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Short communication

Isolation of serum amyloid A protein by small-scale hydrophobic interaction chromatography and two-dimensional electrophoresis

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Abstract

A recently introduced technique to isolate serum amyloid A protein is hydrophobic interaction chromatography combined with two-dimensional electrophoresis with immobilized pH gradients. A modification of the original version of this technique is presented. Mouse serum was subjected to hydrophobic interaction chromatography on a small scale, and the eluate was applied directly to two-dimensional electrophoresis. Simple electropherogramss with optimal resolution of serum amyloid A protein were obtained. The presented technique facilitates isolation of serum amyloid A protein from small blood volumes, and might also be adapted to alternative applications.

Keywords: Amyloid A protein; Serum amyloid A proteins

1. Introduction

The serum amyloid A proteins (SAA) comprise a protein superfamily, that consists of acute phase and constitutive proteins [1]. Acute phase SAA can be used as a disease marker, and it is considered to be the precursor of amyloid A protein [2]. The latter is the main protein component of amyloid deposits, a serious complication of certain longstanding inflammatory diseases, including chronic rheumatoid arthritis. In mouse, the five SAA isotypes, SAA1, SAA2, SAA3, SAA p*I* 5.9 and SAA p*I* 6.15 have been demonstrated (reviewed in [2]).

A recently introduced strategy to isolate SAA is hydrophobic interaction chromatography (HIC) combined with two-dimensional electrophoresis with immobilized pH gradients (2-D IPG) [3]. The advantages of this technique are small serum volumes [4], and optimal resolution and reproducibility [5], the latter allowing comparative analysis [6]. The original version of the 2-D IPG–HIC technique is based on a minimum of 2 ml of serum and octyl Sepharose. Elution is performed with guanidine–HCl, and dialysis and lyophilization is used. The aim of the present study was to reduce the dimensions of the original 2-D IPG–HIC technique. A rapid and simple two-step purification technique is presented. Compared with the original version of the 2-D IPG–HIC

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technique, novel concepts employing both the chromatography and electrophoresis are presented.

2. Experimental

2.1. Reagents and chemicals

Lipopolysaccharide (*Escherichia coli* serotype 026:B6) was obtained from Difco Laboratories (Surrey, UK). Octyl Sepharose CL 4B was obtained from Pharmacia (Uppsala, Sweden).

2.2. Animals

To induce SAA production, C57BL/6J mice received intraperitoneal injections of 50 μg lipopolysaccharide in 100 μl phosphate-buffered saline (PBS). Control animals received 100 μl PBS only. Animals were sacrificed by intraperitoneal injections of thiopentobarbital 24 h after the injection, and blood was collected by cardiac puncture. Pooled sera from three animals of each group, and individual sera, were analyzed.

2.3. Hydrophobic interaction chromatography

A 500-μl polypropylene micro-centrifuge tube (Treff Lab, Degersheim, Switzerland) was used as a micro-column (Fig. 1). The gel bed consisted of 360 μl thoroughly mixed, pre-swollen octyl Sepharose CL 4B. Prior to sample application, the resin was equilibrated with 2 ml PBS, and 250 μl serum was applied and eluted in individual portions. After sample application, the resin was equilibrated with respectively 2 ml PBS, and 2 ml distilled water, and excess resin buffer volume was aspirated through the membrane until no more liquid could be obtained. Elution was performed with 750 μl 8.9 *M* urea–0.11 *M* dithiothreitol–2.5% Triton X-100–2% 2β-mercaptoethanol–bromophenol blue, and the first 500 μl of the eluate was collected. Eluents were frozen, or subjected directly to 2-D IPG. The resin and nylon membrane were replaced between each chromatographic procedure.

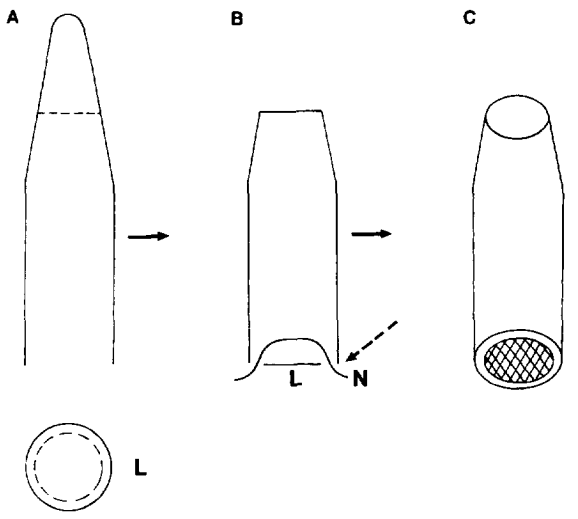

Fig. 1. Schematic representation of the assembly of the micro-column. The tube was used upside down, and a surgical blade was used for excision. Stippled line and arrow indicate points of excision. L=lid, N=nylon membrane. (A) The tube base and lid centre are removed. (B) Nylon monofilament membrane (Sephar, Switzerland) is applied, and the lid is replaced in its original position. Excess nylon membrane is excised along the outer tube circumference. (C) The final version of the column (23×7 mm I.D).

2.4. Two-dimensional electrophoresis, semi-dry blotting and amino acid sequence analysis

Except for the sample composition, these procedures, including silver staining, were performed largely according to the Multiphor II Electrophoresis System manual (Pharmacia 1992), and according to previous experiments [3,7,8]. Immobiline DryStrips were prepared without Triton, acetic acid and ampholytes in rehydration solution. For silver staining, 50 μl HIC eluate was applied per strip, at the anode. For the blotting, 150 or 240 μl HIC eluate was applied. To load 240 μl, two application cups were connected. Immobilon PSQ membranes were used, and transfer was carried out with 10 V/250 mA/5 W/133 mAH per gel. Only those parts of the first dimension gels where SAA was known to be located were used for blotting. Thus, two excised Immobiline DryStrips were applied in parallel on each SDS gel. Five blots of each spot of interest were subjected to N-terminal analysis.

3. Results and discussion

HIC extracted material from pooled, acute phase sera showed five major spots in the pH 6.6–5.5/14 kDa range (Fig. 2). Corresponding spots were seen also in individual acute phase sera, but not in control sera. Direct N-terminal analysis of spots 1, 2 and 3 revealed the seven first positions of the N-terminal mouse SAA sequence [9]. In spot 1, Val and His were found in positions 6 and 7, respectively. In spot 2 and 3, Ile and Gly were found in the same positions. Thus, spot 1 represents mouse SAA1, and spots 2 and 3 represent either SAA2, or SAA p*I* 5.9 [9,2]. The latter isotypes differs only at position 101, where SAA2 contains Ala, and SAA p*I* 5.9 contains Asp. Analysis of spots 4 and 5 yielded no proteins, indicating that these might be N-terminally blocked. These spots might represent yet unidentified, acidic mouse SAA isotypes. To establish the identity of spots 2, 3, 4 and 5 further, complementary amino acid sequence studies are currently being undertaken. Simple, reproducible 2-D electropherograms with excellent resolution of mouse SAA isotypes were obtained, and by incorporating the presented results in a 2-D map, identification of mouse proteins can be enhanced. To our knowledge, the present report is the first description of unmodified eluate being successfully subjected to 2-D electrophoresis. The presented column design allowed rapid flow and aspiration of excess buffer volume, the latter being a crucial factor in the presented technique. Elution was performed with a modified 2-D IPG sample buffer [10], and with volumes correlated to previous experiments [7]. These factors resulted in eluate composition, and protein concentration, highly suitable for the first dimension run. The chromatography could be performed within 15 min, and by avoiding dialysis and lyophilization, the protein loss was minimized.

With respect to the Edman degradation, material from 75 μl serum yielded 50 (spot 1), 30 (spot 2), and 10 (spot 3) pmol SAA per blot. For silver staining and N-terminal analysis, eluents from respectively 25, and 375 μl serum were used. Thus, with the presented material and techniques, 2-D gels and blots containing sufficient material to identify SAA could be prepared within 2 days.

4. Conclusion

A highly efficient purification technique is presented. The methodology facilitates isolation of SAA from small blood volumes, and is of value for characterization and longitudinal studies of SAA in individual specimens where sampling of small blood volumes is pertinent, such as some laboratory animals and paediatric patients. Moreover, the presented

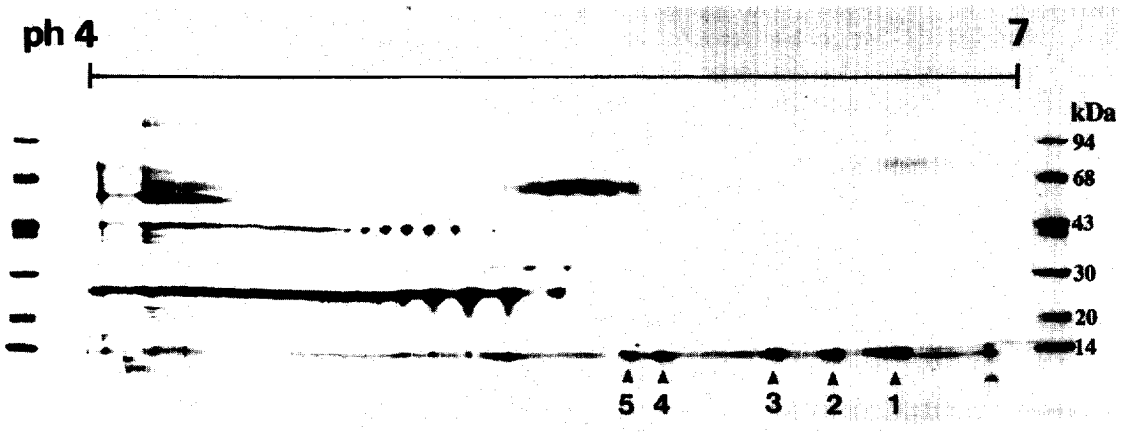

Fig. 2. Photograph of silver stained 2-D gel. Electropherograms shows the separation of HIC extracted proteins from acute phase mouse serum. Immobiline DryStrip pH 4.0–7.0 (18 cm), ExcelGel SDS 8–18 and low molecular mass markers (Pharmacia) were used. Spots of interest are indicated by arrowheads and numbers.

2-D IPG–HIC method can be used to study other hydrophobic proteins, and the presented version can possibly also be modified and adapted to alternative samples, dimensions, and types of affinity chromatography.

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