Journal of Cellular Biochemistry 21:263–275 (1983)

Purification of a Human Urinary Colony-Stimulating Factor

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Colony-stimulating factor (CSF), a protein required for the in vitro formation of colonies composed of granulocytes and/or macrophages, was isolated from the urine of anemic patients by using a seven-step procedure. The purified, homogeneous CSF had a specific activity of 1.9×10^8 U/absorbance unit at 280 nm (AU). This represents an overall purification of 25,330-fold and a total recovery of 3.8%. Upon iodination of the protein, the radioactivity migrated on sodium dodecyl sulfate (SDS) gel electrophoresis as a single peak with an apparent molecular weight of 46,000; reduction with mercaptoethanol caused dissociation to a single component of molecular weight 23,000. Only the dimer is active in stimulating colony formation. Urinary CSF stimulates formation of colonies comprising only macrophages in the mouse bone marrow cell culture assay. A neutralizing antibody raised against mouse L-cell CSF did not neutralize the activity of the urinary CSF but did bind it. This may indicate that the relative positions of antibody binding sites and the active sites are different in these two glycoproteins.

Key words: colony-stimulating factor (CSF), granulocyte/macrophage colonies, hemopoiesis, differentiation, glycoprotein

Colony-stimulating factor (CSF) is a term used to designate a protein or a group of proteins required for the formation of colonies composed of granulocytes and/or macrophages from hemopoietic precursor cells in vitro. Its activity, measured in cultures of bone marrow cells in semisolid medium [1], has been detected in serum, urine, tissue extracts, and medium conditioned by a variety of cells and tissues. All of the purified CSF preparations have been shown, so far, to be glycoproteins.

CSF has been purified or partially purified from L-cell culture supernatant medium [2,3], human lung cell conditioned medium [4], pancreatic carcinoma cells [5], a human T-lymphoblast cell line [6], mouse lung cell conditioned medium [7], and human urine [8,9]. The various CSF preparations exhibit a wide range of molecular properties although they all have the ability to stimulate granulocyte or macrophage-colony formation. For example, CSF from mouse L-cells is a disulfide-linked dimer of molecular weight 70,000; mouse lung cell CSF, on the other hand,

Received February 28, 1983; revised and accepted May 20, 1983.

has a molecular weight of 23,000 and does not appear to be a disulfide dimer. Human pancreatic carcinoma cells contained two distinct species of CSF [5], they are separable by isoelectric focusing and gel permeation; one has a molecular weight of 27,000 and an isoelectric point in the range of 5.8–6.2, while the other has a molecular weight of 50,000 and an isoelectric point in the range of 3.7–4.6. The high molecular weight pancreatic tumor cell CSF has been purified to apparent homogeneity. In some other cases, heterogeneity in molecular properties among different preparations of CSF may arise from the carbohydrate moiety [10].

Human urinary CSF has been highly purified by Stanley et al [8]. However, during the purification process, papain digestion was employed to remove some contaminating proteins. Approximately 70% of the biological activity was lost in the digestion, and the question remained whether the CSF isolated after protease treatment was representative of the native material. Das et al [11] have recently isolated CSF by specifically binding ^{125}I-labeled, partially purified urinary CSF to receptor-bearing cells at 0°C, it was then dissociated from the cells at 37°C. The ^{125}I-CSF obtained was shown to be free of most contaminating ^{125}I-proteins; however, the ^{125}I-CSF was not separated from other unlabeled proteins, thus making chemical characterization of the protein unfeasible.

In this paper, we describe the purification of CSF from human urine and demonstrate that it is a glycoprotein with a molecular weight of 46,000.

MATERIALS AND METHODS

Source of CSF

Urine from anemic patients was collected and processed as described previously [12] by Dr T. Miyake, Kumamoto City, Japan. The particular batch of urine used in this study was a pool collected from three patients with leukemia and two patients with aplastic anemia.

Chemicals

Concanavalin A (ConA)-Sepharose 4B, Sephadex G-150, and sulfopropyl Sephadex C-50 were purchased from Pharmacia, Inc, (Piscataway, NJ). The column packed with TSK G3000 SW was obtained from Toyosoda Manufacturing Co, Ltd, (Tokyo, Japan). PM 10 membranes were from Amicon Corporation, (Lexington, MA), dialysis membranes from Spectrum Medical Industries, Inc, (Los Angeles, CA). DEAE Bio-Gel A and Bio-Gel HT and all the materials for gel electrophoresis (SDS, acrylamide, N,N′-methylene bisacrylamide, N,N,N′,N′-tetramethylethylenediamine) were from Bio-Rad Laboratories (Richmond, CA). Lentil Sepharose was bought from E-Y Laboratories (San Mateo, CA); Na^{125}I from Amersham Corporation (Arlington Heights, IL), and α-methyl-D-mannoside (grade III) from Sigma Chemical Co (St Louis, MO). Standard petri dishes (35 × 10 mm, cat No. 5221R) were from Lux Scientific (Midland, MI). α-Medium and fetal calf serum were purchased from Flow Laboratories (Rockville, MD), Gentamicin was from Schering Corporation (Kenilworth, NJ), methylcellulose (A 4M premium) was from Dow Chemical Company (Midland, MI), and antiserum to L-cell CSF was a gift from Dr R. Shadduck (Montefiore Hospital, Pittsburgh, PA). All other reagents were of reagent grade quality and were purchased from standard sources.

CSF Assay

The method of CSF assay has been described previously [1]. Mouse bone marrow cells were cultured at a concentration of 7.5×10^4 cells/ml in a mixture containing 85% (v/v) α-medium, 15% fetal calf serum, 10 mg/ml of BSA, 8 mg/ml of methylcellulose, and 10^{-4} M β-mercaptoethanol. All media contained 0.5 mg/ml of Gentamicin. One-milliliter aliquots of the cell suspension were incubated in petri dishes (35 × 10 mm) at 37°C in a fully humidified atmosphere of 5% CO_2 and 95% air. Four replicates were run for each point. The colonies were counted on the eighth day under a dissecting microscope. A unit of CSF activity is defined as the amount of CSF which stimulates the formation of one colony in a 1-ml culture under the conditions specified after the background is subtracted.

Electrophoresis

SDS-polyacrylamide gel electrophoresis was done according to Laemmli [13] using 0.25 × 5-cm gel tubes at a constant current of 0.5 mA per tube with bromophenol blue used as a tracking dye. After electrophoresis, the gels were cut into 1 mm slices, eluted with 200 μl of 0.05 M phosphate, pH 7.0, containing 0.05% BSA, overnight and assayed for biological activity. When ^{125}I-CSF was used, the gel slices were counted on a Searle automatic γ-counter.

Iodination

Labeling with ^{125}I was done according to the method of Fraker and Speck [14] with some modification. Iodogen (1,3,4,6-tetrachloro-3α, 6α-diphenylglycouril) was coated evenly on the bottom of a 1-ml conical reaction vial by evaporating 40 μl of Iodogen solution in $CHCl_3$ (1.25 μg/μl). The protein was added in a volume of 5 μl. The reaction was started by introducing 1 μl of $Na^{125}I$ (100 μCi/μl); after 30 sec the solution was transferred to 200 μl of KI solution (10 mg/ml in 0.05 M phosphate buffer, pH 7.0). The unreacted iodide was removed on a Sephadex G-25 column (1.5 × 30 cm) with the same buffer containing 0.05% gelatin.

Morphological Analysis

The study of cell morphology in the assay dishes was conducted as follows: Cells in the colonies were dispersed by adding 1 ml of α-medium to each petri dish containing 1 ml of culture, the mixture was mixed gently with a Pasteur pipette, the cells were centrifuged and washed with α-medium three times, then suspended in the medium and cytocentrifuged onto a microscope slide. The cells were air dried and stained with Wright's stain.

RESULTS

ConA-Sepharose 4B Fractionation

Urine of patients with aplastic anemia is a very good source of erythropoietin, the primary inducer of red blood cell differentiation [12], and is also rich in CSF activity. In order to obtain both erythropoietin and CSF, the CSF in the urine concentrates was adsorbed on ConA-Sepharose 4B, which does not adsorb erythropoietin. A column of ConA-Sepharose 4B (1.5 × 29 cm) was equilibrated with 0.1 M ammonium acetate buffer, pH 5.0, containing 1 mM each of Ca^{2+}, Mg^{2+}, and

Mn^{2+}. One gram of the lyophilized urine concentrates was dissolved in 50 ml of buffer, centrifuged at 10,000 rpm for 15 min to remove any insoluble material, and the supernatant fraction loaded on the column. The column was washed with the same buffer until the A_{280} was less than 0.05; the column was then eluted with 0.1 M α-methyl mannoside in the same buffer. About 90% of the erythropoietin in the crude urine concentrate was recovered in the unbound fraction. The majority of the CSF was adsorbed by ConA and was eluted with 0.1 M α-methyl mannoside. Four such columns were run. We have consistently recovered approximately 2% of the CSF activity in the unbound fraction. When morphological studies were performed on the colonies produced by the two fractions—that bound to ConA-Sepharose, and that not bound—we found that the colonies were composed of only one type of cell, macrophages, when the former fraction was tested. The unbound fraction, on the other hand, stimulated colony formation in which both macrophages and granulocytes were found. The effluent was stored at −20°C for use in the purification of erythropoietin; and the sugar eluate was dialysed extensively against 0.1 M Tris-Cl buffer, pH 7.0, and concentrated by ultrafiltration using a PM 10 membrane. The combined ConA eluates had a volume of 77 ml, which contained a total of 3.2×10^7 units of CSF activity. The average specific activity at this stage was 1.2×10^4 units/AU. The yield was 71% with a purification factor of 1.6.

DEAE Bio-Gel A Chromatography

The pooled ConA eluate was applied to a column of DEAE Bio-Gel A (2.5 × 50 cm) that had been equilibrated with 0.01 M Tris-Cl, pH 7.0. The column was washed with 600 ml of the same buffer and then eluted with a 3-liter linear gradient of 0–0.05 M $CaCl_2$ in 0.01 M Tris-Cl, pH 7.0. The flow was kept constant at 40 ml/hr with a peristaltic pump, 8 ml fractions were collected, and the CSF activity of every fifth fraction was assayed. The results are shown in Figure 1. CSF activity was eluted between a conductivity of 2.80 mmho and 4.80 mmho. The peak fractions (Nos. 155–185) were pooled and concentrated using a PM 10 membrane filter and the concentrate dialysed extensively against 0.01 M sodium acetate buffer, pH 4.0. After dialysis, insoluble material was discarded; the supernatant fraction which contained all of the CSF activity had a volume of 41 ml and 513 AU. The specific activity of pooled fractions 155–185 was 6.4×10^4 units/AU. The total activity recovered was 3.3×10^7 units. This step represented a purification factor of 5.3 with quantitative recovery of the biological activity.

Sulfopropyl-Sephadex Chromatography

In preliminary experiments, pH 5.0 and pH 4.5 were initially chosen for the input buffers of the sulfopropyl (SP)-Sephadex column to avoid prolonged exposure of CSF to low pH. We found that at these pH values CSF was not adsorbed to the column nor was more than 90% of the protein. Adsorption of CSF occurred only when the pH was at 4.0 or lower. Fortunately, CSF remained fully active during this procedure (data not shown).

SP-Sephadex was equilibrated with 0.01 M sodium acetate buffer, pH 4.0, and poured into a column (2.5 × 45 cm). The pool (fractions 155–185) from DEAE-Bio Gel A was applied to this column in 41 ml. The column was washed with 400 ml of the same buffer, and 8.0 ml fractions were collected. Stepwise elution was done with 10 mM, 25 mM, 50 mM, and 100 mM of calcium chloride in 0.01 M sodium acetate,

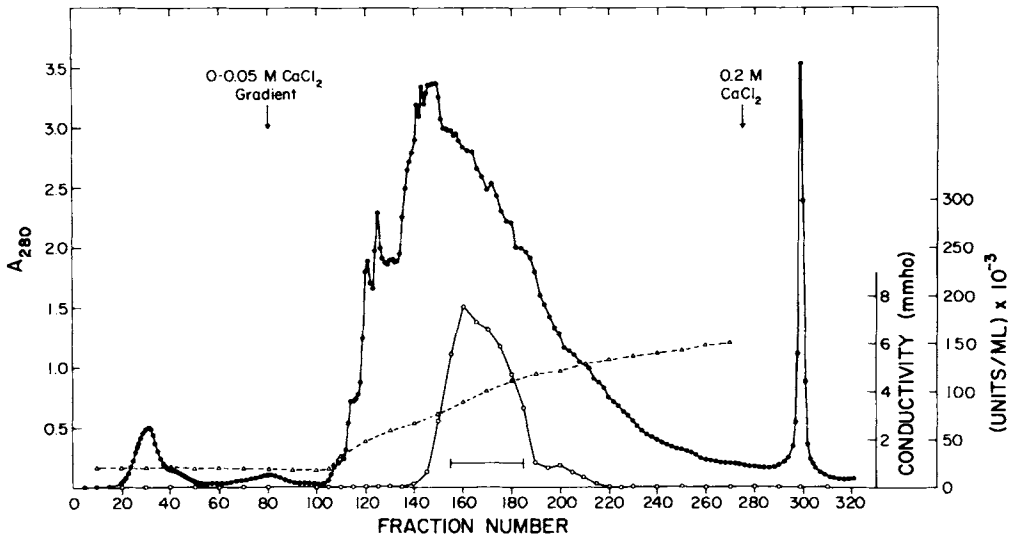

Fig. 1. Chromatography on DEAE-agarose. The column (2.5 × 50 cm) was equilibrated with 0.01 M Tris-Cl, pH 7.0, and the pooled ConA eluate was applied in a volume of 77 ml. The column was washed with 600 ml of the same buffer and then eluted with a 3-liter linear gradient of 0–0.5 M $CaCl_2$ in buffer, 8-ml fractions were collected. ●—●, A_{280}; ○—○, CSF activity; Δ---Δ, conductivity.

pH 4.0; the elution in each step was continued until the effluent A_{280} had decreased to a stable value. The elution profile is shown in Figure 2. About 25% of the biological activity was eluted with 25 mM $CaCl_2$ and about 48% of the biological activity was eluted with 50 mM $CaCl_2$. These two fractions together had 2.4×10^7 units. They were pooled separately, concentrated using a PM 10 membrane. The 25-mM $CaCl_2$ fraction had a specific activity of 1.8×10^5 U/AU, whereas the 50 mM fraction had a specific activity of 3.0×10^5 U/AU. Only the fraction eluted with 50 mM $CaCl_2$ was used for further CSF purification because of its higher specific activity. This step had a purification factor of 4.7.

Sephadex G-150 Chromatography

Sephadex G-150 (medium) was swollen and poured into two 1.6 × 85-cm columns. The two columns were coupled at the bottom, making a column 170 cm long. The column was equilibrated at 4°C with 0.05 M ammonium acetate buffer, pH 7.0, and calibrated wth a mixture of the following proteins of known molecular weight: BSA, ovalbumin, chymotrypsinogen A, and RNase A. The 50 mM $CaCl_2$ eluate of the SP-Sephadex column was dialysed against 0.05 M ammonium acetate, pH 7.0, lyophilized, dissolved in 2 ml of the same buffer, and applied to the column, which was developed at a flow rate of 8 ml/hr, and 1.6 ml fractions were collected. The results are shown in Figure 3. CSF activity was eluted as a sharp peak with Ve/Vo of 1.52, and the apparent molecular weight was estimated to be 85,000 when compared to the standard proteins. Fractions 90–99 were pooled, lyophilized, and redissolved in 4 ml of water. The specific activity was 1.9×10^6 U/AU, and the total

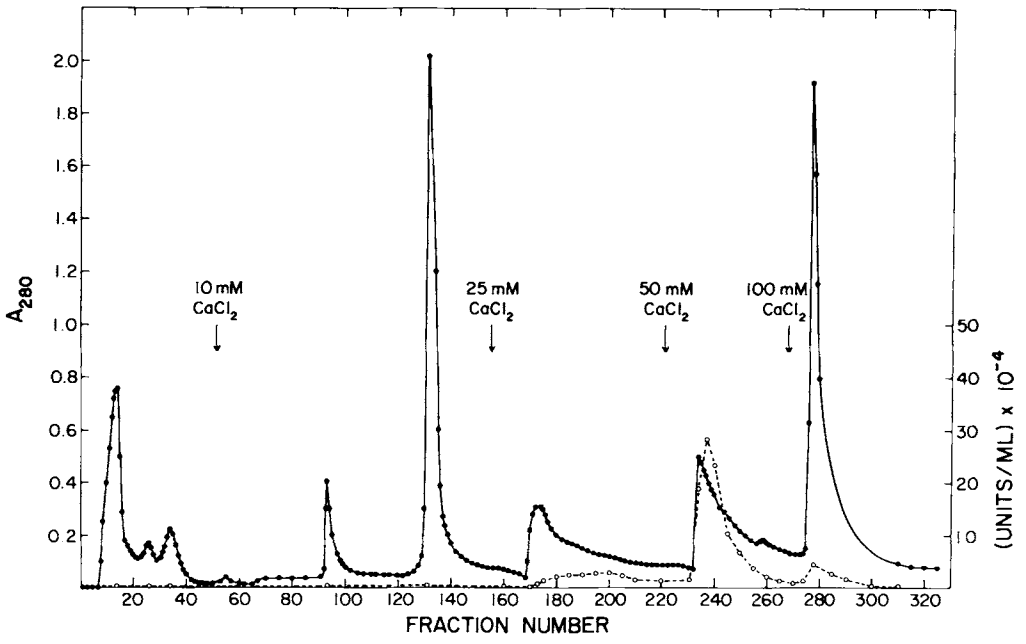

Fig. 2. Chromatography on sulfopropyl Sephadex C-50. The active CSF from DEAE-agarose in a volume of 41 ml was passed through a column of sulfopropyl Sephadex C-50 (2.5 × 45 cm) equilibrated with 0.01 M sodium acetate buffer, pH 4.0. The column was eluted stepwise with increasing concentration of $CaCl_2$ as indicated by arrows; 8.0-ml fractions were collected. ●—●, A_{280}; ○---○, CSF activity.

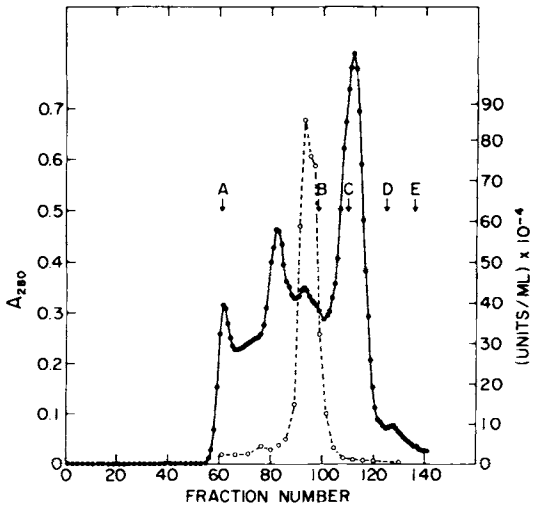

Fig. 3. Gel permeation on Sephadex G-150. The column (1.6 × 170 cm) was run in 0.05 M ammonium acetate buffer, pH 7.0. The CSF in the 50 mM $CaCl_2$ eluate of SP-Sephadex was applied in 2-ml volume at a flow rate of 8 ml/hr, and 1.6 ml fractions were collected. The calibration markers were A, Blue Dextran; B, BSA; C, ovalbumin; D, Chymotrypsinogen; and E, RNaseA. ●--●, A_{280}; ○---○, CSF activity.

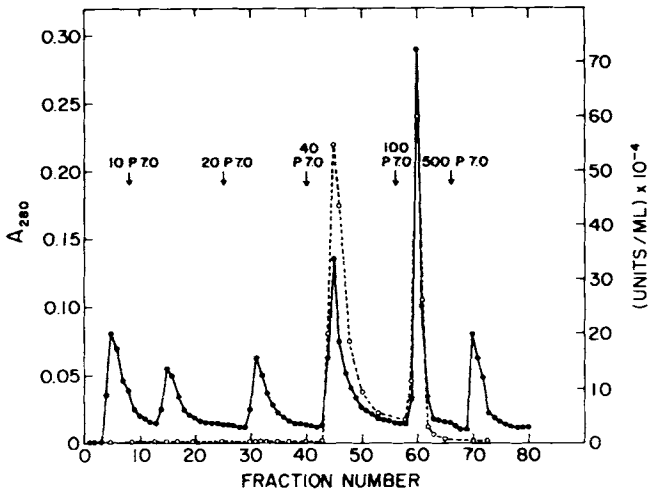

Fig. 4. Chromatography on hydroxyapatite. The column (1.3 × 7 cm) was equilibrated with 5 mM sodium phosphate buffer, pH 7.0, 4 ml of CSF from Sephadex G-150 was applied and 2.5-ml fractions were collected. The arrows indicate buffer changes. 10 P 7.0 (10 mM phosphate, pH 7.0), 20 P 7.0 (20 mM phosphate, pH 7.0), 40 P 7.0 (40 mM phosphate, pH 7.0), 100 P 7.0 (100 mM phosphate, pH 7.0), 500 P 7.0 (500 mM phosphate, pH 7.0). ●—●, A_{280}; ○---○ CSF activity.

activity was 1.0×10^7 units. This represented a purification factor of 6.3 and a yield of 66% for this step.

Hydroxyapatite Chromatography

Bio-Gel HT was packed into a 1.3 × 7 cm column and equilibrated with 5 mM sodium-phosphate buffer, pH 7.0. The active fraction from the Sephadex G-150 column was applied, and stepwise elution was carried out with the following concentrations of phosphate buffer at pH 7.0: 10 mM, 20 mM, 40 mM, 100 mM, and 500 mM. The column was run at 10 ml/hr and 2.5-ml fractions were collected. CSF activity was found in the 40-mM and 100-mM phosphate eluates exclusively (Fig. 4). Of the total of 1.0×10^7 units put on the column, 4.7×10^6 units were recovered in the 40-mM phosphate eluate peak (47% recovery), and 28% of the activity was recovered in the 100-mM phosphate eluate. The 40-mM phosphate fraction had a specific activity of 6.4×10^6 U/AU and the purification factor was 3.4.

Lentil Sepharose 4B Column

Lentil Sepharose 4B was packed into a 1.4 × 2 cm column and equilibrated with 0.05 M Tris-Cl buffer, pH 7.0 containing 1 mM each of Ca^{2+}, Mg^{2+}, Mn^{2+}. The 40-mM phosphate fraction from hydroxyapatite column was loaded directly on the lectin column. The column was washed extensively with the same buffer until the A_{280} was below 0.005 and then eluted with 0.1 M α-methyl mannoside. As can be seen in Figure 5, the biological activity was separated into two fractions; about 30% of the CSF activity was not adsorbed, and the rest adsorbed to the lentil and eluted with α-methyl mannoside. The unbound fraction remained unbound when rechromato-

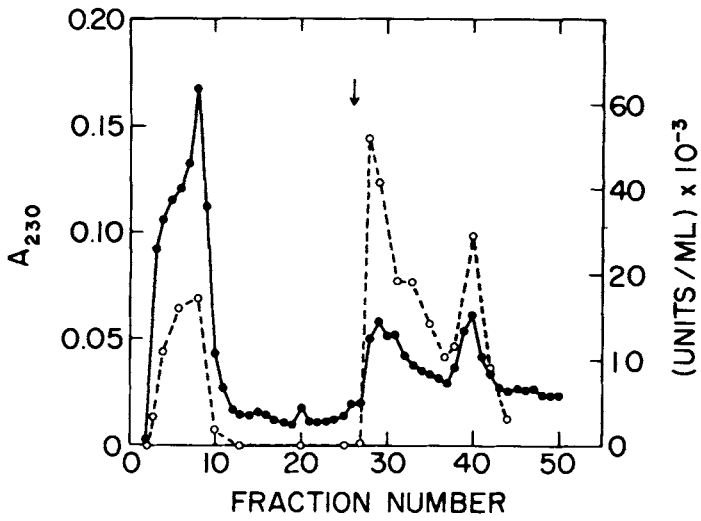

Fig. 5. Chromatography on lentil Sepharose 4B. The column (1.4 × 2 cm) was equilibrated with 0.05 M Tris-Cl, pH 7.0, containing 1 mM each of Ca^{2+}, Mg^{2+}, Mn^{2+}. The arrow indicates the start of the elution with 0.1 M α-methyl mannoside. ●—●, A_{280}; ○---○, CSF activity.

graphed on the lentil column. The two fractions were pooled separately; dialyzed against 0.05 M ammonium acetate buffer, pH 7.0; and lyophilized.

Despite there being a considerable amount of protein in the effluent of the lectin column, the specific activity of the eluate did not increase. When the combined absorbance of eluate and effluent of the lentil column was calculated, we found it to be 30% more than the column input. This suggested that the lectin column shed protein. When the lectin-bound fraction was analyzed on high-performance liquid chromatography (HPLC), using a gel permeation column TSK G3000SW, we found almost all of the absorbance at 280 nm was due to two small molecular weight peaks that were eluted near the bed volume of the column. We did not estimate the molecular weight of these two peaks because they were not within the linear range of the molecular weight vs volume plot. The same two peaks were found when the buffer accompanying the commercial lentil Sepharose 4B was analyzed on the TSK G3000SW column. It is apparent that the excess absorbance came from the lectin column. The eluate of the lentil column was therefore put on the HPLC column to remove the contaminants from the lentil Sepharose 4B. The final product from HPLC had a specific activity of 1.9×10^8 U/AU. The combination of the lentil column and the TSK 3000SW gave a purification factor of 30, and a recovery of 36%.

Summary of Purification

Table I summarizes the purification scheme. The yield and purification factors were calculated with respect to the original urine concentrates.

Polyacrylamide Gel Electrophoresis

The purified CSF was labeled with ^{125}I and electrophoresed on a 10% SDS gel with or without prior reduction with mercaptoethanol. The gels were cut into 1-mm slices and counted for radioactivity. As can be seen in Figure 6, a single radioactive

TABLE I. Purification of Urinary CSF

Step		AU (280 nm)	Total units	Specific activity U/AU	Purification factor	Yield
	Urine Concentrate	6,000	4.5×10^7	7.5×10^3	1	100
I.	ConA-Sepharose	2,542	3.2×10^7	1.2×10^4	1.6	71
II.	DE-Bio Gel A	513	3.3×10^7	6.4×10^4	8.5	73
III.	SP-Sephadex C-50	50	1.5×10^7	3.0×10^5	40	33
IV.	Sephadex G-150	5.3	1.0×10^7	1.9×10^6	253	22
V.	Hydroxyapatite	0.73	4.7×10^6	6.4×10^6	853	10
VI.	Lentil + HPLC sizing column	0.009	1.7×10^6	1.9×10^8	25,330	3.8

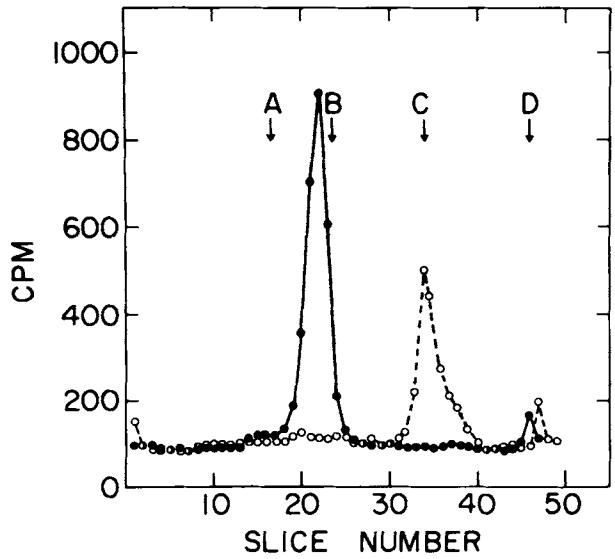

Fig. 6. SDS-polyacrylamide gel electrophoresis of ^{125}I-CSF. The purified CSF was iodinated and run on 10% acrylamide gel electrophoresis. The gels were cut into 1-mm slices and counted for radioactivity. Arrows indicate positions of markers: A, BSA; B, ovalbumin; C, chymotrypsinogen A; D, bromophenol blue. ○---○, CSF in the presence of mercaptoethanol; ●—●, CSF in the absence of reducing agents.

peak was found in both gels. The molecular weight was estimated to be 23,000 for the reduced CSF and 46,000 for the nonreduced protein. No residual radioactivity was detected at the 46,000 molecular weight region when the protein was reduced prior to electrophoresis.

To test whether the monomer is biologically active or not, the step I CSF (ConA eluate) was run on an SDS gel and the activity eluted from the gel slices. A broad activity region with about 33% of the biological activity was recovered with the peak centered at the 46,000 molecular weight region when the protein was not reduced; however, no CSF activity was recovered from the gel slices when the protein was treated with mercaptoethanol prior to electrophoresis.

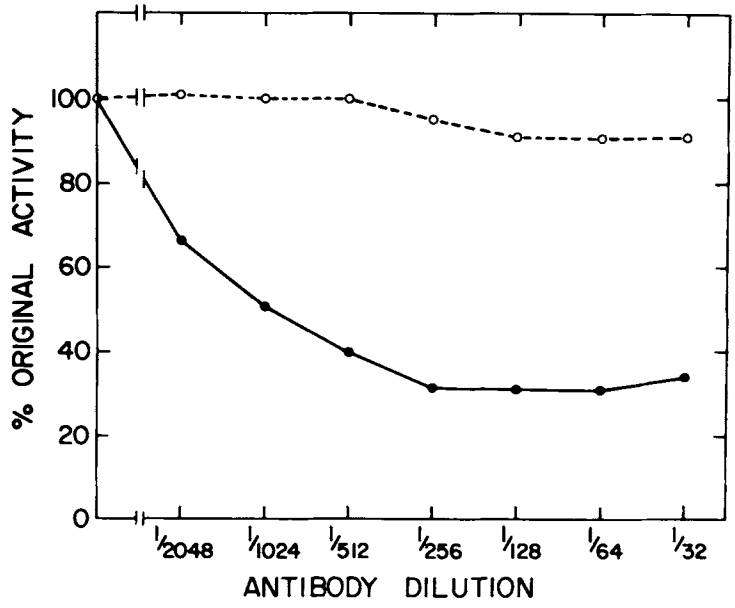

Fig. 7. Inhibition of CSF activity with antibody to L-cell CSF. Urinary CSF (O---O) and endotoxin-treated mouse serum (●—●) were incubated at 37°C for 2 hr with increasing dilutions of antibody to L-cell CSF. The activity was measured and calculated with respect to the original (no antibody).

Other Properties of CSF

The urinary CSF shares some common properties with L-cell CSF [2,3] and urinary CSF-I [8,11]; all three factors stimulate only macrophage-colony formation and are composed of disulfide-linked subunits. Stanley [15] and Shadduck et al [22] have found that antibody to L-cell CSF cross-reacts with urinary CSF-I. It was, therefore, important to test the immunological similarity between L-cell CSF and urinary CSF prepared in this laboratory. The urinary CSF was incubated with different dilutions (1/32 to 1/2,048) of anti-L-cell CSF and assayed for activity. As shown in Figure 7, anti L-cell CSF did not inhibit the activity of urinary CSF to any appreciable extent. We found this to be true even with undiluted antiserum. The same experiment peformed with CSF in serum from mice treated with endotoxin showed that 67% of the colony-forming activity was inhibited.

To test the possibility that the anti-L-cell CSF, though not neutralizing, may still be able to bind to urinary CSF, the stage I CSF was incubated at 37°C for 2 hr with undiluted antiserum; the mixture was then analyzed on TSK G3000SW column. As can be seen in Figure 8, about 15% of the biological activity was shifted to a peak of larger size, suggesting that binding occurred between the immunoglobulin and urinary CSF. When the second peak (fractions 27–31) was reincubated with antiserum to L-cell CSF, and chromatographed on the same column, about 50% of the CSF activity was again found in the first peak of larger molecular weight complex. Normal rabbit serum, on the other hand, did not alter the elution time of CSF activity.

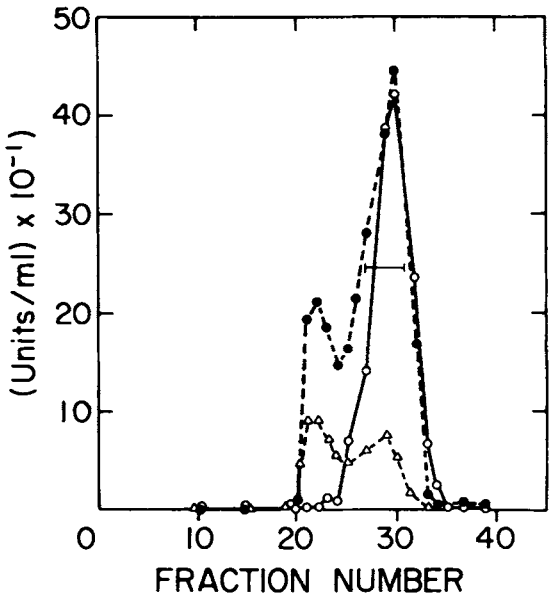

Fig. 8. High-pressure liquid chromatography of CSF and anti-L-cell CSF: CSF complex. The column was equilibrated with 0.15 M ammonium acetate buffer, pH 7.0, and run at a flow rate of 1 ml/min. ○—○, CSF incubated with normal rabbit serum for 2 hr at 37°C; ●---●, CSF incubated with anti-L-cell CSF for 2 hr at 37°C. Fraction 27–31 of this column was pooled, reincubated with anti-L-cell CSF and rerun on the same column (Δ---Δ).

DISCUSSION

Our observation that a small portion of CSF did not bind to ConA column was also found consistently with other CSF preparations, such as medium of human placenta, lung, and peripheral leukocytes [16]. Wu et al [17] have purified two types of CSF, one that binds to ConA and one does not, from human placenta and found them to be different in isoelectric point, molecular weight, and target cell specificity; the CSF eluted from ConA produced colonies that consisted mostly of macrophages, while the CSF not bound caused colony formation consisting predominantly of granulocytes. Our results with human urinary CSF are consistent with those findings. Preliminary studies in this laboratory indicated that the molecular weight of the CSF not bound to ConA, as determined by gel permeation, was less than that of ConA-bound CSF. Human urinary CSF differs appreciably from mouse lung conditioned medium CSF [7], which is ConA-adherent and has been shown to induce colonies of granulocytes and/or macrophages. At low concentrations of mouse lung CSF, only macrophage colonies were found; as the concentration of CSF was increased, the frequency of colonies containing granulocytes also increased. In the case of L-cell CSF, both CSF fractions (ConA effluent and ConA eluate) have identical molecular weights, subunit structures, and antibody specificity; furthermore, both types of CSF induced predominantly macrophage colony formation [2].

The fact that lentil lectin can further separate the ConA-bound proteins into two fractions indicated the subtle difference in the carbohydrate-binding specificity of

these two lectins. Previously, lentil lectin had been thought to have the same specificity as ConA in binding to α-mannosyl and α-glucosyl containing oligosaccharides [18]. It has been shown recently [19] that the presence of fucose attached to the asparagine-linked N-acetylglucosamine residue is essential for binding to lentil lectin but not to ConA. It is clear that heterogeneity exists in the carbohydrate moiety of human urinary CSF.

In the Sephadex G-150 analysis, we found that the activity emerged as a sharp peak with an apparent molecular weight of 85,000. When the CSF of different purification stages was rechromatographed on the same column, the activity eluted consistently at the same position in the elution profile. This observation differs from the findings of Stanley et al [20] with CSF from normal human urine. They found that the cruder CSF tended to form complexes with other components so that the apparent molecular weight decreased considerably from 190,000 to 60,000 on gel permeation column during the course of purification. It should be mentioned that molecular weights by the gel permeation method are generally inaccurate for glycoproteins or any nonspherical macromolecules, but these findings suggest that human urinary CSF is an elongated molecule.

Certain CSF preparations have been shown to stimulate colony formation in cultures of the human leukemic cell line KG-1. Human CSF from placenta conditioned medium and Mo cell conditioned medium, which showed very little or no effect on other human leukemic cell lines, were reported to stimulate synthesis of RNA, DNA, and protein by KG-1 cells [21]. KG-1 cells did not respond to the urinary CSF described here.

The fact that antiserum to L-cell CSF binds the urinary CSF described here clearly indicates that antigenic determinants have similarities between these two glycoproteins. Although the molecular weight of L-cell CSF is considerably larger than urinary CSF (70,000 vs 46,000), structural homologies must exist in these molecules permitting interacting with the same target cell. The observation that the L-cell CSF was neutralized by anti-L-cell CSF while the urinary CSF we prepared remained fully active, even though there was some binding to the antibody, further indicates that the relative positions of the antibody-binding sites and the active sites in these two proteins may be different. Alternatively, if the CSF antibody complex dissociated upon dilution for assay in our experiments and not in those with L-cell CSF, it would also indicate a structural difference.

Motoyoshi et al [9] have partially purified a CSF from normal human urine with a potency of 1×10^6 U/mg; about 1% of that reported here. In a recent report [23], they showed that this CSF had a molecular weight of 70,000 on SDS gel electrophoresis. It stimulated colony formation by unfractionated human bone marrow cells but not by monocyte-depleted human bone marrow cells. Based on these observations, they suggested this CSF preparation did not act on the precursor of the cells in the colonies directly, but did so via the stimulation of monocytes.

Stanley et al [8] have partially purified CSF from normal human urine and estimated it to have a molecular weight of 45,000–60,000 and a specific activity of approximately 1×10^7 colonies/mg. The CSF we described here has a considerably higher specific activity. Recently, they isolated urinary ^{125}I-CSF by binding to receptors and eluting [11] and showed it to be a protein of molecular weight of 42,000 consisting of two subunits linked by disulfide bridges. It had a specific activity of 4×10^7 colonies/mg of iodinated protein. This CSF was named CSF-I. It is the only

CSF present in substantial quantities in normal human urine and induces the in vitro formation of only macrophage colonies. That preparation may be the same as the one described in this paper, but it has been shown by Shadduck et al [22] and Stanley et al [24] that normal human urinary CSF activity is neutralized by the antiserum to mouse L-cell CSF. We found that human urinary CSF activity was not affected by binding to anti-L-cell CSF. The data available are not sufficient to determine whether normal human urinary CSF-I is identical to the CSF described in the paper.

ACKNOWLEDGMENTS

This work was supported in part by grants HD07136, HL 21676, and CA 18375 from the National Institutes of Health. We are indebted to Dr H.P. Koeffler, Department of Medicine, UCLA, for performing experiments of the effect of urinary CSF on the KG-1 cell macromolecular synthesis.

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