Whole Gel Eluter Purification of Crosslinked High Density Lipoprotein and ApoA-I and ApoA-2

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Introduction

Oxidation of human high density lipoprotein (HDL) by the tyrosyl radical results in a series of crosslinked species containing the major apolipoproteins (apos) of HDL, apoA-I and apoA-II. We were interested in purifying each of these crosslinked species in order to assess their abilities to modify cholesterol efflux from cultured cells. We were not successful in separating and purifying them using techniques such as size exclusion HPLC, reversed-phase HPLC, and anion-exchange FPLC®. Tube gel preparative electrophoresis was moderately successful, but was time-consuming and tedious. SDS-PAGE combined with the Bio-Rad Whole Gel Eluter, however, allowed effective separation and collection of the different protein complexes, along with good recovery, without being excessively laborious.

Methods

Preparative electrophoresis was performed with 5 mg of protein from tyrosyl radical-oxidized HDL in a volume of 500 µl, mixed with 250 µl of sample buffer (60 mM Tris-HCl, 2% SDS, 0.1% bromophenol blue, and 25% glycerol). The sample was incubated in a 100 °C water bath for 5 minutes prior to loading into a single preparative well. SDS-PAGE was performed using a 140 x 160 x 1.5 mm 12% gel, at a constant current of 35 mA, for 4–4.5 hr. The running buffer was 25 mM Tris, 192 mM glycine, and 0.1% SDS. Non-delipidated HDL particle proteins migrated with an identical pattern to lipid-free apolipoproteins.

The gel was then placed into the Whole Gel Eluter and eluted at 200 mA for 30 minutes to recover the protein. The elution buffer was 60 mM Tris, 40 mM CAPS, pH 9.4. To assess recovery, the gel was stained with 0.25% Coomassie® blue after elution, and showed virtually no protein remaining. The 30 collected fractions were analyzed for content and purity by SDS-PAGE on 7–20% gradient gels, followed by silver staining, under the same running conditions as stated above.

Since we needed to test the biological activity of our samples, they were concentrated and passed over a 5 x 1.5 cm AG® 11 column, which removed both free and protein-bound SDS. The subsequent cholesterol efflux experiments done with these proteins were published in the *Journal of Biological Chemistry* on July 10, 1998.[1]

Silver stain of fractions collected from a typical elution from 7–20 % gradient gel.

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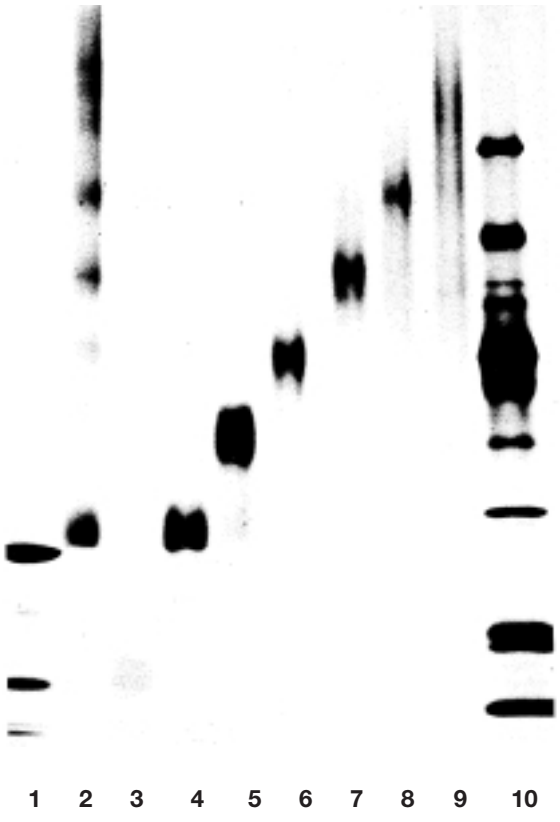

Pooled protein fractions from multiple Whole Gel Eluter runs. Lane 1, control HDL; lane 2, tyrosyl radical-oxidized HDL; lanes 3–9, individual apolipoprotein species isolated from tyrosyl radical-oxidized HDL (MW ranges from approximately 17 to 100 kDa); lane 10, nonreduced low molecular weight standards.

Suggestions

1. When filling the elution chamber, ensure that the channels are equally filled, and fill just enough so that the channels are completely full, but the ridges of the channels are still visible above the surface of the elution buffer. Overfilling past this point makes it difficult to dry the raised areas containing the aspiration ports prior to covering them with the sealing tabs, and causes excessive leakage during elution.
2. When using the template to cut the gel, cut off both left and right sides rather than one side only, since this reduces channel contamination due to the "smile effect".
3. Loading prestained standards in a narrow well next to the preparative well during SDS-PAGE allows monitoring the locations of the proteins of interest as the gel is running, allowing the gel to be stopped at the point of maximum separation of the proteins, even though the actual protein bands in the preparative lane cannot be seen.

References

1 *Wang W. Q., Merriam D. L., Moses A. S. and, Francis G. A., Enhanced cholesterol efflux by tyrosyl radical-oxidized high density lipoprotein is mediated by apolipoprotein AI-AII heterodimers.,* J. Biol. Chem., ***273****, 17391–17398, 1998.*

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