A quick procedure for purification of functional recombinant proteins over-expressed in *E.coli*

Philippe Pognonec, Hiroyuki Kato, Hideki Sumimoto, Marcus Kretzschmar and Robert G. Roeder
Department of Biochemistry and Molecular Biology, Rockefeller University, 1230 York Avenue,
New York, NY 10021, USA

Submitted September 11, 1991

Expression of cloned cDNAs in *E. coli* is a widespread means for obtaining recombinant proteins in large quantities. However, a major inconvenience of the system is that, even though over-expressed, the proteins of interest rarely exceed 20% of the total proteins. Painstaking chromatographic procedures and/or potentially detrimental renaturation steps are thus commonly utilized in order to get a native product sufficiently purified for functional analysis. We present here a high salt lysis and fractionation procedure which makes it possible to obtain purified proteins in soluble native form in about 2 hours. This procedure relies on the observation that most over-expressed proteins are more susceptible to precipitation by ammonium sulfate than are bacterial proteins, probably because of the facilitation of homoaggregate formation by the predominant protein species. It also takes advantage of low temperature growth conditions (which excludes the use of expression systems relying on thermal induction) that usually limit inclusion body formation and increase significantly the proportion of soluble recombinant proteins. The proteins partially purified according to this method have been used successfully in functional assays. However, if purification to near homogeneity is required, a single chromatographic step past this stage appears to be sufficient in most cases.

Cultures are grown and induced at 28°C, in our case using the T7 expression system developed by Studier *et al.* (1). After collection, the bacterial pellet from a 200 ml culture is resuspended in 5 ml of ice-cold lysis buffer (20 mM TRIS pH 7.4, 500 mM NaCl, 10% glycerol, 1 mM EDTA, 1 mM PMSF, 5 µg/ml leupeptine, 1% v/v Aprotinin (Sigma), 0.1% NP40). While kept in an ice-water bath, the lysate is then subjected to sonication (Branson sonifier with microtip, maximum setting) for 20 cycles of 30 second sonication/30 second gentle swirling. The sonicated sample is subjected to two cycles of quick freezing in liquid nitrogen and subsequent thawing in a 37°C water bath. This preparation is centrifuged at 4°C for 10 minutes at 10,000 g. The supernatant is diluted with an equal volume of ice-cold water, and saturated ammonium sulfate is added dropwise with constant mixing to a final volume three times the initial supernatant (resulting in a 33% saturated ammonium sulfate solution). After 15 minutes on ice, the sample is centrifuged at 4°C for 10 minutes at 10,000 g. The pellet is resuspended in 1 ml of lysis buffer by gentle pipetting, centrifuged in a microfuge

for 5 min at 4°C, and the supernatant is diluted with 4 ml of lysis buffer devoid of NaCl, thus bringing the final NaCl concentration to 100 mM.

ACKNOWLEDGEMENTS

This work was supported, in part, by Public Health Service Grants CA42567 and AI27397 to R.G.R., and by the Pew Charitable Trusts to The Rockefeller University. P.P. was supported by an EMBO postdoctoral long-term fellowship, H.K. by a fellowship from the Cancer Research Institute.

REFERENCES

1. Studier, F.W., Rosenberg, A.H., Dunn, J.J. and Dubendorff, J.W. (1990) *Methods Enzymol.* **185**, 60–89.
2. Pognonec, P. and Roeder, R.G. (1991) *Mol. Cell. Biol.* **11**, 5125–5136.
3. Selby, J.M. and Peterlin, B.M. (1990) *Cell* **62**, 769–776.
4. Sumimoto, H., Ohkuma, Y., Sinn, E., Kato, H., Shimasaki, S., Horikoshi, M. and Roeder, R.G. (1991) *Nature* In press.
5. Meyer, R., Hatada, E.N., Hohmann, H.P., Haiker, M., Bartsch, C., Röthlisberger, U., Lahm, H.W., Schlaeger, E.J., Van, L.A. and Scheidereit, C. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 966–970.

Figure. Typical purifications obtained with: A, 43 kDa rUSF (2); B, H₂Tat-coat protein (3); C, TFIIIEβ (4); D, 50 kDa NFκB subunit (5). MW: Molecular Weight markers: 98, 67, 43, 31, 22, 15 kDa. Lane 1: crude supernatant; Lane 2: (NH₄)₂SO₄ supernatant; Lane 3: resuspended (NH₄)₂SO₄ pellet. Arrow heads point to over-expressed products. Lanes of each panel are directly comparable. All cDNAs were expressed in T7 expression vectors from the pET family (1).