Biochemical and Biophysical Research Communications 403 (2010) 97–102

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Efficient hydrolysis of the chemical warfare nerve agent tabun by recombinant and purified human and rabbit serum paraoxonase 1

Manojkumar Valiyaveettil *, Yonas Alamneh, Lionel Biggemann, Iswarduth Soojhawon, Bhupendra P. Doctor, Madhusoodana P. Nambiar *

Closed Head Injury Branch, Center for Military Psychiatry and Neuroscience, Walter Reed Army Institute of Research, Silver Spring, MD 20910, USA

ARTICLE INFO

Article history:
Received 12 October 2010
Available online 30 October 2010

Keywords:
Catalytic bioscavenger
Paraoxonase 1
Chemical warfare nerve agents
Prophylaxis
Medical chemical defense
Tabun

ABSTRACT

Paraoxonase 1 (PON1) has been described as an efficient catalytic bioscavenger due to its ability to hydrolyze organophosphates (OPs) and chemical warfare nerve agents (CWNAs). It is the future most promising candidate as prophylactic medical countermeasure against highly toxic OPs and CWNAs. Most of the studies conducted so far have been focused on the hydrolyzing potential of PON1 against nerve agents, sarin, soman, and VX. Here, we investigated the hydrolysis of tabun by PON1 with the objective of comparing the hydrolysis potential of human and rabbit serum purified and recombinant human PON1. The hydrolysis potential of PON1 against tabun, sarin, and soman was evaluated by using an acetylcholinesterase (AChE) back-titration Ellman method. Efficient hydrolysis of tabun (100 nM) was observed with ~25–40 mU of PON1, while higher concentration (80–250 mU) of the enzyme was required for the complete hydrolysis of sarin (11 nM) and soman (3 nM). Our data indicate that tabun hydrolysis with PON1 was ~30–60 times and ~200–260 times more efficient than that with sarin and soman, respectively. Moreover, the catalytic activity of PON1 varies from source to source, which also reflects their efficiency of hydrolyzing different types of nerve agents. Thus, efficient hydrolysis of tabun by PON1 suggests its promising potential as a prophylactic treatment against tabun exposure.

Published by Elsevier Inc.

1. Introduction

Chemical warfare nerve agents (CWNAs) are of two main classes, G- and V-type [1]. The G-type nerve agents include tabun (GA), sarin (GB), soman (GD), and cyclosarin (GF). These are highly toxic due to their high volatility at room temperature and represent a great inhalation and dermal threat upon exposure. Nerve agents and related compounds irreversibly inhibit acetylcholinesterase (AChE), thereby causing a rapid, life-threatening accumulation of acetylcholine in the cholinergic nervous system [2]. While numerous oximes, including the currently fielded nerve agent antidote, 2-pralidoxime, have been shown to reverse AChE inhibition by some OPs, there is currently no universally effective antidote [3,4]. Reactivation of tabun inhibited AChE is particularly challenging due to the existence of a lone pair of electron on the dimethylamide group of tabun and the structural changes of the gorge of AChE make nucleophilic attack almost impossible [5,6]. An alternative approach for treatment against CWNA poisoning has focused on identification of proteins that can remain stable in circulation for long periods of time while acting as biological scavengers for OPs/CWNAs. For example, prophylactic countermeasures against CWNA exposure using butyrylcholinesterase (BChE) as a bioscavenger to neutralize CWNAs before they reach the target organs have been widely accepted [7–9]. Usually, a stoichiometric bioscavenger, such as BChE, sequesters the OP/CWNAs in a 1:1 ratio, itself representing a limitation. However, catalytic bioscavengers can hydrolyze several OP/CWNA molecules and have broad substrate specificity.

One of the recently discovered catalytic bioscavengers is paraoxonase 1 (PON1), which has the potential to hydrolyze nerve agents [10–12]. It is a 45 kDa arylesterase, synthesized mainly in the liver and secreted in the bloodstream to associate with high density lipoprotein molecules [11,13,14]. PON1 has been described as an efficient catalytic bioscavenger due to its ability to hydrolyze

Abbreviations: PON1, paraoxonase 1; HPON1, human PON1; RPON1, rabbit PON1; rePON1, recombinant PON1; OP, organophosphate; CWNA, chemical warfare nerve agent; DFP, diisopropylfluorophosphate; CPO, chloropyrifos oxon; AChE, acetylcholinesterase; BChE, butyrylcholinesterase; SDS–PAGE, sodium dodecylsulfate polyacrylamide gel electrophoresis; PVDF, polyvinylidenedifluoride; PBST, phosphate buffered saline/0.1% Tween-20; *p*-NPA, *p*-nitrophenyl acetate; DTNB, dithionitrobenzoic acid; 2-HQ, 2-hydroxyquinoline.

* Corresponding authors. Address: 503 Robert Grant Avenue, Walter Reed Army Institute of Research, Silver Spring, MD 20910, USA. Fax: +1 301 319 9404 (M.P. Nambiar).

E-mail addresses: m.valiyaveettil@amedd.army.mil (M. Valiyaveettil), madhusoodana. nambiar@amedd.army.mil (M.P. Nambiar).

0006-291X/$ - see front matter Published by Elsevier Inc.
doi:10.1016/j.bbrc.2010.10.125

OPs and CWNAs [10–12,15–17]. Significant hydrolysis of diisopropylfluorophosphate (DFP), paraoxon, diazoxon, and chloropyrifos oxon (CPO) was reported with recombinant as well as human and rabbit serum PON1 [10,12]. These results were further confirmed *in vivo* and in PON1 knock-out mice, which showed dramatic increase in the sensitivity to CPO toxicity [17]. PON1 has been also reported to hydrolyze sarin, soman, and VX [16,18]. The additional cardioprotective nature and the broad substrate specificity of PON1 make it a candidate of choice for the development of a therapeutic catalytic bioscavenger [13,14].

Limited information is available in the literature about the nature of any molecules which can hydrolyze tabun. The presence of an enzyme in squid nerve has been reported to hydrolyze tabun, DFP, sarin, and soman, with possible implications in disposal and detoxification of nerve gases in the ocean [19]. It has also been reported that soluble fraction of rat and human liver homogenates contains an active enzyme which could hydrolyze several OPs including tabun [20–22]. Since PON1 is mainly synthesized in the liver and secreted in plasma, it was tempting to determine the hydrolysis of different nerve agents by purified PON1. Here, we studied the hydrolysis of tabun, sarin, and soman by purified human and rabbit serum and recombinant human PON1, using an AChE back-titration Ellman method. We also evaluated its catalytic nature to eventually develop as a catalytic bioscavenger for protection against CWNAs.

2. Materials and methods

2.1. Materials

Acetylcholinesterase, acetylthiocholine, dithionitrobenzoic acid, *p*-nitrophenylacetate, Cibacron Blue 3GA agarose, DEAE-Sepharose, Sepharose CL-6B, Concanavalin A-Sepharose, 2-hydroxyquinoline, and PON1 polyclonal antibody were purchased from Sigma (St. Louis, MO). Tris–glycine gradient gels (4–20%), protein molecular weight markers, SDS–PAGE running and transfer buffer were purchased from Invitrogen (Carlsbad, CA). BCA assay kit for protein estimation was purchased from Pierce (Rockford, IL). Centricon 30 microconcentrator and immobilon PVDF membrane (polyvinylidene difluoride) were purchased from Millipore (Billerica, MA). ECL reagent for the development of Western blot was purchased from GE Healthcare (Piscataway, NJ). CWNA experiments were carried out at USAMRICD, Aberdeen Proving Ground, MD.

2.2. Purification of human and rabbit serum and recombinant PON1

PON1 was purified from human and rabbit serum as described earlier [23,24]. Briefly, human or rabbit serum (Innovative Research Inc., Novi, MI) was mixed with Cibacron Blue 3GA-agarose, and the bound PON1 was eluted with 0.1% deoxycholate. The fractions were then loaded on DEAE-Sepharose ion-exchange columns followed by elution with NaCl gradient. Further purification of PON1 was done by Concanavalin A-Sepharose affinity chromatography. The enzyme was eluted with α-methyl mannopyranoside gradient. The final purification of the enzyme was performed by using a Sepharose CL-6B gel filtration column. Human recombinant PON1 was expressed in *Trichoplusia ni* larvae (cabbage looper-worm) and purified by His-tag affinity chromatography in collaboration with Chesapeake PERL (Savage, MD).

2.3. SDS–PAGE

The purity of PON1 from human and rabbit serum and recombinant PON1 was analyzed by SDS–PAGE. Briefly, ~2 μg of the protein samples was incubated with equal volume of 2× Laemmli sample buffer containing reducing agent at 100 °C for 5 min [25]. The samples were resolved on 4–20% gradient Tris–glycine gels at a constant voltage of 100 V. The separated proteins were stained using Coomassie Blue stain and were photographed by using AlphaImager (Cell Biosciences, Santa Clara, CA).

2.4. Immunoblotting

PON1 preparations (~0.1 μg) resolved by SDS–PAGE were transferred onto PVDF membrane. Membranes were blocked with 2% nonfat dry milk in phosphate buffered saline/0.1% Tween-20 (PBST) overnight at 4 °C and incubated with PON1 polyclonal primary antibody (1:1000) for 2 h at room temperature. Membrane was washed three times with PBST followed by incubation with peroxidase-labeled goat anti-rabbit secondary antibody (1:5000) for 1 h at room temperature. Immunoreactivity was detected with an enhanced chemiluminescence method (ECL detection reagent; GE Healthcare) and visualized using FluorChem HD2 system (Cell Biosciences, Santa Clara, CA).

2.5. PON1 activity assay

Aliquots of PON1 samples were incubated with 1 mM of *p*-nitrophenylacetate (*p*-NPA) as substrate in 20 mM Tris–HCl buffer (pH 7.4), containing 1 mM $CaCl_2$. The release of *p*-nitrophenol was measured at 405 nm using Spectramax M5 Spectrophotometer (Molecular Devices, Sunnyvale, CA). One unit of PON1 is defined as the amount of enzyme that will liberate 1 μmol of *p*-nitrophenol per minute at 37 °C.

2.6. Ellman assay

AChE activity was measured spectrophotometrically (Spectramax M5 Spectrophotometer) with a modified Ellman method at 412 nm [26]. The assay mixture consisted of a final volume of 1.0 mM ATCh as substrate and 1.0 mM DTNB as chromogen in 50 mM sodium phosphate buffer (pH 7.4).

2.7. AChE back-titration assay

To evaluate the hydrolytic activity of PON1 against nerve agents, a back-titration of AChE Ellman assay was employed [26]. Briefly, various concentrations of nerve agents (tabun, sarin, and soman) were incubated with 5 ng of AChE for 15 min at room temperature. Residual AChE activity was measured by Ellman assay at 412 nM with a master mix solution containing 1.0 mM ATCh and 1.0 mM DTNB in sodium phosphate buffer (pH 7.4). After determining the concentration of nerve agents required for ~95% inhibition of AChE, various concentrations of human and rabbit serum purified and human recombinant PON1 were incubated with the same concentration of nerve agents (the concentration that inhibit 95% AChE) for 30 min at room temperature. Residual nerve agent in the reaction mixture was determined by incubating with 5 ng of AChE for another 15 min followed by the Ellman assay. Moreover, to determine the catalytic/hydrolytic potential of PON1, a 3-fold concentrated nerve agent than the one used above was added to the reaction mixture and the residual AChE activity was determined. The retention of activity of PON1 after incubation with the nerve agent was determined using *p*-NPA substrate. All the assays were performed 3–5 times in triplicates.

2.8. PON1 inhibition assay

The specificity of PON1 hydrolysis of nerve agents was confirmed by inhibition of the enzymes with a specific PON1 inhibitor, 2-hydroxyquinoline (2-HQ, 10 μM–5 mM), for 15 min at room

Download English Version:

https://daneshyari.com/en/article/10764047

Download Persian Version:

https://daneshyari.com/article/10764047

Daneshyari.com