Biochimica et Biophysica Acta, 1105 (1992) 141–147

BBAMEM 75553

Binding of scatter factor to epithelial cell membrane protein: identification of its receptor

Ansamma Joseph [a], Madhu Bhargava [a], Eliot Rosen [b] and Itzhak D. Goldberg [a]

[a] *Department of Radiation Oncology, Long Island Jewish Medical Center, Long Island Campus for the Albert Einstein College of Medicine, New Hyde Park, NY (USA) and*
[b] *Department of Radiation Therapy, Yale University School of Medicine, New Haven, CT (USA)*

(Received 22 August 1991)

Key words: Scatter factor; Cell membrane protein; Affinity purification; Receptor; (MDCK cell)

Binding of scatter factor (SF) to the surface protein of Madin-Darby canine kidney (MDCK) cells was investigated. The factor has a specific affinity for membrane proteins of MDCK cells and could be purified 10–20-fold using a membrane protein-affinity chromatographic procedure. The binding was pH- and salt-dependent. The factor did not bind to columns prepared with membrane proteins from non responder cells or with bovine serum albumin. Further purification to homogeneity was achieved using reverse phase and immunoaffinity chromatography. The factor dissociated into 92, 62 and 34/32 kDa bands on SDS-PAGE under reducing conditions. A 230 kDa protein band, the receptor-SF complex, was observed when radiolabeled SF was crosslinked to surface proteins of MDCK cells and the complexes were subjected to electrophoresis. The binding of radiolabeled SF to the MDCK cells was decreased in presence of excess unlabeled SF. These observations suggest that the binding of SF to surface proteins of MDCK cells is specific and occurs predominantly to a 150 kDa protein.

Introduction

Scatter factor (SF) is a heterodimeric protein produced by fibroblasts [1–3] and arterial smooth muscle cells [4]. SF is also present in large amounts in human placenta [5,6]. It causes cohesive epithelial cell colonies to scatter and stimulates the migration of individual cells. After treatment with SF, cells spread out, show membrane ruffling, protrude and retract conspicuous processes [7]. Recent findings suggest that SF induced motility in part may be mediated by alterations in protein synthesis, alterations in protein phosphorylation, and cytoskeleton reorganization [8]. Exposure of epithelial cell colonies to SF preparations for 45 min at 4°C or at 37°C followed by vigorous washing and incubation in fresh medium resulted in separation of cells from each other, suggesting the possibility of a specific cell surface receptor mediated interaction [4].

In the present study we have demonstrated that the SF binds to membrane proteins of Madin-Darby canine kidney (MDCK) cells with high affinity and specificity, investigated the characteristics of this binding process, and used this specific binding as a step in the purification of SF. The binding of SF occurred predominantly to a 150 kDa protein and decreased in the presence of excess unlabeled SF.

Materials and Methods

Acrylamide, bisacrylamide, sodium dodecyl sulfate, and cation exchange resin Bio-Rex 70 were purchased from Bio-Rad. Tris-HCl, Triton X-100 and $MgCl_2$ were purchased from Sigma (St. Louis, MO). Fetal calf serum, DMEM and other tissue culture reagents were from GIBCO (Grand Island, NY). CNBr-Sepharose 6B was purchased from Pharmacia.

Cell cultures. Bt 474 and MDCK cells (obtained from ATCC) were grown in DMEM with 10% FCS in presence penicillin-streptomycin as described [5]. All cells were free of mycoplasma.

Scattering assay. Scattering activity was measured on

Abbreviations: BSA, bovine serum albumin; CMP, cell membrane proteins; CNBr, cyanogen bromide; DMEM, Dulbecco's modified Eagle's medium; hpSF, human placental scatter factor; MDCK, Madin-Darby canine kidney; $MgCl_2$, magnesium chloride; $NaHCO_3$, sodium bicarbonate; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate; SF, scatter factor; TBS, 20 mM Tris-HCl (pH 7.5) containing 0.9% NaCl.

Correspondence: M.M. Bhargava, Long Island Jewish Medical Center, Department of Radiation Oncology, 270–05 76th Avenue, New Hyde Park, NY 11042, USA.

colonies of MDCK epithelial cells as described previously [3,4]. Samples to be tested in serum-free DMEM were serially diluted by factors of two in a 96-well titer plate so that each well contains a volume of 150 μl. A suspension of MDCK cells in DMEM-10% FCS was prepared from stock cultures, and 5000 cells in a 150 μl volume were added to each well (total assay volume = 300 μl, final serum concentration = 5%). Plates were incubated at 37°C for 20 h, then stained with crystal violet, and examined by light microscopy. The assay titer of a sample was the highest dilution at which significant scattering effect was observed. This effect consisted of spreading of tight cohesive colonies of MDCK cells, elongation of polygonal cells, and separation into single cells. The activity present at the limiting dilution is defined as 0.5 scattering units/ml.

Preparation of MDCK and Bt 474 cell membrane protein extracts. Bt 474 are epithelial cells which are not scattered by SF. MDCK and Bt 474 cells ($\sim 1 \times 10^7$) were trypsinized, washed in PBS, and lysed in 2 ml distilled water by repeated freezing and thawing. The suspension was spun at 30 000 × *g* for 20 min and the pellet was washed once with PBS. The pellet was then resuspended in 100 mM Tris-HCl buffer (pH 7.5) containing 500 mM NaCl and 1% Triton X-100, vortexed and mixed overnight at 4°C. The suspension was centrifuged at 30 000 × *g* for 20 min at 4°C and the supernatant was used for the preparation of the affinity column. Proteins were quantitated by the dye binding method [9].

Preparation of the affinity column. CNBr-activated Sepharose 6B was swollen in distilled water, washed, and equilibrated with 0.5 M $NaHCO_3$. MDCK cell membrane protein extract was dialyzed against 0.5 M $NaHCO_3$ and then mixed with gel and incubated for 1 h at room temperature and then overnight at 4°C. The gel was washed with 0.25 M $NaHCO_3$ to remove the unbound proteins, and re-equilibrated with 20 mM Tris buffer (pH 6.5) containing 150 mM NaCl [10].

Purification of SF from human placenta. The scatter factor was prepared from human placenta as described before [5] with several modifications. Human placenta was obtained fresh from the delivery room at Long Island Jewish Medical Center after normal and Cesarean deliveries. Approximately 1.5 kg of the tissue was washed with saline (0.9% NaCl), cut into small pieces, and homogenized in a Waring blender for 3 min at low speed followed by 3 min at high speed in 50 mM Tris-HCl buffer (pH 7.5) containing 1 mM PMSF, 2 mM EDTA, and 25 μg/ml gentamycin. The extract was centrifuged at 4°C for 30 min at 30 000 × g in a Sorvall centrifuge and the supernatant was used for the purification of SF. The supernatant was mixed with Bio-Rex 70 resin (0.1 g/ml) in the above buffer and stirred overnight at 4°C. The mixture was filtered, washed, and SF was eluted from the resin using 50 mM Tris-HCl buffer (pH 7.5) containing 800 mM NaCl. The eluates were diluted with buffer so that the final concentration of NaCl was 250 mM. It was then mixed with S-Sepharose and stirred overnight at 4°C. The Sepharose gel was packed into a column, washed and was eluted with a gradient of 0.3–1.2 M NaCl in Tris buffer. SF containing fractions from the affinity column were pooled, concentrated and subjected to FPLC (Pharmacia LKB) using a reverse phase C-18 Pep RPC (HR 10/10 15 μm) column equilibrated with 0.1% TFA in H_2O. The column was washed and the bound SF was eluted using a 0–100% acetonitrile gradient in 0.1% TFA. The fractions containing SF were neutralized with Tris buffer, freeze dried and assayed for SF activity. To obtain highly pure homogeneous preparations of SF with good biological activity, an immunoaffinity purification procedure was used. Antibodies were raised in rabbits against SF purified by reverse phase chromatography. IgG fraction from the antiserum was purified using Protein A-Sepharose and was then coupled to CNBr-activated Sepharose 6B. The fractions containing SF from the above column were pooled and dialyzed against 20 mM Tris-HCl buffer (pH 6.5) containing 150 mM NaCl and applied to the affinity column. The column was washed with the Tris buffer and the bound SF eluted with the immunoaffinity elution buffer (Pierce, Rockford, IL) as described previously [6].

Removal of the non specific binding proteins in SF preparations using a non-responder epithelial cell membrane protein affinity column. An affinity column was prepared with a cell membrane extract from a non-responsive epithelial cell line (Bt 474) by using the above procedures. The SF preparation from the S-Sepharose column was applied to the above affinity column to remove the non-specific proteins that bind to the cell surface components. The unbound proteins were then subjected to column chromatography using the MDCK cell membrane protein affinity column to obtain the SF (see below). About 20% of SF activity was lost in this step.

Affinity chromatography purification of SF. The partially purified SF preparation obtained as described above was applied to the MDCK cell membrane protein affinity column and the eluate was reapplied to ensure optimum binding. After washing to remove the non specifically bound proteins, the bound proteins were eluted from the column with a gradient of (0–5 M) $MgCl_2$ in TBS. The pH of the fractions was adjusted to 7.5, dialyzed against PBS and assayed for SF activity.

Affinity columns for the determination of non-specific binding. Affinity columns were prepared with BSA or MDCK cell lysate supernatant as ligands. MDCK cells were lysed with distilled water by freezing, thawing and by sonication (six times). The proteins in the super-

natant obtained after centrifuging the extract at 30000 $\times g$ for 30 min or BSA were coupled to the CNBr-activated Sepharose 6B as described above.

Preparation of radiolabeled SF. A modification of the method of Greenwood et al. [11,12] was used to iodinate SF. SF was dialyzed against phosphate buffer pH 7.4 at 4°C. Iodination was performed at room temperature. 10 μl (1 mCi) of ^{125}I (carrier free, Amersham) was mixed with 25 μl of 0.5 M sodium phosphate buffer (pH 7.4). The following (in the 0.1 M sodium phosphate buffer (pH 7.4)) were added with stirring; 4–8 μg of SF (200 μl), 20 μg chloramine-T (20 μl) and the mixture was incubated at room temperature for 5 min. 50 μg sodium metabisulfite (50 μl) and 2 mg KI (100 μl) were added to terminate the reaction. The reaction mixture was then applied to a PD-10 (Pharmacia) column equilibrated with 0.03 M Tris-HCl buffer (pH 8.8). 1-ml fractions were collected and monitored for radioactivity (in a Beckman scintillation counter, Model LS 7500) and scattering activity. The fractions containing scatter factor activity and radioactivity were pooled, and mixed with 0.6% BSA in 100 mM Tris-HCl buffer (pH 7.5) in a 2:1 ratio. The specific activity of the labeled SF was $\approx 3 \times 10^4$ cpm/ng.

Binding of ^{125}I to MDCK cells. MDCK cells (number indicated in figure legends) were washed and suspended in PBS containing 25 mM Hepes (pH 7.5) 1 mM $CaCl_2$, 1 mM $MgCl_2$ and 0.1% BSA. Six well collagen coated (Falcon) dishes were used for binding studies. The factor (amounts indicated in figure legends) and the cells were incubated at 4°C for 4 h. The cells were then scraped, washed with cold PBS and dissolved in SDS-PAGE sample buffer (0.1 M Tris-HCl, 2% SDS, 20% glycerol, 0.002% Phenol red (pH 6.8)) and subjected to SDS-PAGE using 7.5% acrylamide. Non specific binding was determined by performing the experiment in the presence of 10 μg/ml of purified unlabeled SF.

Cross linking of ^{125}I to its receptor on MDCK cells. MDCK cells were incubated with ^{125}I-SF as described above, washed thrice with PBS, suspended in TBS containing 100 μg/ml heparin and the suspension incubated for 5 min [13] and washed with PBS again. This was done to remove SF which was bound to heparin-like molecules on the cell surface. Freshly prepared DSS (in DMSO), was added to cells containing 1 ml PBS (final concentration 0.25 mM) and then incubated for 45 min at 4°C for cross linking to occur. Cells were then washed with PBS containing 0.1 mM EDTA, 0.25 M sucrose and 1 mM PMSF and scraped in the same buffer and the suspension was centrifuged in an Eppendorf microcentrifuge. The cell pellet was dissolved in SDS-PAGE sample buffer, boiled for 5 min and subjected to SDS-PAGE (7.5% acrylamide). The gels were dried and kept in contact with Kodak film (XAR-5) at - 80° in the presence of an intensifying screen for 2–3 days and developed.

Results

Purification of SF.

The purification steps are shown in Table I. A 1000-fold purification was achieved by Bio-Rex ion exchange and Sepharose chromatography. A further 10–15-fold purification of SFs was achieved by the affinity chromatography resulting in 10000–15000-fold purification of SF. The steps used in elution of bound hpSF from the membrane affinity column are shown in Fig. 1.

A major 82 kDa band was observed when the purified hpSF preparation was subjected to SDS-PAGE (Fig. 2). When the unstained gel was sliced into 3-mm segments, proteins eluted and assayed for SF activity, only the segment containing the 82 kDa band exhibited activity.

The SF preparation from MDCK affinity step was further purified using a C-18 reverse phase FPLC column. A major protein peak which exhibited scatter factor activity appeared at an acetonitrile concentration of $\approx$ 37% (Fig. 3). The purity of this SF preparation is shown in Fig. 4. When subjected to SDS-PAGE the SF preparations exhibited two protein bands, a minor 90 kDa and a major 82 kDa band. The intensity of the 82 kDa band was 2.6-times higher than the 90 kDa band. (Fig. 4 lane A). Electrophoresis was performed for longer periods to separate the bands. The proteins were eluted from the gel segments containing these protein bands and assayed for activity (method described in Fig. 2 legend). The ratio of the SF activities were 3:4 where as the corresponding protein concentration ratio was 1:2.6. These results suggest that

TABLE I

Purification of scatter factor from human placenta

See text for details.

	Activity units (U $\times 10^6$)	Protein (mg)	Specific activity (U/μg)	Recovery (%)	Purification (x-fold)
Extract	25	$12.5 \cdot 10^3$	0.2	100	1
Bio-rex 70 and sepharose chromatography	12.5	52.5	238	50	1190
Reverse phase FPLC	0.05	0.35	14	–	–
MDCK-CMP affinity chromatography	4.8	2.1	2285	19.2	11425
Immunoaffinity chromatography	1.55	0.31	5000	6.2	17000

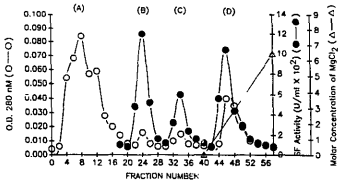

Fig. 1. Affinity chromatography of hpSF. $4 \cdot 10^4$ units of partially purified hpSF was applied to a 4 ml MDCK membrane column. First seven fractions were pooled and reapplied, washed with TBS (pH 6.5). The elution was performed stepwise with 4 column vols of TBS (pH 8.5) (B), followed by TBS (pH 8.5) containing 1 M NaCl (C) and finally with 10 ml of TBS (pH 6.5) and then 0–5 M $MgCl_2$ gradient in TBS (D). Less than 10% of the applied activity was eluted in the pool of 4–12 (A) fractions.

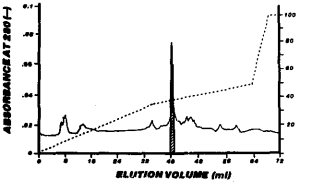

Fig. 3. Reverse phase fast performance liquid chromatography of hpSF. Active fractions from the MDCK membrane affinity column eluates were concentrated to 0.5 ml on Amicon PM10 membranes, was made to 0.1% TFA in H_2O and applied to Pep RPC HR 10/10 C18 (15 μm) column. The column was eluted at 0.5 ml/min with a 70 ml (0–100%) split gradient. Fractions 1 ml were neutralized with TBS (pH 7.5), lyophilized and assayed for MDCK scattering activity. Black stripped peak area marks the fractions which expressed activity.

the specific activity of the 90 kDa band is about 2-times higher than that of the 82 kDa band. On reduction the protein exhibited four bands at 92 kDa, 62 kDa, 34 kDa and 32 kDa each. Although a high degree of purity was attained after the reverse phase chromatography there was considerable loss of activity. Therefore, an immunoaffinity chromatography procedure was used to obtain highly active and pure preparations of hpSF. The specific activity of the purified SF preparations, used in the present studies was 5000 U/μg protein. A single broad band around 82 kDa was observed on SDS-PAGE under non-reducing conditions [6].

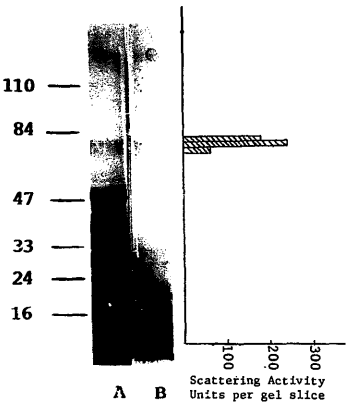

Fig. 2. SDS-PAGE of the membrane affinity purified hpSF. Partially purified hpSF preparations were subjected to SDS-PAGE before (a) and after (b) chromatography using MDCK-membrane protein column. Protein concentrates in the SDS sample buffer were incubated at 37°C and were not boiled to preserve activity. One unstained lane was cut into 1 mm slices and was eluted in sterile PBS at 4°C; by end over end mixing overnight and assayed for scatter activity.

Effect of pH and salt on the binding and elution of hpSF from the MDCK-CMP column

The effect of pH on the binding of SF to MDCK-CMP column is shown in Table II. Higher binding

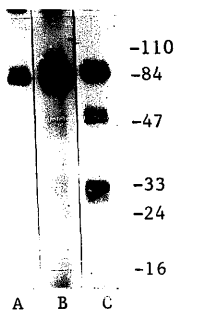

Fig. 4. SDS-PAGE of hpSF. Purified hpSF preparations were labeled with ^{125}I, subjected to SDS-PAGE and radioautography. Lane A and lane B contain non reduced and lane C contains reduced hpSF. Lane A had a shorter exposure to be able to visualize the two bands at 82 kDa and 90 kDa. For details see text.

TABLE II

Effect of pH on binding on hpSF to MDCK cell membrane proteins

$4.2 \cdot 10^4$ units of hpSF were applied to a 3 ml column repeatedly, five times in the buffer of indicated pH, washed with the same pH buffer (15 ml) and eluted with 5 M $MgCl_2$. Eluates were dialyzed against TBS (pH 7.5) and assayed for SF activity.

pH of the binding buffer	SF bound ($U(\times 10^4)$)	SF unbound ($U(\times 10^4)$)	SF recovery (% of the bound)
5.5	4.07	0.12	34
6.5	3.7	0.49	67
7.5	3.5	0.69	65

efficiency was observed at pH 5.5 than at pH 6.5 or pH 7.5. However, the specific activity of SF in the eluates was lower when the binding was done at pH 5.5 instead of pH 6.5 or pH 7.5. The lower recovery of SF at this pH could be due to stronger binding or the reduction in activity of hpSF. Moderate binding and better recoveries were obtained when the samples were applied at pH 6.5. Therefore the binding was done at pH 6.5 in all the subsequent studies.

The effect of salt and pH on the elution of SF bound to the column is shown in Table III. Elution with Tris buffer (pH 8.5) yielded one fourth of the bound activity, with high specific activity. Addition of 1 M NaCl in Tris buffer (pH 8.5) resulted in an additional 5% recovery. Approximately 70% of the bound activity was recovered with a gradient of 0–5 M $MgCl_2$ with SF eluting between 1.5 to 3 M salt concentration (Fig. 1).

Specificity of binding of hpSF to MDCK-CMPs

Non specific binding of SF to the affinity column was determined by using BSA as a ligand. With BSA-affinity column very low binding and recovery (2–4%) was observed. This may represent non-specific binding. Similarly a low binding and recovery (6–8%) of SF was observed from a column prepared with CMPs of a non-responder cell line Bt 474. MDCK cell lysate su-

TABLE III

Effect of salt concentration on recovery of hpSF from MDCK-CMP Sepharose columns

$4.2 \cdot 10^4$ units of hpSF in TBS (pH 6.5) were applied to a 3 ml column repeatedly and eluted with the indicated buffer. About 90% of the activity was bound to the column. The eluate fractions were dialyzed in TBS (pH 7.5) and assayed for SF activity.

Elution buffer	Recovery (%)
TBS, pH 8.5	26–40
TBS, pH 8.5 + 1 M NaCl	30–45
0–5 M $MgCl_2$	68–72

TABLE IV

Binding of hpSF to columns prepared with MDCK-CMP, BSA, Bt-474-CMPs and MDCK-cell lysate supernatant proteins as ligands

$4.2 \cdot 10^4$ units of hpSF in TBS (pH 6.5) were applied on to a 3 ml column and washed with 15 ml of TBS and eluted with 5 M $MgCl_2$. The fractions were dialyzed and assayed for SF activity. For details, see text.

Ligand (10 mg)	Unbound (%)	Recovery (%)
MDCK-CMP	16	68
BSA	72	3
Bt-474 cell membrane protein	76	7
MDCK cell lysate supernatant	58	18

TABLE V

Effect of trypsinization on binding of hpSF to the MDCK cell membrane proteins

$16 \cdot 10^4$ units of hpSF in TBS (pH 6.5) were applied on to a 10 ml column and washed with 50 ml of [illegible] and eluted with 5 M $MgCl_2$. The fractions were dialyzed and assayed for activity.

MDCK cells	Number of cells	Total cell surface proteins (mg)	SF bound ($U(\times 10^4)$)	SF recovered ($U(\times 10^4)$)	Recovery (%)
Scraped	$2 \cdot 10^7$	39	13.7	8.1	59
Trypsinized	$2 \cdot 10^7$	27	12.5	8.2	68

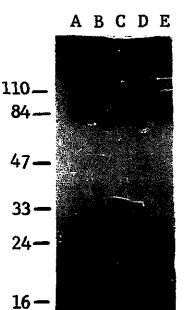

Fig. 5. Displacement of bound ^{125}I-hpSF with increasing amounts of unlabeled hpSF. 10^5 MDCK cells were incubated with 50 ng of ^{125}I-hpSF (A) and with 0.3 (B), 0.6 (C) 1.2 (D) and 2.4 (E) μg of unlabeled SF for 3 h at 4°C. The cells were washed, subjected to SDS-PAGE and autoradiography by methods described in the text.

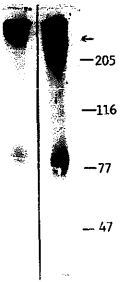

Fig. 6. Identification of a high molecular weight form of hpSF receptor, by SDS-PAGE. 10^5 MDCK cells were incubated with ^{125}I-SF under standard binding conditions, without any other additives (lane B) or in the presence of 100× excess of unlabeled hpSF (lane A). The cells were washed in PBS and then treated with a crosslinking agent DSS (0.25 mM), washed and dissolved in sample buffer and subjected to SDS-PAGE. The gels were dried and processed for autoradiography. A 48 h exposure is shown in the figure. The arrow shows the receptor-hpSF high molecular weight complex.

pernatant proteins exhibited a slightly higher (15–20%) binding and recovery (Table IV).

Effect of trypsin on the binding of hpSF to MDCK-CMP columns

Since trypsin was used to remove the MDCK cells from the plastic, its effect on the relative affinity of hpSF to cell membrane proteins was determined. Two separate affinity columns were prepared with cell membrane proteins of trypsinized and scraped MDCK cells. No significant differences were observed in the binding of hpSF to these columns (Table V).

Displacement of binding of ^{125}I-SF to MDCK cells by unlabeled SF

As can be seen from Fig. 5, there was a concomitant decrease in binding of ^{125}I-SF (82 kDa band) to MDCK cells with increasing amounts of unlabeled SF. A radioactive band was observed on the top of gels; the amount of this band also decreased with the increasing concentration of unlabeled SF. This would suggest that this band is comprised of aggregated form of SF and that it also binds efficiently to the surface of MDCK cells.

Affinity cross linking of ^{125}I-SF to its receptor on MDCK cells

When ^{125}I-SF was cross linked to its receptor using DSS (by methods described above) and the complex subjected to SDS-PAGE and radioautography, a radioactive band with molecular weight of approx. 230 000 dalton was observed (Fig. 6). This band was not present when cells were omitted from the incubation system and also when excess of cold SF was present in the incubation mixture. These data would suggest that this band represents SF-receptor complex and that the approximate molecular mass of the SF-receptor is 250 000 daltons.

Discussion

Purification and subunit structure of hpSF

SF purification from human embryo fibroblasts has been reported recently [14]. The factor migrated as a broad band at an average molecular weight of 64 000 on non-reducing SDS gels, and all parts of this band exhibited the same specific activity.

Gherardi et al. [1] have purified SF from conditioned medium of ras transformed NIH 3T3 cells to homogeneity by a different method. The mouse scatter factor is a 62 kDa peptide which dissociates into 57 and 30 kDa subunits on reduction. A 90 kDa peptide was reported to be present in only certain preperations. Our scatter factor preparations from human placenta always exhibit a 92 kDa component when subjected to SDS-PAGE under reducing conditions. This could represent unprocessed SF. Partial amino acid sequence data derived from tryptic peptides of mouse [5,17] and human [14] fibroblast derived SFs indicate significant sequence homology with rat and human HGFs. Recently SF was shown to be identical to HGF. The two subunits arise from a precursor protein by a specific proteolytic cleavage [15].

The data presented here suggests that both the unprocessed and the processed forms exhibit biological activity when they are still cross linked by disulfide bridges. Biological activity could not be detected in peptides eluted from the 92, 62 and 32 kDa bands separated under reducing conditions. These findings are in agreement with those of Gherardi et al. [1] and Rosen et al. [5] who have demonstrated that sulfhydryl groups of SF are required for its biological activity. Weidner et al. [14] have shown that the upper portion of the single diffused 64 kDa band on reduction generates the 62 and 34/32 bands, and the lower band mainly consists of the 92 kDa band. Peptide map comparisons and sequence analysis data has also demonstrated that the 62 and 34/32 bands are derived from the 92 kDa band. These results suggest that SF preparations from human placenta and conditioned medium of human embryo fibroblasts may consist of a mixture of processed (cleaved by a specific protease but still linked by disulfide bonds) and unprocessed (uncleaved) forms and that both forms are biologically active.

'Scatter factor like' activity of antibodies to cell adhesion molecules and integrins

SF-induced scattering involves dissociation of cells from each other and from extracellular matrix components. Cell adhesion molecules and integrins are involved in cell adhesions and cell–substratum interactions. Antibodies to cell adhesion molecules (CAMs) [18] and integrin [19], a receptor for several extracellular matrix proteins, also causes 'scatter factor like' effects. Whether SF acts through regulating CAMs or integrins remains to be determined.

Binding of hpSF to cell surface proteins and identification of a SF-receptor

The binding of hpSF to the columns prepared from MDCK-CMPs was dependent on the pH. Binding was better at pH 5.5 than at pH 7.5 but the recovery was low. Elution with 5 M $MgCl_2$ releases all the bound proteins. This lowers the specific activity of the SF in this fraction. The lower pH (3.5) of $MgCl_2$ and the high (1.5–3 M) salt concentration, may account for the loss of activity under these conditions. A loss of 30% activity at pH 3 for SF has been reported earlier [4]. Therefore, for the highest specific activity, the elution with TBS (pH 8.5) was considered optimal; however, for the higher total recovery of SF, elution was performed with a gradient of 0–5 M $MgCl_2$.

Recent findings have shown that SF is identical to hepatocyte growth factor, a mitogen for hepatocytes. A receptor for HGF has been partially identified [13] and characterized [16]. The molecular mass of the receptor for SF (150 kDa), (derived from SF-receptor complex of 230 minus 80 kDa, the molecular mass of SF) observed in the present studies is very similar to that observed by these investigators. Our results suggest that MDCK cell surface proteins exhibits two levels of affinity towards scatter factor. The elution of SF at near neutral pH and low salt from the affinity column may represent low-affinity binding while the elution at higher pH and higher salt may represent high-affinity binding. HGF exhibits a low affinity binding site (for heparin or heparin like molecules) and a high-affinity specific binding site (to its receptor) for the surface components of hepatocytes [16].

The factor does not show any significant binding to the BSA or to non-responsive (Bt 474) cell membrane proteins. The small amount of binding and recovery observed with MDCK-cell lysate supernatant proteins could be due to the presence of some SF binding protein(s) in the cytoplasm or due to incomplete removal of cell membrane proteins from the cell lysate supernatant.

The binding characteristics of SF to CMPs of MDCK cells reported here may be useful in devising experiments related to binding of SF to MDCK cells. The information could also be useful for the purification of receptor(s) for SF using SF-affinity chromatographic procedures. Advantages of affinity chromatographic procedures are obvious, it renders better total recoveries, purer preparations, and the affinity column could be used repeatedly. With increasing interest in scatter factor and other motility factors, the procedure described above may be useful in the purification and determination of binding characteristics of these factors.

Acknowledgement

This work was supported by a grant from Joel Finkelstein foundation and by a fellowship award to Ansamma Joseph from Long Island Jewish Medical Center. Dr. Rosen was supported by USPH CA50516, ACS Grant BE7, and Established investigatorship American Heart Association.

References

1 Gherardi, E., Grey, J., Stoker, M., Perryman, M. and Furlong, R. (1989) Proc. Natl. Acad. Sci. USA 86, 5844–5848.
2 Stoker, M. and Perryman, M. (1985) J. Cell Sci. 77, 209–223.
3 Stoker, M., Gherardi, E., Perryman, M. and Gray, J. (1987) Nature 327, 239–242.
4 Rosen, E.M., Meromsky, L., Setter, E., Vinter, D.W. and Goldberg, I.D. (1990) Invasion Metastasis 10, 49–64.
5 Rosen, E.M., Kacinski, B.M., Romero, R., Setter, E. and Goldberg, I.D. (1990) Biochem. Biophys. Res. Commun. 168, 1082–1088.
6 Bhargava, M.M., Li, Y., Joseph, A., Pendergast, M., Hofmann, R., Rosen, E.M. and Goldberg, I.D. (1991) in Cell Motility Factors (Goldberg, I.D. and Rosen, E.M., eds.), pp. 63–75, Birkhauser-Verlag, Geneva.
7 Warn, R.M. and Dowrick, P. (1989) Nature 340, 186–187.
8 Rosen, E.M., Meromsky, L., Goldberg, I.D., Bhargava, M.M. and Setter, E. (1990) J. Cell. Sci. 96, 639–649.
9 Bradford, M. (1976) Anal. Biochem. 72, 248–254.
10 Kowal, R. and Parsons, R.G. (1980) Anal. Biochem. 102, 72–76.
11 Greenwood, F.C., Hunter, W.M. and Glova, J.S. (1963) Biochem. J. 89, 114–123.
12 Frolik, C.A., Wakefield, L.M., Smith, D.M. and Spoon, M.B. (1984) J. Biol. Chem. 259, 10995–11000.
13 Zarnegar, R., DeFrances, M.C., Oliver, L. and Michalopoulos, G. (1990) Biochem. Biophys. Res. Commun. 173, 1179–1185.
14 Weidner, K.M., Arakaki, N., Hartmann, G., Vandekerckehove, J., Weingart, S., Rieder, H., Fenaisch, C., Tsubouchi, H., Hishida, T., Daikuhara, Y. and Birchmeier, W. (1991) Proc. Natl. Acad. Sci. USA 88, 7001–7005.
15 Nakamura, T., Nishizawa, T., Hagiya, M., Seki, T., Shimonishi, M., Sugimura, A., Tashiro, K. and Shimizu, S. (1989) Nature 342, 440–443.
16 Bottaro, D.P., Rubin, J.S., Faletto, D.L., Chan, A.M.L., Kmiecik, T.E., Vandewoude, G.F. and Aaronson, S.A. (1991) Science 251, 802–804.
17 Gherardi, E. and Stoker, M. (1990) Nature 346, 228.
18 Behrens, J., Birchmeier, W., Goodman, S.L. and Imhof, B.A. (1985) J. Cell. Biol. 101, 1307–1315.
19 Pulido, R., Elices, M.J., Campanero, M.R., Osborn, L., Schiffer, S., Garcia-Pardo, A., Lobb, R., Hemler, M.E. and Sanchez-Madrid, F. (1991) J. Biol. Chem. 266, 10241–10245.