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Amino acid residues at the N- and C-termini are essential for the folding of active human butyrylcholinesterase polypeptide

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ABSTRACT

Human serum butyrylcholinesterase (HuBChE) is currently the most suitable bioscavenger for the prophylaxis of highly toxic organophosphate (OP) nerve agents. A dose of 200 mg of HuBChE is envisioned as a prophylactic treatment that can protect humans from an exposure of up to 2 × LD_{50} of soman. The limited availability and administration of multiple doses of this stoichiometric bioscavenger make this pretreatment difficult. Thus, the goal of this study was to produce a smaller enzymatically active HuBChE polypeptide (HBP) that could bind to nerve agents with high affinity thereby reducing the dose of enzyme. Studies have indicated that the three-dimensional structure and the domains of HuBChE (acyl pocket, lip of the active center gorge, and the anionic substrate-binding domain) that are critical for the binding of substrate are also essential for the selectivity and binding of inhibitors including OPs. Therefore, we designed three HBPs by deleting some N- and C-terminal residues of HuBChE by maintaining the folds of the active site core that includes the three active site residues (S198, E325, and H438). HBP-4 that lacks 45 residues from C-terminus but known to have BChE activity was used as a control. The cDNAs for the HBPs containing signal sequences were synthesized, cloned into different mammalian expression vectors, and recombinant polypeptides were transiently expressed in different cell lines. No BChE activity was detected in the culture media of cells transfected with any of the newly designed HBPs, and the inactive polypeptides remained inside the cells. Only enzymatically active HBP-4 was secreted into the culture medium. These results suggest that residues at the N- and C-termini are required for the folding and/or maintenance of HBP into an active stable, conformation.

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1. Introduction

Plasma-derived cholinesterases (ChEs) are effective bioscavengers of highly toxic organophosphorus compounds (OP) including pesticides and nerve agents [1]. Exogenously administered plasma-derived human butyrylcholinesterase (HuBChE) protects animals from multiple LD_{50}s of OP nerve agents without any toxic effects or performance decrements [2–4]. Thus, HuBChE is currently in advanced development as a prophylactic treatment for nerve agent toxicity. A dose of 200 mg of HuBChE is estimated to protect humans from an exposure of up to 2 × LD_{50} of soman [5]. However, the production of native HuBChE in such quantities is expensive and requires large amounts of plasma. This limited availability and administration of multiple doses of large quantities of this stoichiometric bioscavenger make this pretreatment difficult.

One approach for reducing the dose of this enzyme is to design a small molecule polypeptide bioscavenger that can be produced in large quantities using molecular biology techniques. HuBChE is a globular protein made up of four identical polypeptides of 574 amino acids each. Previous studies have shown that deletion of 45 C-terminal amino acids resulted in secretion of active HuBChE monomers [6]. Therefore, in this study, the production of smaller HuBChE polypeptides (HBPs) was attempted by deleting more amino acid residues from both N- and C-termini. The active site S198 in HuBChE is also the site for the binding of OPs. This was clearly demonstrated by isolating a HuBChE nonapeptide (FGESAGAAS)

Abbreviations: AChE, acetylcholinesterase; BHK, baby hamster kidney cells; BTC, butyrylthiocholine iodide; ChE, cholinesterase; CHO, Chinese hamster ovary cells; DTNB, 5,5′-dithiobis 2-nitrobenzoic acid; HBP, HuBChE polypeptide; HEK, human embryonic kidney cells; HuBChE, human butyrylcholinesterase; OP, organophosphorus compounds.

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that includes the active site Ser modified by various OP and non-OP inhibitors [7]. Results of site-directed mutagenesis, molecular modeling, and X-ray crystallography studies suggest that the architecture of the active-site gorge is important for the binding of OPs to ChEs. The structural features of HuBChE that maintain the folds of the active site are contained in the polypeptide region between residues 61 and 478 of HuBChE [8–12]. Accordingly, three HBPs that lack amino acid residues from either N- or C-terminus (HBP-1$^{(1-478)}$, HBP-2$^{(61-529)}$, HBP-3$^{(156-529)}$) were designed. HBP-4$^{(1-529)}$ that lacks 45 residues from C-terminus but known to have BChE activity was used as a control. The cDNAs for the HBPs containing signal sequences were synthesized and cloned into different mammalian expression vectors, which were used for transfecting different cell lines. BChE activity secreted into the culture medium and in cell lysates was tested.

2. Materials and methods

2.1. Reagents and cells

Gene juice, nano juice and pTriEx-4Neo plasmid were purchased from EMD Millipore Chemicals (San Diego, CA). pcDNA™3.1/V5-His TOPO TA expression kit, Lipofectamine 2000, Lipofectamine Plus, Lipofectamine LTX Plus and 293fectin transfection reagents were obtained from Life Technologies, Inc. (Gaithersburg, MD). Plasmid containing HuBChE (pGS-HuBChE) gene was generous gift from Dr. Oksana Lockridge (University of Nebraska, Omaha, NE). Rabbit polyclonal antibodies to purified plasma-derived HuBChE were produced by Spring Valley Laboratories, Inc., Woodline, MD.

2.2. Designing of HBPs

Molecular dynamics simulations and X-ray crystal structure analysis of HuBChE show that the structural features of HuBChE that are required for effective binding of OPs are largely contained in the 61–478 polypeptide region. The essential amino acid residues involved in OP-binding are contained in five peptide fragments (61–88, 108–148, 187–236, 285–336, and 384–478) [11–12]. These peptides were identified by deleting residues surrounding the active site while still maintaining its architecture. The resulting molecule contained essential binding subsites: (1) three-pronged oxyanion hole, (2) pi-cation site, (3) acyl-binding pocket, (4) gorge rim residues, (5) E197 that makes hydrogen bonds directly or through water molecules with residues at the catalytic site, and (6) D70, that plays an important role in promoting substrate binding and in controlling the dynamics and structural organization of water molecules. Based on this information, (1) HBP-1 was designed by deleting 96 amino acid residues from C-terminus (479–574), (2) HBP-2 was designed by deleting 60 amino acid residues from N-terminus (1–60) and 45 amino acid residues from C-terminus (530–574), (3) HBP-3 was designed by deleting 155 residues from N-terminus (1–155) and 45 amino acid residues from C-terminus (530–574) of the HuBChE protein (Figs. 1 and 2). HBP-4 that lacks 45 residues from C-terminus (530–574) was used as a control.

2.3. Cloning and expression of recombinant HBPs

Genes encoding HBPs containing the signal peptide and Kozak sequence at their N-terminus were synthesized (Retrogen, Inc., San Diego, CA). A signal peptide that drives the secretion of

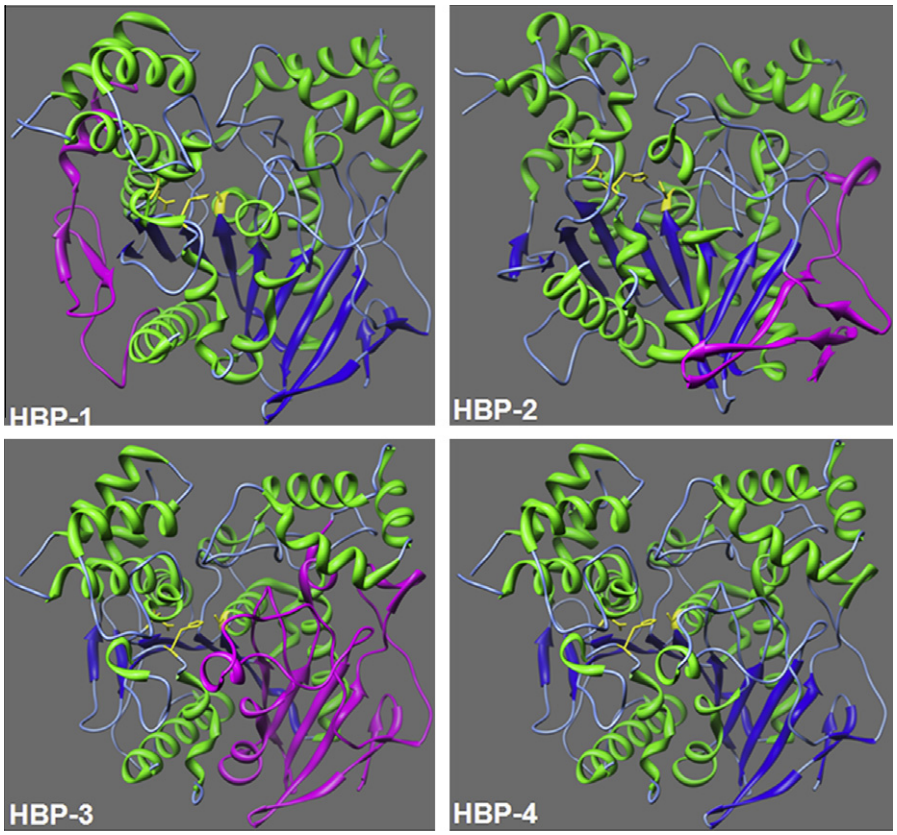

Fig. 1. Ribbon diagrams or predicted structures of HBPs. Sequences were selected based on the X-ray crystal structure of HuBChE (PDB ID: 1P0I), and N- and C-terminus regions forming small self-folded domains and predicted to be not required for HuBChE activity were deleted (shown in purple). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)