Rapid Screening of Mini Rotofor® Fractions Using the Experion™ Automated Electrophoresis System

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Introduction

The Experion automated electrophoresis system and Pro260 analysis kit provide sizing and quantitation data for up to ten protein samples in ~30 min, with a detection sensitivity that is similar to Coomassie Blue-stained polyacrylamide gels requiring several hours to run and stain. The Experion system can thus be used to rapidly screen samples obtained from a number of preparative techniques.

In order to take full advantage of the rapid screening afforded by the Experion system, utilizing a sample in its existing buffer is ideal, rather than have to exchange the sample into another buffer prior to analysis. While it is recognized that high salt concentrations compromise the electrokinetic injection and downstream separation of proteins by the Experion system, resulting in low sensitivity or other anomalous results, the effects of extreme pH are not as well understood. Indeed, initial attempts at analysis of fractions generated by liquid-phase isoelectric focusing (IEF) on a mini Rotofor cell, which covered a pH range of 2.5–10, showed that proteins in the fractions at the extreme pHs, particularly those with pH below 5, were not sized correctly. Here, we describe a simple modification to the sample buffer that allows rapid and accurate analysis of protein samples fractionated with the mini Rotofor cell.

Methods

Test Samples:

- Bio-Rad broad range protein ladder (catalog #161-0317)
- Human serum proteins (~25 mg) separated in mini Rotofor cell in IEF buffer (3.5 M urea, 1 M thiourea, 2% CHAPS, 2% Bio-Lyte® ampholytes)

Automated Electrophoresis:

- Separation on the Experion system using the Experion Pro260 analysis kit

SDS-PAGE:

- Separation using Criterion™ 4–20% Tris-HCl precast gels and the Criterion cell. Staining with Bio-Safe™ Coomassie stain and imaging on a GS-800™ calibrated densitometer.

Results

Initial analysis of fractionated human serum with the Experion system showed that protein fractions at the most extreme pH values were not represented correctly following electrophoresis. This effect was most evident in the fractions with pH < 5 (Figure 1). Figure 1 also demonstrates that this effect can be easily remedied. A simple modification to enable correct electrophoresis of proteins in all mini Rotofor fractions is to add 25% by volume of 1.5 M Tris-HCl, pH 8.8 (catalog #161-0798) to the fractions.

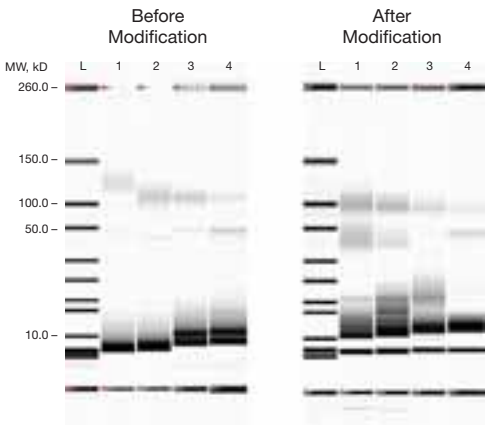

Fig. 1. Analysis of fractionated serum samples on the Experion automated electrophoresis system before and after modification. Simulated gel images of the Pro260 ladder (lane L) and fractions 1–4 (pH 2.6 to 4.2) (lanes 1–4). Note that the 260 kD upper marker (internal marker added to all samples) and 50–125 kD proteins seen in lanes 1 and 2 after modification (and by SDS-PAGE) are not resolved without modification.

Figure 2 shows the effect of addition of various amounts of Tris-HCl, pH 8.8 to fractionated serum samples at pH 2.5. To better visualize the effect of modification, this acidic fraction, which contains little protein relative to others, was spiked with a broad range protein standard. Various amounts of 1.5 M Tris-HCl, pH 8.8 were added. Without this addition, these samples exhibited poor separation and detection of high molecular weight proteins on the Experion system. However, addition of >0.25 M Tris allowed separations most similar to those of the ladder diluted in water and the best resolution of high molecular weight proteins.

To demonstrate the effectiveness of the modification for more complex protein samples, human serum from either normal or diabetic patients were fractionated on a mini Rotofor cell and the 20 fractions collected were analyzed; the fractions covered a pH range of 3–11. The fractions were separated and analyzed by SDS-PAGE (Figure 3) and with the Experion system (Figure 4). For analysis with the Experion system, 1 µl 1.5 M Tris-HCl, pH 8.8 was added to each 3 µl sample. Results from the first ten fractions, containing the bulk of the protein, are shown to illustrate the effective separation and detection of the internal 260 kD marker in all of these samples.

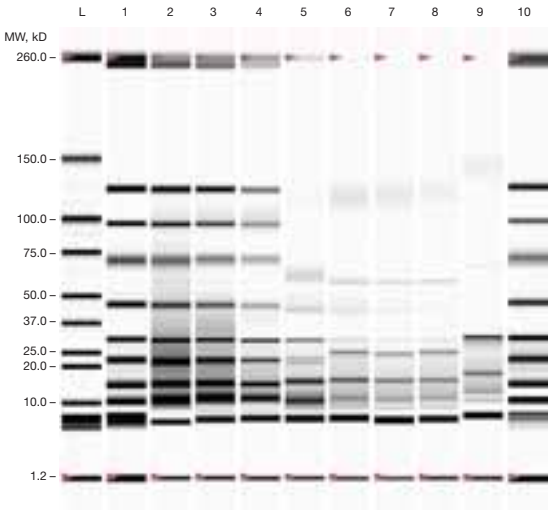

Fig. 2. Simulated gel image generated by the Experion system showing the effect of increasing amounts of Tris-HCl, pH 8.8 on the separation of a broad-range protein standard. Lane L, separation of the Pro260 ladder, lanes 1 and 10, broad-range standard diluted in water; lanes 2–8, standard in IEF buffer (pH 2.5) with Tris-HCl, pH 8.8 added to final concentrations of 0.37 M, 0.25 M, 0.17 M, 0.11 M, 0.07 M, 0.05 M, 0.03 M; lane 9, standard in IEF buffer, pH 2.5.

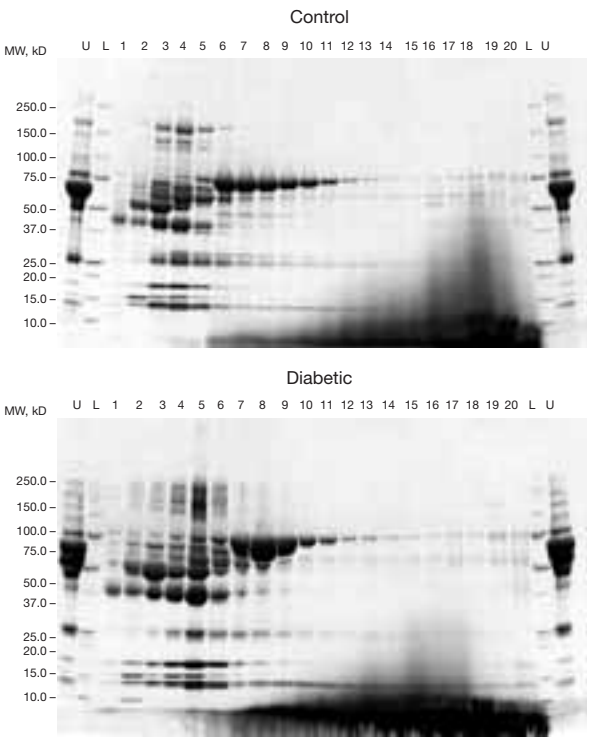

Fig. 3. SDS-PAGE analysis of fractions obtained from separation of human serum from control and diabetic patients on the mini Rotofor cell. Lanes U, unfractionated serum; lane L, Precision Plus Protein™ standards; lanes 1–20, fractions 1–20, respectively.

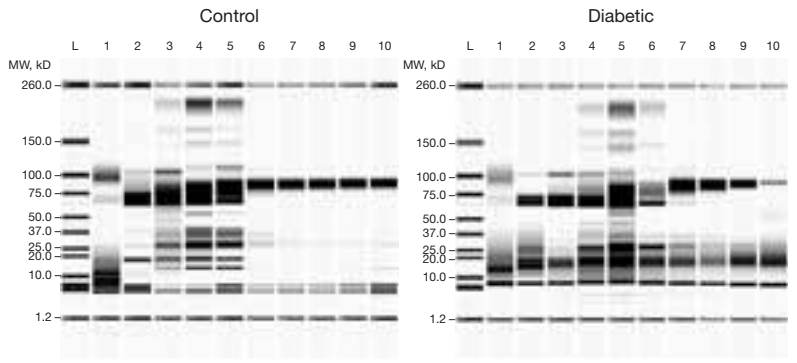

Fig. 4. Experion analysis of fractions obtained from separation of human serum from control and diabetic patients on the mini Rotofor cell. Lanes L, Pro260 ladder; lanes 1–10, fractions 1–10, respectively. Prior to analysis, 1 µl 1.5 M Tris-HCl, pH 8.8 was added to each 3 µl sample.

Conclusions

The Experion system can be used to rapidly screen fractions derived from a mini Rotofor run. The only modification to the fractions required is to adjust the pH by addition of Tris-HCl, pH 8.8 to a final concentration of 0.25 M. With this simple sample modification, fractions from a Rotofor separation can be analyzed in less time with fewer reagents using the Experion system. In addition to its speed, this approach uses only minimal amounts of the fractionated material, leaving the rest available for analysis by 2-D gel electrophoresis, mass spectrometry, or other techniques.