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SHORT COMMUNICATION

High Resolution Continuous Elution Electrophoresis for the Evaluation of Low Abundance Serum Proteins

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SUMMARY

Background: **The evaluation of low abundance biomarkers in the circulating low molecular weight serum proteome is an important source of information. Techniques for sample preparation to remove high abundant proteins and to enrich the low molecular weight fraction are usually required prior to novel biomarker detection.**
Methods: **A continuous elution electrophoresis was used to separate the low molecular weight serum proteins from the high abundance serum proteins, such as albumin and immunoglobulins. Centrifugal concentration, SDS-PAGE, and total protein staining were performed to analyze eluted protein fractions.**
Results: **Consecutive concentrated serum protein fractions demonstrate separation at a high resolution of 1 - 2 kDa below 20 kDa.**
Conclusions: **Continuous elution electrophoresis is an adequate method to eliminate high abundance proteins which interfere with the detection of low abundance biomarkers in the low molecular weight proteome and to enrich its proteins for subsequent detection and clinical evaluation.**
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KEY WORDS

continuous elution electrophoresis, serum biomarkers, low abundance serum proteome

INTRODUCTION

The circulating low molecular weight proteome constitutes a rich source of diagnostic information, as proteins secreted or discharged into the circulation by various organs and tissues represent clinically relevant biomarkers and their determination is a valuable tool for risk stratification, diagnosis, and treatment [1]. The overwhelming majority of the total protein composition is constituted by a limited number of high-molecular weight, high abundant proteins, such as immunoglobulins and albumin. The detection of low molecular weight (LMW) biomarkers is technically challenging, as they are often of low abundance, and their detection is interfered with by the presence of high abundance proteins [2]. Thus, the detection of LMW biomarkers requires prior separation from high abundant proteins and, ideal-

ly, enrichment. Here, we describe a continuous electrophoresis system for the separation and enrichment of LMW serum proteins of low abundance that results in a particularly high resolution of protein fractions of 1 - 2 kDa.

MATERIALS AND METHODS

A PrepCell 491 device (cat. no. 170-2925) was setup according to manufacturer instructions and connected with a BioLogic LP controller (731-8300) as well as a fraction collector (741-002) and a buffer recirculation pump (170-2926, all from BioRad). A large gel tube assembly (170-2933, BioRad, unless stated otherwise) was used for the casting of a preparative SDS-PAGE separating gel of 10 cm height and 14% acrylamide monomer concentration, followed by a 4% stacking gel of 1 cm height. The gel was allowed to polymerize OVN at RT, with a buffer cooled in an ice-bath recirculating through the core at 70 mL/minute. Two hundred µL human serum, corresponding to 14.56 mg total protein, were mixed with 200 µL Laemmli-buffer containing β-mercaptoethanol and denatured for 5 minutes at 95°C on a thermo-block. After complete assembly of the PrepCell with the gel assembly, upper and lower electrophoresis chamber, elution chamber with supporting frits and dialysis membrane, and loading of electrophoresis buffer (tris/glycine/SDS-buffer), the sample was loaded on top of the stacking gel. The PrepCell was placed into a chromatography refrigerator at 4°C (Tritec 602-1, Hannover, Germany) and the electrophoresis was started at 150 VDC, with a maximum of 40 mA and 20 W. A buffer was recirculated through the cooling core and the lower electrophoresis chamber. The migration front of the sample was visible as a blue, even, horizontal disc in the gel. After 5 hours, the migration front reached the bottom of the gel, and the elution was started at a flow rate of 1 mL/minute. Consecutive fractions of 13 mL were collected in 15 mL tubes automatically by the fraction collector. The assembled system is shown in Figure 1. The fractions were loaded into 15 mL centrifugal filters with a MWCO of 3 kDa (Vivaspin 15R, Sartorius) and centrifuged at 2,300 g for 2.5 hours, after which the concentrate volume had reached < 200 µL. A precast Mini-Protean TGX 4-20 % Stain-free Gel (456-8093) was assembled in an electrophoresis cell (165-8033) and, after mixing with 10 µL Laemmli buffer and denaturation, 15 µL of the concentrate or 0.7 µL human serum were loaded as well as 1.5 µL of protein standards for molecular weight determination (161-0377). The SDS-PAGE was performed at 180 V constantly for approximately 35 minutes and total protein image- as well as multichannel image-acquisition (for the detection of the fluorescent molecular weight marker) was done with the ChemiDoc MP Imaging System (170-8280). The molecular weights of the bands were determined with the Image Lab Software (Version 5.2.1, build 11).

RESULTS

SDS-PAGE and total protein staining of consecutive concentrated serum protein fractions eluted from the cell demonstrate separation at a high resolution of 1 - 2 kDa below 20 kDa (Figure 2A) The bands are more intense than their counterparts in non-separated human serum, as they eluted from a volume of 200 µL serum (14.56 mg protein), as opposed to 0.7 µL (50.96 µg protein) of the non-separated serum (Figure 2B). Further, a new band at 16.4 kDa, which is not present in the non-separated serum, is observed in the concentrate of fraction 19 (Figure 2B). The weak bands of higher molecular weight, which are visible in the SDS-PAGE analysis of fractions 9 to 13, and in fraction 19, are protein fractions eluted from the supporting frits of the dialysis membrane, and do not actually belong to the sample. Washing of the supporting frits between PrepCell runs with TBST further reduces these bands. It was noted that a failure of the buffer recirculation through the lower electrophoresis chamber, or an increase in the temperature of the buffer, caused an irregular elution of the protein fractions with a significant reduction in the resolution of the separation. It is also noteworthy that the UV-detector, with which protein fractions could supposedly be monitored during elution, did not detect the protein fractions demonstrated in Figure 2. Re-use of electrophoresis buffer reduced the mean current during a run, resulting in a significantly longer time required for the migration front to reach the bottom of the gel. The parts which connect the tubes between the compartments of the PrepCell are susceptible to damage due to minor stress, it is therefore advisable to keep replacements.

DISCUSSION

The separation and fractionation of human serum by this continuous electrophoresis system constitutes an adequate method to eliminate high abundant proteins, and to enrich low abundant proteins of the LMW proteome. This procedure of serum sample preparation is valuable, as it enables and improves subsequent detection of biomarkers by various analytical techniques, such as Western blotting, immunoprecipitation, mass spectrometry and/or microplate-based assays. Without prior separation by continuous electrophoresis and enrichment by pooling, and depending on the sensitivity of these techniques, low abundant biomarkers would escape detection and then be inaccessible to clinical evaluation.

The evaluation of low abundance biomarkers in the circulating LMW proteome is an important source of information. The present report shows an excellent separation and enrichment of low molecular weight serum proteins not reported elsewhere and supports recommendations of continuous elution electrophoresis as an adequate method to eliminate high abundance proteins

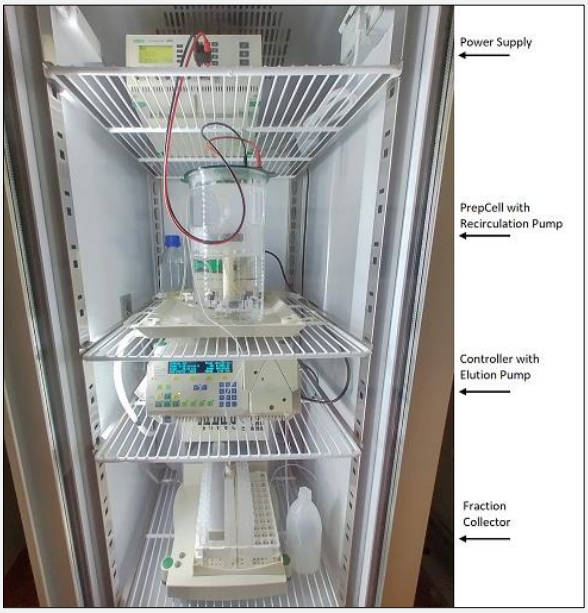

Figure 1. The high resolution continuous preparative electrophoresis system consists of a power supply, the preparative electrophoresis cell (PrepCell) with a buffer recirculation pump, a controller with an elution pump, and a fraction collector, all set up in a chromatography refrigerator.

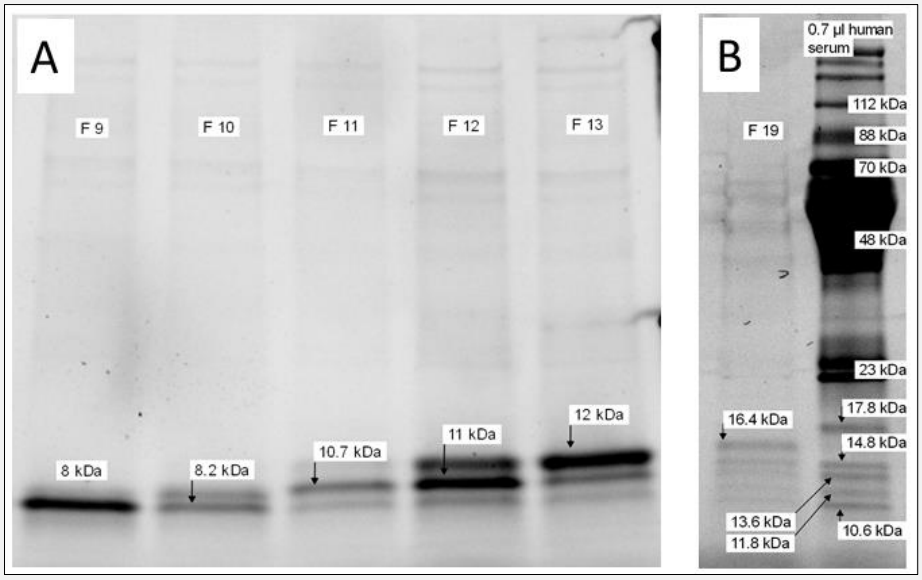

Figure 2. A: Evaluation of protein fractions obtained by continuous elution electrophoresis by SDS-PAGE and total protein staining. Consecutive concentrated serum protein fractions (F = fraction 9 - 13) eluted from the cell demonstrate separation at a resolution of 1 - 2 kDa below 20 kDa. B: F19 demonstrates the 16 kDa fraction of 200 µL (14.56 mg total protein) human serum. The comparison of the band pattern between fraction 19 and 0.7 µL (50.96 µg) non-separated human serum demonstrates almost complete removal of high abundance proteins and a new band of 16.4 kDa in fraction 19. In non-separated serum, immunoglobulin light chains are at approximately 23 kDa, and heavy chains are at 48 kDa.

which interfere with the detection of low abundance biomarkers in the LMW proteome and to enrich LMW proteins for subsequent detection and clinical evaluation [3,4]. It is likely that this technique will help to discover new biomarkers as well as aid in the detection and quantitative determination of known biomarkers for which no assay exists.

Ethics Statement:
The study was conducted in accordance with the ethical standards of the WMA Declaration of Helsinki and its ethical principles for medical research involving human subjects. An anonymous residual blood sample, obtained after performing routine laboratory tests, was used. The procedure was exempt from Title 45, Title 21 and HIPAA/IRB/consent requirements (receiver and/or the study investigator cannot link private information to the individual from whom the material was obtained) and written informed consent and ethical approval was therefore waived by the IRB of the Paracelsus Medical University.

Data Availability:
High resolution image source files were deposited at the Harvard Dataverse and can be accessed via:
https://doi.org/10.7910/DVN/1LZIHT

Declaration of Interest:
The authors declare that no potential conflict of interest with respect to the research, authorship, and publication of this article exists.

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