Purifying natively folded proteins from inclusion bodies using sarcosyl, Triton X-100, and CHAPS

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We describe a rapid, simple, and efficient method for recovering glutathione S-transferase (GST)- and His6-tagged maltose binding protein (MBP) fusion proteins from inclusion bodies. Incubation of inclusion bodies with 10% sarcosyl effectively solubilized >95% of proteins, while high-yield recovery of sarcosyl-solubilized fusion proteins was obtained with a specific ratio of Triton X-100 and CHAPS. We demonstrate for the first time that this combination of three detergents significantly improves binding efficiency of GST and GST fusion proteins to glutathione (GSH) Sepharose.

Proteins are usually engineered to be overexpressed in Escherichia coli as fusion proteins, commonly with glutathione S-transferase (GST) (1). His6 tag (2,3), small ubiquitin-like modifier (SUMO) (4,5), thioredoxin (6), and maltose binding protein (MBP) (3,7). These fusion systems promise increased solubility of target proteins, except for the His6 tag, with single-step purification efficiency. However, many recombinant proteins, especially those of eukaryotic origin, aggregate or become packaged into inclusion bodies (8–11). Refolding recombinant proteins from inclusion bodies can be challenging and yields of correctly folded proteins can be low (2,12).

We observed a number of proteins that readily formed inclusion bodies in E. coli, even with optimized conditions. The problem is amplified when the cells are grown in M9 minimal media, which is required to isotonically label proteins for nuclear magnetic resonance (NMR) and x-ray crystallographic studies. The increased insolubility when using M9 minimal media compared with Luria Bertani (LB) media may be the result of differences in the cellular environments of overexpressed proteins, or may be protein-specific (13,14).

Lysis buffers containing 0.3–2% sarcosyl (Cat. no. 61207-5000; Acros, Morris Plains, NJ, USA) have been used to solubilize GST and other proteins expressed in bacteria grown in LB media (10,11,15–17), but are less effective when using minimal media. Three cysteine-rich zinc binding domains (RING, Bbox1, and Bbox2) from Midline1 (MID1), a microtubule-associated ubiquitin E3 ligase, readily form inclusion bodies when expressed as GST fusion proteins in M9. We found these typical amounts of sarcosyl were insufficient to solubilize these GST fusion proteins and a related MBP fusion protein, MBP-RBCC (RING-Bbox-coiled-coil domains of MID1) (Figure 1A). Therefore, we tried higher percentages of sarcosyl (up to 10%) in the lysis buffer, and obtained 40–70% of the GST fusion proteins in the solubilized extract based on the intensities of the protein band following SDS-PAGE. However, we determined that soaking the insoluble pellet (containing essentially 100% insoluble GST fusion RING, Bbox1, Bbox2, or MBP-RBCC) from 5–10 g lysed cells in 2 mL ST buffer (50 mM Tris, 300 mM NaCl, 5 mM ZnCl2, 10 mM β-mercaptoethanol) with 10% (w/v) sarcosyl for 6–24 h effectively and efficiently solubilized >95% of the proteins from the pellet (Figure 1, A and B). We found 10% sarcosyl to be optimal because higher concentrations were too viscous, leading to difficulty in subsequent purification steps. The solubilizing effects of the sarcosyl actually decreased when the concentration of sarcosyl was >10% (data not shown). This simple approach of using 10% sarcosyl was effective in solubilizing at least six different proteins tested, all of which formed inclusion bodies even when fused to His6-MBP and His6 tag. Of note, we observed that the majority of other proteins found in the pellet were also solubilized with 10% sarcosyl (Figure 1, A and B), suggesting that this methodology is broadly applicable.

Even though sarcosyl can solubilize GST fusion proteins, purifying these proteins in the presence of the detergent can be challenging and difficult (12). Consistent with previous reports (10,11,15), we observed that GST and our GST fusion proteins could not be affinity-purified even in 0.3% sarcosyl. To overcome the problem of high sarcosyl concentrations, the 10% sarcosyl–solubilized pellet solution was diluted with the lysate to yield a 2% sarcosyl solution, or to 1% with a variety of common buffers. In each case, solubility of the overexpressed protein was maintained, although at lower sarcosyl concentrations (<1%), some proteins began to precipitate. Proteins in the soluble extract with 2–10% sarcosyl can be stably stored at 4°C for a week before affinity purification.

To facilitate efficient glutathione (GSH) Sepharose (Cat. no. 17-5132-01; Sigma-Aldrich, St. Louis, MO, USA) affinity purification, 4% Triton X-100 and 40 mM CHAPS were added to the 2% sarcosyl solution (Figure 1C). To the solution that contained 1% sarcosyl, the addition of 2% Triton X-100 and 20 mM CHAPS resulted in similar binding efficiency (data not shown). While this ratio was used in these studies, 3% Triton X-100 and 30 mM CHAPS also worked well (data not shown). All three detergents resulted in significantly greater binding of GST to the GSH Sepharose (Figure 1C). Both Triton X-100 and CHAPS had a greater synergistic effect with sarcosyl than either alone, and neither worked alone to yield solubile GST fusion RING, Bbox1, or Bbox2 proteins.

To understand how these detergents work, we performed kinetic assays with GST (Figure 2A). The activity of purified GST (horse liver; Sigma Aldrich) at a concentration of 0.01 unit/µl in phosphate buffer (100 mM KH2PO4, 1 mM EDTA, pH 6.5) was assayed by its conjugation of GSH to 1-chloro-2,4-dinitrobenzene (CDNB), according to manufac-
In the presence of all three detergents. Adding either Triton X-100, or CHAPS had no effect on the NMR spectra of free Bbox2 or Bbox2 with 1% sarkosyl.

Figure 2. Native folding of detergent solubilized proteins. (A) The enzymatic activity of purified GST (from horse liver) was measured in the presence of sarkosyl with increasing amounts of Triton X-100 and CHAPS to emphasize how GST behaves in the three detergents. Colorimetric changes, as result of formation of a GSH-CNDB adduct, was measured at 340 nm. (B) Superposition of a portion of the $^{1}H$-$^{15}N$ HSQC spectra of $^{15}N$-labeled MID1 Bbox2 in the presence (red) and absence (green) of 1% sarkosyl. The full spectrum in the absence of detergent is shown in panel C (green). The weaker signals of the spectrum in red indicate slower Bbox2 tumbling rates, mostly likely due to sarkosyl molecules encapsulating the protein. (C) Superposition of the $^{1}H$-$^{15}N$ HSQC spectra of Bbox2 in the presence (red) and absence (green) of 1% sarkosyl, 2% Triton X-100 and 20 mM CHAPS. Bbox2 remained natively folded in the presence of all three detergents. Adding either Triton X-100, or CHAPS had no effect on the NMR spectra of free Bbox2 or Bbox2 with 1% sarkosyl.
not shown). The spectra of these proteins in the presence of 1% Sarkosyl, 2% Triton X-100, and 20 mM CHAPS were similar to natively folded GST and MBP. It is important to note that proteins denatured by urea, guanidine hydrochloride, or heat could not be refolded with just these three detergents. An intrinsically unstructured protein also remained unstructured in the three detergents (data not shown).

As the ingredients for minimal media are relatively expensive and yet essential for isotopically labeling proteins for structural studies, it is important to maximize, in milligrams amounts, the yield of soluble folded protein. We therefore tested the protocol with one His6-tagged and seven His6-MBP fusion proteins, including one that contained two disulfide bonds. Incubation of the His6-tagged FMN/NAD-dependent trehalose oxidoreductase from Sinorhizobium meliloti (His6-Thub) with either 5% or 10% Sarkosyl resulted in >75% (Figure 1D) and >95% soluble protein (data not shown), respectively. Subsequent dilution to 1% Sarkosyl enabled efficient affinity purification of His6-Thub with Ni2+ resin (Cat. no. 30410; Qiagen, Valencia, CA, USA). Similarly, the interleukin binding protein with disulfide bonds was also successfully folded in the presence of all three detergents (data not shown). While some of the His6-MBP fusion proteins required the pellets to be incubated with 10% Sarkosyl, others were soluble with 1% Sarkosyl in the lysis buffer. Even though the His6-MBP fusion proteins could be purified with Ni2+ resin in the presence of 1% Sarkosyl, the addition of Triton X-100 and CHAPS increased the binding. Based on our NMR spectra of solubilized protein purified in this manner, we believe it is important to have all three detergents to maximize yields.

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**Competing interests**

The authors declare no competing interests.

**References**


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