Increasing the Catalytic Efficiency of Butyrylcholinesterase through Surface Loop Modifications

Authors: Gil M. Repa, Kirstin Poindexter, Krishna Bhattarai, Haobo Jiang, Jing Liu, Dr. Carey Pope*
Department of Veterinary Health Sciences-Physiological Sciences, Oklahoma State University

Abstract

Organophosphorus compounds (OPs) are highly neurotoxic anticholinesterase agents. Their widespread availability and potency make them attractive for use in chemical terrorism and warfare. Developing safe and efficient methods for counteracting the effects of OP intoxication remains a priority. When given prophylactically, the G117H mutant of butyrylcholinesterase (BChEG117H) is able to catalytically hydrolyze OPs before they reach target organs. However, its widespread use as a treatment for OP intoxication is limited by several factors, including its extremely low substrate-turnover rate. To address this, we hypothesized structural changes to BChEG117H in the form of surface loop modifications would increase the enzyme's catalytic efficiency. Data on butyrylthiocholine substrate hydrolysis is presented for BChEG117H and wild-type butyrylcholinesterase ($BChE_{wt}$).

Keywords: Organophosphorus compound, Nerve agent, Acetylcholinesterase, Butyrylcholinesterase, Bioscavenger

Introduction

Organophosphorus compounds are a continued threat due to their widespread availability and potency. Members of this class of chemicals include the widely used insecticides chlorpyrifos and malathion, as well as the nerve agent sarin, a potent toxicant previously used in both chemical warfare and chemical terrorism. OPs derive their toxicity from their ability to bind to the active site serine residue of acetylcholinesterase (AChE) (Mileson *et al.* 1998). Excessive inhibition of AChE results in an accumulation of the neurotransmitter acetylcholine at cholinergic synapses. Subsequently, an overstimulation of the cholinergic receptors on the post-synaptic cell occurs. This leads to a disruption in cholinergic neurotransmission, which may present as muscle paralysis and seizures, with death usually caused by respiratory failure. There is a need to protect individuals from the toxicological effects elicited by OP poisoning.

Typically, OP intoxication is treated with administration of atropine (a muscarinic receptor antagonist), and an oxime (to reactivate inhibited AChE) (Eddleston *et al.* 2002). This therapeutic strategy is effective but is limited by several factors, including the relatively short circulation times of the drugs compared with the covalent long-term inhibition of the enzyme. Also, the drugs' difficulty in passing the blood brain barrier renders them unable to reach inhibited AChE in the central nervous system; individuals are often left with long-term neurological deficits. Therefore, alternative treatment strategies are currently under investigation.

It has been previously demonstrated that administration of BChE as a bioscavenger, i.e. a circulating protein to deactivate OP molecules before they reach their target tissue, is effective in counteracting the debilitating consequences of OP intoxication with little side effects (Raveh *et al.* 1993). Widespread application of this approach is limited, as BChEwt stoichiometrically interacts with OPs, i.e. one molecule of enzyme inactivates one molecule of OP. Creating a catalytic enzyme for OP degradation would drastically improve its efficiency, and widen its application as a treatment for OP intoxication. The G117H mutant of BChE has the desired effect, yet still has a relatively low substrate-turnover rate (Lockridge *et al.* 1997). This, combined with its ability to hydrolyze a variety of OPs makes it an ideal starting enzyme for further development. Improving the efficiency of BChEG117H would make its administration a more effective treatment strategy for OP poisoning.

In a number of enzymes, peptide residues leading from the surface of the protein to the active-site region have been shown to influence catalytic efficiency (Agarwal 2005). It is proposed that these surface loops create a pathway for energy transfer and promote thermodynamic coupling of solvent to the active site residues. It is further hypothesized that the amount of energy transferred is proportional to the number of peptides making up the surface loop,

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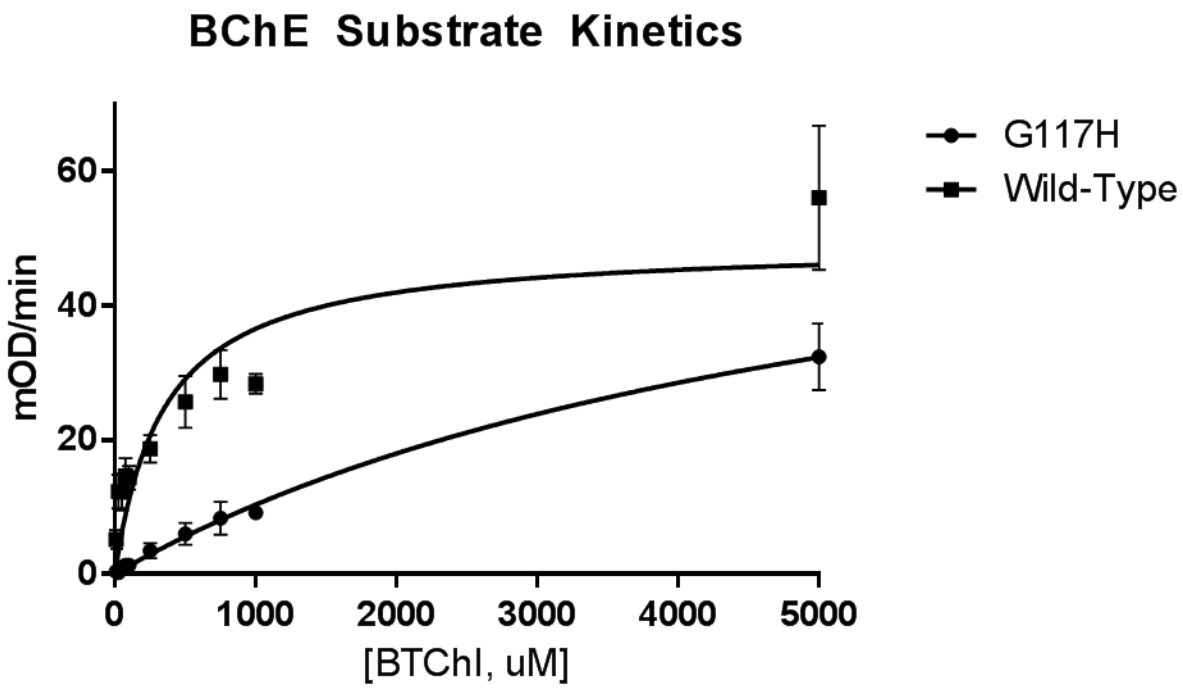

Figure 1 *- Catalysis of butyrylthiocholine by $BChE_{wt}$ and $BChE_{G117H}$ as influenced by substrate concentration [10 µM-5 mM].*

and may be related to the specific types of amino acids involved. Thus, increasing the number of surface loop peptides, or modifying their composition in recombinant enzymes could provide a mechanism for enhancing catalysis.

The aim of this study is to examine the effects of systematic modifications of surface loop residues on the catalytic efficiency of BChE. We will use computational studies to select for mutants with the greatest predicted catalytic efficiency against OP substrates that will then guide the expression of recombinant mutants. The ultimate goal is to develop a more effective catalytic bioscavenger for countering OP toxicity.

Methods

Wild-type and G117H mutant BChE were expressed in live cell cultures, and the resultant enzyme collected, purified, and stored at -80° C. BChE activity was evaluated photometrically using the Ellman (1961) method. To evaluate substrate kinetics, varying concentrations of the substrate butyrylthiocholine (10 µM - 5 mM) in Tris-EDTA is incubated with BChE. As butyrylthiocholine is hydrolyzed, the resultant thiocholine reacts with DTNB (final concentration: 100 µM) to produce a yellow ion. Monitoring the increase in absorbance at 37° C and 412 nm over 5 minutes allows calculation of enzyme kinetic constants Km, Vmax.

Progress to Date

Figure 1 shows catalysis of butyrylthiocholine by BChEwt and BChEG117H as influenced by substrate concentration [10 µM - 5 mM]. Data from multiple replicates were analyzed using an extra sum of squares F test (α=.05). Analysis of the data in Figure 1 shows that the maximal velocity of BChEG117H was not statistically different than that of $BChE_{wt}$ (p=.4324). However, the Km of BChEG117H was statistically different than that of $BChE_{wt}$ (p=.0001).

Discussion and Future Work

Due to the difference in Km, it is evident that the binding affinity of butyrylthiocholine to BChEG117H is reduced, yet the maximal reaction velocity is unchanged. In the presence of an OP substrate, however, it is expected that $BChE_{wt}$ will have no activity, while activity increases with BChEG117H mutants expressing more peptides in the surface loops. Future work will include evaluation of the kinetic parameters of BChEG117H against that of $BChE_{wt}$ in the presence of an OP substrate, evaluation of the kinetic parameters of G117H variants with increased surface loop size and number, and determination of the stability of G117H variants relative to BChEwt and BChEG117H.

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