Chemico-Biological Interactions 203 (2013) 85–90

Contents lists available at SciVerse ScienceDirect

Chemico-Biological Interactions

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

Proteomic analysis of adducted butyrylcholinesterase for biomonitoring organophosphorus exposures

Judit Marsillach [a,b], Edward J. Hsieh [b], Rebecca J. Richter [a,b], Michael J. MacCoss [b], Clement E. Furlong [a,b,*]

[a] Dept. of Medicine (Division of Medical Genetics), University of Washington, 98195 Seattle, WA, USA
[b] Dept. of Genome Sciences, University of Washington, 98195 Seattle, WA, USA

ARTICLE INFO

Article history:
Available online 2 November 2012

Keywords:
Organophosphates
Mass spectrometry
Protein adducts
Butyrylcholinesterase

ABSTRACT

Organophosphorus (OP) compounds include a broad group of toxic chemicals such as insecticides, chemical warfare agents and antiwear agents. The liver cytochromes P450 bioactivate many OPs to potent inhibitors of serine hydrolases. Cholinesterases were the first OP targets discovered and are the most studied. They are used to monitor human exposures to OP compounds. However, the assay that is currently used has limitations. The mechanism of action of OP compounds is the inhibition of serine hydrolases by covalently modifying their active-site serine. After structural rearrangement, the complex OP inhibitor-enzyme is irreversible and will remain in circulation until the modified enzyme is degraded. Mass spectrometry is a sensitive technology for analyzing protein modifications, such as OP-adducted enzymes. These analyses also provide some information about the nature of the OP adduct. Our aim is to develop high-throughput protocols for monitoring OP exposures using mass spectrometry.

© 2012 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Organophosphorus (OP) compounds were first described at the beginning of the 19th century by Jean Pierre Boudet, when he generated traces of "phosphoric ether" as a result of combining alcohol and phosphoric acid [1]. However, it was not until 1848 that Franz Anton Voegeli created the first OP compound, triethyl phosphate. Just 5 years later, Philippe de Clermont described the synthesis of the first OP compound with anticholinesterase properties, the tetraethyl pyrophosphate (TEPP) [1]. In the 1930s, OPs were reported to be toxicants for mammals and insects, a property that triggered military interest and influenced the evolution of OPs into the highly toxic nerve agents sarin, soman and tabun, synthesized by Gerhard Schrader, considered the "father of modern OP compounds" [2]. After World War II, OP compounds saw use as a major class of insecticides (e.g., chlorpyrifos, malathion and parathion), as originally intended by Schrader. In addition to their utility as agriculture chemicals and warfare agents, OP compounds have also been used in industry as plasticizers, flame retardants, fuel additives, and lubricants, or even in medicine as therapeutic agents [3].

OPs are one of the most common causes of poisoning worldwide, with 3 million cases of pesticide poisonings per year, resulting in 220,000 deaths [4]. Their broad use results in many cases in environmental or occupational human exposure, causing a variety of adverse health effects [5]. As a consequence, research on biomarkers for biomonitoring human exposures to OPs has become an area of extensive investigation.

2. Mechanism of action of OP compounds

OPs comprise a large and diverse family of compounds, many of which can be hydrolyzed in the presence of water or by specific enzymes, resulting in detoxification. OP insecticides are mainly derivatives of phosphoric or thiophosphoric acid, containing usually two alkoxy substituent groups and a third substituent known as the "leaving group", as it is displaced when the OP interacts with serine hydrolases [6].

The mechanism of action of OP compounds involves the progressive inhibition of serine hydrolases [7], the main target being acetylcholinesterase (AChE). Their inhibitory action on AChE at nerve synapses seems to be responsible for their toxicity toward both invertebrates and mammals [8]. AChE (EC 3.1.1.7, accession #P22303) is a tetrameric serine esterase present in many tissues, particularly in central and peripheral nervous tissue where it terminates nerve impulse transmission by hydrolyzing the neurotransmitter acetylcholine at nerve synapses. AChE is also found on red blood cells (RBCs), where its function is unclear. In vertebrates, butyrylcholinesterase (BChE) can also hydrolyze acetylcholine.

* Corresponding author. Address: Dept. of Medicine (Division of Medical Genetics), University of Washington, 1959 NE Pacific St., HSB Room I-204A, Box 357720, 98195 Seattle, WA, USA. Tel.: +1 206 543 1193; fax: +1 206 685 4696.
E-mail addresses: jmarsi@uw.edu (J. Marsillach), edhsieh@uw.edu (E.J. Hsieh), rrichter@uw.edu (R.J. Richter), maccoss@uw.edu (M.J. MacCoss), clem@u.washington.edu, clem@uw.edu (C.E. Furlong).

0009-2797/$ - see front matter © 2012 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.cbi.2012.10.019

BChE (EC 3.1.1.8, accession #P06276) is also a tetrameric serine esterase present almost ubiquitously and in plasma. BChE is also inhibited by OP compounds, although this inhibition has no cholinergic symptoms and no known biological effect [8,9]. The physiological function of BChE is still not clear, although some studies suggest that it could act as an AChE backup [10–12]. This certainly appears to be the case in the AChE knockout mouse generated by Lockridge and colleagues [10]. Furthermore, BChE, as a stoichiometric scavenger, may protect from synthetic and naturally occurring poisons by preventing AChE inhibition by these toxins [13].

3. Biomonitoring OP exposures

Biomonitoring refers to the assessment of human exposure to chemicals and health risk by measuring the chemicals or their metabolites in body fluids, such as blood or urine [14]. Early detection of exposures can enable interventions before severe symptoms occur and follow-up of the recovery of the intoxicated subject.

The term biomarker refers to biological substances that are indicators of exposure or disease [15], and that can be measured by laboratory techniques [16]. There is an increasing interest in identifying and characterizing new biomarkers for a better health assessment.

Blood cholinesterases have long served as biomarkers of OP exposure, since they were the first OP targets discovered. It has been shown that inhibition of RBC AChE can be used as a valuable surrogate for inhibition of neuronal AChE [17,18], although plasma BChE is more sensitive to some OPs than RBC AChE. Furthermore, both cholinesterases are found in blood, an ideal matrix for biomarker analysis.

In 1961, Ellman et al. reported a colorimetric enzymatic assay that is currently the standard method for determining occupational or accidental OP exposures [19]. The Ellman assay uses acetylthiocholine or butyrylthiocholine for determining AChE or BChE activity, respectively. Other methods for monitoring cholinesterases have been reported [20], but the Ellman assay has remained the method of choice due to its advantages, being an inexpensive, simple and rapid assay. Despite the advantages, the Ellman assay has also some important drawbacks. It is not an accurate assay at low levels of inhibition (20% or less) [17,21–23]. OPs causing delayed or chronic toxicity without significant inhibition of cholinesterases cannot be monitored by this method [7,24]. In addition, it requires a pre-exposure determination of baseline activity level in order to overcome intra- and inter-individual activity variability, and for more accurate assessment of low level inhibition [25,26]. The pH of the buffers used in the assay and the concentration of substrate can also be critical in obtaining consistent results [20]. Furthermore, the temperature of sample storage is also an important issue. Spontaneous reactivation of the inhibitor-enzyme complex is observed at ambient temperature with some OP inhibitors [20]. OP inhibitions are not irreversible until a structural reorganization, "aging", of the inhibitor-enzyme complex happens (see Section 4). Another limitation is the impossibility of identifying the OP compound responsible for the observed inhibition. Moreover, because the replacement of inactive enzyme by *de novo* synthesized protein can occur rapidly, this method is less amenable for retrospective analyses [22,23]. When comparing plasma BChE *vs.* RBC AChE, AChE determination can present further disadvantages, resulting from difficulties in reproducible pipetting and washing of the RBCs, or from a potential interference by hemoglobin [20,27]. In terms of enzyme availability, humans have ten times more BChE than AChE [28], plasma is an easier matrix to handle than RBCs and, as noted above, BChE may be more sensitive to inhibition by some OPs than AChE. Despite these disadvantages, RBC AChE has a longer half-life than plasma BChE (33 *vs.* 11 days, respectively) [29], enabling evaluation of exposures after longer periods of time.

4. OP binding to the active-site serine and proteomics

Inhibition of serine hydrolases by OPs is mainly due to the formation of a covalent bond between the phosphor-alkoxy moiety of the OP compound and the hydroxyl residue of the catalytic serine of the active site of these enzymes, with the resulting release of the OP "leaving group". The OP adduct can suffer further dealkylation through a process called "aging", probably assisted by residues near the active site of the enzyme. Although the initial covalent bond is usually reversible, the aging (loss of one alkoxy group) enhances the stability of the OP adduct bound to the enzyme, preventing the dissociation of the enzyme-inhibitor complex [12]. The OP-adducted protein will remain in circulation for a length of time dependent on the half-life of the enzyme, generally much longer than the resident time of the OP or its metabolites.

The fact that serine hydrolases are modified by OPs provides another means for identifying the specific OP exposures with the use of liquid chromatography and tandem mass spectrometry (LC–MS/MS) to detect and quantify modified (OP-adducted) proteins. LC–MS/MS is ideal for the identification of a wide variety of protein modifications. It is a highly sensitive and precise technique that can perform accurate mass readings, allowing effective identification of different adducts with similar or identical masses. LC–MS/MS can quantify adducted and non-adducted enzymes, making it suitable for quantitative retrospective analysis. It does not require a baseline activity measurement for the individual, eliminating the need of pre-exposure bleeds. Part of the original OP structure is released during the reaction of the OP with the active-site serine and another part during the aging process; therefore MS may provide ambiguous or limited identification of the specific OP of exposure. LC–MS/MS also has some limitations or disadvantages, such as being time consuming, costly, limited by reactivation, and requiring well-trained personnel [16,23].

Application of MS to the study of OP exposures was first reported in 2001, using *in vitro* inhibited equine serum BChE [30]. The first "*in vivo*" application came just one year later [31], and since has become a field of intense research [32]. MS has also proven to be useful for the identification of new biomarkers of OP exposure [33]. Both LC and MALDI-TOF MS have been reported to be useful in the identification of OP exposures from either insecticides or nerve agents [21,33–39]. Most of these studies have focused on BChE, since it is an abundant plasma protein that is easy to obtain and has been extensively studied as a biomarker of OP exposure. The methods described in these studies involve either pure BChE or partial purification of OP-adducted BChE, followed by proteolytic digestion (e.g., pepsin, trypsin or chymotrypsin) and LC–MS/MS or MALDI-TOF analysis. Even though MS provides high sensitivity analyses, plasma is still a very complex matrix and purification of the biomarker protein (or modified peptide) facilitates analysis. One reported partial purification of BChE was achieved by affinity chromatography, which is time consuming, requires specific resins and a large volume of sample, and thus limits the standardization of the assay for high-throughput. Other studies, including those from our laboratory, that also monitor OP exposures by LC–MS/MS, have described the use of immunomagnetic beads coupled to a specific anti-BChE antibody (Fig. 1), a procedure that should overcome the limitations of affinity chromatography for biomarker purification [15,40–42]. Immunomagnetic separation (IMS) protocols are faster, require smaller quantities of starting material and can be easily adapted for automated high-throughput analysis. The affinity column approach,

Download English Version:

https://daneshyari.com/en/article/5848287

Download Persian Version:

https://daneshyari.com/article/5848287

Daneshyari.com