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Identification of human serum proteins detectable after Albumin removal with Vivapure® Anti-HSA Kit

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Introduction

The analysis of the complete proteome is a major interest of many researchers. Of particular importance are human bodily fluids such as serum or plasma due to their ease of accessibility and the potential for the identification of disease biomarkers and drug treatment surrogate markers. However, a remaining major challenge in the analysis of blood proteins is the high dynamic range of the protein species, which can show differences of up to 12 orders of magnitude. In serum the primary component is serum albumin, representing 60-72% of the total protein content.

On two dimensional (2D) gels, the presence of albumin has a number of deleterious effects, including masking many proteins located in the area of albumin itself and rendering the detection of low abundant proteins present throughout the gel difficult. The selective removal of albumin would therefore improve the detection of all proteins throughout the gel and provide access to quantification and identification of the lower abundance proteins, commonly accepted to be excellent sources of new biomarkers.

Here, we describe the removal of albumin from human serum with the Vivapure Anti-HSA Kit. This kit is based on unique antibody fragments coupled to a low binding, cross-linked agarose. Using this resin, the albumin is bound efficiently with negligible non-specific removal of other sample proteins. Gel electrophoresis of a human serum sample treated with this kit resulted in the detection of additional and lower abundance proteins compared to untreated sample. A number of these newly detected proteins were then identified by MALDI-TOF mass spectrometry.

Materials and Methods

General method for albumin removal with Vivapure Anti-HSA Kit:

The anti-HSA resin was mixed to get a uniform suspension prior to pipetting. 1 ml of the slurry was transferred to a 2.2 ml tube, centrifuged at 400 x g for 2 min, and the supernatant discarded. 40 µl of human serum was diluted with 460 µl of binding buffer and then added to the tube containing the HSA-affinity resin. The sample / HSA-affinity resin slurry was incubated on a rotary shaker for 30 min at room temperature. The shaker was adjusted to assure gentle mixing of sample and resin. Two clarification spin columns were filled with 500 µl of the incubated sample solution. The sample was centrifuged at 400 x g for 2 min and the flow through (sample depleted of albumin) was collected. Further unbound material was recovered from the resin, by adding 200 µl binding buffer to each spin column. After incubation for an additional 2 min on a rotary shaker, the samples were centrifuged at 400 x g for 2 min. After a second washing step the flow through material was collected and pooled.

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Preparation of samples prior to 2D gel electrophoresis

The samples were concentrated and desalted with Vivaspin 500 concentrators, 10 MWCO. The albumin depleted sample (900 µl) was concentrated successively with a Vivaspin 500 device by centrifugation at 12,000 x g to reach a final volume of about 90 µl, discarding the flow through. The concentrator was refilled with ultrapure water for desalting and centrifuged to a sample volume of about 90 µl. Finally, this procedure was repeated with 2D gel sample buffer and the samples were concentrated to reach a final volume of 30 µl.

2D gel electrophoresis

Sample buffer was added to the serum samples to yield a final concentration of 7 M Urea, 2 M Thiourea, 70 mM DTT, 2% Ampholyte 2-4 (Serva), 50 mM KCl, 25 mM Tris including protease inhibitors. Approximately 350 µg of total protein was loaded onto each gel. High resolution NEPHGE 2D gel electrophoresis according to O'Farrell [i] was performed similar to the protocol of Klose and Kobalz [ii] using a pH gradient from pH 2-11 in first dimension. A 15% acryl-amide/bisacryl-amide concentration was used for the 20x30 cm size slab gels in the second dimension that ran under SDS-PAGE conditions [iii]. The gels were stained overnight with Sypro® Ruby (Molecular Probes, Leiden, Netherlands) fluorescent stain according to the manufacturer's instructions [iv].

MALDI-TOF analysis

Selected proteins of interest were excised from the gel and digested with sequencing grade modified trypsin (Roche, Mannheim, Germany). Mono-isotopic masses (M+1) of tryptic fragments were measured on an Ultraflex MALDI-TOF instrument (Bruker Daltonics GmbH, Bremen, Germany). The spectra obtained were calibrated against the mass peaks derived from autolytic trypsin fragments. A mass list of the first of each set of mono-isotopic peaks was searched against the NCBInr protein database (National Center for Biotechnology Information, Bethesda, U.S.A.) using Mascot software (Matrix Science Ltd., London, U.K.) [v].

Results and Discussion

The 2D gels of human serum prepared before (Gel A) and after (Gel B) removal of albumin with the Vivapure Anti-HSA Kit are shown below in figure 1.
The albumin depleted serum sample contains only traces of albumin (<5% of the albumin compared to the unprocessed serum) and clearly shows a highly increased resolution of proteins in the high molecular weight range as well as in the acidic area at about 65 kDa. Importantly, the amount of detectable protein spots was enhanced 5 fold, as demonstrated in figures 1, over the gel's entirety, and figure 2 in the region of albumin.

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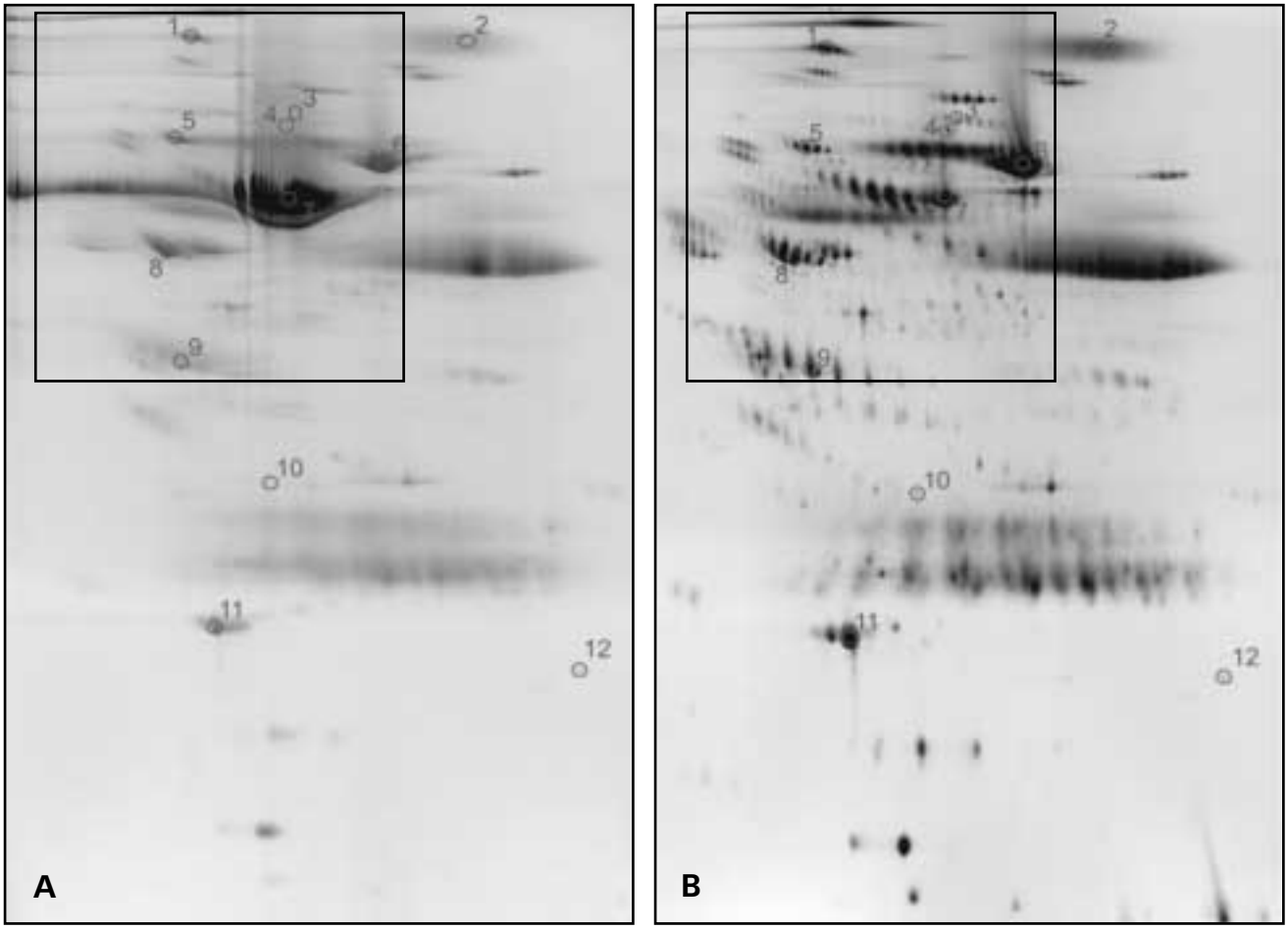

Figure 1:
Eurogentec's high resolution 2D gels of human serum: (A) unprocessed, (B) albumin depleted with Vivapure anti-HSA kit. Numbers indicate protein spots that were excised and identified by MALDI-TOF MS, see Table 1 and [vi] .

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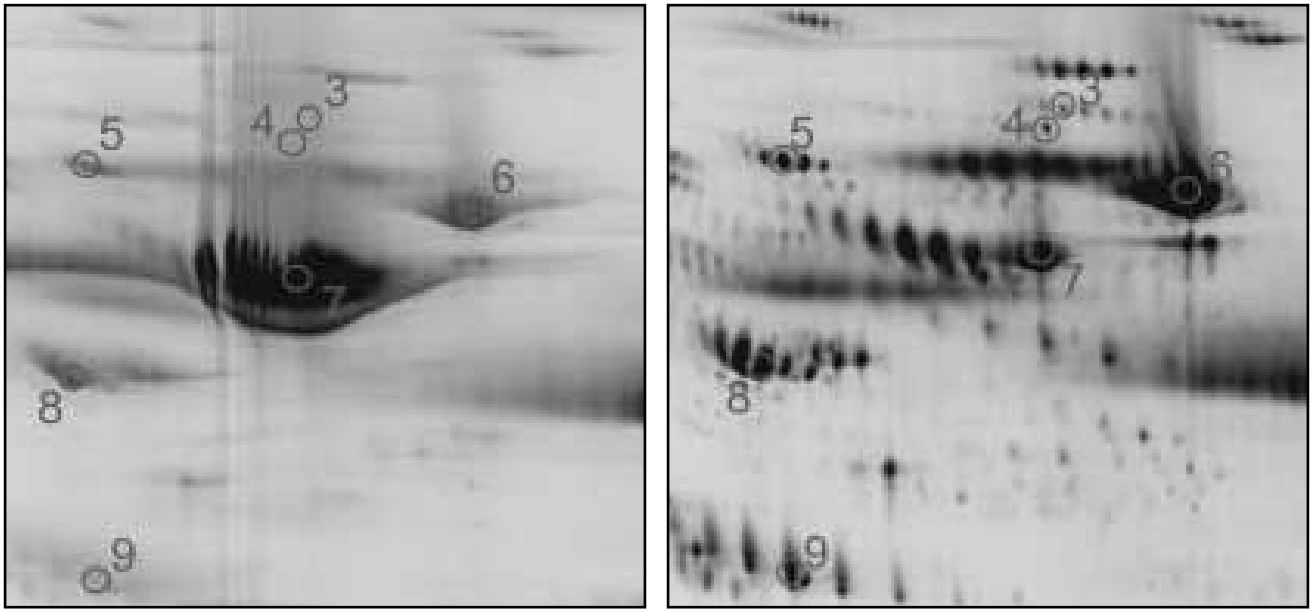

Figure 2:
Enlarged area of the albumin portion of the 2D gels shown in figure 1. Remaining albumin is marked by an arrow in figure 2B.

A number of proteins (circled spots shown in figure 1) were selected for protein identification to demonstrate the effect of albumin removal on the resulting 2D gel [vi]. Identified proteins are listed in table 1.

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Spot	Identification from Vivapure processed gel	Detectability in unprocessed sample
1	Ceruloplasmin	Poorly resolved
2	Lamin B2	Detectable
3	Gelsolin	Not detectable
4	alpha 2 Macroglobulin	Not detectable
5	alpha-1-ß-Glycoprotein	Poorly resolved
6	Transferrin	Detectable
7	Albumin	Detectable
8	alpha-1-Antitrypsin	Poorly resolved
9	Apolipoprotein A3	Poorly resolved
10	Apolipoprotein E	Not detectable
11	Apolipoprotein A1	Detectable
12	Complement component 8, gamma polypeptide	Not detectable

Table 1:
Selected proteins from the albumin depleted serum, identified by MALDI-TOF MS.

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A number of proteins not detected in the untreated sample were detected and identified from the treated sample (see table 1). For example the actin-severing protein gelsolin with a calculated MW of 85 kDa is masked by albumin in untreated human serum gel. After albumin removal it appears clearly resolved. In the basic, low molecular weight portion of the gel the gamma polypeptide of the complement component 8, was identified. C8_ is a member of the lipocalins, a family of proteins that bind small hydrophobic ligands. This protein was not detected in the 2D gel of the unprocessed sample but due to the higher loading capacity for low abundance proteins from samples treated with the albumin removal kit it was identified.

Conclusions

The Vivapure Anti-HSA Kit for Human Albumin Depletion provides a highly specific, antibody fragment based resin that shows negligible cross-reactivity with other human proteins. It provides a fast protocol with easy to use spin columns for parallel processing of e.g. serum, plasma, synovial or cerebrospinal fluid. The kit is very flexible regarding the amount of albumin to be removed due to the usage of resin in combination with spin columns. The quality of the 2D gel results is significantly improved yielding more protein identifications in the area of albumin as well as for low abundance proteins. This kit is anticipated to prove useful in the comparison of samples from diseased and healthy patients for the detection of disease and surrogate biomarkers.

References

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