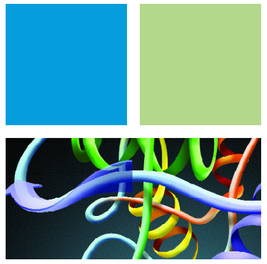

ProteoMiner™ Technology Enriches Interleukins and Low-Abundance Proteins From Tissue Leakage for Serum Proteome Studies

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Introduction

Human serum contains interleukins and proteins, such as retinol binding protein and myelin basic protein, from tissue leakage. These proteins are often present at very low concentrations ranging from several picograms to just a few nanograms per milliliter of serum. The status of these proteins reflects the physiological condition of the human body and these proteins may be used as disease biomarkers for clinical diagnosis or prognosis. However, it is extremely difficult to detect them due to the presence of high-abundance common serum proteins, such as albumin, immunoglobins and their complements. ProteoMiner is a protein enrichment technology that uses a combinatorial hexapeptide library bound to a chromatographic support to remove high-abundance serum proteins and enrich low-abundance proteins.

To determine whether ProteoMiner technology could facilitate the detection of tissue leakage proteins in serum, we made an artificial serum by mixing 17 serum proteins (albumin, IgG, haptoglobin, retinol binding protein, myelin basic protein, troponin, IL-8, IL-2, etc.) representing the common serum proteins, tissue leakage proteins, and interleukins at the concentrations reported in normal human serum. This artificial serum sample was fractionated by the ProteoMiner technology. The proteins in the ProteoMiner bead-bound fraction were presented for either 1- and 2-D gel electrophoresis followed by mass spectrometric (MS) protein identification or for Western blotting to detect the low-abundance proteins, using the unfractionated artificial serum as control.

Methods

The proteins selected for this study are listed in Table 1.

All proteins were purchased from Sigma-Aldrich and mixed as stock solutions to result either in equimolar sets of 500 ng/ml of each protein or in a set with concentrations close to physiological levels in human blood, ranging from 50 mg/ml to 10 pg/ml (see Table 1 for details).

Table 1. The serum proteins used in the artificial serum and their concentrations.

Lane Number	Protein	pI	Molecular Mass, kD	Concentration in Serum, pg/ml	Concentration in Equimolar Mixture, pg/ml
1	Albumin	3.5–4.5	68	5×10^{13}	5×10^{8}
2	IgG	5.0–10.0	150	1×10^{10}	5×10^{8}
3	Fetuin	5.2	39	1×10^{10}	5×10^{8}
4	Apo-Transferrin	6.9	75	5×10^{7}	5×10^{8}
5	A1-antitrypsin	5.3	45	2×10^{7}	5×10^{8}
6	A2-macroglobulin	6.0	160	2×10^{7}	5×10^{8}
7	Haptoglobulin	6.1	45	1×10^{7}	5×10^{8}
8	Retinol binding protein	5.0	16	5×10^{5}	5×10^{8}
9	Ferritin	5.7	20	2×10^{5}	5×10^{8}
10	Myoglobin	7.2	17	5×10^{4}	5×10^{8}
11	Thyroglobulin	5.4	303	3×10^{4}	5×10^{8}
12	C-peptide	3.4	3.0	5×10^{3}	5×10^{8}
13	Myelin basic protein	11.2	18	5×10^{3}	5×10^{8}
14	Troponin	9.8	25	1×10^{3}	5×10^{8}
15	IL-8	9.1	11	100	5×10^{8}
16	Calcitonin	8.0	3.6	30	5×10^{8}
17	IL-2*	6.8	4.7	10	5×10^{8}

* This is a mixture of IL-2 and albumin (65 kD) but only albumin could be detected.

ProteoMiner Protocol

1 ml of sample was treated with ProteoMiner beads according to instructions. The sample was mixed with the beads and vortexed for 2 hr, washed with PBS buffer, and eluted with 3 x 100 µl acidic urea CHAPS buffer. The procedure was repeated four times so that a total of 5 ml of artificial serum was fractionated.

1-D Electrophoresis

Criterion™ Tris-HCL 4-20%, 12+2 well gels were used for 1-D electrophoresis of the stock solutions of each protein, the 1-D run of the crude and artificial serum treated with ProteoMiner, and for Western blots.

2-D Electrophoresis

For all 2-D runs, 100 µg of sample was loaded on 11-cm ReadyStrip™ IPG strips 3-10 NL, followed by a second dimension using Criterion 4-20% gel. Flamingo™ fluorescent gel stain was used to visualize the proteins prior to fluorescent imaging on a Molecular Imager® PharosFX™ imaging system.

Results

Figure 1 shows all 17 protein samples run on a 1-D gel with a loading concentration of 0.5 µg per lane. Although purity is estimated to be above 80% for all samples, significant byproducts are visible. Figures 2 and 3 show 2-D electrophoresis of the same proteins in artificial serum mixed either in concentrations close to physiological levels (Figure 2) or in equimolar amounts (Figure 3). Figure 4 represents 2-D electrophoresis of proteins in artificial serum mixed in concentrations close to physiological levels (as in Figure 2) and treated with ProteoMiner beads. This 2-D gel is as complex as a natural serum sample. However, most spots represent albumin fragments as determined by MS.

Figure 5 shows the artificial serum mix with and without ProteoMiner treatment on a 1-D gel and provides clear evidence of the enrichment in the low molecular mass range. Western blot (Figure 6) confirms that retinol binding protein is barely detectable in the artificial mix but generates a sharp band in serum treated with ProteoMiner beads.

Conclusions

- ProteoMiner treatment can detect tissue leakage proteins such as retinol binding protein, which is present at 500 ng/ml
- ProteoMiner treatment results in a ~10x enrichment of low-to-medium abundant proteins
- Future work will demonstrate enrichment of the other tissue leakage proteins in artificial serum mixture through Western blots and 1-D electrophoresis in real serum samples

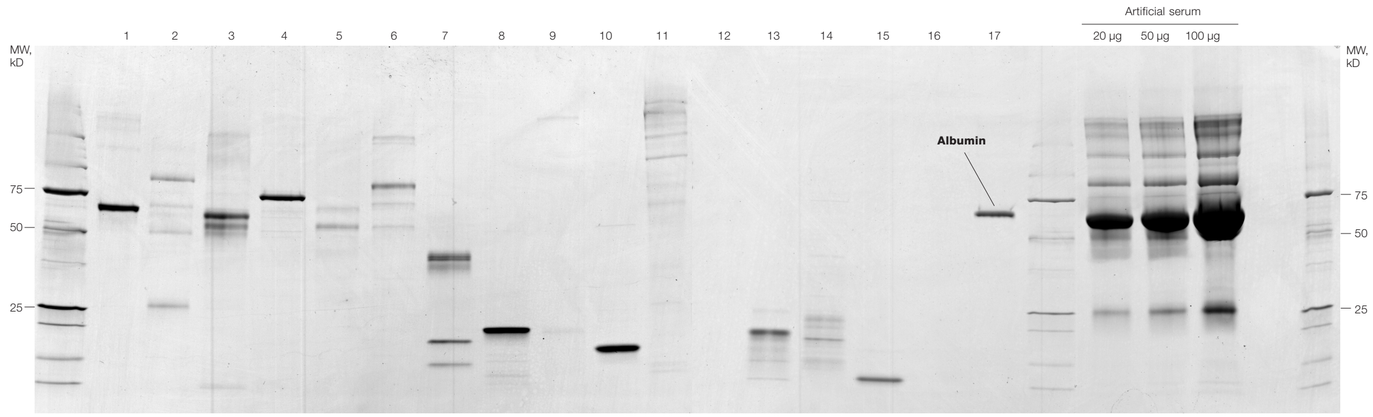

Fig. 1. 1-D electrophoresis of proteins used for the artificial serum mixture. Lane numbers 1-17 correspond to lane numbers in Table 1.

Fig. 2. 2-D electrophoresis of 100 µg of crude artificial serum mixture with concentrations close to physiological levels.

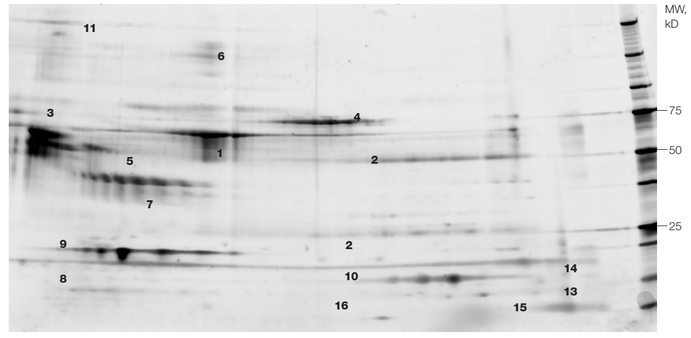

Fig. 3. 2-D electrophoresis of proteins used for the artificial serum mixture in equimolar amount (500 ng each) with 8 µg of total protein.

Fig. 4. 2-D electrophoresis of 100 µg of artificial serum mixture processed with ProteoMiner technology.

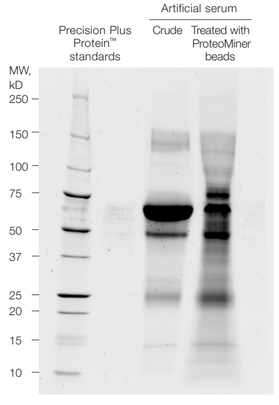

Fig. 5. 1-D electrophoresis of artificial serum mixture, crude or treated with ProteoMiner beads. 5 µg of protein was loaded in each lane.

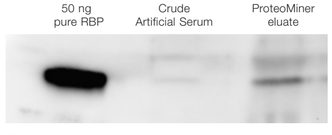

Fig. 6. Western blot with antibody against retinol binding protein. Amounts of protein loaded per lane were 0.8, 100 and 100 µg respectively.