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Short Communication

Rapid and simple method for the determination of α_1-acid glycoprotein in serum by column liquid chromatography

Satoshi Kishino, Zhai S. Di, Mitsuru Sugawara, Ken Iseki and Katsumi Miyazaki

Department of Pharmacy, Hokkaido University Hospital, School of Medicine, Hokkaido University, Kita-14-jo, Nishi-5-chome, Kita-ku, Sapporo 060 (Japan)

Shigeru Kakinoki, Akikazu Nomura and Akira Kitabatake

Department of Cardiovascular Medicine, School of Medicine, Hokkaido University, Kita-15-jo, Nishi-7-chome, Kita-ku, Sapporo 060 (Japan)

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ABSTRACT

A rapid and simple method for the determination of α_1-acid glycoprotein (AAG) in serum was developed by using an anion-exchange column for clean-up of serum and a hydroxyapatite column for high-performance liquid chromatography (HPLC). A good correlation was observed between this HPLC method and the conventional radial immunodiffusion method. The method may also be used to determine the AAG concentration in the serum of experimental animals.

INTRODUCTION

The serum glycoprotein α_1-acid glycoprotein (AAG) is an acute-phase reactant that increases following cancer [1], myocardial infarction [2] and congestive heart failure [3]. Several investigators have observed that AAG binds to a number of basic antiarrhythmic drugs, including lidocaine [4], quinidine [5], propranolol [6] and disopyramide [7]. The rapid determination of AAG in serum is therefore essential for the investigation of rapid changes in AAG levels and/or the relationship between serum-unbound drug fractions and pharmacological effects during treatment.

Several methods for the assay of AAG in plasma or serum have been reported. Radial immunodiffusion utilizing antibody against AAG has been widely used to determine AAG in serum [8 - 12] because of its high specificity. This method, however, is time-consuming and is not easily applicable to experimental animals, as the antibodies against AAG of animals are not commercially abailable. Sugiyama *et al.* [13] reported a simple fluorimetric method for serum using a cationic

Correspondence to: Dr. K. Miyazaki, Department of Pharmacy, Hokkaido University Hospital, School of Medicine, Hokkaido University, Kita-14-jo, Nishi-5-chome, Kita-ku, Sapporo 060, Japan.

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fluorescent dye, auramine O. Although this method has the advantage of requiring only a short time (at most 20 min), a tendency to underestimate AAG concentrations was reported. Funae *et al.* [14] reported a chromatographic separation of AAG from α_1-antitrypsin in plasma by high-performance liquid chromatography (HPLC) using a hydroxyapatite column. This method, however, requires dialysis and affinity chromatography for the clean-up of plasma and separation of AAG and α_1-antitrypsin, and is time-consuming in clinical use. In this paper, we describe a rapid and simple method for the determination of AAG in serum by using an anion-exchange column for clean-up of serum and a hydroxyapatite column for HPLC.

EXPERIMENTAL

Materials

Serum samples were obtained from fifteen healthy and drug-free male subjects (24–55 years old) and were refrigerated at −80°C until used. Commercial human AAG (Lot 57F-9319) was purchased from Sigma (St. Louis, MO, USA). All other chemicals were of analytical-reagent grade and used without further purification. A Toyopak DEAE M anion-exchange column (Tosoh, Tokyo, Japan) was washed with 5 ml of distilled water before use.

Clean-up of serum and HPLC procedure

A 250-μl aliquot of serum was diluted to 50 ml with 0.01 *M* citrate–phosphate buffer (pH 4.0) (buffer A), and then the whole mixture was loaded on to the DEAE M column. After washing the column with 5 ml of buffer A, AAG was eluted with 1 ml of 0.15 *M* citrate–phosphate buffer (pH 4.0) (buffer B), and then 50 μl of the eluate were injected into the HPLC system (Model 638-50, Hitachi, Tokyo, Japan) equipped with a Hitachi Model 638-41 multi-wavelength UV detector set at 280 nm. The separation was achieved on an A-7610 hydroxyapatite column (10 × 0.76 cm I.D.) (Koken, Tokyo, Japan) with a linear gradient of potassium phosphate buffer (pH 5.2) at a flow-rate of 1.0 ml/min; 0.35 *M* phosphate buffer was added to 0.01 *M* phosphate buffer linearly up to 50% in 10 min.

Electrophoresis

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed on a slab separating gel 1.0 mm thick containing 12% acrylamide. The protein samples were incubated with 0.9% mercaptoethanol for 10 min in a boiling water-bath.

RESULTS AND DISCUSSION

Fig. 1 shows the effect of the concentration of buffer B on the elution of AAG from the DEAE M column. The elution of AAG increased linearly with increasing concentration of buffer and reached a plateau at concentrations above *ca.* 0.1 *M*. Moreover, in the serum sample, the protein elution profile was similar to that of standard AAG. Above pH 4.0, an increase in interfering peaks in the HPLC profile was observed.

Fig. 2 shows hydroxyapatite HPLC profiles of (A) standard AAG and (B) serum from a healthy subject. The sample for HPLC was obtained by pre-treatment with a DEAE M column as mentioned previously. The peak at 11 min in Fig. 2B was homogeneous and the retention time coincided with that of the standard AAG. Moreover, in

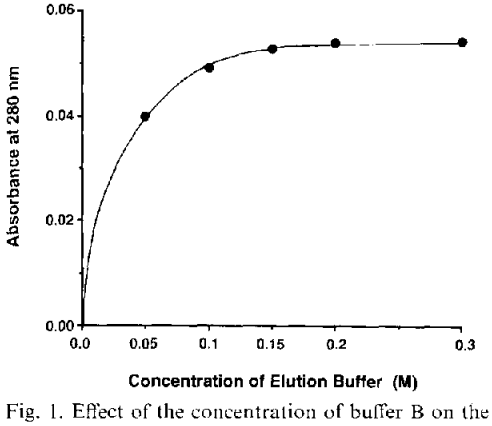

Fig. 1. Effect of the concentration of buffer B on the elution of AAG from the DEAE M column: 0.25 mg of AAG in 50 ml of buffer A was loaded on to the column. After washing the column with 5 ml of buffer A, AAG was eluted with 1 ml of various concentrations of buffer B.

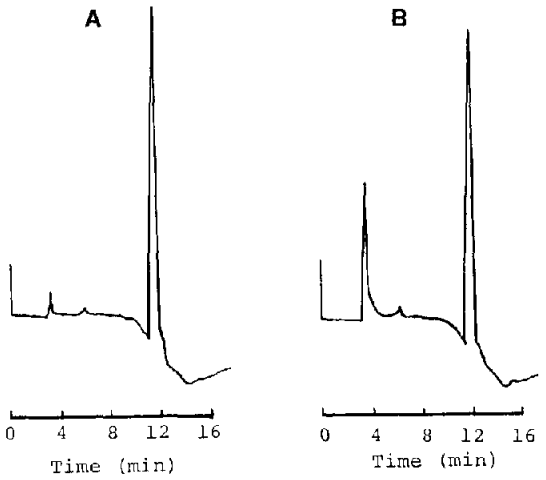

Fig. 2. Hydroxyapatite HPLC profiles of the eluate from the DEAE M column. (A) Standard AAG; (B) serum from a healthy subject.

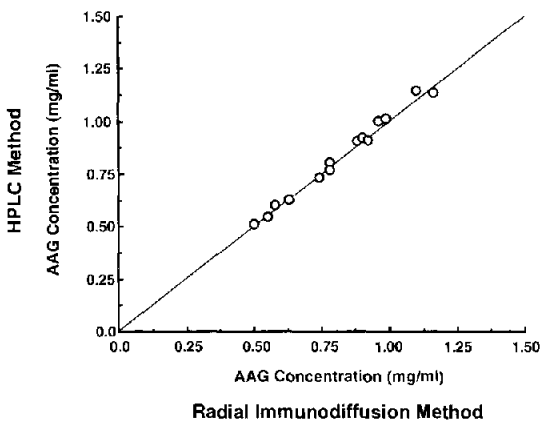

Fig. 3. Relationship between AAG concentrations in serum from fifteen healthy subjects determined by the radial immunodiffusion method and by the HPLC method. The regression line is $y = 1.009x + 0.004$ ($r = 0.9956$).

SDS-PAGE the peak at 11 min obtained from the serum samples showed a single band and its electrophoretic mobility was in agreement with that of standard AAG (data not shown).

For a rat serum sample, a similar chromatographic profile could also be obtained by using the same clean-up and HPLC procedure.

A calibration graph obtained according to the procedure described under Experimental was constructed. A linear relationship between the standard AAG concentration (x) and the peak height (y) was observed over the concentration range 0.5–2.5 mg/ml AAG and the regression line was $y = 125.7x + 1.7$ ($r = 0.9993$). The coefficient of variation at 0.5 mg/ml AAG was 3.7% ($n = 8$) and the limit of detection was 0.05 mg/ml AAG.

Using the proposed HPLC method and the conventional radial immunodiffusion method, the AAG concentrations in the serum of fifteen healthy subjects were determined. As shown in Fig. 3, a good correlation between the two methods was observed.

It is concluded that it is possible to determine rapidly (maximum 30 min) and precisely serum AAG concentrations in individual patients by the proposed HPLC method. Moreover, it can also be used to determine AAG concentrations in the serum of experimental animals.

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