Serum Amyloid P Component Purification for Imaging of SAA Amyloidosis

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Background

Systemic AA amyloidosis, a serious complication of many chronic inflammatory disorders, is caused by the persistent stimulation and overproduction of acute-phase reactant Serum Amyloid A (SAA) protein. Abnormal polymerization of SAA protein leads to aggregation of insoluble fibrillar protein deposits, which may adversely affect multiple organ systems.[1] In Europe, diagnostic imaging techniques have been developed for AA amyloidosis based on Serum amyloid P component (SAP). SAP, a normal circulating plasma protein universally present in amyloid deposits, can be radioiodinated with I-123 to allow anatomical localization of amyloid in patients by whole-body scintigraphy.[2] With the goal of developing a similar diagnostic tracer for the clinic, SAP was selected for pre-clinical evaluation. In the work described herein, the protein was isolated from human serum and a trial radiolabeling with I-125 was performed.

Chemistry

The purification of SAP protein from human serum was based on previous methods with several modifications.[3] Heat inactivated human sera was mixed with an affinity media in the presence of calcium buffer solution to induce SAP binding. The media was washed exhaustively with buffer before being eluted with EDTA. Additional purification using a preparative size-exclusion column was necessary to achieve the purified product. Purified SAP was radiolabeled with I-125 using the Iodogen method.

Experimental

General. Pooled normal human AB serum was purchased from Innovative Research, Inc. (Novi, MI). Sepharose 4B and a XK50/60 column were purchased from GE Healthcare. A Labscale

tangential flow filtration (TFF) system and 30kDa Pellicon XL device were purchased from Millipore (Billerica, MA). Preparative and analytical TSK3000 gel filtration columns were purchased from Tosoh BioSciences (Montgomeryville, PA). A gel filtration chromatography protein standard was purchased from Bio-Rad (Hercules, CA). NativePAGE, NuPAGE gels and protein standards were purchased from Invitrogen (Carlsbad, CA). Purified SAP was purchased from Lee BioSolutions Inc. (St. Louis, MO) and used as a standard for comparison. Buffer reagents were purchased from Sigma-Aldrich (St. Louis, MO) and used without further purification. A GE Healthcare AKTApurifier FPLC and Beckman Coulter HPLC were utilized for protein purification and characterization, respectively. MALDI-TOF mass spectrometry was performed on a Shimadzu Biotech Axima-CFP Plus. HPLC analysis of the radiolabelled product was performed on a Beckman Coulter HPLC equipped with an in-line IN/US β-Ram Model 4 radiodetector.

Purification of SAP from Human Serum

1. Affinity Chromatography: 1L of sera was heat inactivated at 56°C for 30 minutes and stored at 4°C before use. Sepharose-4B was equilibrated overnight with Tris-NaCl-$CaCl_2$ buffer (0.01M Tris-HCl, 0.15M NaCl, 4mM $CaCl_2$, pH=7.5). Sepharose 4B (1L) in Tris-NaCl-$CaCl_2$ buffer was then mixed with 1L of sera and shaken for 24 hours at 4°C. The sera-Sepharose 4B mixture was loaded into a XK50/60 column and washed with 4L Tris-NaCl-$CaCl_2$ buffer using a flow rate of 100ml/hr until the UV detector reading at 280nm approached 0mAU. The retained protein was eluted from the column with 2L Tris-NaCl-EDTA (0.01M Tris-HCl, 0.15M NaCl, 5mM EDTA, pH 7.5) using a flow rate of 100ml/hr. The protein was concentrated to 40ml by TFF and a concentration of 1.42mg/ml was determined by BCA assay.

2. Preparative Size-Exclusion Chromatography (SEC): A preparative SEC column TSKgel 3000SW (21.5 x 60cm) was used for further purification. A Bio-Rad protein standard was used to determine the retention time of the SAP fraction. A scout run was done and each peak was manually collected and tested on an analytical SEC column to decide which fraction was SAP. Afterwards, 5ml of sample was loaded and collected manually according to the retention time and UV reading. A total of eight purifications were done on the AKTA purifier.

3. Analytical Size-Exclusion Chromatography (SEC): This procedure was done on a Beckman Coulter HPLC using a TSKgel SuperSW3000 (4.6 x 300mm) column. 5ul Bio-Rad protein standard and 5ul commercial SAP was tested for accurate SAP retention time measurement, and compared with our purified sample (Figure 1) after each purification procedure from the preparative SEC column.

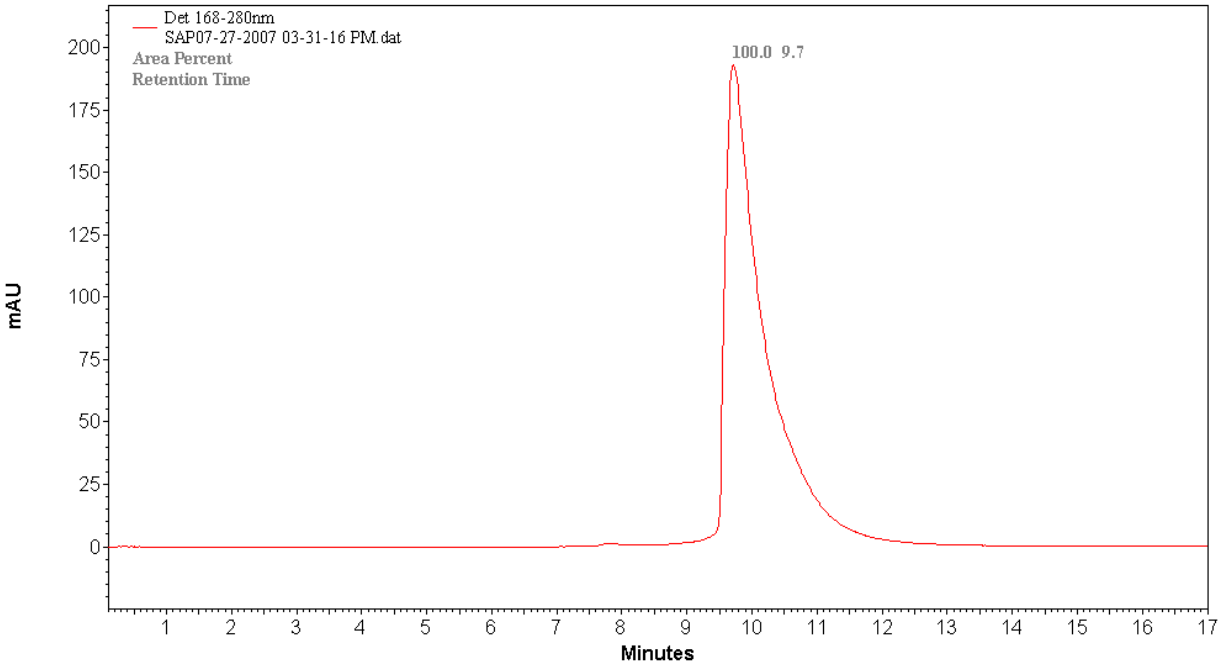

Figure 1. Analytical chromatogram of purified SAP

4. BCA protein assay: Total purified SAP was determined by BCA protein assay using a commercial kit from Pierce. Briefly, the working reagent was prepared by mixing reagents A and B in a 50:1 ratio. 2ml of working reagent was then mixed with either 100ml of bovine serum albumin standard or purified SAP followed by incubation of the samples at 37C for 30mins. Bovine serum albumin standards were prepared by serial dilution between 2mg/ml to 25ug/ml. The UV absorbance at 562nm was read from Spectrophotometer and it allows the protein quantification. 9.04mg of SAP was purified after the gel filtration chromatography.

5. Native and SDS gel characterization of purified SAP: Purified SAP together with commercial SAP were evaluated by Native-PAGE and SDS-PAGE. For NativePAGE, 20ul of sample in

sample buffer was loaded and the samples were run at 150V, 12mA for 90mins. A total of 5ul Nativemark unstained protein standard was used for MW determination. For SDS-PAGE, 20ul of samples in sample buffer with reduced reagents were heated at 95C for 5 minutes before loading and the samples were run at 200V, 120mA for 35 minutes. 5ul of Novex sharp pre-stained protein standard was used for MW determination. Identical band patterns were observed on both NativePAGE (Figure 2) and SDS-PAGE (Figure 3) for our purified and commercial SAP. A single band at 25KD was seen on SDS-PAGE.

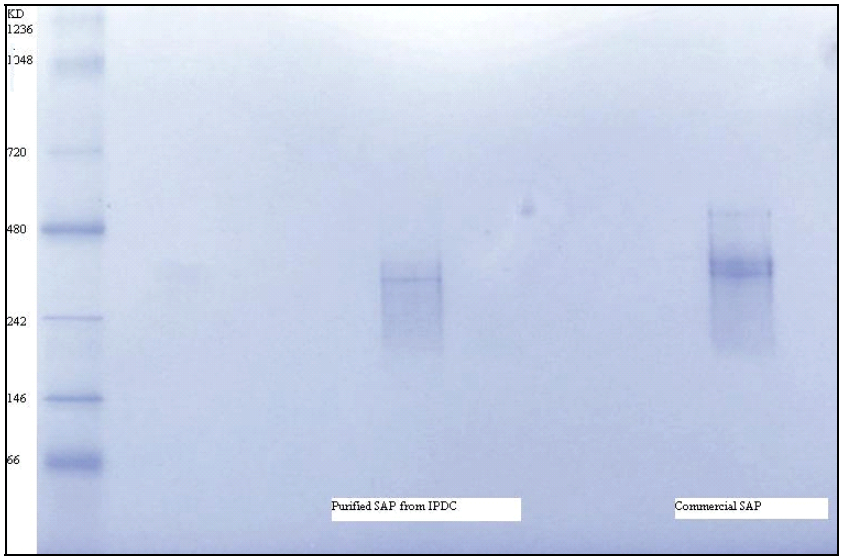

Figure 2. Native-PAGE of purified SAP

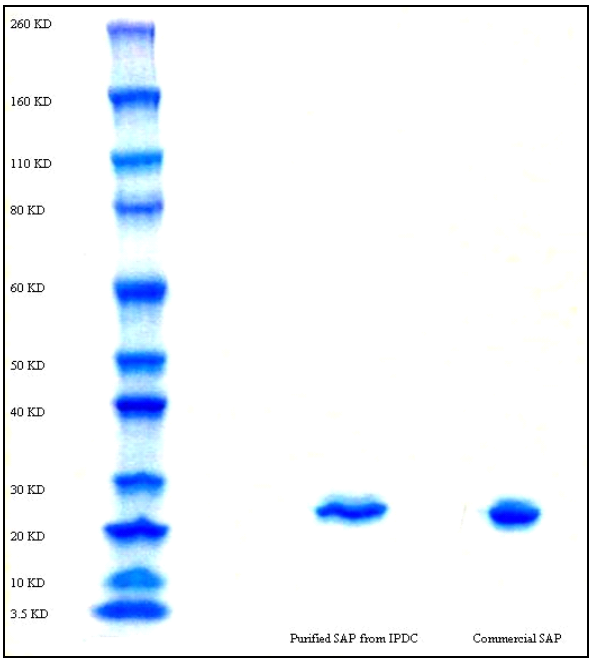

Figure 3. SDS-PAGE of purified SAP

6. MALDI-TOF: AXIMA from SHIMADZU was used for measuring the exact molecular weight of SAP (Figure 4) and sinapinic acid was used as the matrix for MALDI-TOF testing. Briefly, 1ul of a mixture of matrix with protein samples were loaded onto each well of a MALDI-TOF plate and allowed to dry at room temperature. The positive linear mode was used and a peak of 25 kD was observed at a power of 92.

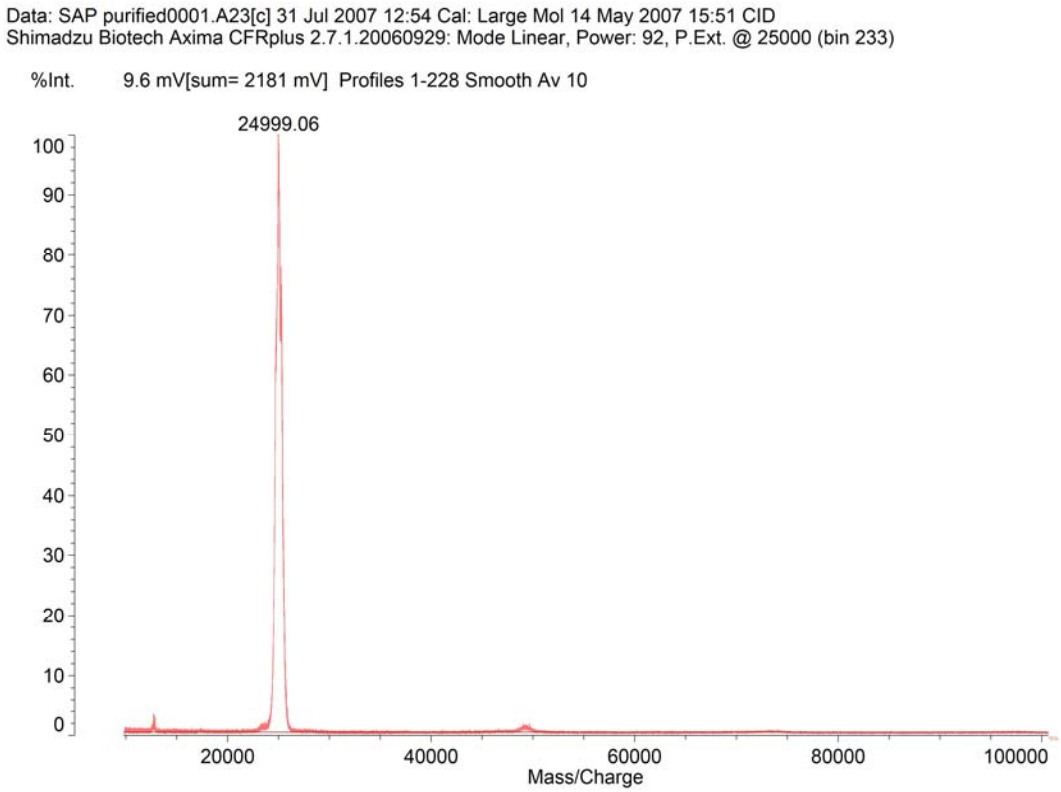

Figure 4. MALDI-TOF MS spectrum of purified SAP

I-125 Radiolabeling of SAP.

To an iodogen coated tube, 100uL of SAP (1.42 mg/mL) was added, followed by 700µCi of sodium iodide (I-125). The reaction was carried out for 15 minutes and then quenched using 30uL of saturated tyrosine solution. The labeled protein was eluted in ten 1mL fractions using a PD-10 desalting column, which was equilibrated with Tris/NaCl/EDTA buffer. 1% BSA was added to the solution to prevent radiolysis during storage. The radiochemical yield was 30% and the radiochemical purity was over 90%.

References

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