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Rapid, simple and effective technical procedure for the regeneration of IgG and HSA affinity columns for proteomic analysis

Short Communication

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Summary. In plasma and serum, the presence of high-abundance proteins can overwhelm the signals of low-abundance proteins, which then become undetectable either by two-dimensional gels or chromatographic techniques. Therefore, depletion of abundant proteins is a prerequisite to detect low-abundance components. Furthermore, the regeneration of pre-purification tools could be money-saving. We applied an affinity chromatography kit to remove albumin and the immunoglobulin chains from plasma and propose a simple and effective technical procedure for the regeneration of these affinity columns.

Keywords: Body fluids – HSA/IgG Removal kit – Proteomics – Two-dimensional electrophoresis

Introduction

Proteomics analysis of plasma or serum is influenced by the presence of high abundance proteins such as human serum albumin (HSA) and immunoglobulins (IgG). Removal of these proteins eliminates about 75% of total plasma proteins (Anderson and Anderson, 2002), allowing the visualization and analysis of the remaining proteins by two-dimensional electrophoresis or other chromatographic techniques. A variety of depletion methods for the specific removal of these high-abundant proteins have been developed. Björhall et al. (2005) showed that, based on reproducibility and binding specificity, the ProteoExtract HSA/IgG Removal kit® (Merck Biosciences) displayed the best results among disposable (single-use) products. This kit is based on the combination of a new affinity resin which is highly specific for albumin and a unique immobilized protein A polymeric resin, which binds to the Fc region of the IgG. Single-use kits are not very attractive, for costs problems, to small academic laboratories. Reusability of analytical tools is an important prerequisite for kit evaluation.

In this paper, we report a method to regenerate the ProteoExtract HSA/IgG removal affinity columns. We show data on the reproducibility of 2-DE maps obtained using new and recycled columns and on the depletion efficiency of affinity columns after regeneration steps.

Materials and methods

Materials

Immobilized pH-gradient (IPG) strips (24 cm), pH range 4–7, IPG buffer, dithiothreitol and iodoacetamide were purchased from Amersham Biosciences (Uppsala, Sweden). Methanol, acetic acid, tris, glycerol and glycine were from Carlo Erba (Rodano, MI, Italy). Acrylamide and ammonium persulfate were from Eurobio (Courtaboeuf Cedex, France). All other chemicals were from Sigma (St. Louis, MO, USA). HSA and IgG affinity columns were obtained from Merck Biosciences (Nottingham, United Kingdom).

Plasma samples were from human controls, EDTA-treated and were stored at −80 °C until use.

HSA and IgG removal and affinity column regeneration protocol

The ProteoExtract Albumin/IgG Removal kit contains 12 disposable gravity-flow columns pre-packed with 450 ml of a resin mix (albumin-removal resin plus the protein A IgG-removal resin). The resin binding capacity of each column is 0.7 mg IgG and 2 mg albumin.

Before the use, each column was prepared adding 850 µl of sodium phosphate buffer 0.1 M pH 7.5 and allowing it to pass the resin bed by

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gravity flow, following the manufacturer's instructions. Then, 50 µl of plasma were diluted in 400 µl of sodium phosphate buffer 0.1 M pH 7.5, applied to the affinity column, to accomplish the specific binding of HSA and IgG (1st use) and the eluate was collected together to 1200 µl of sodium phosphate buffer 0.1 M pH 7.5, used to wash the column.

For column regeneration, the bound HSA fraction was eluted with 1.2 ml of 2.5 M NaCl in 0.1 M sodium phosphate buffer pH 8.0, whereas the bound IgG fraction was eluted with 1 ml of 0.25 M citric acid. Thereafter, the columns were washed with 2 ml of 0.1 M sodium phosphate buffer pH 7.5 before the 2nd use.

Then, another 50 µl of the same plasma sample were diluted and loaded to the regenerated columns and the sample was processed as described above (2nd use).

After this second use of the columns, the bound HSA and IgG fractions were removed as described, and the columns regenerated another time as described above, for a 3rd use.

Two-dimensional electrophoresis

All depleted plasma samples after each use were treated for 2-DE electrophoresis as follows. Four volumes of ice-cold acetone were added to the samples, then they were incubated on ice for 15 min. The proteins were precipitated after centrifugation for 10 min at 12,000 × *g* in a centrifuge at 4 °C. The supernatant was discarded and the air-dried pellet was resuspended with a solution of 8 M urea, 2% CHAPS, 0.5% IPG buffer, 1% DTT. An amount of 200 µg of proteins was loaded onto 24 cm IPG strips providing a 4–7 pH range on an Ettan™ IPGphor Isoelectric Focusing Unit (Amersham Biosciences, Sweden) for 38 kVh. After IEF completion, the strips were equilibrated for 15 min in a buffer containing 6 M urea, 30% glycerol, 2% SDS, 50 mM Tris–HCl pH 8.8 and 1% DTT, then for 15 min in a similar buffer containing 2.5% iodoacetamide instead of DTT. The second dimension was carried out in an Ettan DALT six Large Vertical Electrophoresis System (Amersham Biosciences), on a 12% polyacrylamide gel. The gels were stained with 0.1% Coomassie Brilliant Blue G250. Electrophoresis was conducted at 75 V constantly for 18 h. The protein patterns in the gels were recorded as digitalized images using an Epson Expression 1680 Pro scanner (Seiko-EPSON Corp., Japan) with 16 bit dynamic range and 300 dpi resolution. Gel image matching was performed using the Proteomweaver® software (BioRad, CA).

Protein assays

Protein concentration of untreated and depleted plasma samples were measured using Bradford assay (Bradford, 1976). HSA and IgG concentrations were determined by turbidimetric methods using the Cobas Fara Analyzer (Roche, Germany).

Results and discussion

We compared 2-DE maps obtained from non depleted plasma samples (A), plasma samples after ProteoExtract HSA/IgG depletion using new columns (B), one time recycled columns (C) and two times recycled columns (D).

On visual examination, 2-DE gels obtained using new and recycled columns appeared to be almost identical and equally depleted (Fig. 1). After spot matching, the following parameters were taken into account to estimate 2-DE gel-to-gel reproducibility: number of detected spots, percentage of spots matched, which is indicative of qualitative differences among gels and coefficient of variation ($CV\% = SD * 100/\text{mean}$) of protein spot intensities, value that reflects the quantitative differences (Barry et al., 2003). We used these parameters in intra- and inter-groups (A–D) comparisons.

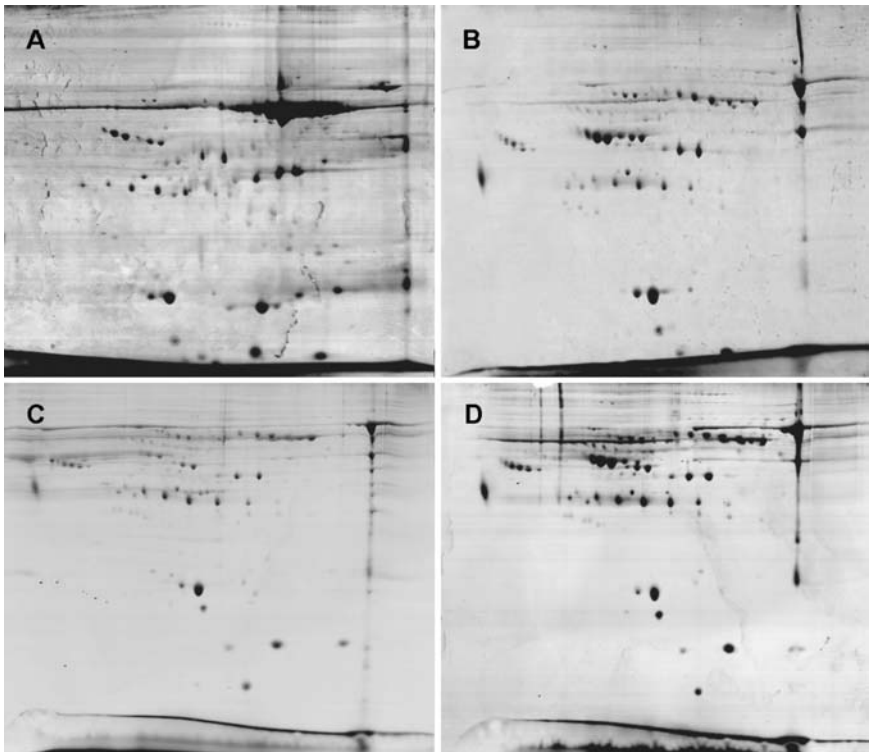

Fig. 1. 2-DE maps obtained from non-depleted plasma samples (**A**), plasma samples after ProteoExtract HSA/IgG depletion using new columns (**B**), one time recycled columns (**C**), or two times recycled columns (**D**)

Table 1. Intra-group analysis data obtained from depleted plasma samples (A), plasma samples after ProteoExtract HSA/IgG depletion using new columns (B), one time recycled columns (C) and two times recycled columns (D)

	A	B	C	D
Number of detected spots (mean ± sem)	73 ± 8	99 ± 10	88 ± 10	100 ± 13
Protein recovery	–	17.9%	16.7%	16.9%
Efficiency of matching (%)	60%	69%	77%	70%
Coefficient of variation (CV)	40.6%	28%	29.3%	26.6%

There was no significant difference between the efficiency of matching and the CV values comparing data obtained from 2-DE gels of depleted plasma using new columns (B), one time recycled columns (C) and two times recycled columns (D) (Table 1).

Both by using new and recycled columns, the protein recovery and the number of detected spots of all depleted plasma were almost the same (Table 1).

For the regeneration of the affinity columns, albumin was eluted with 2 ml of 2.5 M NaCl in 0.1 M sodium phosphate buffer at pH 8.0 and IgG were eluted with 1 ml of 0.25 M citric acid. To investigate the depletion efficiency of affinity columns after regeneration, we determined HSA and IgG concentrations in these solutions.

Due to the high dilution factor, the concentration of IgG in the eluates was below the detection limit of the turbidimetric method, as already observed by Björhall et al. (2005). However, we found the same quantity of HSA in all the eluates, thus suggesting that the depletion efficiency of the affinity columns is well conserved even up to two regeneration steps.

The inter-group comparison was done using all data obtained from groups B, C, and D. 2-DE gels obtained using recycled columns, as compared with those obtained using new columns, showed a similar matching efficiency in the intra-group analysis, but with a small increment (from 28 to 35%) in the spot intensities' CV.

In conclusion, the ProteoExtract HSA/IgG Removal kit can be reused at least twice with a good performance. However, as a safe rule, we would suggest to compare 2-DE gels obtained from the same procedure, and not to compare protein-depleted samples obtained using new columns with protein-depleted samples obtained using recycled columns.

A major advantage of recycling these affinity columns is the possibility to use the same column many times, therefore increasing the proteic amount of the depleted sample, lowering costs.

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