Chemico-Biological Interactions xxx (2016) 1–10

Contents lists available at ScienceDirect

Chemico-Biological Interactions

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

Understanding the non-catalytic behavior of human butyrylcholinesterase silent variants: Comparison of wild-type enzyme, catalytically active Ala328Cys mutant, and silent Ala328Asp variant

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ARTICLE INFO

Article history:
Received 31 January 2016
Received in revised form
15 March 2016
Accepted 4 April 2016
Available online xxx

Keywords:
Human butyrylcholinesterase
Natural variant
Silent variant
Myorelaxant abnormal response
Succinylcholine
Mivacurium
Prolonged apnea
Molecular dynamics
Binding free energy
Umbrella Sampling

ABSTRACT

Conformational dynamics of wild-type human butyrylcholinesterase (BChE), two mutants of residue Ala328, the catalytically active Ala328Cys, and the catalytically inactive (silent) Ala328Asp, and their interactions with butyrylcholine were studied. The aim was to understand the molecular mechanisms by which point mutations may lead to silent BChE variant or alter catalytic activity. Importance of BChE natural variants is due to medical consequences, i.e. prolonged apnea, following administration of the myorelaxant esters, succinylcholine and mivacurium.

Comparison of molecular dynamics (MD) simulations for the three model systems showed that: 1) the active mutant Ala328Cys mutant has some changes in configuration of catalytic residues, which do not prevent binding of butyrylcholine to the active site; 2) in the naturally-occurring silent variant Ala328Asp, the Asp328 carboxylate may either form a salt bridge with Lys339 or a H-bond with His438. In the first case, the Ω-loop swings off the gorge, disrupting the π-cation binding site and the catalytic triad. In the second case, binding of cationic substrates in the catalytic center is also impaired. MD simulations carried out in 0.15 M NaCl, close to physiological ionic strength conditions, favored the second situation. It was seen that Asp328 forms a H-bond with the catalytic triad His438, which in turn disrupts the catalytic machinery. Therefore, we concluded that the Ala328Asp variant is not catalytically active because of that dramatic event. Computational results, consistent with *in vitro* biochemical data and clinical observations, validate our MD approach.

1. Introduction

Human butyrylcholinesterase (BChE, EC. 3.1.1.8) is a sialoglycoprotein whose 3D structure is similar to that of acetylcholinesterase (AChE, EC. 3.1.1.7) [1]. The BChE monomer is composed of 574 amino acids. BChE is present in numerous tissues and plasma where its concentration is about 50 nM. The tetrameric form is the major component of the human plasma BChE [2]. Unlike AChE that plays a key role in the cholinergic system in terminating the action of the neurotransmitter acetylcholine, BChE has no clear physiological functions. However, BChE hydrolyzes numerous carboxylesters and reacts with carbamyl- and phosphyl-esters (progressive inhibition). Thus, BChE is of pharmacological and toxicological importance. In particular the plasma enzyme, plays a role in catabolism of ester-containing drugs (e.g. succinylcholine, mivacurium, aspirin) and poisonous esters (e.g. cocaine, heroin), xenobiotics (e.g. carbamate pesticides, organophosphate pesticides, cresylsaligenin phosphate — the active metabolite of the flame retardant tri-*o*-cresylphosphate, and chemical warfare nerve

Abbreviations: AChE, acetylcholinesterase; BCh, butyrylcholine; *BCHE*, butyrylcholinesterase gene; BChE, butyrylcholinesterase enzyme; EDA, essential dynamics analysis; H-bond, hydrogen bond; MD, molecular dynamics; PAS, peripheral anionic site; PDB, Protein Data Bank; PMF, potential of mean force; QM, quantum mechanics; R_g, radius of gyration; SASA, solvent-accessible surface area; SMD, steered, molecular dynamics; US, umbrella sampling; wt, wild type.

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http://dx.doi.org/10.1016/j.cbi.2016.04.007
0009-2797/