Journal of Molecular Catalysis B: Enzymatic 99 (2014) 169–175

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

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

Review

Expression of organophosphorus hydrolase in *Escherichia coli* for use as whole-cell biocatalyst

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ARTICLE INFO

Article history:
Received 17 July 2013
Received in revised form
24 September 2013
Accepted 24 September 2013
Available online 11 October 2013

Keywords:
Organophosphorus hydrolase
Whole-cell biocatalyst
Recombinant expression
Secretion
Surface-display

ABSTRACT

Among the various removal strategies against neurotoxic organophosphorus (OP) compounds, a live catalyst using whole-cell microorganisms has been considered as a useful scavenger with the virtue of cost-effectiveness and elimination efficiency. To over the catalytic activity by wild-type isolates, the expression of organophosphorus hydrolase (OPH) has been attempted in *Escherichia coli* for the extended detoxification efficacy to OP chemicals. However, early studies had unsatisfactory results, like low enzyme production levels due to the formation of insoluble inclusion bodies from the recombinant enzyme produced within the cells, and a bottleneck caused by the cell membrane of gram-negative strain *E. coli* acting as a permeability barrier to substrate diffusions. To date, various approaches have been suggested as effective solutions for overcoming these limits. This review will outline current studies, and their critical findings, on the successful expression of OPH in *E. coli* for the effective recombinant *E. coli* whole-cell biocatalysts.

Contents

1. Introduction

Synthetic organophosphorus (OP) compounds are widely used as pesticides, plasticizers, and air fuel ingredients. The percentage of OP chemicals as pesticides is approximately 38% of the total pesticides used globally [1]. These compounds are also mainly present in chemical nerve agents, estimated at ~200,000 tons of world widely stored amounts [2]. The toxicity of OP compounds is related with its effects on acetylcholinesterase (AChE, acetylcholine acetyl hydrolase; EC 3.1.1.7) in the progression of impulse transmission at cholinergic synapses. Even the neurotransmitter acetylcholine should be hydrolyzed by AChE activity after a nerve impulse, OP chemicals disturb the normal breakdown of acetylcholine by blocking AChE activity with phosphorylation at serine 203 in the catalytically active center, and cause the accumulation of acetylcholine in the synaptic cleft [1,3]. Aroused overstimulation in the nervous system by OP compounds are effective for the control of targeted pests but are also acute toxicity factors to non-target organisms, such as mammals.

Organophosphorus hydrolase (OPH, also referred to as parathion hydrolase; EC 3.1.8.1), a representative enzyme hydrolyzing OP chemicals, was initially reported from *Flavobacterium* sp. ATCC 27551 [4] and *Brevundimonas diminuta* MG (formerly *Pseudomonas diminuta* MG) [5]. Native OPH was identified as a membrane-bound homodimeric metalloenzyme and the precursor form of this protein has a signal sequence of 29 amino acids at its N-terminus for membrane targeting [6–11]. This enzyme contains two Zn^{2+} ions per molecule as native metals and these cations can be substituted with other metal ions such as Cd^{2+}, Ni^{2+}, Mn^{2+} or Co^{2+} showing the highest enhanced catalytic activity [12,13]. The *opd* (organophosphorus degrading) gene encoding OPH is located in each indigenous plasmids (43 kb sized pPDL2 for

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http://dx.doi.org/10.1016/j.molcatb.2013.09.023

Flavobacterium sp. ATCC 27551 and 66 kb sized pCMS1 for *B. diminuta* MG, respectively) as highly conserved forms [5,14–16]. With the removal of a variety of OP contaminants, the OPH has been known as a broad-spectrum phosphotriesterase due to the hydrolyzing capability against various phosphorus-ester bonds such as P—O, P—CN, P—F and P—S bonds with quite different catalytic rates [17].

In recent years, numerous microorganisms have been isolated with the practical capacity to degrade compounds previously considered non-degradable [18]. Having whole-cells produce the degrading enzyme has important advantages on the preparation of simple- and low-cost catalysts compared with other biocatalyst preparations, such as the purified enzyme and/or the immobilized enzyme on physical structures for recycled applications [19]. The potential for decontamination can be enhanced by several features provided by whole-cell microbial systems, such as the stable maintenance of enzyme catalytic activity within the cells, an easier regeneration of required cofactors, or the possible secondary transformation of chemicals through multiple enzymes or metabolic pathways for degradation to less- or non-toxic degradation components [20]. Nevertheless, the slow growth rate and insufficient population of native isolates capable of detoxification, or the low levels of enzyme expression under the native promoter can be disadvantageous, resulting in reduced bioremediation efficiencies [21–23]. In addition, most microorganisms producing OPH have been reported as gram-negative bacteria thereby the produced enzyme is placed within the cells [1]. This trait is also causes inefficient degradation rates of targeted OP xenobiotics, due to the disturbance by cell membrane as a permeability barrier in substrate uptake [24]. To overcome these limits, the expression of OPH in *Escherichia coli* was proposed for the recombinant whole-cell biocatalysts with improved catalytic activities.

2. Expression of organophosphorus hydrolase in *E. coli*

The study by Mulbry and Karns [10] can be exemplified as the initial performance showing the confronting problems of OPH expression in *E. coli*. The expression of *opd* gene from *Flavobacterium* sp. ATCC 27551 was attempted under the exogenous *E. coli lacZ* promoter. Though the strain *E. coli* has numerous advantages for the high-level production of heterologous proteins as an efficient and cost-effective host strain [25], the expression of recombinant OPH was at significantly low levels and most of the produced enzymes were located in the cytosol, unlike in wild-type strain. This result aroused the interest on the improper procession of produced protein, thus a study on whether the deletion of coding region for the signal peptide could increase the production of recombinant OPH in *E. coli* extracts was performed. However, the low activity level was also observed using the *lacZ-opd* fusion without the 29-residue signal peptide, although the activity was a 2-fold improvement over enzymes with the signal peptide.

Similar patterns of OPH expression in *E. coli* were reported by Serdar and Gibson [26]. OPH expression was attempted with a DNA fragment containing the *opd* gene from *B. diminuta* MG, and the constructed recombinant *E. coli* strain showed the meaningful production of OPH. However, although the *opd* gene was expressed under the *lac* promoter of a high copy number plasmid vector pUC7, the activity level ($\sim$0.19 μmol min^{-1} mg^{-1} in crude cell extracts) was about 10-fold less than those in the native strain *B. diminuta* MG. Later, shorting the coding region for signal peptide from the *opd* gene improved the expression of a soluble and mature formed OPH under the phage lambda P_L promoter in *E. coli* FM5. Furthermore, the OPH activity was enhanced approximately 20-fold more with Co^{2+}, and this cation was hypothesized to influence either the expression of *opd* gene itself or the stabilization of produced enzyme [11]. Since then, Manavathi et al. [27] suggested that divalent cations, such as Zn^{2+} or Co^{2+}, could enhance the specific activity through improving the protein folding of expressed recombinant OPH, not by inducing the transcriptional activation.

Based on the inverted repeat sequences in the signal coding region of *opd* gene, Pandey et al. [28] presumed this region would influence the formation of a stable stem loop structure in the generated mRNA transcript and that the stabilized secondary structure of *opd* mRNA might be responsible for the insufficient initiation of translation process. To examine this possibility, they constructed a recombinant plasmid containing the *opd* gene from *Flavobacterium* sp. ATCC 27551 and examined the OPH expression levels. Consequently, when deleting the signal peptide, approximately 3-fold increased expression level of recombinant OPH was observed. These enhanced values were further confirmed through mutations in the inverted repeat sequences for reducing the folding energy (ΔG values) of stem structure. Although the expression level was compared using the arbitrary units based on Western blotting, this study suggested a relation between mRNA secondary structure and OPH expression in *E. coli*.

The research trends for the interrelationship between mRNA structural stability and protein production [29,30] was also reflected in our study on the expression of recombinant OPH in *E. coli* [31]. When using the signal sequence of xylanase (XynB) from *Streptomyces thermocyaneoviolaceus* which was predicted to have a more stable secondary structure in mRNA transcripts than native 29 amino acids signal peptide, the *opd* gene from *Flavobacterium* sp. ATCC 27551 was not expressed. On the other hand, the structural *opd* gene without any signal peptide was successfully expressed as soluble forms under the T7 promoter, and secreted extracellularly in *E. coli* BLR(DE3).

3. Surface-expression of organophosphorus hydrolase in *E. coli*

The cell envelope of gram-negative bacteria, like *E. coli*, consists of the cytoplasmic membrane, periplasm and outer membrane [32]. In *E. coli* strain containing the intracellular-expressed recombinant OPH, the complexly structured cell membrane would be a barrier with the substrate-transports, leading to the limit of whole-cell catalytic efficiency [21]. Due to this issue, protein expression on the surface of *E. coli* using carrier proteins could be an alternative strategy for enhancing the catalytic activities.

The lipoprotein-outer membrane protein A (Lpp-OmpA) fusion is composed of a short N-terminal region containing the signal peptide as well as the first nine amino acid residues from the major *E. coli* outer membrane lipoprotein (Braun's lipoprotein, Lpp), and the transmembrane domain of outer membrane protein A (OmpA, amino acid residues 46–159) [33,34]. This chimeric fragment serves as a carrier protein for the surface expression of a C-terminally fused target protein (passenger). The Lpp domain has a role in targeting and anchoring of the tribrid to the outer membrane, and the OmpA domain is needed for the surface array of the passenger [35]. The successful surface display of OPH in *E. coli* was established using this hybrid system by Richins et al. [24]. The expressed recombinant protein was anchored by Lpp-OmpA fusion onto the bacterial cell surface, with more than 80% of OPH activity on the cell surface. These kinds of whole cells possessed a 7-fold higher degrading efficacy to OP compound parathion than whole cells expressing intracellular OPH at similar levels. Since then, Kaneva et al. [36] investigated about the various factors which might influence the degradation efficacy of recombinant *E. coli* expressing Lpp-OmpA-OPH on the bacterial surface. The production of active OPH was highly host-specific with tight regulation governed by the *tac* promoter. In addition, OPH activity was dependent on growth