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Effective non-denaturing purification method for improving the solubility of recombinant actin-binding proteins produced by bacterial expression

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ABSTRACT

Bacterial expression is commonly used to produce recombinant and truncated mutant eukaryotic proteins. However, heterologous protein expression may render synthesized proteins insoluble. The conventional method used to express a poorly soluble protein, which involves denaturation and refolding, is time-consuming and inefficient. There are several non-denaturing approaches that can increase the solubility of recombinant proteins that include using different bacterial cell strains, altering the time of induction, lowering the incubation temperature, and employing different detergents for purification. In this study, we compared several non-denaturing protocols to express and purify two insoluble 34 kDa actin-bundling protein mutants. The solubility of the mutant proteins was not affected by any of the approaches except for treatment with the detergent sarkosyl. These results indicate that sarkosyl can effectively improve the solubility of insoluble proteins during bacterial expression.

1. Introduction

The actin cytoskeleton is involved in numerous cellular processes, including morphogenesis, endocytosis, cell division, and cell–cell signaling [1]. It also participates in several pathologies and diseases such as cancer and neurodegeneration [2,3]. The structure and function of the actin cytoskeleton are regulated by several actin-binding proteins (ABPs) that include actin-cross-linking and -bundling proteins [4], and more than one hundred ABPs exist [5]. To understand the function of a particular ABP, it is necessary to identify its actin-binding site, which usually involves biochemical analysis of recombinant truncated mutants [6–12].

Bacterial expression is commonly used to produce and purify recombinant and truncated mutant proteins. One major setback is that expressed proteins are often aggregated or packaged in inclusion bodies, making purification very difficult [13,14]. Although the precise reason is not known, there are several factors that can promote insolubility [15]. When a heterologous gene is introduced into a bacterial cell, its expression control mechanism is likely lost as the microenvironment (i.e., pH, presence of cofactors, and folding mechanisms) of the original host differs from that of the bacterial cell [16].

Soluble and recombinant proteins can be purified from inclusion bodies by two steps. In this method, denaturants such as urea and guanidine hydrochloride denature soluble proteins within inclusion bodies, followed by their refolding [17]. However, this method is time-consuming and inefficient [18–20]. There are several non-denaturing approaches that can increase the solubility of recombinant proteins that include using different bacterial cell strains, altering the time of induction, lowering the incubation temperature, and employing different detergents for purification such as Triton X-100 and sarkosyl [13,21–23].

In this study, we compared several non-denaturing protocols to express and purify two insoluble 34 kDa ABP mutants. We describe herein a convenient and effective non-denaturing purification method for insoluble truncated mutant proteins, which is important in the structural and functional characterization of ABPs.

Abbreviations: ABP, actin-binding protein; PCR, polymerase chain reaction; LB, Luria broth; IPTG, isopropyl-β-D-thiogalactopyranoside; OD, optical density; TEM, transmission electron microscopy.

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2. Materials and methods

2.1. Cloning of DNA mutants into the protein expression vector

The 34 kDa gene (GenBank accession no. U32112) was amplified by polymerase chain reaction (PCR) using the synthesized *Dictyostelium discoideum* DNA (pGEM-T Easy-34 kDa) as a template (COSMOGENTECH, Rep. Korea). The gene was inserted downstream of the T7 promoter of the expression vector pET-28a(+) (Novagen, USA), and the resulting construct expressed residues 1-297 of the 34 kDa protein. After verifying the DNA sequence, plasmid DNA was used as a template for constructing the 34 kDa DNA mutants. The *D. discoideum* 34 kDa DNA mutants were amplified by PCR using primers containing a stop codon in the middle of the amplified DNA fragment (Table 1). The PCR was performed in a DNA Engine Dyad peltier thermal cycler (Bio-Rad, USA) using five cycles of denaturation at 98 °C for 10 s, annealing at 55 °C for 30 s, and chain extension at 68 °C for 4 min 30 s. After the PCR, the products were treated with 2 μl of DpnI for 2 h to digest the unwanted methylated DNAs. To recover the digested template and DpnI, the samples were precipitated with 100% ethanol and incubated for 2 min. The DNA was then washed with 70% ethanol and collected using 10 μl of TE buffer (10 mM Tris, 1 mM ethylene diamine tetraacetic acid, pH 8.0). The resulting construct pET-28a(+) was transformed into host *Escherichia coli* DH5α(DE3) cells, and stable transformants were selected on LB plates supplemented with 50 μg/ml kanamycin. From the selected transformants, plasmid DNA was isolated and transformed into expression host *E. coli* BL21(DE3), Rosetta(DE3), and Rosetta-gami2(DE3) cells.

2.2. Bacterial cultures and protein expression

E. coli BL21(DE3), Rosetta(DE3), and Rosetta-gami2(DE3) cells carrying recombinant pET-28a(+) were grown in LB medium at 37 °C with shaking at 180 rpm in the presence of 50 μg/ml kanamycin until the optical density at 600 nm (OD A_{600}) reached 0.5–0.6. Protein expression was induced by the addition of IPTG to a final concentration of 1 mM. After 4 h, the cells were centrifuged at 3000 × g for 10 min, washed once with the buffer solution containing 50 mM sodium acetate, 2 mM $MgCl_2$, 1 mM ethylene glycol tetraacetic acid, 20 mM 3-morpholinopropane-1-sulfonic acid, pH 7.0 (referred to as 34 kDa buffer hereafter), and centrifuged again at 3000 × g for 10 min. The cell pellets were stored at −80 °C.

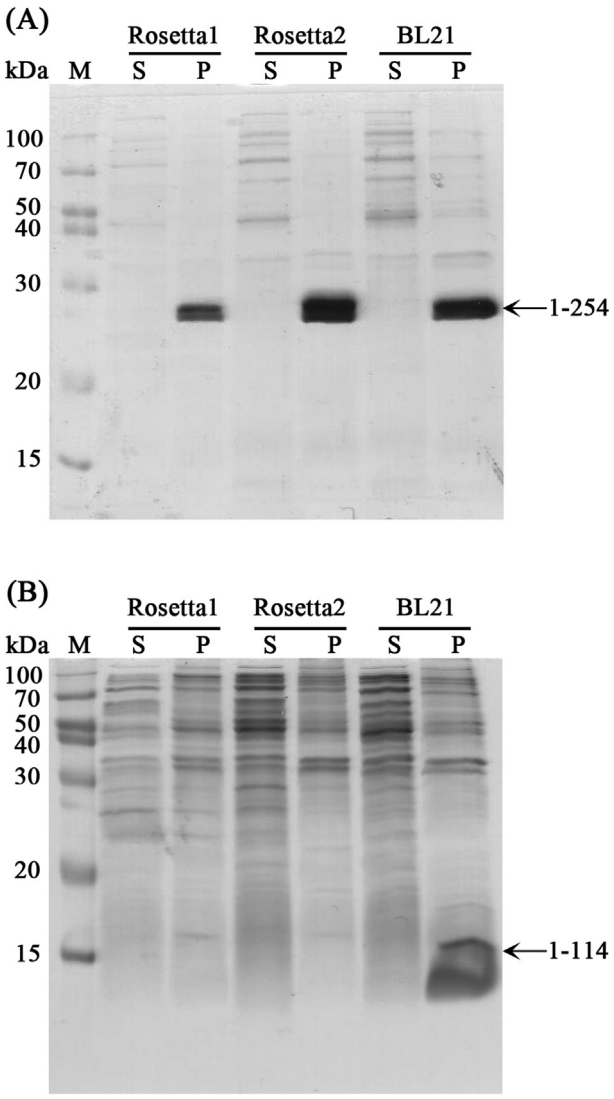

Fig. 1. Validation of expression of the truncated actin-binding mutant proteins. 1-254 (A) and 1-114 (B) mutant proteins produced in three different *E. coli* strains, namely, Rosetta (DE3; Rosetta1), Rosetta-gami2 (DE3; Rosetta2), and DE3 (BL21). S, supernatant (soluble form of the protein); P, pellet (insoluble form of the protein). Arrows point to the 1-254 and 1-114 mutant proteins.

Table 1
Lists of strains, plasmids and primers used in the study.

	Relevant genotype/phenotype	Source
Strain		
DH5α	F− *Φ80lacZΔM15 Δ(lacZYA-argF) U169 recA1 endA1 hsdR17* (*rK*−, *mK*+) *phoA supE44 λ− thi-1 gyrA96 relA1*	Invitrogen
BL21(DE3)	*fhuA2* [*lon*] *ompT gal* (*λ DE3*) [*dcm*] Δ*hsdS λ DE3* = *λ sBamHIo* Δ*EcoRI-B int*::(*lacI*::*PlacUV5*::*T7 gene1*) *i21* Δ*nin5*	Novagen
Rosetta(DE3)	F^- *ompT* $hsdS_B(r_B^- m_B^-)$ *gal dcm* (DE3) pRARE (Cam^R)	Novagen
Rosetta-gami2(DE3)	Δ(ara-leu)7697 ΔlacX74 ΔphoA PvuII phoR araD139 ahpC galE galK rpsL (DE3) F′[lac^+ $lacI^q$ pro] gor522::Tn10 trxB pLysSRARE (Cam^R, Str^R, Tet^R)	Novagen
Plasmid		
pGEM®-T Easy	Am^R, *E.coli* cloning vector	Promega
pET28a(+)	Km^R, *E. coli* expression vector	Novagen
34 kDa (1-254)	Km^R, wild type 34 kDa(1-254) in pET28a(+)	This study
34 kDa (1-114)	Km^R, wild type 34 kDa(1-114) in pET28a(+)	This study
Primer	**Sequence (5′ to 3′)**	**Length (bp)**
34 kDa WT	F: taagaaggagatataccatggcagaaacaaaagttgcaccaa	42
	R: gtggtggtggtggtgctcgagtttcttttgtggaccgtatc	42
34 kDa 1-254	F: cgtactgcttcaaagtaatatggtggtgctgct	33
	R: agcagaccaccatattactttgaagcagtacg	32
34 kDa 1-114	F: ttctgtgaagatccataaaacaaaaactatgca	33
	R: tgcatagtttttgttttatggatcttcacagaa	33