



## Research paper

# A novel expression cassette delivers efficient production of exclusively tetrameric human butyrylcholinesterase with improved pharmacokinetics for protection against organophosphate poisoning



Stanislav Terekhov<sup>a,1</sup>, Ivan Smirnov<sup>a,1</sup>, Tatiana Bobik<sup>a,b,1</sup>, Olga Shamborant<sup>a</sup>, Marina Zenkova<sup>c</sup>, Elena Chernolovskaya<sup>c</sup>, Danil Gladkikh<sup>c</sup>, Arkadii Murashev<sup>d</sup>, Igor Dyachenko<sup>d,e</sup>, Viktor Palikov<sup>d,e</sup>, Yulia Palikova<sup>d,e</sup>, Vera Knorre<sup>a</sup>, Alexey Belogurov Jr.<sup>a,b</sup>, Natalie Ponomarenko<sup>a</sup>, G. Michael Blackburn<sup>f</sup>, Patrick Masson<sup>g,h</sup>, Alexander Gabibov<sup>a,\*</sup>

<sup>a</sup> Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Miklukho Maklaya 16/10, Moscow, 117997, Russian Federation

<sup>b</sup> Kazan Federal University, Combinatorial Chemistry and Neurobiology Laboratory, 18 Kremlyovskaya Str, Kazan, 420008, Republic of Tatarstan, Russian Federation

<sup>c</sup> Institute of Chemical Biology and Fundamental Medicine, Siberian Branch of the Russian Academy of Sciences, 8 Lavrentiev Avenue, Novosibirsk, 630090, Russian Federation

<sup>d</sup> Branch of the Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, 6 Prospekt Nauki, Moscow Region, Pushchino, 142290, Russian Federation

<sup>e</sup> Pushchino State Natural-Science Institute, Pushchino, 142290, Russian Federation

<sup>f</sup> Krebs Institute, Department of Molecular Biology and Biotechnology, University of Sheffield, Western Bank, Sheffield, S10 2TN, United Kingdom

<sup>g</sup> Kazan Federal University, Neuropharmacology Laboratory, 18 Kremlyovskaya Str, Kazan, 420008, Republic of Tatarstan, Russian Federation

<sup>h</sup> Institute of Structural Biology, DYNAMOP, 71 Avenue des Martyrs CS 10090, 38044, Grenoble Cedex 9, France

## ARTICLE INFO

## Article history:

Received 31 May 2015

Accepted 30 July 2015

Available online 1 August 2015

## Keywords:

Organophosphorus poisoning  
Butyrylcholinesterase  
Matrix Attachment Region (MAR)  
Paraoxon  
Mammalian expression system

## ABSTRACT

Butyrylcholinesterase is a stoichiometric bioscavenger against poisoning by organophosphorus pesticides and nerve agents. The low level of expression and extremely rapid clearance of monomeric recombinant human butyrylcholinesterase (rhBChE) from bloodstream ( $t_{1/2} \approx 2$  min) limits its pharmaceutical application. Recently (Ilyushin et al., PNAS, 2013) we described a long-acting polysialylated recombinant butyrylcholinesterase (rhBChE-CAO), stable in the bloodstream, that protects mice against 4.2 LD<sub>50</sub> of VR. Here we report a set of modifications of the initial rhBChE expression vector to improve stability of the enzyme in the bloodstream and increase its production in CHO cells by introducing in the expression cassette: (i) the sequence of the natural human PRAD-peptide in frame with rhBChE gene via “self-processing” viral F2A peptide under control of an hEF/HTLV promoter, and (ii) previously predicted *in silico* MAR 1-68 and MAR X-29 sequences. This provides fully tetrameric rhBChE (4rhBChE) at 70 mg/l, that displays improved pharmacokinetics ( $t_{1/2} = 32 \pm 1.2$  h, MRT =  $43 \pm 2$  h). 3D Fluorescent visualization and distribution of <sup>125</sup>I-labeled enzyme reveals similar low level 4rhBChE and rhBChE-CAO accumulation in muscle, fat, and brain. Administered 4rhBChE was mainly catabolized in the liver and breakdown products were excreted in kidney. Injection of 1.2 LD<sub>50</sub> and 1.1 LD<sub>50</sub> of paraoxon to BALB/c and knockout BChE<sup>-/-</sup> mice pre-treated with 4rhBChE (50 mg/kg) resulted in 100% and 78% survival, respectively, without perturbation of long-term behavior. In contrast, 100% mortality of non-pre-treated mice was observed. The high expression level of 4rhBChE in CHO cells permits consideration of this new expression system for manufacturing BChE as a biopharmaceutical.

© 2015 Published by Elsevier B.V.

## 1. Introduction

Chemical and nuclear weapons gave many dramatic war episodes in the 20th century. The Geneva Protocol, signed in 1925,

\* Corresponding author.

E-mail address: [gabibov@ibch.ru](mailto:gabibov@ibch.ru) (A. Gabibov).

<sup>1</sup> Authors who equally contributed to this study.