Appl Microbiol Biotechnol
DOI 10.1007/s00253-016-7407-x

BIOTECHNOLOGICALLY RELEVANT ENZYMES AND PROTEINS

Extensive hydrolysis of phosphonates as unexpected behaviour of the known His_6-organophosphorus hydrolase

Ilya V. Lyagin[1,2] · **Mariia S. Andrianova**[3] · **Elena N. Efremenko**[1,2]

Received: 9 December 2015 / Revised: 10 February 2016 / Accepted: 19 February 2016

Abstract The catalytic activity of hexahistidine-tagged organophosphorus hydrolase (His_6-OPH) in hydrolytic reactions of methylphosphonic acid (MPA) and its monoesters and diesters being decomposition products of R-VX was demonstrated for the first time. The catalytic constants of enzyme in such reactions were determined. The mechanism of C–P bond cleavage in the MPA by His_6-OPH was proposed. Such reaction was estimated to be carried out with the soluble and nanocapsulated forms of His_6-OPH. His_6-OPH was demonstrated to be capable of degrading the key organophosphorus components of reaction masses (RMs) that are produced by the chemical detoxification of R-VX and RMs are multisubstrate mixtures for this enzyme. The kinetic model describing the behaviour of His_6-OPH in RMs was proposed and was shown to adequately fit experimental points during degradation of the real samples of RMs.

Keywords Organophosphorus compound · Phosphonate · Mechanism · Enzymatic degradation · Hexahistidine-tagged organophosphorus hydrolase

Electronic supplementary material The online version of this article (doi:10.1007/s00253-016-7407-x) contains supplementary material, which is available to authorized users.

✉ Elena N. Efremenko
elena_efremenko@list.ru

[1] Chemistry Department, Lomonosov Moscow State University, Lenin Hills, 1, building 3, Moscow 119991, Russia

[2] Institute of Biochemical Physics RAS, Kosygin St. 4, Moscow 119334, Russia

[3] SMC Technological Center MIET, Proezd No. 4806, 5, Moscow Zelenograd 124498, Russia

Introduction

Phosphonates are a class of organophosphorus compounds (OPCs) with a C–P bond that is quite resistant to chemical hydrolysis and thermal degradation (Freedman and Doak 1957). Phosphonates include biogenic (2-aminoethylphosphonic acid (Horiguchi and Kandatsu 1959), alafosfalin and fosfomycin (Hilderbrand 1983), methylphosphonic acid (MPA) (Metcalf et al. 2012), etc.) and anthropogenic compounds. The latter includes organophosphorus chemical warfare agents (OP CWAs) classified as nerve agents (VX, R-VX, sarin, soman, etc.) (Munro et al. 1999). Their detoxification yields less toxic MPA esters, which are resistant to decomposition even in soils, where they can be found after decades at depths over 1 m (Small 1984).

According to the Organisation for the Prohibition of Chemical Weapons (OPCW) (Organisation for the Prohibition of Chemical Weapons 2014), over 78 % of Category 1 CWAs had been destroyed by 2014, including 90 and 78 % of the stockpile in USA and Russia, accordingly. The remaining CWAs are scheduled for disposal by the end of 2023 and 2015 in USA and Russia, respectively. In Russia, the remaining CWAs mostly include OP nerve agents. R-VX, the most toxic of these, makes up a half of the stockpile. So, the lethal doses are 0.14, 0.05 and 0.008 mg/kg for soman, sarin and VX, respectively (Gupta 2009).

The method employed in Russia for the chemical destruction of R-VX stockpiles involves injecting 3–7 % (*v*/*v*) aqueous acid or base solution as hydrolysing agent into a piece of munitions containing up to 90–93 % (*v*/*v*) OP CWA without removing the CWA from munitions and allowing the reaction to proceed over 80 to 100 days (Utkin et al. 2009). The resulting reaction masses (RMs) containing a variety of phosphonates (0.1 wt.% R-VX, 2 wt.% O,O′-(diisobutyl)methylphosphonate (DIBEMPA), 54.4 wt.%

Published online: 02 March 2016