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RESEARCH ARTICLE

Expression and characterization of a codon-optimized butyrylcholinesterase for analysis of organophosphate insecticide residues

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Abstract

Organophosphate insecticide residues on vegetable, fruit, tea and even grains are primary cause of food poisoning. Organophosphate compounds can cause irreversible inhibition of the activity of acetylcholinesterase and butyrylcholinesterase (BChE, EC 3.1.1.8), which are both candidates for rapid detection of organophosphate pesticides. To develop an easy-to-handle method for detecting organophosphate pesticides using BChE, *BChE* from human was optimized according to the codon usage bias of *Pichia pastoris* and successfully expressed in *P. pastoris* GS115. The codon-optimized cDNA shared 37.3% of the codon identity with the native one. However, the amino acid sequence was identical to that of the native human butyrylcholinesterase gene (*hBChE*) as published. The ratio of guanine and cytosine in four kinds of bases ((G+C) ratio) was simultaneously increased from 40 to 47%. The recombinant hBChE expression reached a total protein concentration of 292 mg mL^{-1} with an activity of 14.7 U mL^{-1}, which was purified 3.2×10^3-fold *via* nickel affinity chromatography with a yield of 68% and a specific activity of 8.1 U mg^{-1}. Recombinant hBChE was optimally active at pH 7.4 and 50°C and exhibited high activity at a wide pH range (>60% activity at pH 4.0 to 8.0). Moreover, it had a good adaptability to high temperature (>60% activity at both 50 and 60°C up to 60 min) and good stability at 70°C. The enzyme can be activated by Li^+, Co^+, Zn^{2+} and ethylene diamine tetraacetic acid (EDTA), but inhibited by Mg^{2+}, Mn^{2+}, Fe^{2+}, Ag^+ and Ca^{2+}. Na^+ had little effect on its activity. The values of hBChE of the Michaelis constant (K_m) and maximum reaction velocity (V_m) were 89.4 mmol L^{-1} and 1 721 mmol min^{-1} mg^{-1}, respectively. The bimolecular rate constants (K_i) of the hBChE to four pesticides were similar with that of electric eel AChE (EeAChE) and higher than that of horse BChE (HoBChE). All values of the half maximal inhibitory concentration of a substance (IC_{50}) for hBChE were lower than those for HoBChE, but most IC_{50} for hBChE were lower than those for EeAChE except dichlorvos. The applicability of the hBChE was further verified by successful detection of organophosphate insecticide residues in six kinds of vegetable samples. Thus, hBChE heterologously over-expressed by *P. pastoris* would provide a sufficient material for development of a rapid detection method of organophosphate on spot and produce the organophosphate detection kit.

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1. Introduction

One of the most concerns in our daily life is food safety. Foods and food materials are vulnerable to be contaminated by foodborne pathogenic microorganism, illegal food additive, heavy metals and pesticides. Organophosphate compounds are broad spectrum insecticides used for crop protection to control agricultural pests. The primary toxicity of these compounds results in irreversible inhibition of nervous system enzyme, for example, cholinesterase (ChE, EC 3.1.1.8). ChE serves as a key enzyme mainly distributed in the central and peripheral nervous systems, plasma, liver and kidney, where it regulates the level of choline as well as transmits nerve impulse by catalyzing the hydrolysis of choline (Massoulié *et al*. 1993). In terms of different substrates, cholinesterase is classified in acetylcholinesterase (AChE, EC 3.1.1.7) and butyrylcholinesterase (BChE, EC 3.1.1.8). AChE functions in cholinergic nerve catalyzing the hydrolysis of acetylcholine, whereas BChE is considered as a toxic scavenger and substitute for AChE (Li *et al*. 2000; Mesulam *et al*. 2002).

BChE, which is also called pseudocholinesterase, belongs to the serine esterase family. It is mainly distributed in serum and liver, and even little in muscle and brain (Taylor and Radic 1994). Compared with the catalytic specificity of AChE, BChE possesses a broad hydrolytic spectra of choline ester substrates (Taylor and Radic 1994) because of the weak steric hindrance in the active center (Harel *et al*. 1992; Ordentlich *et al*. 1993; Radic *et al*. 1993). The function of BChE is to protect AChE from inactivation and to hydrolyze many esters, amides and peptides (Small *et al*. 1996). Moreover, it involves in some metabolic processes of some drugs and even cell growth process (Lockridge 1990; Alber *et al*. 1994; Mattes *et al*. 1997). In particular, as the target of organophosphorus pesticide (OP) and carbamate pesticide (CB), BChE could be inhibited by pesticides to detect OP and CB residues (Boublik *et al*. 2002; Pritchard *et al*. 2004; Vakurov *et al*. 2004). However, BChE purified from tissues or blood is both time and cost intensive. Therefore, *BChE* is highly necessary for heterologous expression in a new host fleetly and economically.

Pichia pastoris is now widely used for heterologous production of recombinant protein owing to its high level expression, efficient secretion, superior post-translational modification and potential to dense cultivation (Macauley *et al*. 2005; Mchunu *et al*. 2009). Due to the difference of codon usage between the host and their original organisms, researchers have utilized codon optimization to increase expression of a variety of protein (Wang *et al*. 2008; Zhu *et al*. 2011). Moreover, the recombinant plasmid pPIC9K/nhBChE of the native human BchE gene (*hBChE*) constructed by our research group expressed poorly in *P*. *pastoris* after repeated experiments.

Therefore, in this assay, we optimized the codon usage of hBChE cDNA fragment based on *P*. *pastoris* preferred codons to increase the production level. As a result, *hBChE* was successfully expressed in *P*. *pastoris* with a high-level expression. The biochemical characteristics of the recombinant enzyme were also determined.

The study is to produce a codon-optimized butyrylcholinesterase with low-cost preparation for achieving promising biomaterials to detect organophosphate insecticide residues. The expressed enzyme could be an ideal tool for detection of insecticides in agricultural products and their derivatives.

2. Results

2.1. Synthesis of codon-optimized *hBChE* and bioinformatics analysis for gene optimization

We successfully synthesized a codon-optimized *hBChE* with 6×His-Tag at N-terminal according to the prefer codons of *P*. *pastoris*. Here, it is clear that the native gene containing tandem rare codons leads to poor expression. So, we changed the codon usage frequency in *P*. *pastoris* by upgrading the codon adaptation index (CAI), which is a measurement of the relative adaptiveness of the codon usage of a gene compared with the codon usage of highly expressed genes, from 0.79 to 0.87 (Fig. 1). Moreover, the ratio of guanine and cytosine in four kinds of bases ((G+C) ratio) increased from 0.40 to 0.47 and unfavorable peaks were optimized to prolong the half-life of the mRNA (Fig. 2). The secondary structures, which affect ribosomal binding and stability of mRNA, have been removed as well. In addition, optimization process has successfully screened and removed the splice sites, polyadenylation signal, instability elements, and all the *cis*-acting sites that may have a negative influence on the expression rate. Finally, the codon-optimized cDNA (*hBChE*) shared 37.3% of codon identity with the native one, however, amino acid sequence of the synthetic gene was aligned with amino acid sequence of the native one (Fig. 3).

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