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


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 μM 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 dodecyl 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 μg 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, lyophylized 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 efficiency 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.

REFERENCES


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