Solubilization of RhRnBp and Peysn5 by protein fusion in *Eshcherichia coli*

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Abstract

RhRnBp and Peysn5 are the proteins related to carbohydrate synthesis. RhRnBp originated form human was expressed as inclusion body in E. coli. Peysn5 originated form actinomadura was expressed as low level and inclusion body in E. coli. Ub, Trx, MalE and NusA is used as fusion partner to RhRnBp and Peysn5. The solubility of all fusion protein is NusA > MalE > Trx > Ub. Expression level of RhRnBp fusions in 37℃ is higher than that in 25℃. However in the case of Peysn5, Expression levels in 25℃ were higher. MalE fusion had highest activity in RhRnBp fusions. There were no activity in Peysn5.

Introduction

When high-expression level is achieved, recombinant proteins are frequently expressed in *Escherichia coli* as insoluble protein aggregates termed "inclusion body". There were many tries for minimization of inclusion body production. These are the control of cell incubation temperature and concentration of inducer and usage of additives. Recently, protein fusion with highly soluble proteins is regarded attensively. The first trial of fusion technique for solving inclusion body is thioredoxin fusion. GST, MBP and NusA were also used as fusion partner.[1)2)] Ubiquitin was used as fusion partner low molecular weight peptide.[3)]

RhRnBp and Peysn5 is the proteins related to carbohydrate synthesis. RhRnBp originated form human is expressed as inclusion body in E. coli. Peysn5 originated form actinomadura is expressed as low level and inclusion body

In this study, Ub, Trx, MalE and NusA were fused translationally to RhRnBp and Peysn5. Soluble expression levels of fusion proteins were checked. Biological activitys is also checked.

Materials and methods

Strains and vectors- Used strains were E. coli (DH5α), E. coli (BL21). Used vectors were pET43.1a, pET32a, pMal-c2x, pET::ub, pACYC::ub, pET24ma::rhrnbp, pKK223-3::peysn5 and pET24ma::rffH. Constructed vectors are pET::ub::rhrnbp, pET32a::rhrnbp, pMal-c2x::rhrnbp, pET43.1a::rhrnbp, pET::ub::peysn5, pET32a::peysn5, pMal-c2x::peysn5, pET43.1a::peysn5.

Pasmid construction- rhrnbp and peysn5 was amplified by PCR and inserted to pGEM-T vector. pGEM-T::rhrnbp and pGEM-T::peysn5 is cutted restriction enzyme BamHI and HindIII. pET43.1a, pET32a, pMal-c2x, pET::ub was cutted by restriction enzyme BamH and HindIII. rhrnbp and peysn5 inserted to pET43.1a, pET32a, pMal-c2x, pET::ub by BamHI and Hind III sites.

Cell culture- E. coli cells with plasmids were innoculated to 2 mL LB media containing 100ppm ampicilin and cultured over night at 37℃. 2mL cultured cells wer transformed to 50 mL LB media amicilin and cultured at 37℃ and 25℃. Promoter was induced by IPTG at O.D. 0.6. Cells are more cultured for 6 hr.

Making cell lysate- Cultured cells were harvested by centrifugation at 4000rpm, washed twice by sonication buffer(50 mM Tris-Cl, 200 mM NaCl, 1 mM EDTA, 0.5 mM PMSF, 2.8 mM β-mercaptoenthanol). Cells are resuspended by 10 mL sonication buffer. Resuspended cells were sonicated for 10 min, centrifuged at 10,000 rpm. Soluble fracttion was stored at -70℃. Debris was washed twice by sonication buffer, solved into 10 mL 1% SDS solution.

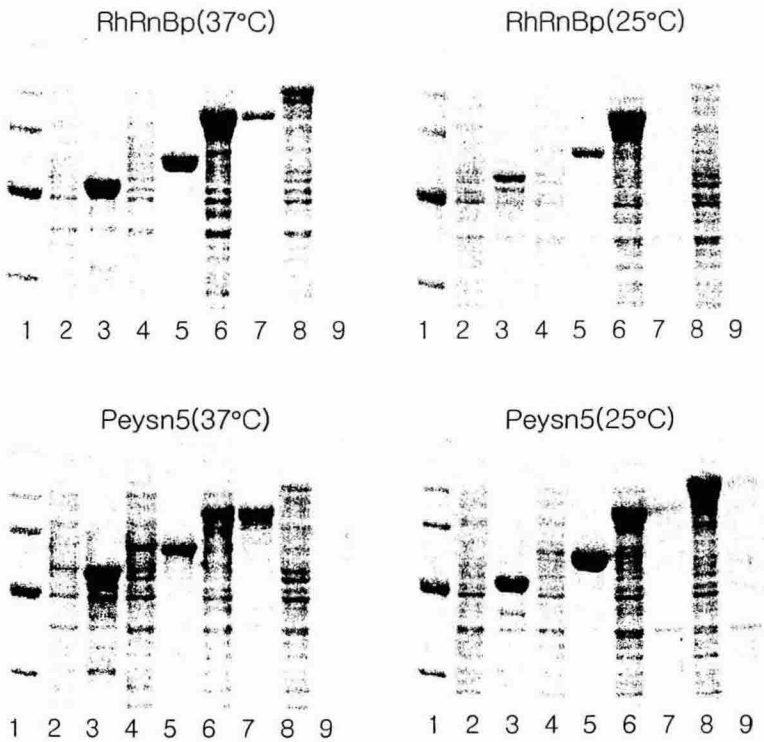
SDS-PAGE analysis- Soluble and insolube fractions were loaded to 10% SDS-PAGE Gel. The gels were stained with coomasie blue.

Activity assay-The reaction solution for epimerization was 100mM GlcNAC, 10mM MgCl2 and 5mM ATP in 100mM pH7.5 Tris-HCL buffer at 30℃. The reaction solutiotion for TDP-glucose synthesis was 5mM dTTP and 20mM glucose-1-phosphate. Activity was measured by HPLC.

Result and Discussion

The ratio of the soluble fraction to insoluble fraction was in the order of Ub < Trx < MBP < NusA. Expression level of RhRnBp fusion proteins at 37℃ is higher than that at 25℃.(Figure 1) In case of Peysn5, the tendency was reverse.

Activity of fusion proteins was highest in MBP-RhRnBp. Peysn5 fusion proteins did not give any activities.(Table 1)



Fiqure 1. 10% SDS-PAGE gel. 1, 10 lane : protein maker; 2, 3 lane : ubiquitin fusion; 4, 5 lane : Trx fusion; 6, 7 lane : MBP fusion; 8, 9 lane : NusA fusion; 2, 4, 6, 8 lane: soluble fraction; 3, 5, 7, 9 lane: insoluble fraction

Table 1. relative activity of fusion proteins(protein expression in 37℃)

fusion partners / target proteins	Ubiquitin	thioredoxin	maltose binding protein	NusA
RhRnBp	nd	nd	1	0.2
Pcysn5	nd	nd	nd	nd

Reference

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