PREPARATION OF TISSUE CULTURE SERUM LACKING α_2-MACROGLOBULIN

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1. Introduction

α_2-Macroglobulin (α_2M) is a plasma glycoprotein with mol. wt > 700 000 [1]. Although a variety of interesting activities have been ascribed to this molecule [2–6], its functional importance undoubtedly resides in its ability to bind and inhibit essentially all active endopeptidases [7]. Because of its uniquely broad specificity as a protease inhibitor, the presence of α_2M may provide plasma with a buffer against invasion and activation by foreign proteases [7,8]. α_2M is found in close association both with the vascular endothelium of blood vessels [8] and with other protease-secreting cells [9,10], suggesting that it may function to regulate the activity of cellular hydrolases. Cell culture would appear to offer a means for evaluating this important conclusion. However, the observation that cells in culture contain α_2M derived from the serum in the growth medium [11] complicates this approach.

We have taken advantage of the affinity of concanavalin A (Con A) for glycoproteins to obtain a highly purified, active preparation of α_2M from fetal bovine serum (FBS). Con A affinity chromatography is rapid and readily adaptable to large scale purification of the molecule and yields as a by-product serum selectively depleted of α_2M. This sera should prove useful for studying the production and metabolism of α_2M by cells in culture.

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2. Materials and methods

FBS was purchased from Reheis Chemical Co. (Phoenix, AZ), Con A and soybean trypsin inhibitor (SBTI) from Miles Laboratories (Elkhart, IN), and α-methyl glucoside from Sigma (St Louis, MO). α_2M activity was determined according to [12] by measuring the rate of hydrolysis of benzyl-DL-arginine-*P*-nitroanilide (Schwartz/Mann, Orangeburg, NY) by trypsin (3 × crystallized; Worthington Biochemical Corp., Freehold, NJ) at 410 nm in the presence of SBTI and various fractions of FBS. Con A (5 mg/ml in 0.1 M phosphate buffer, pH 7.4) was coupled to activated Sepharose 4B (Pharmacia, Piscataway, NJ) as in [13]. Coupling efficiency varied from 49–82%. A 30 ml column of settled Con A–Sepharose was prepared, equilibrated with 0.1 M NaCl, 0.1 M Tris, pH 7.4 and used in these experiments. Sepharose 6B chromatography was conducted at 4°C in a column (4 × 96 cm) equilibrated with 0.1 M Tris–HCl buffer, pH 7.4. The flow rate was 33 ml/h and 6 ml fractions were collected. Immunoelectrophoresis was performed in 1.5% agar in 0.1 M barbital buffer, pH 8.2. The slide was developed against antisera to native FBS, dried overnight at 30°C, and stained with amido black. Protein concentrations were determined using Lowry's method [14]. All other chemicals and reagents were of the highest grade available.

3. Results

When Con A was added to FBS, a flocculant precipitate was formed. The amount of protein in the precipitate increased with the concentration of the

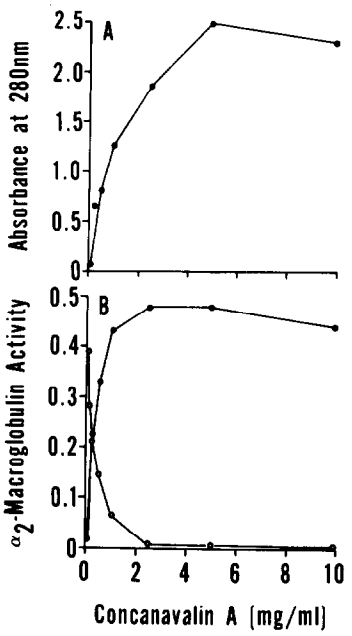

Fig.1. Fractionation of FBS with Con A. Increasing amounts of Con A were incubated with 1 ml aliquots of FBS for 18 h at 4°C. The precipitates were collected by centrifugation (13 000 × *g* for 10 min at 4°C), dissolved in 0.1 M α-methyl glucoside, and tested for α_2M activity. (A) Total precipitated protein. (B) Accumulation of α_2M activity in the pellet (•——•); loss of α_2M activity from the supernatant (○——○).

lectin until the ratio of Con A to total serum protein was ~ 0.1 (fig.1A). The insolubilized protein represented considerably less than 10% total protein in FBS since part of the material in the precipitate was Con A itself. α_2M was efficiently removed from the serum by Con A and could be recovered quantitatively in the pellet after the mixture was centrifuged (fig.1B). More than 95% α_2M activity was removed from FBS when the serum was passed through a column of Con A–Sepharose (fig.2). α_2M apparently bound to the column under these conditions and could be desorbed with α-methyl glucoside. α_2M activity was recovered in a fraction that contained < 4% protein applied to the column.

The fractions resulting from Con A affinity chromatography of FBS were analyzed by chromatography on Sepharose 6B (fig.3). All α_2M activity in FBS was associated with a single peak of rapidly migrating protein having an est. mol. wt 700 000 (fig.3A). Both

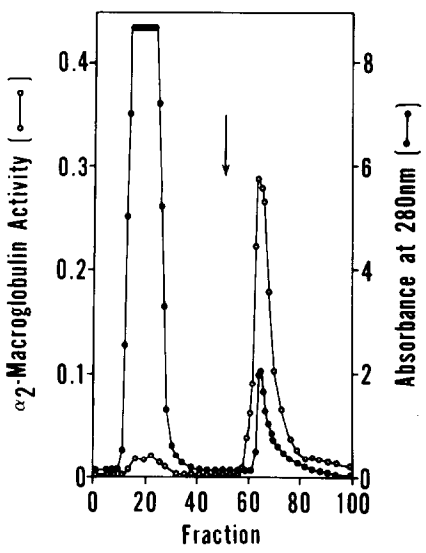

Fig.2. Representative profile for the purification of α_2M from FBS by Con A affinity chromatography. In a typical experiment, 20 ml FBS was applied to the top of the column, the column was washed with equilibration buffer until eluant absorbance returned to the baseline value, and the bound material was desorbed with 0.1 M α-methyl glucoside in the same buffer. The flow rate was ~ 4 ml/h, and 2 ml fractions were collected. The arrow indicates addition of the hapten, α-methyl glucoside.

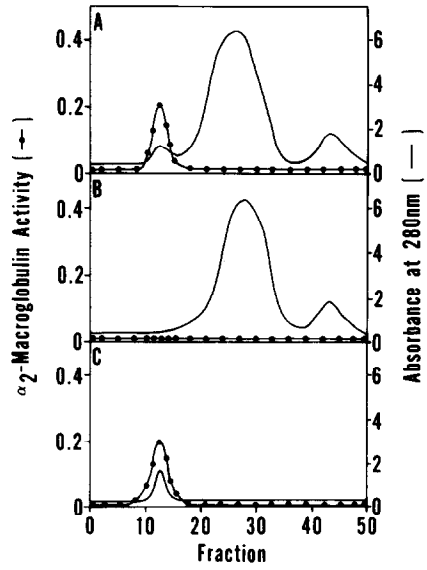

Fig.3. Profiles of various fractions of FBS chromatographed on Sepharose 6B. (A) Native FBS. (B) FBS-pre-run through Con A affinity column. (C) Material in FBS that was eluted from the Con A affinity column with α-methyl glucoside.

the protein peak and the α_2M activity were absent in FBS that had been passed through the Con A column (fig.3B) and present in the fractions eluting from the Con A column with α-methyl glucoside (fig.3C). When these fractions were analyzed by polyacrylamide gel electrophoresis [15], a single protein band was detected (data not shown).

To further characterize the product isolated from FBS by Con A affinity chromatography, the material was subjected to immunoelectrophoresis and then to absorption against antisera to native FBS. A single precipitin band was detected after such treatment (fig.4). When whole FBS was treated similarly, multiple bands were observed. In addition, antisera prepared against Con A-purified α_2M recognized a single protein in FBS but no proteins in FBS that had been passed through the Con A column (unpublished observations).

4. Discussion

Although it is apparent that α_2M functions to limit the activity of a number of circulating proteases [1,7], its role in modulating the activity of cellular hydrolytic enzymes remains uncertain [10,11,16]. The presence of α_2M in the culture medium and its ingestion by cultured cells [11] has made it difficult to study this question or to examine factors that regulate the production and metabolism of this important molecule. Con A affinity chromatography selectively removes α_2M from FBS, a serum widely employed in cell culture. This approach thus affords a convenient method for constructing media that should still support cell growth but will no longer contain α_2M, the molecule to be studied. The use of α_2M-depleted FBS as a supplement to cell culture media should provide a simple and direct system for defining the importance of α_2M in various cellular processes.

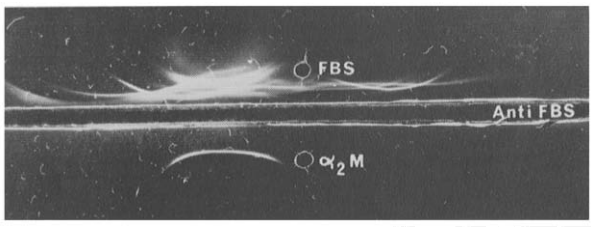

Fig.4. Immunoelectrophoresis of native FBS and of α_2M purified by Con A affinity chromatography.

A precipitate was noted to form when phytohemagglutinin or Con A was added to various adult sera and that α_2M was among the precipitated proteins [17]. We have extended these earlier findings by demonstrating that fractionation of fetal bovine serum by Con A affinity chromatography provides a simple new approach for purifying α_2M. This single-step procedure for the isolation of α_2M from FBS sets it apart from the complicated approaches devised for orther, more complex sera [18,19]. The relative ease and rapidity of the technique reduces the possibility that the molecule will be exposed to plasma proteases. The efficiency of the approach makes possible the isolation of large amounts of purified α_2M, a feature that should facilitate biochemical studies of the structure of unmodified α_2M.

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