BENCHMARKS

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

Preparative Electrophoresis: An Improved Method for the Isolation of Human Recombinant Apolipoprotein A-I

BioTechniques 23:778-779 (November 1997)

Protein expression is a powerful technique to study the functional role of proteins both in vivo and in vitro. Various systems have been established to produce proteins in milligram quantities; however, the purification of many expressed proteins commonly results in trace amounts. We report on an improved method for the isolation of apolipoprotein A-I (apoA-I) using the Model 491 Prep Cell (Bio-Rad, Munich, Germany). This system has been previously used for similar protein isolation problems (3,7). We expressed apoA-I using a baculovirus system in Chinese hamster ovary (CHO) cells and in *E. coli* in excess (8). Apolipoproteins such as apoA-I are amphiphilic, and their purification is known to be difficult because of their amphipathic properties. Several purification methods have been used for isolating apoA-I (1,2,8,9); however, none of these isolation procedures has succeeded in separating proteins with the difference of 2 kDa in their molecular weight. Our method combines the advantages of easy handling, high efficacy and rapid purification.

Human recombinant apoA-I was expressed in transfected CHO cells containing the human metallothionein II promoter (4,5). Briefly, cells were cultured in Ham's F-12, grown to 80% confluency, washed extensively with phosphate-buffered saline (PBS) and then cultured in serum-free medium (CHO-S-SFM II; Life Technologies, Berlin, Germany) for 70 h. ApoA-I expression was induced using 70 μ M ZnSO₄. Medium was harvested, centrifuged and concentrated 20-fold by ultrafiltration with Ultrafree®-15 (Millipore, Bedford, MA, USA).

We used an improved procedure for large-scale preparation of recombinant proteins, the Model 491 Prep Cell, which enables preparative sodium do-

decyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). A 5-cm-high 12% (30:0.8 acrylamide/bisacrylamide) separating gel was poured into the 28-mm-diameter tube of the used gel apparatus. A 0.5-cm-high 3.5% stacking gel was loaded on the top of the separating gel. The concentrated expression medium containing 150 μ g protein was diluted 2:1 with sample buffer (252 mM Tris-HCl, pH 6.8, 40% glycerol, 8% SDS, 0.01% bromophenol blue [BPB] and 15% β -mercaptoethanol) and incubated for 5 min at 95°C. Sample (2.0 mL) was loaded. Running time was 6 h at constant power (12 W). The elution buffer (25 mM Tris-HCl, 200 mM glycine, 0.1% SDS) was pumped at 0.8 mL/min. By the time the BPB reached the bottom of the gel, 3-mL fractions were collected. Samples were analyzed by 15% SDS-PAGE and visualized by silver staining (Novex, San Diego, CA, USA) and by immunoblot analysis, using a polyclonal affinity-purified goat anti-human apoA-I primary antibody. We used a biotin-labeled, affinity-purified rabbit anti-goat IgG (Kirkegaard & Perry, Rockville, MD, USA) as a secondary antibody. The blot was stained with 4-chloro-1-naphthol after incubation with a streptavidin-biotinylated horseradish peroxidase complex (Amersham International plc, Bucks, England, UK). Figure 1 illustrates the silver-stained SDS polyacrylamide gel of collected fractions. The apoA-I appears in the fractions 15–25 (28 kDa). This was confirmed by immunoblot analysis (not shown). Isolated fractions were dialyzed against 50 mM NH₄HCO₃ extensively, lyophilized and stored at -80°C for further analysis. This isolation procedure was very reproducible. The efficiency of the apoA-I yield was 50%–70%; therefore, this method is able to purify apoA-I in a single step from crude extract.

As shown in Figure 1, this preparative electrophoresis method shows a high-resolution capacity in separating proteins. Proteins with a difference as low as 2 kDa in their molecular weight can be separated by this method. Under optimized conditions, we were able to separate the commonly seen 26-kDa apoA-I fragment from the 28-kDa intact apoA-I (Figure 2). To separate the fragments of apoA-I, a degraded apoA-

I sample was loaded on the gel, and electrophoresis was performed as described above. Collected fractions were analyzed by SDS-PAGE (Figure 2) and immunoblot analysis (Figure 3). The purified 26-kDa fragment of apoA-I appears in fraction 24 at a concentration of 0.2 µg/mL. Purified intact apoA-I (28 kDa) appears in fractions 34–38 at concentrations of 1–2 µg/mL.

Compared with other preparative electrophoresis methods, the Model 491 Prep Cell has better resolution in separating proteins and does not require the additional step of electroelution. This method is applicable for the purification of almost any type of protein, resulting in an increased purification ef-

ficiency compared to column chromatography and ultracentrifugation.

In summary, this report describes an improved method for the isolation of recombinant apoA-I that has been used satisfactorily in our laboratory for protein structure function studies. This method is easy to perform, saves time and is very efficient. It can be used under both native and denaturing conditions. After optimization, this preparative procedure separates proteins with a 1–2-kDa difference in size. The use of the Model 491 Prep Cell is a significant improvement to previously described methods for purifying apoA-I and may also be applicable for the isolation of other recombinant proteins.

Figure 1. Silver-stained SDS polyacrylamide gel (12%) of eluted fractions using the Model 491 Prep Cell. The loaded samples represent medium of CHO cell-expressed recombinant human apoA-I. Mw: molecular weight standard; C: concentrated cell culture supernatant; lanes 3–18: fractions 1, 5, 10, 15, 20, 25, 35, 40, 45, 55, 62, 68, 75, 82, 90, 97 and 105; S: purified human high-density lipoprotein (HDL) standard.

Figure 2. Silver-stained SDS polyacrylamide gel (15%) of eluted fractions. Separation of the 26-kDa fragment from the 28-kDa intact apoA-I. Mw: molecular weight standard; C: concentrated cell culture supernatant; lanes 4–12: fractions 1, 20, 24, 26, 28, 32, 34, 36 and 38.

Figure 3. Immunoblot analysis of SDS-PAGE products (Figure 2) of eluted fractions after separation of fragments from apoA-I using the Model 491 Prep Cell. Mw: molecular weight standard; C: concentrated cell culture supernatant; lanes 4–12: fractions 1, 20, 24, 26, 28, 32, 34, 36 and 38.

REFERENCES

1. Brissette, L., N. Cahuzac-Bec, M. Desfor- ges, J.-L. Bec, Y.L. Marcel and E. Rassart. 1991. Expression of recombinant human apolipoprotein A-I in Chinese hamster ovary cells and *Escherichia coli*. *Protein Expr. Purif.* 2:296-303.
2. Hughes, T.A., M.A. Moore, P. Neame, M.F. Medley and B.H. Chung. 1988. Rapid quantitative apolipoprotein analysis by gradient ultracentrifugation and reversed-phase high performance liquid chromatography. *J. Lipid Res.* 29:363-376.
3. Li, Z., C.D. Smith, J.R. Smolley, J.H. Bridge, J.S. Frank and K.D. Phillipson. 1992. Expression of the cardiac Na⁺-Ca²⁺ exchanger in insect cells using a baculovirus vector. *J. Biol. Chem.* 267:7828-7833.
4. Mallory, J.B., P.J. Kushner, A.A. Protter, C.L. Cofer, V.L. Appleby, K. Lau, J.W. Schilling and J.-L. Vigne. 1987. Expression and characterization of human apolipoprotein A-I in Chinese hamster ovary cells. *J. Biol. Chem.* 262:4241-4247.
5. Remaley, A.T. and J.M. Hoeg. 1995. Polarized secretion of apoA-I and apoA-II by transfected MDCK cells. *J. Lipid Res.* 36:407-413.
6. Schmidt, H.H.-J., A.T. Remaley, J.A. Stonik, R. Ronan, A. Wellmann, F. Thomas, L.A. Zech, H.B. Brewer, Jr. and J.M. Hoeg. 1995. Carboxy-terminal truncation alters apolipoprotein A-I in vivo catabolism. *J. Biol. Chem.* 270:5469-5475.
7. Treuheit, M.J., A. Ataei, E.T. Wallick and T.L. Kirley. 1993. Purification of the alpha and beta subunits of (Na,K)-ATPase by continuous elution electrophoresis. *Prep. Biochem.* 23:375-387.
8. Weinberg, R., C. Patton and B. DaGue. 1988. Analytic and preparative separation of apolipoproteins A-I, A-II, and A-IV by reverse phase high pressure liquid chromatography. *J. Lipid Res.* 29:819-824.
9. Weisweiler, P., C. Friedl and M. Ungar. 1987. Isolation and quantitation of apolipoproteins A-I and A-II from human high-density lipoproteins by fast protein liquid chromatography. *Clin. Chim. Acta* 169:249-254.

Address correspondence to Hartmut H.-J. Schmidt, Abteilung Gastroenterologie und Hepatologie, OE 6852, Medizinische Hochschule Hannover, D-30623 Hannover, Germany.

Received 11 December 1996; accepted 8 May 1997.

**Hartmut H.-J. Schmidt,
Janine Genschel, Regina Haas
and Michael P. Manns**
*Medizinische Hochschule
Hannover
Hannover, Germany*