Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 5844–5848, August 1989
Cell Biology

Purification of scatter factor, a fibroblast-derived basic protein that modulates epithelial interactions and movement

ERMANNO GHERARDI*, JULIA GRAY*†, MICHAEL STOKER*†‡, MARION PERRYMAN*†, AND ROBERT FURLONG*†

*Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge, CB2 1QP, England; and †Imperial Cancer Research Fund Laboratories, Lincoln's Inn Fields, P.O. Box 123, London, WC2A 3PX, England

Communicated by J. B. Gurdon, April 17, 1989 (received for review March 11, 1989)

ABSTRACT **Scatter factor is a fibroblast-derived protein that causes separation of contiguous epithelial cells and increased local mobility of unanchored cells. Highly purified scatter factor has been obtained by a combination of ion-exchange and reverse-phase chromatography from serum-free medium conditioned by a *ras*-transformed clone (D4) of mouse NIH 3T3 fibroblasts. Under nonreducing conditions scatter factor has a pI of ≈9.5 and migrates in SDS/polyacrylamide gels as a single band at ≈62 kDa from which epithelial scatter activity can be recovered. Treatment with reducing agents destroys biological activity and is associated with the appearance of two major bands at ≈57 and ≈30 kDa. Whether both the 57-kDa and 30-kDa polypeptides are required for biological activity remains to be established. All the activities observed in crude medium conditioned by cells producing scatter factor are retained by highly purified preparations of scatter factor. These include (*i*) increased local movement, modulation of morphology, and inhibition of junction formation by single epithelial cells and (*ii*) disruption of epithelial interactions and cell scattering from preformed epithelial sheets. These changes occur with picomolar concentrations of purified scatter factor and without an effect on cell growth.**

Cell movement is restricted by the interaction with basement and extracellular membrane proteins (1) and, in certain tissues, by cell–cell interactions that involve cell-specific adhesion molecules (2). Movement of epithelial cells is further limited by cell–cell (desmosomes, tight and gap junctions) and cell–substratum (hemidesmosomes) junctional systems. Cytokines are also involved in the regulation of cell movement, as it appears that certain growth factors, including nerve growth factor (3), platelet-derived growth factor (4), and epidermal growth factor (5, 6) may stimulate cell movement as well as cell growth.

There is increasing evidence, however, for a new group of cytokines that regulate cell movement with little or no effect on cell growth. Pioneering studies by Yoshida *et al.* (7) indicated that certain mouse and rat hepatoma lines and mouse and human leukemias produced a protein of 70 kDa that was chemotactic for the producer cells as well as for other tumor cells but not for polymorphonuclear cells (7). More recently, another motility factor has been isolated from serum-free medium conditioned by the human melanoma line A2058. This 55-kDa protein has both chemotactic and chemokinetic activity for the producer cells (but not for polymorphonuclear cells) and has been designated autocrine motility factor (AMF) (8). *ras*-transformed derivatives of mouse NIH 3T3 fibroblasts also produce AMF and respond to it and, interestingly, normal NIH 3T3 firboblasts, which do not produce AMF, are able to respond to the AMF secreted by *ras*-tranformed cells (8). A factor similar to AMF has been isolated from serum-free medium conditioned by a highly metastatic clone (MTLn3) of rat mammary adenocarcinoma (9). This factor (53 kDa) is chemotactic and chemokinetic both for the producer cells and, to a reduced extent, for a less metastatic clone of rat mammary adenocarcinoma. Recently, another motility factor produced by fetal fibroblasts and by fibroblasts from patients with cancer has been identified (10). This factor is thought to be responsible for the high migration rate exhibited by the producer cells in collagen matrices and can also stimulate the migration of high-density cultures of normal adult fibroblasts (10).

A feature common to the factors described by Yoshida *et al.* (7), Liotta *et al.* (8), Atnip *et al.* (9), and Schor *et al.* (10) is that they appear to stimulate the motility of the producer cells in an autocrine mode.

In our laboratory we have identified a motility factor different, in several respects, from the ones described above. The factor is present in medium conditioned by normal embryo fibroblasts and certain normal and transformed fibroblast lines and affects the motility of epithelial cells, thus acting in a paracrine rather than autocrine manner (11, 12). In the presence of this factor epithelial colonies expand and cells are scattered; therefore, the factor has been designated "scatter factor."

We have now been able to obtain highly purified preparations of scatter factor and we report here that purified factor can inhibit or disrupt epithelial interactions at picomolar concentrations in the absence of any effect on cell growth.

MATERIALS AND METHODS

Cell Cultures and Conditioned Medium. Madin–Darby canine kidney (MDCK) cells were maintained and propagated as described (11). The *ras*-transformed D4 clone of NIH 3T3 cells was grown in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS). For preparation of serum-free conditioned medium, 5×10^7 cells were seeded in 200 ml of DMEM with 10% FBS into 2-liter roller bottles and cultured for 5 days with one medium change. At day 5, cells were washed twice with 100 ml of phosphate-buffered saline and incubated for 48 hr in 200 ml of serum-free DMEM. At day 7, the medium was harvested and the culture discarded. Serum-free conditioned medium was centrifuged at 5000 × *g* for 15 min and concentrated ≈50-fold with an Amicon P30 membrane before dialysis against Mono S buffer A (0.05 M Mes/0.25 M NaCl, pH 6.0). After dialysis the sample was centrifuged at 18,000 × *g* for 15 min, filtered on 0.2-μm filters (Millex GS, Millipore) pretreated with bovine serum albumin (BSA), and used for chromatography.

Fast Protein Liquid Chromatography. FPLC of medium conditioned by *ras*-transformed D4 NIH 3T3 fibroblasts was performed with the cation- and anion-exchange (Mono S and Mono Q) and C_8 reverse-phase (ProRPC) columns (Phar-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: BSA, bovine serum albumin; FBS, fetal bovine serum.

‡To whom reprint requests should be addressed.

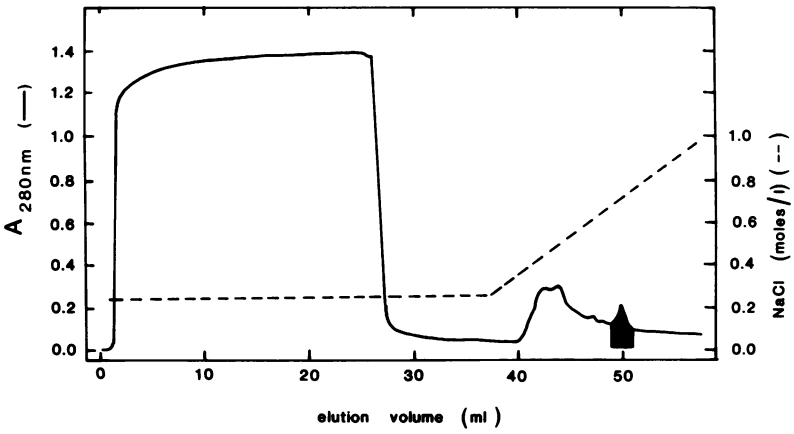

FIG. 1. Cation-exchange FPLC of medium conditioned by *ras*-transformed D4 NIH 3T3 cells. Concentrated, serum-free conditioned medium (166.1 mg of protein) in 24 ml of Mono S buffer A (0.05 M Mes/0.25 M NaCl, pH 6.0) was loaded on a Mono S HR 5/5 column equilibrated in buffer A at a flow rate of 1 ml/min. Bound proteins were eluted with a linear gradient of NaCl (0.25–1.0 M; broken line) and 1-ml fractions were collected. Fractions containing MDCK scattering activity (Mono S fraction) are shown in black. Recovery of protein and MDCK scatter activity from the column are shown in Table 1.

macia LKB). Chromatography was performed at room temperature. The conditions used for elution of scatter activity from these columns are described in detail in the legends to Figs. 1–3.

Electrophoresis and Protein Assay. SDS/PAGE was performed in gel slabs 1.5 mm thick. Total monomer concentration in the separating gel was 15% and the acrylamide/*N,N'*-methylenebisacrylamide weight ratio was 29:1. Reduced samples were prepared by boiling for 2 min in 5% 2-mercaptoethanol/2% SDS prior to electrophoresis. Size markers were myosin heavy chain (205 kDa), β-galactosidase (116 kDa), phosphorylase *b* (97 kDa), BSA (66 kDa), egg albumin (45 kDa), and carbonic anhydrase (29 kDa). Isoelectric focusing was carried out in polyacrylamide gel rods (0.5 × 9 cm); total monomer concentration was 7.5% and the acrylamide/methylenebisacrylamide ratio was 28.6:1.4. The following catolyte and anolyte solutions were used: 0.02 M NaOH and 0.01 M H_3PO_4 (pH 3.5–10), 0.01 M ethanolamine and 0.01 M DL-glutamic acid (pH 5–8), and 0.01 ethylenediamine and 0.01 M Hepes (pH 8–10.5). Protein was measured by absorbance at 280 nm with BSA as standard and a value of $A_{280}^{1\,cm} = 0.65$ for a 1-mg/ml solution of BSA.

Biological Assays. These were carried out as described (11). In brief, MDCK scattering activity was assayed in 96-well plates by using serial 2-fold dilutions of test fractions in 0.15 ml of DMEM containing 5% FBS. MDCK cells (3000 per well) were then added in 0.15 ml of DMEM with 5% FBS and the plates were incubated overnight at 37°C. Cell scattering was assessed after fixation and staining. The highest dilution of the sample at which scattering could be observed (the titer) was divided by 0.3 to obtain the units of scatter activity per milliliter. Time-lapse cinematography was carried out at 90-sec intervals overnight using 35-mm dish cultures at 37°C.

RESULTS

We reported earlier (12) that two clones of mouse 3T3 fibroblasts (J2 and D4) released epithelial scatter activity indistinguishable from that released by the MRC5 strain of human embryo fibroblasts, which had been used in previous studies (11, 13). Thus a fast-growing *ras*-transformed derivative of the D4 clone was chosen for production and purification of the factor.

ras-transformed D4 NIH 3T3 fibroblasts were cultured in medium containing 10% FBS to a density of ≈1.5×10^5 cells per cm^2 and then switched to serum-free medium for 2 days. Serum-free, concentrated conditioned medium was loaded on an FPLC cation-exchanger (Mono S) column at pH 6.0 under conditions optimized for binding of scatter activity to the column and elution of the bulk of the protein in the unbound fraction. Epithelial scatter activity, measured with MDCK cells in a microtiter well assay (11), was eluted at ≈0.7 M NaCl (Fig. 1, black area). Typical recoveries of protein and scatter activity from Mono S chromatography are shown in Table 1. Specific activity increased from 3.7 units/μg of protein in unprocessed conditioned medium to ≈2 units/ng of protein (Mono S fraction) with a purification factor of >500. Rechromatography of this fraction on Mono S increased the specific activity to 3–5 units/ng (data not shown).

Further purification of scatter factor was achieved by FPLC anion-exchange chromatography on a Mono Q column at pH 8.0. From this column MDCK scatter activity was eluted as a broad peak at 0.15–0.20 M NaCl (Fig. 2). The fraction obtained from sequential chromatography on Mono S and Mono Q is referred to hereafter as the Mono Q fraction. Recovery of epithelial scatter activity from the Mono Q column was consistently >50% and the specific activity ranged between 5 and 10 units/ng, but the activity was very unstable (see below). Scatter factor partially purified on a Mono S column (Mono S fraction) or on Mono S and Mono Q columns (Mono Q fraction) was further purified by reverse-phase chromatography on a C_8 (ProRPC) column from which MDCK scatter activity was eluted as a sharp peak at ≈50% acetonitrile (Fig. 3).

SDS/PAGE of scatter factor purified by ion-exchange and reverse-phase chromatography is shown in Fig. 4. Under nonreducing conditions a major band was present at ≈62 kDa (Fig. 4, lane a). Under reducing conditions two bands were present, at ≈57 and ≈30 kDa (lane b). Preliminary experiments have indicated that the N terminus of the 30-kDa protein is blocked. A third, minor band at ≈90 kDa was seen

Table 1. Recovery of protein and MDCK scatter activity after cation-exchange FPLC of conditioned medium (CM) from *ras*-transformed NIH 3T3 cells

	Volume, ml	Scatter activity: Units × 10^{-3} per ml	Scatter activity: Units × 10^{-6}	Scatter activity: %	Protein: μg/ml	Protein: mg	Protein: %	Specific activity, units/μg
CM	1345	1.7	2.29	100.0	457	614.8	100.0	3.7
Processed CM*	24	68.3	1.64	71.6	6920	166.1	27.0	9.9
Mono S, unbound	31	0	0	0	5000	155.0	25.2	0
Mono S fractions 50 + 51	2	983.0	1.97	86.0	495	1.0	0.2	1985.9

*Concentrated, dialyzed, and filtered.

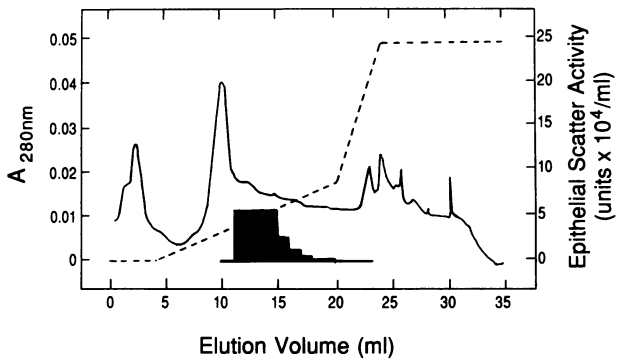

FIG. 2. Anion-exchange FPLC of partially purified scatter factor. Two milliliters of Mono S fraction (0.42 mg of protein; 4.4×10^5 units of MDCK scatter activity) was dialyzed against 0.05 M Tris Cl (pH 8.0) and loaded on a Mono Q HR 5/5 column equilibrated in the same buffer at a flow rate of 1 ml/min. Bound proteins were eluted with a gradient of NaCl (0–1.0 M; broken line) and 1-ml fractions were collected. The black histogram shows the fractions containing MDCK scatter activity. Recovery of activity from the column was 64%.

in some, but not all, preparations. The actual specific activity of scatter factor purified by ion-exchange and reverse-phase chromatography (ProRPC fraction) was difficult to establish because the amount of protein recovered from the reverse-phase column was very low and could not be measured accurately. Densitometric scanning of nonreduced samples in silver-stained gels calibrated with known amounts of BSA indicated that the specific activity of the ProRPC fraction could be in excess of 50 units/ng. However, it is possible that the amount of protein in the final fraction was underestimated, since it is known that some proteins (including several glycoproteins) do not stain well with silver. If so, the specific activity of purified scatter factor could be significantly lower than 50 units/ng, although it must be at least 2 units/ng based on the A_{280} of the Mono S fraction (Table 1).

MDCK scatter activity could be recovered from the nonreduced 62-kDa band after elution by diffusion in 0.05 M NH_4HCO_3/1 mM SDS, pH 7.8 (Fig. 5). No scatter activity could be recovered from the reduced 57-kDa or 30-kDa bands (data not shown), a finding consistent with our previous observation that reductive alkylation of crude factor preparations is associated with complete loss of biological activity

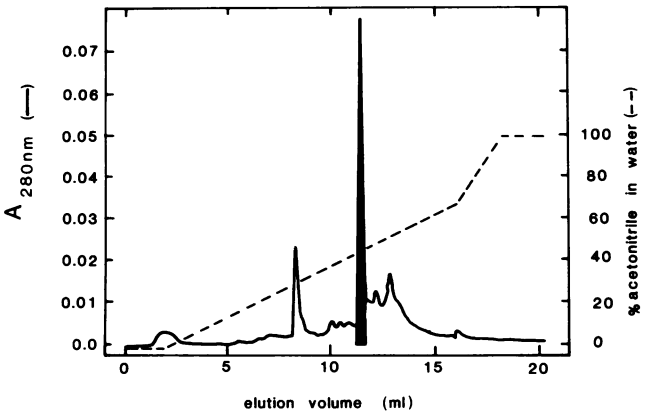

FIG. 3. Reverse-phase FPLC of partially purified scatter factor. One milliliter of Mono S fraction (0.55 mg of protein, 7.0×10^5 units of MDCK scatter activity) was adjusted to 0.1% trifluoroacetic acid (pH 2.5) and loaded on a ProRPC HR5/10 column equilibrated in 0.1% trifluoroacetic acid at a flow rate of 0.2 ml/min. Bound proteins were eluted with a gradient of acetonitrile (broken line), and fractions were freeze-dried and resuspended in 1.0 ml of phosphate-buffered saline for assay of activity. The peak of activity is shown in black. Recovery of activity from the column was 15.6%.

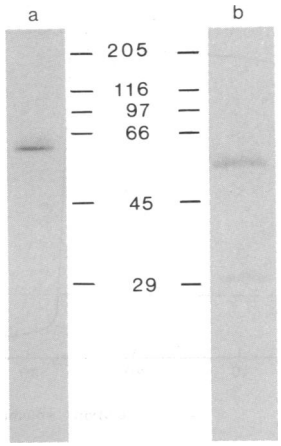

FIG. 4. SDS/PAGE of purified scatter factor. The peak of MDCK scatter activity from the ProRPC column (freeze-dried and stored at −20°C for 3 days) was dissolved in 100 μl of SDS lysis buffer (without 2-mercaptoethanol) and incubated at room temperature for 30 min. The sample was then divided in two and half of it (nonreduced) was loaded directly on a 15% polyacrylamide gel containing SDS (14). To the other half (reduced), 2.5 μl of 2-mercaptoethanol was added and the sample was heated at 100°C for 2 min before loading. The gel was stained with the Bio-Rad silver staining kit. The mobility of size markers (kDa) is indicated.

(15). Isoelectric focusing of nonreduced Mono S or ProRPC fractions on pH 3.5–10 or pH 5–8 gel rods followed by elution of scatter activity from gel slices showed that the peak of scatter activity was present at the top of the gel and had an apparent pI of ≈8.5 (data not shown). The presence of the activity at the top of the gel was not due to aggregation of the factor, since addition of 6 M urea or Triton X-100 and 6 M urea to the sample and gels did not alter significantly the peak of scatter activity. Scatter factor entered gels focused in a narrower pH interval (8–10.5) and migrated with an apparent pI of ≈9.5 (data not shown). These data are consistent with the strong binding of scatter activity to the cation exchanger (Fig. 1).

Stability of scatter factor varied greatly according to the stage of purification. We reported earlier (15) that scatter activity in unprocessed conditioned medium was stable for months at 4°C or −20°C. Stability decreased with purification, and although Mono S fractions have been stored for weeks at −20°C with only occasional loss of activity, the fraction obtained after sequential chromatography on Mono S and Mono Q was very unstable (>90% of the activity was

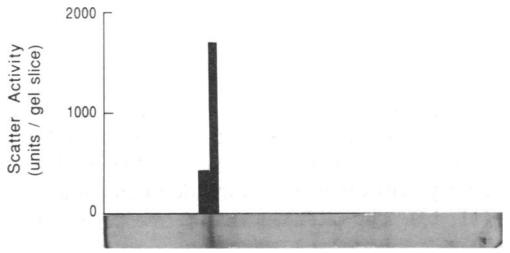

FIG. 5. Recovery of MDCK scatter activity after nonreducing SDS/PAGE. A 1.2-ml aliquot of partially purified scatter factor (Mono Q fraction) containing 1.1×10^5 units/ml was loaded in a 6-cm sample slot of a 15% polyacrylamide gel containing SDS. When electrophoresis was completed half the gel was silver-stained and the other half was used for elution of MDCK scatter activity from the gel. For this, 0.5-cm gel strips were cut in 3-mm slices and these were incubated overnight in 1 ml of 0.05 M NH_4HCO_3/1 mM SDS, pH 7.8, at 37°C with shaking. MDCK scatter activity was assayed directly on gel eluates at dilutions of 1:8 or higher. Lower dilutions were toxic and could not be assayed. Recovery of MDCK activity after electrophoresis and elution from the gel was 23.3%.

lost after 2 hr regardless of the storage temperature). This loss of activity could be entirely prevented by BSA (1 mg/ml) but not glycerol or sucrose (20%, vol/vol), suggesting that it was probably due to adsorption. Fractions obtained from reverse-phase chromatography were freeze-dried immediately and found to be active in the MDCK assay up to 4 weeks after freeze-drying, but the stability of these fractions has not been carefully investigated.

When purified scatter factor (ProRPC fraction) was assayed in MDCK cells by the standard method, the same changes were observed as in assays of unprocessed conditioned medium or partially purified factor—namely, cell separation and alteration in morphology (Fig. 6). The time course of the changes in MDCK cells following exposure to purified scatter factor, when observed by time-lapse cinematography, was also similar to that found with crude conditioned medium. The observed changes included an immediate expansion of colony area, followed 5 hr later by progressive separation of the cells, with increased membrane ruffling, increased local motility with rapid extension and withdrawal of pseudopodia, and consequent change from a rounded to a multipolar shape.

We previously reported (11) a small inhibition of growth of MDCK cells accompanying the above changes after exposure to conditioned medium containing the factor. This inhibitory activity was lost on purification. Highly purified factor (ProRPC fraction) caused a small increase in the thymidine incorporation of MDCK cells, but not 3T3 cells, in the presence of 5% FBS (Table 2). However, cell counts showed no significant change in growth of either MDCK or 3T3 cells after exposure to highly purified factor (Table 2).

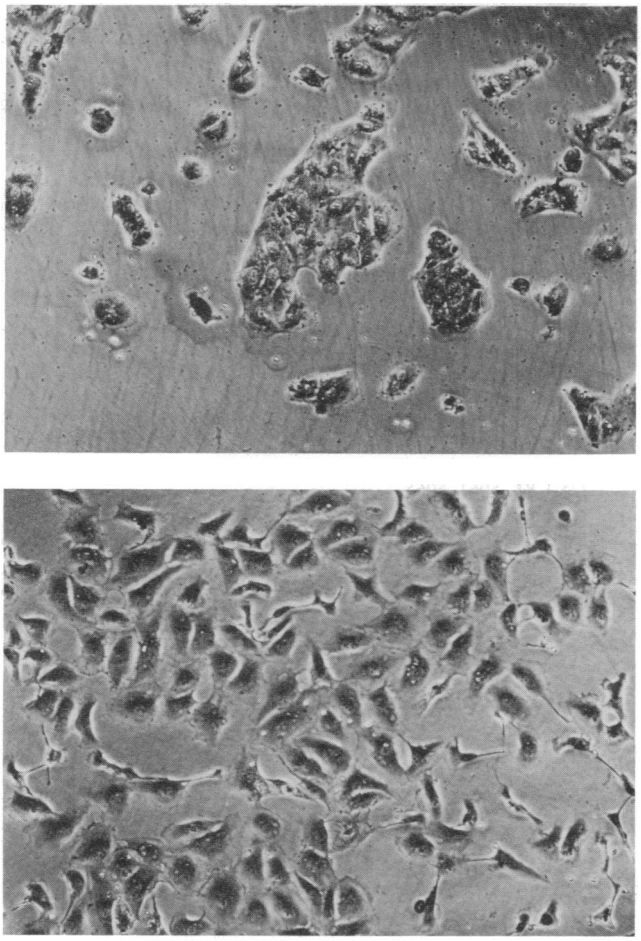

FIG. 6. Distribution of MDCK cells in wells of assay plates without (*Upper*) and with (*Lower*) 8 units of purified scatter factor (ProRPC fraction). Assay was performed as described (11). Cells were fixed and stained with methylene blue. (×100.)

Table 2. Effect of purified scatter factor (SF) on DNA synthesis and growth of MDCK and 3T3 cells

Cells	Addition	DNA synthesis,* cpm per well	Cell count,† no. × 10^{-4}
MDCK	BSA (100 μg/ml)	2126 ± 62	129 ± 6.2
	SF (100 units/ml)	2663 ± 103	ND
	SF (1000 units/ml)	2465 ± 88	118 ± 7.3
3T3	BSA (100 μg/ml)	2501 ± 166	20 ± 3.2
	SF (100 units/ml)	2700 ± 153	ND
	SF (1000 units/ml)	2613 ± 11	20 ± 2.4

*Cells (3000 in 0.1 ml of DMEM with 5% FBS) were added to wells in triplicate. Forty-eight hours later, phosphate-buffered saline (0.1 ml) containing BSA (1 mg/ml) with or without SF was added. After incubation for 48 hr, [^{3}H]thymidine was added (5 μCi/ml, 1 μg/ml final concentration; 1 μCi = 37 kBq). Five hours later the medium was removed, the cells were washed, and trichloroacetic acid-insoluble radioactivity was measured by liquid scintillation. Values are means ± SEM of three wells.

†Replicate cultures of MDCK or 3T3 cells (seeded at 5 × 10^4 per 35-mm dish) were grown in DMEM with 5% FBS for 48 hr before addition of phosphate-buffered saline containing BSA (1 mg/ml) with or without purified SF. Forty-eight hours later the cells were suspended in 0.25% trypsin/EDTA and counted in a hemocytometer chamber. Values are means ± SEM of three dishes. ND, not done.

DISCUSSION

We previously reported (12, 15) that epithelial scatter activity in unprocessed conditioned medium was associated with a protein peak of 50 kDa as judged by gel filtration chromatography in 6 M guanidinium chloride or 8 M urea. The experiments reported here indicate that in highly purified preparations of scatter factor, the activity is associated with a single protein band of ≈62 kDa after SDS/PAGE of nonreduced samples (Fig. 4, lane a). This band contains all the MDCK scatter activity recovered from the gel (Fig. 5). Treatment with reducing agents gives two inactive bands at ≈57 and ≈30 kDa (Fig. 4, lane b).

We have not obtained sufficient amounts of the 57-kDa and 30-kDa proteins to attempt reconstitution of scatter activity. Therefore, we do not know whether both proteins are required for biological activity. The 57-kDa and 30-kDa proteins copurify on ion-exchange, reverse-phase, and heparin-Sepharose columns, and the ratio between the two proteins, as judged from SDS/PAGE, does not change during purification. Yet the polypeptide composition of highly purified preparations of scatter factor is intriguing, since it is difficult to reconcile the patterns observed under nonreducing and reducing conditions. There are two possible explanations for the data obtained. The first is that the unreduced band of 62 kDa is a mixture of two distinct species that happen to copurify: the protein that migrates at 57 kDa under reducing conditions and a dimer of the 30-kDa protein. This seems unlikely because it would imply that the apparent molecular mass of the 57-kDa band would be higher under nonreducing conditions than under reducing conditions. The second explanation is that the band at 62 kDa under nonreducing conditions (biologically active scatter factor) is a heterodimer of the 57-kDa and 30-kDa proteins. The expected molecular mass of this complex would be much higher (87 kDa) than found (62 kDa), but this could be explained by the presence of intra- and interchain disulfide bonds. There is, in fact, an absolute requirement for intact disulfide bonds for epithelial scatter activity and these could greatly reduce the apparent size of the complex.

Several other properties of scatter factor have emerged during the course of this study. No epithelial scatter activity was detected in the conditioned medium of *ras*-transformed D4 NIH 3T3 cells incubated with tunicamycin (0.03–1.0 μg/ml), suggesting that the factor may contain N-linked carbohydrates important either for secretion or for activity. Recently, Rosen *et al.* (16) found that scatter factor bound to concanavalin A-Sepharose and could be dissociated from the lectin column with buffers containing methyl α-D-mannoside or methyl α-D-glucoside (16). The same authors reported that scatter factor bound strongly to immobilized heparin and that soluble heparin (≥5 μg/ml) could inhibit scatter activity in the MDCK assay (16), a finding which suggests that matrix components could modulate the activity of scatter factor *in vivo*.

The cellular pathways that are triggered by scatter factor and that result either in the inability of single epithelial cells to form stable intercellular interactions or in the dissociation of preformed epithelial sheets are as yet unknown. "Scattering" of MDCK cells has been produced with a monoclonal antibody (Arc-1) directed to a uvomorulin-related antigen (17, 18), although the concentration of antibody required to induce cell dissociation was ≈10,000 times higher than the concentration of scatter factor sufficient to disperse MDCK cells (2–3 pM, calculated by assuming a molecular mass of 62 kDa for the active factor). Biological activity at the concentration of scatter factor active in tissue culture would certainly suggest the presence of high-affinity binding sites on the surface of cells responsive to the factor, and the availability of highly purified factor should now enable us to test this hypothesis. Furthermore, binding-competition experiments with antibodies directed to antigens involved in cell–cell contact (such as the Arc-1 monoclonal antibody) may provide preliminary evidence about the nature of the cell surface components involved in the response to scatter factor. Studies in bacterial cells (19, 20) and slime molds (21, 22) have revealed that exogenous signals affecting cell movement are mediated by membrane receptors and induce rapid changes in protein methylation and/or phosphorylation. It is possible that similar events are involved in the response to scatter factor. The possibility that the factor acts as a specific protease cannot be ruled out, although we have not been able to inhibit activity with purified α_2-macroglobulin (0.03–100 μg/ml) or diisopropyl fluorophosphate (0.003–1.0 mM).

We reported earlier (12) that production of scatter factor was restricted to embryo fibroblasts and certain fibroblast cell lines, suggesting a role of scatter factor in embryogenesis. This suggestion has been supported recently by two lines of evidence. (*i*) Implantation of fibroblasts secreting scatter factor near the early primitive streak of chicken embryo induced the formation of a second primitive streak, whereas the implantation of nonyielding cells had no effect (23). (*ii*) MDCK scatter activity has been detected in FBS but not in newborn calf serum when fractionated by cation-exchange chromatography (E.G. and M.S., unpublished observations). However, postnatal rat and mouse fibroblasts still release MDCK scatter activity (M.P. and M.S., unpublished data), and a recent report demonstrated that calf aortic smooth muscle cells release an epithelial scattering activity indistinguishable from that secreted by human embryo fibroblasts (16). Therefore, further studies will be required to clarify the sites of synthesis and activity of scatter factor *in vivo*.

We thank Dr. M. Crumpton for his interest in this work and for critical advice and Drs. L. Crawford, L. Buluwela, J. Greenberg, and E. Rosen for reading the manuscript. We are grateful to A. Hales, I. Rae, and their colleagues, who provided most of the conditioned medium used in these studies.

1. Ruoslahti, E. & Pierschbacher, M. D. (1987) *Science* **238**, 491–497.
2. Edelman, G. M. (1986) *Annu. Rev. Cell. Biol.* **2**, 81–116.
3. Levi-Montalcini, R. (1987) *EMBO J.* **6**, 1145–1154.
4. Grotendorst, G. R., Seppä, H. E. J., Kleinman, H. K. & Martin, G. R. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 3669–3672.
5. Blay, J. & Brown, K. D. (1985) *J. Cell. Physiol.* **124**, 107–112.
6. Barrandon, Y. & Green, H. (1987) *Cell* **50**, 1131–1137.
7. Yoshida, K., Ozaki, T., Ushijima, K. & Hayashi, H. (1970) *Int. J. Cancer* **6**, 123–132.
8. Liotta, L. A., Mandler, R., Murano, G., Katz, D. A., Gordon, R. K., Chiang, P. K. & Schiffmann, E. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 3302–3306.
9. Atnip, K. D., Carter, L. M., Nicolson, G. L. & Dabbous, M. Kh. (1987) *Biochem. Biophys. Res. Commun.* **146**, 996–1002.
10. Schor, S. L., Schor, A. M., Grey, M. & Rushton, G. (1988) *J. Cell Sci.* **90**, 391–399.
11. Stoker, M. & Perryman, M. (1985) *J. Cell Sci.* **77**, 209–223.
12. Stoker, M., Gherardi, E., Perryman, M. & Gray, J. (1987) *Nature (London)* **327**, 239–242.
13. Stoker, M. (1984) *J. Cell. Physiol.* **121**, 174–183.
14. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
15. Stoker, M. & Gherardi, E. (1987) *Factors Affecting Epithelial Interactions*, Ciba Foundation Symposium 125 (Wiley, Chichester), pp. 219–239.
16. Rosen, E. M., Goldberg, I. D., Kacinski, B. M., Buckholz, T. & Winter, D. W. (1989) *In Vitro Cell Dev. Biol.* **25**, 163–173.
17. Imhof, B. A., Vollmers, P., Goodman, S. L. & Birchmeier, W. (1983) *Cell* **35**, 667–675.
18. Behrens, J., Birchmeier, W., Goodman, S. L. & Imhof, B. A. (1985) *J. Cell Biol.* **101**, 1307–1315.
19. Springer, M. S., Goy, M. F. & Adler, J. (1979) *Nature (London)* **280**, 279–284.
20. Springer, M. S. & Zanolari, B. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 5061–5065.
21. Green, A. A. & Newell, P. C. (1975) *Cell* **6**, 129–136.
22. Rahmsdorf, H. J., Malchow, D. & Gerisch, G. (1978) *FEBS Lett.* **88**, 322–326.
23. Ireland, G. W., Stern, C. D. & Stoker, M. (1987) *J. Anat.* **152**, 223–224.