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High-level expression of soluble recombinant proteins in *Escherichia coli* using an HE-maltotriose-binding protein fusion tag

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ABSTRACT

Recombinant proteins are commonly expressed in prokaryotic expression systems for large-scale production. The use of genetically engineered affinity and solubility enhancing fusion proteins has increased greatly in recent years, and there now exists a considerable repertoire of these that can be used to enhance the expression, stability, solubility, folding, and purification of their fusion partner. Here, a modified histidine tag (HE) used as an affinity tag was employed together with a truncated maltotriose-binding protein (MBP; consisting of residues 59–433) from *Pyrococcus furiosus* as a solubility enhancing tag accompanying a tobacco etch virus protease-recognition site for protein expression and purification in *Escherichia coli*. Various proteins tagged at the N-terminus with HE-MBP(Pyr) were expressed in *E. coli* BL21(DE3) cells to determine expression and solubility relative to those tagged with His6-MBP or His6-MBP(Pyr). Furthermore, four HE-MBP(Pyr)-fused proteins were purified by immobilized metal affinity chromatography to assess the affinity of HE with immobilized Ni^{2+}. Our results showed that HE-MBP(Pyr) represents an attractive fusion protein allowing high levels of soluble expression and purification of recombinant protein in *E. coli*.

1. Introduction

As a product of DNA recombinant technology, fusion proteins expressed in several host organisms have been developed as a class of novel biomolecules with multifunctional properties and comprehensive applications in biological research [1]. Compared with eukaryotic expression systems, *Escherichia coli* remains the dominant host for producing recombinant proteins due to its advantageous, rapid, inexpensive, and high-yield protein production, together with its well-characterized genetics and variety of available molecular tools [2,3]. However, protein insolubility, conformation, stability, and structural flexibility, as well as low purification yields and host-cell toxicity, remain challenges that must be resolved when the *E. coli* bacterial system is used to express recombinant proteins [4]. Despite the aforementioned issues associated with *E. coli* recombinant-protein production, the cost benefits, ease of use, and scale make it essential to design new strategies for the production of recombinant soluble protein in this host [3].

Fusion tags are proteins or peptide molecules capable of soluble expression in *E. coli* and commonly used to facilitate target-protein expression, resistance to proteolytic degradation, solubility, and purification [5–7]. Widely used fusion tags can be divided into affinity tags and solubility tags. Affinity tags used for protein isolation and purification include hexahistidine (His6), maltose-binding protein (MBP) [8], glutathione-S-transferase) [9], and Strep-tag II [10]. Solubility tags for soluble-protein production include MBP [11], N-utilization substance A [12], thioredoxin A [13], and small ubiquitin-related modifier (SUMO) [5]. Some protein tags, such as glutathione-S-transferase and MBP, can also function as promoters of both affinity and solubility. Additionally, numerous solubility enhancing tags are employed with affinity tags to enhance protein solubility and yield and simplify the purification procedure. Protease-cleavage sites located between the fusion partner and target protein are invariably employed to remove the fusion partner from the final protein to counteract potential interference with attainment of proper structure and function by the target protein [3]. Nevertheless, the effectiveness of a fusion protein

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is not always a given for every protein target [14], and many proteins frustratingly remain insoluble or difficult to purify regardless of which of these fusions are used. For example, a His-tag used as a fusion partner for protein expression affects the formation of inclusion bodies [15]. Therefore, new effective tags are needed to increase the accumulation of soluble recombinant proteins expressed in *E. coli* while also assisting purification.

The HEHEHE-tag is a modified hexahistidine tag in which every second histidine residue is replaced by a more hydrophilic glutamate, which still allows efficient purification by immobilized metal ion affinity chromatography (IMAC) [16]. It is plausible that substitution of a His-tag with a HEHEHE-tag could represent an effective strategy for addressing the issue of inclusion bodies formed by recombinant proteins. In this study, hence, an (HE)7-tag used as an affinity tag was incorporated with maltotriose-binding protein, a presumptive solubility tag isolated from *Pyrococcus furiosus* (WP_011013078) [MBP(Pyr)], and located adjacent to a tobacco etch virus (TEV) protease-recognition site as a new fusion tag [HE-MBP(Pyr)] for protein expression and purification in *E. coli*. His6-MBP [17] was used to compare the expression and purification yields of HE-MBP(Pyr) when fused to the protein of interest.

2. Materials and methods

2.1. Strains, vectors, and reagents

E. coli strain BL21(DE3), previously preserved in our laboratory, was used for cloning and expression. The plasmids used for expression (Table 1) were preserved or constructed in our laboratory. The pUC57-HE-MBP(Pyr)-Nb plasmid was codon-optimized and synthesized by GenScript (Nanjing, China). Restriction endonucleases and PageRuler pre-stained protein ladder were purchased from Thermo Fisher Scientific (Waltham, MA, USA). The SanPrep column plasmid mini-prep kit, SanPrep column DNA gel extraction kit, and SanPrep column PCR product purification kit were purchased from Sangon (Shanghai, China). T4 DNA ligase was purchased from Takara Bio (Dalian, China). High-affinity Ni-NTA resin was obtained from GenScript.

2.2. Construction and verification of recombinant plasmids

The pUC57-HE-MBP(Pyr)-Nb and pET-21b vectors were digested with *Nde*I/*Xho*I and ligated to obtain the pET21b-HE-MBP(Pyr)-Nb recombinant plasmid as the carrier for other recombinant plasmids. Further, HE-MBP(Pyr) was replaced with either His6-MBP(Pyr) or His6-MBP. The gene fragments encoding H5HA10, PCV2b, NLSFMD, HAFnt, Cago60, PCV2VHHC3, CdiGMP499 (*Caulobacter vibrioides*), CdiGMP026 (*Phenylobacterium zucineum*), Prop acid OAS, and FMDV98, respectively, were digested with *BamH*I/*Xho*I and inserted into the *BamH*I/*Xho*I-digested pET21b-HE-MBP(Pyr), pET21b-His6-MBP(Pyr), and pET21b-His6-MBP vectors. All recombinant plasmids were transformed into *E. coli* strain BL21(DE3) cells. Positive clones were identified by restriction endonuclease reaction and verified by DNA sequencing.

Table 1
Expression and solubility levels of HE-MBP(Pyr)- and His6-MBP tag-fused proteins.

Protein	Expression, %		Solubility, %	
	HE-MBP(pyr)	His_6-MBP	HE-MBP(pyr)	His_6-MBP
Nb	60	60	80	30
PCV2b	50	40	70	60
Cago60	60	50	80	50
PCV2VHHC3	60	60	80	60
CdiGMP499	50	40	60	40
CdiGMP026	20	0	40	0
Prop acid OAS	60	60	80	30
FMDV98	70	50	70	30
H5HA10	50	50	80	60

The expression level (%) of fusion protein was calculated based on the density ratio of the target fusion protein to the total *E. coli* -expressed-proteins.
The solubility level (%) was calculated based on the density ratio of soluble fusion-protein to total fusion protein expression.

2.3. Protein expression and solubility identification

Verified transformants were cultured overnight at 37 °C and diluted 1:100 in Luria–Bertani broth culture containing 100 μg/mL ampicillin. When the optical density at 600 nm reached 0.6, 0.5 mM of isopropyl β-D-thiogalactopyranoside (IPTG) was added to induce protein expression for 12 h–16 h at 25 °C. Cells were collected by centrifugation at 8000*g* for 10 min, resuspended in phosphate-buffered saline (pH 7.4), and disrupted by sonication on ice, followed by ultracentrifugation for 20 min at 4 °C. The soluble and insoluble fractions of recombinant proteins were analyzed by 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) under denaturing conditions to appraise the relative expression level and solubility of each fusion protein. Expression and solubility levels were determined using an Amersham Imager 600 (GE Healthcare, Pittsburgh, PA, USA) and calculated as described previously [18].

2.4. Protein purification

Ni-NTA affinity chromatography was performed for the purification of HE-MBP(Pyr)-, His6-MBP(Pyr)-, or MBP(Pyr)-tagged proteins. Induced cells measured by the optical density at 600 nm(OD_{600} = 0.2) were harvested by centrifugation at 8000*g* for 10 min at 4 °C and dissolved in ice-cold lysis buffer [50 mM Na_2HPO_4, 300 mM NaCl, and 10 mM imidazole (pH 8.0)]. The supernatant obtained by sonication and subsequent ultracentrifugation was incubated for 1 h at 4 °C with shaking at 200 rpm along with 200 μL pre-equilibrated Ni-NTA resin. The resin was allowed to settle completely by gravity, and aliquots of the supernatant were aspirated. Buffers containing increasing concentrations of imidazole (15, 20, 25, 30, 40, 50, 75, 100, 250, 300 and 500 mM) were used at 10-fold the column volume to elute proteins bound to the Ni-NTA resin. All fractions were collected and analyzed by 12% SDS-PAGE under denaturing conditions to evaluate the affinity of the HE tag relative to the His6 tag.

3. Results

3.1. Construction of the recombinant plasmids

To test the effects of the HE-MBP(Pyr) tag on the expression and solubility of *Nb*, *H5HA10*, *PCV2b*, *NLSFMD*, *HAFnt*, *Cago60*, *PCV2VHHC3*, *CdiGMP499* (*C. vibrioides*), *CdiGMP026* (*P. zucineum*), *Prop acid OAS*, and *FMDV98*, the DNA fragments were isolated by digestion and ligated into similarly digested pET21b-HE-MBP(Pyr), pET21b-His6-MBP, and pET21b-His6-MBP(Pyr) vectors (Fig. 1). The HE tag represents a modified His-based tag containing 7 × histidine-glutamate repeats (HEHEHEHEHEHE) suitable for IMAC purification [16]. The MBP(Pyr) tag consists of a maltotriose-binding protein derived from *P. furiosus* (GenBank accession no. WP_011013078), and the nucleic acid sequence of the combined HE-MBP(Pyr) tag was codon-optimized for expression in *E coli* (Fig. S1). The TEV protease-recognition site was inserted adjacent to the HE-MBP(Pyr) tag to enable efficient separation during purification.