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Purification of glutathione S-transferase fusion proteins as a non-degraded form by using a protease-negative *E.coli* strain, AD202

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Recombinant proteins prepared by genetic engineering are utilized for various purposes in molecular biology such as production of specific antibodies or investigation of the mechanism of protein–DNA or protein–protein interactions (1). There are several methods commonly used to produce recombinant proteins in bacteria or insect cells (2). Expression of a recombinant protein in *E.coli* as a fusion protein with glutathione S-transferase (GST) is one of the most popular and easiest methods (3). However, we often encounter the serious problems that some fusion proteins are rapidly degraded or cannot be solubilized in bacterial cells.

To prepare a polyclonal antibody against the γ chain of the high affinity IgE receptor (FcεRIγ), we constructed GST-FcεRIγ, which was composed of the GST gene joined to the cytoplasmic portion of the FcεRIγ cDNA. In initial experiments, we utilized two commonly used bacteria strains, DH5α and TG1, for production of the fusion protein. Although the GST-fusion protein was induced with isopropyl-β-D-thiogalactopyranoside and solubilized in the supernatant of the bacterial lysate after sonication, the fusion protein was rapidly cleaved at the fusion joint between the GST and FcεRIγ during purification (Fig 1.A). This cleavage could not be prevented by the addition of several available protease inhibitors such as phenylmethyl sulfonyl fluoride, aprotinin, leupeptin, bestatin and ethylenediaminetetraacetic acid (data not shown). Several trials for alternative methods such as shortening the induction time, changing the incubation temperature, or lysis with freezing and thawing method instead of sonication did not improve the results at all (data not shown).

To overcome this problem, we then used several protease negative *E.coli* strains (Table 1). In one of these strains, AD202, which is defective in *ompT* encoding an outer membrane-associated protease (4), the fusion protein was not cleaved at all and recovered from bacterial lysate with an expected molecular size (Fig. 1A). The prevention of degradation was observed not only with this construct but also with other GST-fusion proteins including GST-CD3ε (Fig. 1B). It is noteworthy that a fusion protein, GST-CD3ζ, which could not be detected in DH5α, could be induced and purified by using AD202 (Fig. 1C). These results suggest that an extremely unstable fusion protein such as GST-CD3ζ can be stabilized in AD202. We next examined the efficiency of this strain by using a larger fusion protein construct. A 75 kD GST-CTBP1, which contains a coding frame of a single-stranded DNA and RNA binding protein of the yeast, was purified efficiently in AD202, whereas most of the products were degraded in DH5α (Fig. 1D). This suggests that AD202 is also effective for fusion proteins as large as 75 kD and for those encoding nucleic acid binding proteins. From these observations, we conclude that AD202 is one of the most appropriate strains for the purpose of producing GST-fusion proteins in *E.coli*.

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Table 1. *E.coli* strains used in this study and their efficiency of purification of GST-fusion proteins

Strains	phenotype	% degradation GST-FcεRIγ	GST-CD3ε	GST-CD3ζ	GST-CTBP1
DH5α		100	91	100	97
JE7852	*lon*-100	100	89	N.T.	N.T.
C600 *lon*-	*lon*-100	100	75	N.T.	N.T.
AD202	*ompT*::Tn5	0	8	52	56

The degradation of the fusion proteins was evaluated as the percentage of the degraded form among the total fusion protein by densitometric analysis of the stained bands on the gel. N.T.: not tested.

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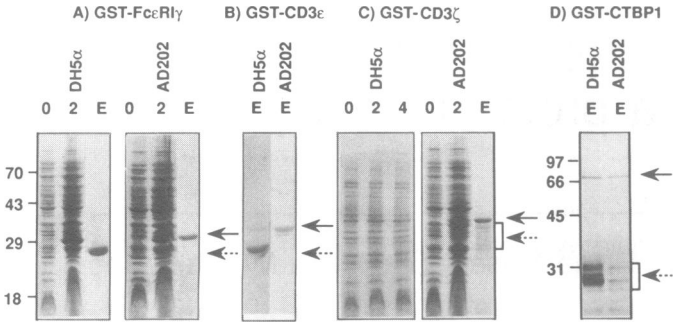

Figure 1. Induction and purification of GST-fusion proteins by using two different *E.coli* strains. Bacterial lysates were prepared after induction for the indicated periods (0, 2 and 4 hr). The fusion proteins were purified as eluates (indicated as 'E') from glutathione−sepharose beads. The whole lysates or eluates were analyzed on 12% SDS−PAGE followed by staining with Coomassie blue (**A**, **B** and **C**) or silver (**D**). The fusion proteins with the expected molecular size (bald arrow) and the degraded size (dotted arrow) were indicated. Molecular size of GST, GST-FcεRIγ, GST-CD3ϵ, GST-CD3ζ and GST-CTBP1 were 26 kD, 31 kD, 33 kD, 39 kD and 75 kD, respectively. Molecular weights of protein standards are indicated at the left margin.

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