Benchmarks

BENCHMARKS

Benchmarks are brief communications that describe helpful hints, shortcuts, techniques or substantive modifications of existing methods.

Preparation of Functional Recombinant Protein from *E. coli* Using a Nondetergent Sulfobetaine

BioTechniques 29:1166-1167 (December 2000)

Expression of foreign proteins in *E. coli* is frequently stymied by the formation of insoluble inclusion bodies. To recover the insoluble protein, the inclusion body is usually solubilized with a denaturing reagent such as urea and guanidine HCl, followed by dialysis to renature the target protein. Nondetergent sulfobetaines (NDSB) are a family of zwitterionic compounds carrying a sulfobetaine hydrophilic group and a hydrophobic group that is not long enough for micelle formation. Recently, NDSBs have been used as the solubilizing and stabilizing reagents for protein purification and renaturation (2,6–8). Our previous trials on preparation of GST-C/EBP β from *E. coli* did not recover active GST-C/EBP β using either the Sarkosyl detergent (4) or the regular denaturation-renaturation protocol (1). In this study, we tested the effect of NDSB 201 on renaturation of GST-C/EBP β purified from *E. coli* and found that NDSB 201 facilitates the preparation of active recombinant GST-C/EBP β from inclusion bodies.

To express GST-C/EBP β fusion protein, cDNA encoding the rodent C/

Table 1. Representative Yield of Functional GST-C/EBP β ^a

	GST-C/EBP β	Percentage ^b
Total expressed protein	2200 μ g	100%
Renatured protein	1125 μ g	51.1%
Functional protein	1047.3 μ g	47.6%

^aExpression is performed in a 500-mL culture.
^bProtein yield relative to the total expressed protein.

EBP β was placed downstream of the GST fragment in pGEX5T-1. The resultant construct was transformed into BL21(DE3)pLysS for protein expression. A positive colony was inoculated into 25 mL LB medium containing 100 μ g/mL ampicillin and incubated at 37°C overnight. Next day, the saturated culture was placed into 500 mL culture medium and incubated at 37°C until the cell density reached A₆₀₀ = 0.8. Protein expression was induced with 0.5 mM IPTG at 37°C for 2 h. The bacteria were spun down and resuspended in the lysis buffer (50 mM Tris, pH 7.5, 250 mM NaCl, 1% Triton® X-100, and 5 mM DTT). Cells were lysed by one cycle of freezing and thawing plus a brief sonication in the presence of the complete, EDTA-free protease inhibitor cocktail (Roche Molecular Biochemicals, Mannheim, Germany). The lysate was then treated with 20 μ g/mL DNase I at 37°C for 1 h. The insoluble inclusion body was spun down and washed twice with 1% Triton X-100. The inclusion body was dissolved in the denaturation solution (6 M guanidine HCl, 25 mM DTT, and 50 mM Tris, pH 7.5) at 4°C for 1 h. The protein concentration of solubilized sample was then adjusted to 1 μ g/ μ L with the denaturation solution. For renaturation, the solubilized sample was quickly diluted with a tenfold volume of renaturation solution (1 M NDSB, 0.2 M NaCl, 1 mM DTT, and 50 mM Tris, pH 7.5) and incubated at 4°C for 1 h. The renatured sample was dialyzed against double-distilled water at 4°C overnight. The dialyzed sample was concentrated with PEG 20 000 and purified with glutathione agarose. The purified GST-C/EBP β fusion protein was eluted with the elution buffer (20 mM reduced glutathione, 120 mM NaCl, and 100 mM Tris, pH

8.0), dialyzed against 20 mM Tris (pH 7.5), and stored at -20°C.

We tested the efficiency of NDSB 201 in the renaturation of GST-C/EBP β . We reasoned that correctly folded GST-C/EBP β fusion protein will bind to the glutathione agarose beads and be eluted with the reduced glutathione. As shown in Figure 1A, the eluted GST-C/EBP β analyzed by SDS-PAGE appeared to match the predicted size. To further test the bioactivity of the renatured GST-C/EBP β , we performed the electrophoretic mobility shift assay (EMSA) and the GST pull-down assay. For EMSA, a C/EBP β responsive element (FPII, 5'-TTGTTGC-TCAACATGTTGA-3') derived from the rat pregnancy-specific glycoprotein gene, *rnCGM3*, was used as the probe (3). The radiolabeled FPII fragment was incubated with C/EBP β translated in vitro and the renatured GST-C/EBP β , respectively. As shown in Figure 1B, lanes 3 and 7, a specific DNA-protein complex was formed with both protein samples because unlabeled FPII could compete with complex formation (lanes 2 and 6). Moreover, antibody specific to C/EBP β was able to abolish the formation of the complex between FPII and the C/EBP β translated in vitro or the renatured GST-C/EBP β (Figure 1B, lanes 4 and 8). Antibody specific to GST was also able to prevent the formation of the FPII-GST-C/EBP β complex (Figure 1B, lane 5). Because C/EBP β interacts with the p50 subunit of NF κ B (5), we also performed pull-down assays using the renatured GST-C/EBP β and ³⁵S-methionine-incorporated p50. As shown in Figure 1C, p50 specifically interacted with GST-C/EBP β (lane 3) but not GST (lane 2). These results suggested that preparation of active GST-C/EBP β from inclusion

Figure 1. Functional characterization of renatured GST-C/EBPβ. (A) Purified GST (0.3 μg) and GST-C/EBPβ (0.1 μg) proteins were analyzed by SDS-PAGE and visualized by Coomassie® Brilliant Blue R250 staining. Numbers on the left indicate the protein molecular mass markers in kDa. (B) C/EBPβ EMSA with GST-C/EBPβ and C/EBPβ translated in vitro. Two micrograms of GST-C/EBPβ or 1 μL C/EBPβ translated in vitro was incubated with radiolabeled FPII fragments in EMSA. The competitor oligonucleotide is present at 100-fold excess over the amount of the target DNA. Antibody specific to C/EBPβ (C-19, Santa Cruz Biotechnology, Santa Cruz, CA, USA) or GST (Z-5; Santa Cruz Biotechnology) was preincubated with the reaction mixture for 20 min on ice before the addition of probe. The reaction mixtures were analyzed by electrophoresis on a 5% non-denaturing polyacrylamide gel. The arrow indicates the DNA-protein complex formed. (C) Pull-down assay with ³⁵S-methionine-incorporated p50. Approximately equal molar GST (lane 2) or GST-C/EBPβ (lane 3) was incubated with ³⁵S-methionine-incorporated p50 and glutathione agarose beads in the binding buffer (20 mM HEPES, pH 7.5, 100 mM NaCl, 0.5% NP40, 5 mM MgCl₂, 0.1 mM EDTA, 100 μg/mL BSA, and 1 mM DTT) at 4°C for 2 h. The reactions were terminated by washing with the binding buffer eight times and analyzed by SDS-PAGE and fluorography. Numbers on the left indicate the protein molecular mass markers in kDa. One-tenth of the p50 used in each reaction was shown in lane 1. As a control, lane 4 is the reaction containing only labeled p50 and glutathione agarose beads.

bodies is facilitated with NDSB 201. Table 1 shows the yield from a 500-mL culture. As shown, the yield of the functional GST-C/EBPβ is 47.6% of the total expressed proteins. We have also compared the efficiency of NDSB 201 with other NDSBs (NDSB 195 and 256) on the preparation of active GST-C/EBPβ. We found that NDSB 195 indeed resulted in a better yield than NDSB 201 or 256 of the correctly folded GST-C/EBPβ (data not shown). However, NDSB 201 is sufficient to meet our experimental purposes in terms of cost effectiveness. Therefore, we suggest testing the efficiencies of different NDSBs in the refolding of the protein of interest in a pilot study before a large-scale purification is performed.

REFERENCES

1. Ausubel, F.M., R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidman, J.A. Smith, and K. Struhl. 1992. Current Protocols in Molecular Biology. Greene Publishing Associates/Wiley-Interscience, New York.
2. Benetti, P.H., S.I. Kim, D. Chaillot, M. Canonge, T. Chardot and J.C. Meunier. 1998. Expression and characterization of the recombinant catalytic subunit of casein kinase II from the yeast *Yarrowia lipolytica* in *Escherichia coli*. Protein Expr. Purif. 13:283-290.
3. Chen, H., B. Lin, C.L. Chen, P.F. Johnson, and J.Y. Chou. 1996. Role of the transcription factor C/EBPβ in expression of a rat pregnancy-specific glycoprotein gene. DNA Cell Biol. 14:681-688.
4. Frangioni, J.V. and B.G. Neel. 1993. Solubilization and purification of enzymatically active glutathione S-transferase (pGEX) fusion proteins. Anal. Biochem. 210:179-187.
5. LeClair, K.P., M.A. Blonar and P.A. Sharp. 1992. The p50 subunit of NF-κB associates with the NF-IL6 transcription factor. Proc. Natl. Acad. Sci. USA 89:8145-8149.
6. Ochem, A.E., D. Skopac, M. Costa, T. Rabilloud, L. Vuillard, A. Simoncsits, M. Giacca, and A. Falaschi. 1997. Functional properties of the separate subunits of human DNA helicase II/Ku autoantigen. J. Biol. Chem. 272:29919-29926.
7. Vuillard, L., C. Braun-Breton, and T. Rabilloud. 1995. Non-detergent sulphobetaines: a new class of mild solubilization agents for protein purification. Biochem. J. 305:337-343.
8. Vuillard, L., D. Madern, B. Franzetti, and T. Rabilloud. 1995. Halophilic protein stabilization by the mild solubilizing agents non-detergent sulfobetaines. Anal. Biochem. 230:290-294.

This work was supported by grants to H.C. from Academia Sinica of Taiwan. Address correspondence to Hungwen Chen, Assistant Professor, Institute of Biological Chemistry, Academia Sinica, Nankang, Taipei 115, Taiwan. e-mail: hwchen@gate.sinica.edu.tw

Received 12 June 2000; accepted 5 September 2000.

Yichuen Chong¹ and Hungwen Chen^{1,2}

¹National Taiwan University

²Academia Sinica

Taipei, Taiwan