticides. Mass spectrometry analysis of the plasma BChE peptide can be used to determine whether a person was exposed to OP and what kind of OP he or she was exposed to [4–6].

The 12 most abundant proteins in plasma make up 96% (by weight) of the total plasma proteins. Their concentrations range from 50 mg/ml (albumin) to 1 mg/ml (apolipoprotein A) [7]. Human BChE concentration in blood is 4.2×10^{-3} mg/ml. Even though mass spectrometry technologies have advanced to high sensitivity, purification steps are still necessary to identify a low abundant protein like BChE in a complex plasma sample [8].

Here we describe a fast and simple affinity purification method for plasma BChE that produces BChE sufficiently pure that it can be identified by mass spectrometry. The purification procedure consumes as little as 1 ml of plasma. A similar procedure has been described by Fidder et al. [5]. However, our purification is substantially simpler and has the potential to be developed into a high throughput method. The procedure was applied to samples of human plasma treated with the nerve agents soman and sarin. Peptides from BChE, isolated from 1 ml of plasma, were identified including the OP-labeled active-site peptide. The identity of the OP-labeled active site serine peptide was confirmed by MS/MS spectrum.

* Corresponding author. Tel.: +1 5106432394.
E-mail addresses: heli@berkeley.edu (H. Li), pym.masson@free.fr (P. Masson).

0009-2797/$ – see front matter © 2008 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.cbi.2008.04.027

2. Experimental methods

2.1. OP treatment of human plasma

Human plasma was treated with 200 μM soman or sarin, reducing BChE activity to zero. Soman and sarin were provided by CEB (Vert-le-Petit, France). Only trace amounts of intact soman and sarin remained in the plasma after 24 h at room temperature. Samples were stored at −80 °C. Plasma was cleared of solids and fat by centrifugation.

2.2. Procainamide-Sepharose micro column purification of plasma BChE

Procainamide-Sepharose gel, custom made by Dr. Yacov Ashani [9], bound 34 μmol of procainamide per milliliter gel. 0.2 ml (0.4 ml of 1:1 slurry in 50% ethanol) of procainamide gel was packed into a 1.5 ml microfuge spin column (Princeton Separations, Adelphia, NJ). The column was equilibrated with 2 ml of 20 mM potassium phosphate pH 7.0 buffer. 1 ml of cleared, OP-treated plasma was allowed to flow through the column by gravity flow at a flow rate of 1 ml/10 min. The column was washed 4 times with 1 ml of 0.2 M NaCl in 20 mM potassium phosphate pH 7.0 buffer. Each wash time was reduced to less than 1 min by briefly centrifuging the column. BChE was eluted with 0.5 ml of 1 M sodium chloride in 20 mM potassium phosphate pH 7.0 buffer or alternatively with 0.5 ml of 0.2 M procainamide.

2.3. Nondenaturing gradient gel electrophoresis

A four-to-thirty percent, polyacrylamide, nondenaturing, gradient gel, 0.75 mm thick, was prepared in a Hoefer SE6000 gel apparatus (Hoefer Scientific Instruments, San Francisco, CA; presently part of GE Healthcare). Electrophoresis was at 250 V constant voltage for 16 h at 4 °C. 10 μl samples were mixed with 50% glycerol in 0.1 M Tris/Cl, pH 7.5 to a final glycerol concentration of 10%. The gel was first stained for BChE activity using the Karnovsky & Roots method [10], and then stained with Coomassie blue R-250 (Fisher Scientific).

The BChE intended for mass spectrometry was reduced from 0.5 to 0.06 ml and desalted in an Amicon YM10 centrifugal filter with a molecular weight cutoff of 10 kDa (Millipore, Billerica, MA) before it was loaded on the nondenaturing gel. A nondenaturing gel rather than an SDS gel was used because only the nondenaturing gel separates BChE from albumin.

2.4. Protein in-gel digestion and peptide extraction

The protein band corresponding to the position of BChE was cut out and digested with trypsin [11]. Peptides were extracted from the gel and dissolved in 5% acetonitrile, 0.1% formic acid for mass spectrometry analysis.

2.5. ESI LC–MS/MS analysis of tryptic peptides

A FAMOS autosampler in conjunction with a SWITCHOS and ULTIMATE capillary liquid chromatography system (LC Packings Dionex, Sunnyvale, CA) was used to deliver peptides to a QTrap hybrid quadrupole, linear ion trap mass spectrometer model 2000 (Applied Biosystems, Foster City, CA). Peptides were eluted from a Vydac C18 nanocolumn (Grace Vydac, Southborough, MA) at a flow rate of 300 nl/min, using an acetonitrile gradient containing 0.1% formic acid. The acetonitrile increased from 5 to 60% in 60 min. Detailed instrument settings and data collection protocols are described by Schopfer et al. [12].

MASCOT (Matrix Science, Boston, MA) was used for database searching to identify proteins [13]. Mascot search parameters: IPI human database, enzyme is trypsin; allow for one missed cleavage; fixed modification consists of carbamidomethylated cysteine; variable modifications consist of oxidized methionine, dehydrated serine, and methylphosphonylated serine; mass values are monoisotopic; peptide mass tolerance ±2.0 amu; fragment mass tolerance ±1.0 amu. Similar results were obtained if the peptide mass tolerance was ±1.2 amu and fragment mass tolerance was ±0.6 amu. The Analyst filter was set to allow charge states +1 to +5, the "Discard ions with charge of 5+ or higher" was deactivated; "Determine charge state from Survey scan" was deactivated "Remove peaks with intensity—% of highest" was set to 0.

3. Results and discussion

3.1. A single-step procainamide affinity purification recovers 70% of the starting BChE

1 ml of control plasma was applied onto a 0.2 ml procainamide-Sepharose column packed into a microfuge spin column. As shown in Table 1, 94% of the BChE was retained on the column and 70% of the starting BChE was recovered by elution with 1 M NaCl. The time elapsed from loading the sample to elution was 15–30 min.

Table 1
Procainamide affinity purification of BChE from 1 ml plasma

Fraction	Composition	Volume (ml)	BChE activity (μ/ml)
Loading	Plasma	1	2.95
Flow-through	Plasma eluted during loading	1	0.17
Wash-off	0.2 M NaCl	1	0.17
	0.2 M NaCl	1	0.06
	0.2 M NaCl	1	0.04
	0.2 M NaCl	1	0.03
Elute	1 M NaCl	0.5	4.15