

Improved method for making nondenaturing composite gradient gels for the electrophoretic separation of lipoproteins

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Abstract Nondenaturing gradient gel electrophoresis continues to be used widely for resolution and characterization of lipoprotein subclasses. Methods for making such gels in the laboratory have been published, but occasionally samples do not display uniform mobilities for all lanes in a laboratory-made gel. To help overcome this limitation, we recommend a modification—addition of a sucrose gradient—that significantly improves within-gel variation in protein mobility.—Rainwater, D. L., P. H. Moore, Jr., and I. O. Gamboa. Improved method for making nondenaturing composite gradient gels for the electrophoretic separation of lipoproteins. *J. Lipid Res.* 2004. 45: 773–775.

Supplementary key words gradient gel electrophoresis • lipoprotein separation • human • baboon

Extensive literature supports the hypothesis that knowledge of lipoprotein size distributions provides a more detailed indication of lipoprotein metabolism and a more accurate prediction of cardiovascular disease risk (1). Lipoprotein size distributions have been measured by a number of physicochemical methods, perhaps most commonly by gradient gel electrophoresis. Previously, we have described a highly reproducible method designed to make nondenaturing acrylamide gradient gels for the electrophoretic separation of plasma HDLs (2) and LDLs (3). More recently, we described a composite gradient gel that enabled the simultaneous analysis of both LDLs and HDLs (4). Estimates of peak diameter and fractional absorbance made from the composite gel were strongly correlated with those made using the respective dedicated gel formats (4). Recently, however, we began to notice that the gels have run unevenly on occasion; that is, samples appeared to run different distances depending on location in the gel (generally, samples in the outside lanes

tended to run faster than those in the interior lanes, leading to a “frowning” appearance). The problem was observed in the LDL portion of the gradient and did not seem to affect mobilities of HDLs in the areas of higher acrylamide concentrations.

In a previous study, Rhigetti et al. (5) reported that convective flows are imprinted in the final gel structure, but this effect can be minimized by use of a sucrose gradient during the gelling process. Accordingly, we modified our published protocol and tested whether the addition of a sucrose gradient might solve the problem of differential mobilities for different lanes in a gel.

Composite acrylamide gradient gels were made exactly as described previously (4) except that, to some high limit solutions, we added sucrose (10 g/l). The two types of gels will be referred to as –sucrose and +sucrose. Briefly, the gradient was made with two solutions: a low limit solution (3% acrylamide with 4% cross-linker) and a high limit solution (31% acrylamide with 5.5% cross-linker and $\pm 10\%$ sucrose). Gels were cast in groups of 16 and allowed to polymerize for 3 h prior to storage at 4°C for no more than 1 month before use. Serum and plasma samples from baboons and humans, respectively, were subjected to electrophoresis at 3,000 v · h (using electrophoresis chambers supplied by CBS Scientific) and staining with Sudan black B. These samples were run as part of ongoing studies in this laboratory. Following restoration of the original gel shape and size in the electrophoresis buffer, we subjected the gels to densitometry using an LKB-Ultroscan XL laser densitometer with GelScan XL software.

Studies involving samples from humans were approved by the Institutional Review Board of the University of Texas Health Science Center at San Antonio, and all subjects gave written informed consent. Studies involving samples from baboons were approved by the Institutional

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Animal Care and Use Committee at Southwest Foundation for Biomedical Research, which is certified by the Association for Assessment and Accreditation of Laboratory Animal Care International.

LDLs show considerable interindividual and interspecies variation in mobility. Therefore, to assess within-gel variation, we measured in each lane the relative mobility of a slow refractile band that is observed in both human and baboon samples (6). This refractile band was chosen because its size is similar to that of smaller LDLs (~ 20 nm in diameter), and because its mobility behavior corresponds to that of LDL in the lane. **Figure 1** illustrates this correspondence in lanes from a $-$ sucrose gel having similar size LDLs (the refractile band is designated aMG in the figure). The panel from a $+$ sucrose gel illustrates the considerable interindividual variation of LDL mobility that is often observed.

The refractile band is probably due to a large molecular size protein, such as α_2 -macroglobulin (7). We tested that α_2 -macroglobulin occurs in this location by immunoblotting procedures. A series of different baboon and human samples (loaded in an alternating pattern) were subjected to electrophoresis in a $+$ sucrose gel. Proteins from the gradient gel were transferred to nitrocellulose paper as described (8) and detected using a polyclonal antibody directed against human α_2 -macroglobulin (Fitzgerald Industries International) and horseradish peroxidase-conjugated secondary antibody (Vector Laboratories) with staining by 3-amino-9-ethyl-carbazole and H_2O_2 (9). The antiserum detected a major band in each baboon and human sample; the bands were identical in size (i.e., mobility) and occurred in the location of the refractile band

(data not shown), consistent with the suggestion that the refractile band may in fact be α_2 -macroglobulin (6, 7).

In the present study, we measured the distance (in millimeters) from gel top to the refractile band (detected as a small peak in the absorbance profile) in each sample lane across the gel using the GelScan XL software. We made such a measurement for all samples in 200 gels, half $-$ sucrose and half $+$ sucrose gels. To avoid the possibility of chamber-specific consistency, we only made measurements of the lanes from one gel (of four) from each electrophoretic run. The 200 gels were derived from a total of 54 separate gel castings. On average, the 20 nm refractile band ran 28.2 mm (SD = 1.6 mm, $N = 2133$ lanes measured) into the gel in this gradient. Analysis of migration distance in an interior lane from each of the gels revealed no difference between gel types (means were 28.2 mm and 28.0 mm for $-$ sucrose and $+$ sucrose, respectively; $P = 0.56$ by ANOVA). In contrast to the $+$ sucrose gels, however, the $-$ sucrose gels appeared to show a substantially greater range of migration distances across the lanes of a gel. To quantify this difference between gel types, we calculated the difference between the mobility extremes of the refractile band for each of the 200 gels (i.e., within-gel mobility difference). The average within-gel mobility differences for the two types of acrylamide gradients were 1.64 ± 0.08 mm for $-$ sucrose and 0.85 ± 0.05 mm for $+$ sucrose gels. ANOVA indicated this was a significant difference ($P < 0.001$). **Figure 2** shows a frequency histogram of within-gel mobility difference for $-$ sucrose and $+$ sucrose gels and illustrates the approximately 50% improvement when we used sucrose in the gradient.

In conclusion, our extensive experience with the two gel types suggests that the inclusion of a 0–10% sucrose gradient during the formation of an acrylamide gradient generates significantly superior gels in terms of within-gel consistency of mobility for a large refractile protein. **Fig. 1**

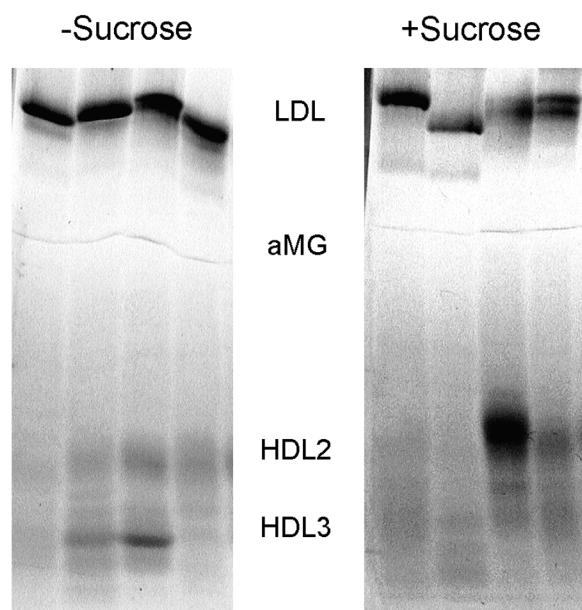


Fig. 1. Human plasma samples run on $-$ sucrose and $+$ sucrose gels. Plasma samples were run as described in the text and detected with Sudan black B. Indicated are the LDL and HDL regions and the faint refractile band (labeled aMG).

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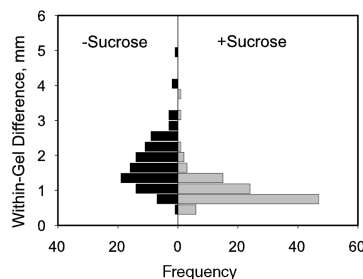


Fig. 2. Frequency histogram for within-gel mobility difference of a 20 nm refractile band occurring in sample lanes from 100 $-$ sucrose (black bars) and 100 $+$ sucrose gels (gray bars). Following electrophoresis, the distance from gel top to the refractile band (detected as a small absorbance peak) in each lane was determined using the GelScan XL software. Within-gel mobility difference was calculated as the difference between the two extremes of migration distance for each of the 200 gels.

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