Degradation of nerve agents by an organophosphate-degrading agent (OpdA)

R.M. Dawson [a,*], S. Pantelidis [a], H.R. Rose [a],
S.E. Kotsonis [b]

[a] *DSTO Melbourne, PO Box 4331, Melbourne VIC 3001, Australia*
[b] *Orica Australia Pty Ltd., 1 Nicholson Street, Melbourne VIC 3000, Australia*

Abstract

Enzyme-catalysed degradation of the nerve agents tabun, sarin, ethyl sarin and soman by three variants of an organophosphate-degrading enzyme was studied at low concentrations of nerve agent. The concentration of nerve agent at a given time was determined by its ability to inhibit the enzyme acetylcholinesterase. Experiments were conducted in 96-well microtitre plates. Values of the ratio of k_{cat} (turnover number) to K_m (Michaelis–Menten constant) were calculated. For tabun, this value (for the most effective OpdA variant) exceeded any value published to date for other enzymes. The value was within an order of magnitude for the highest value reported for sarin, but there appears to be no published value for ethyl sarin for comparison. The OpdA enzymes were relatively inefficient in degrading soman.
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1. Introduction

Enzymes have considerable potential for decontamination and detoxification of organophosphorus anticholinesterase compounds, such as pesticides and the nerve agent class of chemical warfare agents [1,2]. They may also be used for destruction of nerve agent stockpiles, in "active" fabrics and filter elements for personal protection, and in sensors for chemical warfare agents [3]. The decontamination solutions currently fielded for use against nerve agents (DS2 and bleach) are corrosive in nature and result in hazardous waste. Enzyme-mediated decontamination is non-toxic, noncorrosive and environmentally compatible [1]. In Australia, an enzyme product known as Landguard[TM] OP-A is marketed to clean up organophosphate insecticide-contaminated water, in particular sheep dips

([4]; http://www.orica-landguard.com). Many of the enzymes known to hydrolyse organophosphorus esters are known as organophosphorus hydrolase (OPH; EC 3.1.8.1; alternative name phosphotriesterase (PTE)) or organophosphorus acid anhydrolase (OPAA; EC 3.1.8.2). Landguard[TM] OP-A does not fit neatly into either of these categories, and is known as an organophosphate-degrading agent (OpdA). The first OpdA was described in 2002 [5], and its genetics characterised in 2003 [6]. OpdA enzymes, like OPH/PTE, hydrolyse organophosphates by cleavage of a P–O (or P–S) bond with formation of hydrolysis products that are of low toxicity [7]. The mechanism has been confirmed in numerous studies, e.g. references [8–11]. Recently, mutants of OpdA have been prepared and have shown enhanced activity towards organophosphorus insecticides; the activity is superior to that of OPH in some cases [8,9]. In the present paper, the ability of Landguard[TM] OP-A to inactivate the G-type nerve agents tabun (commonly abbreviated as GA), sarin (GB), soman (GD) and ethyl sarin (GE) was investigated. In addition to Landguard[TM] OP-A itself, two mutants of the enzyme that had been designed to enhance its activity against organophosphorus pesticides were also evaluated.

* Corresponding author. Tel.: +61 3 9626 8477; fax: +61 3 9626 8410.
E-mail addresses: ray.dawson@dsto.defence.gov.au
(R.M. Dawson), sue.pantelidis@dsto.defence.gov.au (S. Pantelidis),
harry.rose@dsto.defence.gov.au (H.R. Rose), steven.kotsonis@orica.com
(S.E. Kotsonis).

2. Materials and methods

2.1. Materials

The nerve agents were synthesised in-house (DSTO) and were ≥95% pure. Stock solutions (0.1 mg/ml) were prepared in acetonitrile and were diluted into phosphate-buffered saline pH 7.4 (PBS) just before use. After use, they were decontaminated with 0.1 M NaOH. PBS was prepared by adding the contents of a commercial sachet to 1 l of Milli-Q distilled water. This product, and all other reagents, were obtained from Sigma–Aldrich, Australia. Nunc-Immuno 96-microwell flat bottom plates were obtained from In Vitro Technologies, Melbourne, Australia. Landguard[TM] OP-A (designated OpdA mark I) and its two mutants (OpdA mark II and mark III) were products of Orica, Australia. All three variants have a molecular weight of approximately 38.6 kDa, and were supplied as a powder. The actual enzyme represented 6% of the powder in the cases of mark I and mark II and 2.5% for mark III. Stock solutions of 50 g/l were stored at 4 °C and diluted in water prior to use.

2.2. Methods

Reactions and assays were performed in 96-well plates. Aliquots (20 μl) of nerve agent (see Table 1 for final concentration) were placed in wells, and 50 μl Landguard[TM] OP-A in water (designated OpdA from here on) was added per well at time zero and at eight subsequent 1-min intervals. The plate was shaken on a plate-shaker between additions of OpdA. At 10 min, eel acetylcholinesterase (0.6 units per ml; 50 μl) was added to each well and incubated with shaking for 10 min, after which the acetylcholinesterase substrate (acetylthiocholine; final concentration 2.4 mM, with 0.3 mM 5,5′-dithio bis(2-nitrobenzoic acid)) was added. The rate of increase of absorbance at 412 nm was measured with a BioTek Synergy HT plate reader over 10 min [12]. Appropriate controls were run. The rate of increase of absorbance in the presence of acetylcholinesterase and absence of nerve agent was in the range 0.10–0.15 abs/min. A number of wells received nerve agent of different concentrations, but no OpdA, enabling a calibration plot to be obtained (see Section 2.3).

2.3. Analysis

Nerve agents inhibit acetylcholinesterase irreversibly (in the time frame of the experiment [13]). A plot of abs/min vs. log (nerve agent concentration) for the 10-min acetylcholinesterase–nerve agent incubation was found to be linear over most of the inhibition range (see Fig. 1 for an example). This enabled the concentration of non-degraded nerve agent to be determined after a given time of incubation with OpdA (1–9 min). The limit of detection of the nerve agents was found to be approximately 2 pmol for GA (tabun) and 40, 7.5 and 120 fmol for GB (sarin), GD (soman) and GE (ethylsarin), respectively. The initial concentration of nerve agent was chosen such that approximately 90% inhibition of acetylcholinesterase occurred over 10 min in the absence of OpdA. This concentra-
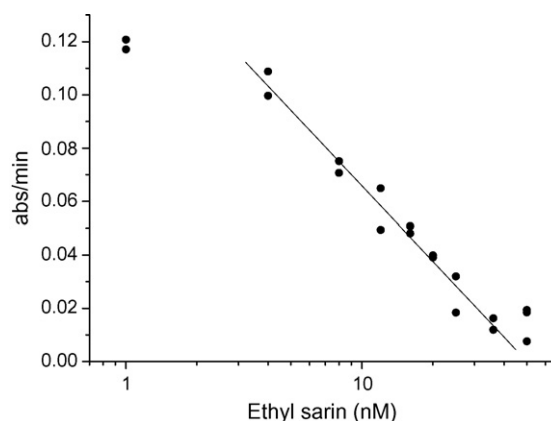

Fig. 1. Calibration plot: acetylcholinesterase activity (rate of increase of absorbance at 412 nm) vs. log (concentration of nerve agent (ethyl sarin)).

tion is shown in Table 1. Degradation of nerve agent by OpdA was measured for three concentrations of OpdA, these concentrations being selected such that significant degradation (>50%) occurred during the 10-min incubation in each case. It was possible to study each of the three concentrations of OpdA in duplicate on the same 96-well plate (using six of the eight rows), together with the calibration plot in duplicate (the remaining two rows). The first-order rate constant for degradation of nerve agent (see below) was found to be directly proportional to the concentration of OpdA.

Because substantial depletion of the OpdA substrate (nerve agent) occurs during the experiment, analysis of the Michaelis–Menten kinetics by initial rates is not applicable. The kinetics for a progress curve were therefore applied. The equation [14] is

$$V_{max}t = K_m \ln\left(\frac{S_0}{S}\right) + (S_0 - S) = K_m \ln\left(\frac{S_0}{S}\right) + P \qquad (1)$$

where S_0 and S are the concentration of substrate at time zero and time t, respectively, P is the concentration of product (degraded nerve agent) at time t, and K_m and V_{max} are the Michaelis–Menten kinetic constants.

Rearranging,
$$\frac{V_{max}}{K_m}t = \ln\left(\frac{S_0}{S}\right) + \frac{P}{K_m} \qquad (2)$$

If the assumption is made that $P \ll K_m$ (see Section 4), the equation reduces (after rearrangement) to

$$\ln S \approx \ln S_0 - \left(\frac{V_{max}}{K_m}\right)t \qquad (3)$$

A plot of $\ln S$ vs. t is therefore linear with a slope of V_{max}/K_m. Designating the slope as the first-order rate constant k_{obs}, and recalling that $V_{max} = k_{cat}[E]$, where k_{cat} is the turnover number and [E] is the concentration of enzyme (OpdA), enables the ratio of k_{cat} to K_m to be determined [3]:

$$\frac{k_{cat}}{K_m} = \frac{k_{obs}}{[E]} \qquad (4)$$

The ratio k_{cat}/K_m is a measure of the efficacy of OpdA, since the higher the turnover number (k_{cat}) and the higher the affinity of