IDENTIFICATION AND PURIFICATION OF GLIAL GROWTH FACTOR

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Abstract

Cultured rat Schwann cells are stimulated to divide by a protein growth factor, present in extracts of bovine brain and pituitary, which we have named glial growth factor (GGF). Two lines of evidence indicate that GGF activity in both brain and pituitary resides in a protein of $M_r = 31,000$. (1) Four independently isolated monoclonal antibodies that immunoprecipitate the activity react with an antigen of this molecular weight in sodium dodecyl sulfate (SDS)-polyacrylamide gels. (2) After SDS-polyacrylamide gel electrophoresis of partially purified preparations, mitogenic activity on Schwann cells is recovered at this molecular weight. GGF has been purified approximately 100-fold to apparent homogeneity from bovine pituitary anterior lobes by a combination of column chromatography steps and preparative SDS gel electrophoresis. Purified human platelet-derived growth factor, a molecule with properties similar to those of GGF, is inactive on Schwann cells and therefore appears to be distinct.

The growth, division, and survival of animal cells in culture are dependent on the presence of growth factors and hormones. These molecules are often present in animal sera, tissue extracts, and other undefined medium supplements. For some cell types it has been possible to replace the general serum requirement with an appropriate mixture of purified growth factors (see, for example, Bottenstein et al., 1979). It has been suggested that the development of new cell culture systems will lead to the identification of new growth factors of biological significance (Ross and Sato, 1979).

We have described the use of immunological methods to obtain pure populations of rat Schwann cells from the neonatal sciatic nerve (Brockes et al., 1977, 1979). These cells divide very slowly in a conventional tissue culture medium containing 10% fetal calf serum, but they are strongly stimulated by an activity present in extracts of bovine brain and pituitary (Raff et al., 1978; Brockes et al., 1979). This activity is not detectable in extracts of non-neural tissue and is not mediated by a variety of purified growth factors and pituitary hormones (Raff et al., 1978). In a previous report (Brockes et al., 1980), the activity was partially purified from extracts of the bovine pituitary by a combination of cation exchange chromatography and gel filtration. The most purified fraction still displayed significant heterogeneity, but further electrophoretic analyses by native and sodium dodecyl sulfate (SDS) gels indicated that the activity was associated with a basic protein of $M_r = 30,000$ which readily dimerized. In addition to its action on Schwann cells, this molecule was active in stimulating division of cultured astrocytes of the rat corpus callosum and of fibroblasts, but not of oligodendrocytes and macrophage-like microglia (Brockes et al., 1980). We have also reported on the activity in bovine brain (Brockes and Lemke, 1981; Brockes et al., 1981) which exhibits a reproducible variation in specific activity between regions and which is indistinguishable from that of the pituitary by electrophoretic and chromatographic criteria. In view of its localization to the nervous system, and its action on Schwann cells and astrocytes, the molecule has been named glial growth factor (GGF).

In this paper we describe two new lines of evidence which substantiate our earlier identification of the active species. In addition, we describe a method which permits purification of GGF to apparent homogeneity and demonstrate its distinction from a previously characterized growth factor which is related in structure and function.
Materials and Methods

Materials. Lyophilized bovine anterior lobes, rats, Schwann cell tissue culture media and plastics, and radioisotopes were all purchased as described previously (Brookes et al., 1980). Protein A was from Pharmacia and was iodinated according to the method of Moore et al. (1982). Hybridoma materials were prepared or purchased as described by Moore et al. (1982). NIH 3T3 cells were a gift of Dr. Rex Risser, University of Wisconsin, Madison. Purified human platelet-derived growth factor was a gift of Drs. Elaine Raines and Russell Ross, University of Washington, Seattle.

Cell culture. Rat Schwann cells were dissociated from neonatal sciatic nerve, purified by immunoselective methods, and maintained as described previously (Brookes et al., 1977, 1979, 1980). NIH 3T3 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal calf serum (FCS).

Materials and methods. Female BALB/c mice were immunized with partially purified fractions of GGF complexed with poly(I),poly(C) as an adjuvant as described previously (Lemke and Brookes, 1981). Spleen cells from responding animals were fused to SP-2/O myeloma cells as described by Moore et al. (1982). Culture supernatants from the resulting hybridomas were screened for GGF binding by assaying their ability to precipitate growth factor activity from solution as follows: 40 μl of culture medium (or an appropriate dilution) were incubated with 10 μl of CM-cellulose fraction GGF (Brookes et al., 1980) at 400 μg/ml for 4 hr at 37°C. Ten microliters of a solution of normal mouse IgG (60 μg/ml in HMEM + 10% FCS) and 10 μl of a solution of affinity purified rabbit anti-mouse IgG (400 μg/ml) were then added. The mixture was incubated at 37°C for 30 min and then overnight at 4°C. After centrifugation at 20,000 × g for 4 min at 4°C, 20 μl of the resulting supernatant were tested in the microwell proliferation assay (see Lemke and Brookes, 1981).

Antibody reactions with partially purified GGF fractions resolved on SDS gels were performed essentially as described by Moore et al. (1982), except that anti-mouse IgG was not used and all reactions were carried out at pH 8.5 to permit 125I-protein A binding to monoclonal IgG; antibody (Ey et al., 1978); anti-GGF antibody E IgG, purified to near homogeneity from ascites fluid by DEAE-cellulose chromatography was used at 0.9 μg/ml. Autoradiography was performed using Kodak XAR-5 x-ray film with an intensifying screen. Exposures were carried out at -70°C for 18 to 28 hr.

Analytical SDS gel electrophoresis. Small-scale (5.5 × 7.0 × 0.1 cm, 1.5-cm stack) SDS gel electrophoresis was conducted according to the methods of Laemmli and Favre (1974), but with modified concentrations of bis-acrylamide (0.11% for 12.5% acrylamide gels and 0.09% for 15% acrylamide gels). Gels were stained either with 0.2% Coomassie blue (in 50% methanol, 10% acetic acid) or according to the silver staining procedure described by Morrissey (1981). Samples to be analyzed for migration of growth factor activity were solubilized in 0.5 vol of sample buffer (80 mM Tris-HCl, pH 6.8, 10% glycerol, 2% SDS) by heating at 37°C for 2 to 3 min in the absence of disulfide reducing agent. After electrophoresis, the sample lane was excised from the gel and sliced into 2.5-mm segments, and the segments were placed into sterile snap-cap tubes and washed with 1 ml of sterile phosphate-buffered saline (PBS) for 30 min at room temperature by vigorous agitation. The PBS was replaced with 1 ml of fresh buffer, and a second wash was performed for an additional 10 min. The PBS was then removed and replaced with 150 μl of HMEM containing 10% FCS. Proteins were eluted from the segments by shaking overnight at 4°C. Fifteen to 20 μl of each eluate were assayed in the standard microwell proliferation assay.

Large-scale purification of GGF. CM-cellulose fractions of GGF were prepared from 20,000 and 10,000 lyophilized anterior lobes as described previously (Brookes et al., 1980). In brief, tissue was extracted at pH 4.5, fractionally precipitated with ammonium sulfate, and chromatographed through batch elution from CM-cellulose. This material was then processed in two alternative ways. In procedure I (20,000 lobes, starting material), the CM-cellulose fraction was reapplied to a 200-ml CM-cellulose column equilibrated with 0.1 M sodium phosphate, pH 6.0 (P buffer) + 0.025 M NaCl, and then eluted with a 2-liter linear gradient from 0.025 M to 0.210 M NaCl in P buffer. Active fractions (see Fig. 4A) were pooled (total volume = 550 ml), precipitated with 309 gm of ammonium sulfate, resuspended in P buffer + 0.4 M NaCl, and chromatographed on an AcA 44 Ultrogel column exactly as described previously (Brookes et al., 1980). Active fractions (see Fig. 4B) were pooled, applied to a 5.5-ml column of phosphocellulose P11 equilibrated with P buffer + 0.4 M NaCl, and then eluted with a 60-ml linear gradient from 0.4 M to 0.85 M NaCl in P buffer. GGF was purified from active fractions of the phosphocellulose elution by small-scale SDS gel electrophoresis and elution by the procedure of Mendel-Hartvig (1982). In procedure II (10,000 lobes, starting material), the CM-cellulose fraction (in P buffer + 0.35 M NaCl) was applied directly to a 25-ml phosphocellulose column, equilibrated with P buffer + 0.35 M NaCl, and then eluted with a 200-ml linear gradient from 0.35 M to 0.85 M NaCl, in P buffer. Active fractions from this run were pooled and concentrated to 3.5 mg/ml in an Amicon cell (total...
protein recovered = 35 mg). Six-milligram portions of this material were then purified by successive preparative gel electrophoresis under native and denaturing conditions. Native gel electrophoresis was performed in slab gels (10 cm x 12.5 cm x 0.7 cm, 2.5-cm stack) using the buffer system of Reisfeld et al. (1962), modified as described previously (Brookes et al., 1980). Samples were prepared by mixing them with 1/5 vol of 1.5% agarose at 40°C for 1 min, applied to a preformed well in the native stacking gel, and allowed to solidify. Electrophoresis was conducted at 50 mA at 4°C for approximately 7 hr, after which time a gel slice from 0.38 to 0.6 mobility relative to cytochrome c was excised and cut into fine segments. The segments were placed in a 15-ml tube with 5 ml of 2X PBS and shaken 15 min. The PBS was removed and 5 ml of elution buffer (20 mM Tris-HCl, pH 6.8, 0.5% SDS) were added. Proteins were then eluted from the segments by shaking overnight at room temperature. The elution buffer was removed, an additional 5 ml of buffer was added, and a second overnight elution was performed. The two eluates were pooled, lyophilized, and adjusted to approximately 2 ml with distilled water. Glycerol was added to 10% final concentration, and the sample was heated to 37°C for 2 to 4 min before application to second-dimension SDS gels. Large-scale (10 cm x 12.5 cm x 0.5 cm, 12.5-cm stack) SDS gel electrophoresis was carried out at room temperature at 25 mA for approximately 18 hr. Resolved proteins were visualized by staining the gel with Coomassie blue (2% in 20 mM Tris-HCl, pH 6.8) with several changes. The GGF band was identified by its migration relative to carbonic anhydrase and excised with a scalpel. The gel slice was incubated in SDS running buffer with 0.05% bromphenol blue for approximately 30 sec and then positioned on top of a second SDS slab (4 cm x 12.5 cm x 0.6 cm) for electrodifusion exactly as described by Mendel-Hartvig (1982). Eluted proteins were collected in 80 mM Tris-HCl, pH 8.7, 30% glycerol, 0.5% SDS.

Results

Molecular identification of GGF by monoclonal antibodies. Two new lines of evidence support our earlier tentative identification of GGF as a protein with an apparent Mr of 31,000 when analyzed by SDS gel electrophoresis (Brookes et al., 1980).

After immunizing with partially purified fractions from a large-scale purification of bovine pituitary GGF, we derived four mouse monoclonal antibodies apparently directed against this activity (Lemke and Brookes, 1981). An assay of one of these reagents (termed antibody ER) is shown in Figure 1. A partially purified fraction of GGF was incubated with culture medium from cells secreting ER, and immune complexes were then precipitated after addition of an appropriate amount of anti-mouse IgG followed by centrifugation. The resulting supernatant was assayed for GGF activity in the standard Schwann cell proliferation assay. Immunoglobulin from clone ER but not clone 4-F7 produced a dose-dependent depletion of activity (Fig. 1). This depletion of activity was only demonstrable after indirect immunoprecipitation of immune complexes (Lemke and Brookes, 1981). The four independent clonal isolates that showed this reactivity all secreted immunoglobulin of the IgG subclass as determined by Ouchterlony double diffusion analysis with subclass-specific antisera.

The antigen reactive with these reagents was investigated by running partially purified fractions of GGF on SDS gel electrophoresis and incubating with each of the monoclonal antibodies after the method of Burridge (1978). Bound IgG, was detected by reaction with [125I]labeled protein A at pH 8.5 (Ey et al., 1978), followed by autoradiography. The results of such an experiment with antibody ER are shown in Figure 2, A and B. Although many protein species were detectable on the Coomassie blue-stained gel (Fig. 2A), the antibody reacted strongly and specifically with a single minor band of Mr = 31,000 (Fig. 2B). Each of the four independent monoclonal isolates reacted in the same way. There was evidence of very minor diffuse reactivity with protein species in the Mr = 15,000 to 30,000 range and at the dye front in some but not all preparations (see Fig. 2B), which may be a consequence of degradation. These antibodies also recognize a 31,000-dalton antigen in partially purified GGF preparations from bovine caudate nucleus and from whole chick brain (data not shown).

Molecular identification of GGF by SDS gel electrophoresis. We have found that GGF activity can be recovered from SDS gels run in the absence of disulfide reducing agents. Figure 3A shows an experiment in which a partially purified preparation of GGF (phosphocellulose
fraction 48; see Fig. 4C) was analyzed on a 12.5% SDS gel, using the standard Laemmli discontinuous buffer system. One lane was silver stained to detect proteins, and the other was sliced into segments which were washed in buffer and then eluted into tissue culture medium (see “Materials and Methods”). GGF activity was detected as a single peak of $M_r = 31,000$. No activity was recovered if the gels were run in the presence of disulfide reducing agents such as 2% β-mercaptoethanol.

GGF activity is detectable in bovine brain (Raff et al., 1978) and shows a regional variation in specific activity with a particularly high level in the caudate nucleus (Brockes and Lemke, 1981; Brockes et al., 1981). The activity in the caudate is indistinguishable from that in the pituitary by the criteria of native gel electrophoresis at pH 4.5 and ion exchange chromatography on phosphocelulose (Brockes and Lemke, 1981). When partially purified preparations of caudate GGF were analyzed by SDS gel electrophoresis, a major peak of activity was detected at 31,000 daltons (Fig. 3B). In addition, a minor peak of activity was detectable at 56,000 daltons in this experiment. This species, which constitutes less than 10% of the activity in the 31,000-dalton peak when corrected for the logarithmic dosage relationship (Lemke and Brockes, 1981; see Fig. 4D), has also been detected at low and variable levels in some less purified preparations of the pituitary activity. The existence of aggregates of the 31,000-dalton species is considered further under “Discussion.”

Large-scale purification of bovine pituitary GGF. We have purified the 31,000-dalton species of GGF to apparent homogeneity by combining the previously described procedures of column chromatography and native gel electrophoresis (Brockes et al., 1980), together with a final step of preparative SDS gel electrophoresis. In the procedure outlined in Table I, the CM-cellulose fraction (see Brockes et al., 1980) from 20,000 lyophilized anterior lobes was further purified by gradient elution from CM-cellulose (Fig. 4A), gel filtration on AcA 44 Ultrogel (Fig. 4B), and elution from phosphocelulose (Fig. 4C). Peak fractions from the phosphocellulose column gave plateau stimulation of Schwann cells at a concentration of 200 ng/ml (Fig. 4D), indicating a purification factor of approximately 10,000-fold with a yield of 3% (Table I). After SDS gel electrophoresis, approximately 10% of the protein (Fig. 4D) and all of the activity (Fig. 3A) resided in a 31,000-dalton band. This species could be further purified by preparative SDS gel electrophoresis following elution from the column by the elution procedure of Mendel-Hartvig (1982) (Fig. 4D), yielding approximately 4 µg/1,000 anterior lobes.

In an alternative procedure which employs fewer steps, the CM-cellulose fraction (Brockes et al., 1980) from 10,000 anterior lobes was chromatographed on phosphocelulose essentially as in Figure 4C and then was purified by preparative native gel electrophoresis at pH 4.5 (Fig. 4E) using the buffer system of Reisfeld et al. (1962), as described earlier (Brockes et al., 1980). The pH 4.5 fractions were passively eluted from the gel slices and further purified by SDS gel electrophoresis to yield the 31,000-dalton species (Fig. 4F). The material resulting from this procedure (Fig. 4F) showed significant activity in the Schwann cell proliferation assay (after dilution into medium with 1 mg/ml of BSA and extensive dialysis to remove SDS), but at a concentration that was consistent with the loss of 95% of the activity due to the multiple electrophoretic steps.

Comparison with the platelet-derived growth factor (PDGF). PDGF has been purified from platelet lysates by virtue of its mitogenic effect on fibroblasts or 3T3 cells (Ross et al., 1979; Antoniades, 1981; Deuel et al., 1981; Heldin et al., 1981a). It shares several properties with GGF as defined in this paper and previously (Brockes and Lemke, 1981). Both are basic proteins of $M_r = 31,000$ that are functionally inactivated by disulfide reducing agents, are relatively heat stable, and act on fibroblasts. To further investigate their relationship, we assayed a preparation of purified human PDGF (Raines and Ross, 1982) for its ability to stimulate DNA synthesis in cultured rat Schwann cells. The preparation was active (Fig. 5) on mouse NIH 3T3 cells in the range of 1 to 20 ng/ml as previously reported (Raines and Ross, 1982), but gave no significant stimulation of Schwann cells at
Figure 3. Identification of GGF activity after SDS-polyacrylamide gel electrophoresis. A, Electrophoresis in a 12.5% gel of approximately 1 μg of a phosphocellulose fraction of GGF from the bovine pituitary (~10% pure; see below). Samples were solubilized (without disulfide reduction) and electrophoresed as described under "Materials and Methods." The sample lane was excised and sliced into 2.5-mm segments, and the proteins were eluted from each segment into medium (see "Materials and Methods"). The GGF activity eluted from the gel slices was measured in the Schwann cell proliferation assay. The silver-stained profile of unreduced proteins and the migration of reduced molecular weight markers are from parallel lanes of the same gel. B, Electrophoresis in a 12.5% gel of 30 μg of a phosphocellulose fraction of GGF prepared from the caudate nucleus of the bovine brain (0.3% pure). Electrophoresis, elution of proteins, and analysis were as for A, except that the proteins were visualized with Coomassie blue.

Discussion

The two lines of evidence presented in this paper provide strong support for the identification of GGF activity with a 31,000-dalton protein. Our earlier evidence for this was based on native gel electrophoresis of a partially purified preparation and was suggestive but not conclusive. The ability of a set of four monoclonal antibodies to both specifically precipitate GGF activity from solution and bind to the 31,000-dalton species is independent evidence for the association of mitogenic activity with this molecule. It might still be argued, however, that GGF activity derives from a smaller component that is adventitiously associated with the 31,000-dalton protein. This possibility is made very unlikely by the demonstration that growth factor activity is recovered at 31,000 daltons after denaturing electrophoresis in SDS.

Although we now consider this association to be established, the presence of oligomers is not fully understood. In previously reported gel filtration experiments (Brockes et al., 1980), the major peak of growth factor activity was observed to migrate with an apparent Mr of 56,000 (GGF dimer), whereas in procedure I, activity was observed predominantly at Mr = 31,000 (GGF monomer). We have found that the GGF dimer is largely removed as a result of the gradient elution from CM-cellulose of this latter procedure; if subjected to SDS gel electrophoresis under nonreducing conditions, material from pool a (Fig. 4A) gives a major peak of activity at Mr = 56,000 (data not shown). Significant dimerization of GGF has not been detected in the more highly enriched fractions (AeA 44(Fig. 4B) and phosphocellulose P11 (Fig. 5A)) subsequently prepared from pool b (Fig. 4B).

The two purification procedures that are reported above result in apparently homogeneous preparations of GGF, but ones whose bioactivity is significantly reduced after the preparative SDS electrophoresis steps. The
Figure 4. Steps in the large-scale purification of GGF. A, Gradient elution of the CM-cellulose fraction from CM-cellulose. The batch-eluted CM-cellulose fraction (1090 mg) was applied to a CM-cellulose column and eluted with a linear NaCl gradient as described under “Materials and Methods.” Fractions were collected and assayed at 3 μg/ml in the Schwann cell proliferation assay. Two pools were made and pool b was used for further purification (the properties of pool a are considered under “Discussion”). B, Elution profile of the (gradient eluted) CM-cellulose fraction after gel filtration on AcA 44 Ultrogel. The CM-cellulose fraction (210 mg) was chromatographed on a calibrated column of AcA 44 as described under “Materials and Methods.”
Figure 4, D–F

Fractions were collected and assayed at 0.5 μg/ml. Fractions 36 to 40 were pooled for further purification. C, Elution profile of the AcA 44 fraction from phosphocellulose. The AcA 44 fraction (19 mg) was applied to a phosphocellulose column and eluted with a linear NaCl gradient as described under “Materials and Methods.” Fractions were collected and assayed at 0.2 μg/ml; no activity was detectable in the flow-through. D, Dose response curve and further purification of the phosphocellulose fraction. The phosphocellulose fraction (fraction 49 of C) was diluted in medium and assayed in the Schwann cell proliferation assay.

Inset i, Proteins of this fraction (1 μg) were analyzed on a 12.5% SDS gel followed by silver staining. The 31,000-dalton GGF band accounts for approximately 10% of the protein as determined densitometrically, and can be purified to apparent homogeneity by excision from the gel and subsequent elution (see “Materials and Methods”). ii, Eluted material (0.1 μg) is shown after SDS gel electrophoresis followed by silver staining. E, Profile of phosphocellulose fractions after native gel electrophoresis at pH 4.5. The phosphocellulose fraction (6 mg), derived as described in the text, was subjected to polyacrylamide gel electrophoresis at pH 4.5 as described under “Materials and Methods.” The migration of GGF activity relative to cytochrome c was determined previously on pilot scale gels. A slice of cytochrome c = 0.38 to 0.5 was excised and eluted as described under “Materials and Methods.” A parallel lane of the pH 4.5 gel was sliced into 3-mm segments which were eluted into medium and assayed in the Schwann cell proliferation assay. The activity profile in the parallel lane is shown together with the region (diagonally lined bar) excised from the sample lane. F, SDS gel electrophoresis. Shown in a is the native gel eluate as resolved by electrophoresis through a preparative scale SDS-polyacrylamide slab gel (see “Materials and Methods”). The GGF band (arrowhead) was identified after staining with Coomassie blue by its migration to a position just above that for carbonic anhydrase. A gel slice containing this band was excised from the slab, and GGF was eluted according to the procedure of Mendel-Hartvig (1982). An analytical SDS gel of the eluted material (0.2 μg) is shown in b.
second procedure, which employs a combination of native gel electrophoresis at pH 4.5 and SDS gel electrophoresis, is similar in design to that employed by Barde et al. (1982) in the isolation of a neurotrophic factor from pig brain and is a modification of the two-dimensional analytical procedure that we used previously (Brockes et al., 1980). Attempts to purify GGF by using the monoclonal antibodies as immobilized affinity reagents have thus far been largely unsuccessful. Although growth factor activity is purified approximately \(10^5\)-fold and the estimated potency of highly purified GGF is comparable to that of other purified mitogenic growth factors, it might still be formally argued that the final preparation of the 31,000-dalton band is not homogeneous and contains one or more components of identical molecular weight which co-purify with GGF through the multiple steps of the purification procedures. For this reason, the homogeneity is termed "apparent." Amino acid sequence analysis of the purified preparation followed by the derivation of antisera to synthetic peptides, or the cloning and subsequent manipulation of the gene coding for this protein are clearly important elements of a definitive identification of the GGF molecule.

Although we have not undertaken an investigation of the mechanism of action of GGF at the cellular level, some of our data bear on this question. The equilibrium dissociation constant of the putative Schwann cell receptor for GGF can, for example, be estimated from the midpoint of dose response curves of highly purified fractions (such as the 10% pure preparation of Fig. 4D) to be approximately \(10^{-10}\) M. This value is comparable to that exhibited by high affinity cell receptors for epidermal growth factor (Carpenter et al., 1975) and PDGF (Heldin et al., 1981b; Bowen-Pope and Ross, 1982), and indicates that GGF acts in a "hormonal" concentration range.

Although PDGF and GGF are strikingly similar, our results (Fig. 5) clearly demonstrate a functional disparity with respect to their action on Schwann cells. Additionally, GGF, unlike PDGF, appears to be a single polypeptide, in that treatment with disulfide reducing agents does not alter the observed molecular weight of the purified protein on SDS gels (data not shown) or the binding of anti-GGF antibodies to the 31,000-dalton antigen (Fig. 2). It seems possible, nevertheless, that these molecules are members of a family of growth factors, some of whose target cells (e.g., fibroblasts) overlap. The lack of effect of PDGF on Schwann cells is consistent with the relatively weak mitogenicity of 10% serum on these cells. It points to the importance of Schwann cell proliferation as a distinctive test for specific growth factors—in our studies we have found no defined mitogen other than GGF which stimulates their division. The relation of GGF to the cell surface mitogen of cultured neurites and central nervous system axolemma (Salzer and Bunge, 1980; De Vries et al., 1982) awaits chemical and immunochemical characterization of the latter.

### Table I
**Summary of purification**

The activity was purified from 20,000 lyophilized anterior lobes as described in the text. The fold purification through the phosphocellulose step was determined from the displacement of dose response curves (see Lemke and Brockes, 1981).

<table>
<thead>
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<th>Step</th>
<th>Total Protein (mg)</th>
<th>Recovery of Activity (%)</th>
<th>Fold Purification</th>
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<tr>
<td>Crude extract</td>
<td>400,000</td>
<td>100</td>
<td>2</td>
</tr>
<tr>
<td>Ammonium sulfate fraction</td>
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<td>CM-cellulose (gradient elution)</td>
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<tr>
<td>AcA 44 Ultrogel gel filtration</td>
<td>19</td>
<td>6.8</td>
<td>1,250</td>
</tr>
<tr>
<td>Phosphocellulose</td>
<td>1.1</td>
<td>3.1</td>
<td>10,000</td>
</tr>
<tr>
<td>SDS gel electrophoresis</td>
<td>0.09*</td>
<td>0.43*</td>
<td>190,000*</td>
</tr>
</tbody>
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\*This is an estimate based on protein recoveries (as determined by calibrated cellophane stained gels) in small scale elutions by the procedure of Mendel-Hartvig (1982).

\*This is an estimate based on activity recoveries in small-scale elutions by the procedure described under "Materials and Methods" and illustrated in Figure 3.

\*This figure is based on the observation that GGF accounts for 10% of the protein in the phosphocellulose fraction.

Figure 5. Comparison with PDGF. Purified human PDGF was diluted to varying concentrations with HMEM + 0.5 mg/ml of BSA and assayed for mitogenic activity simultaneously on both Schwann cells and NIH 3T3 fibroblasts. Proliferation assays were performed in microwells with \(^{3}H\)UdR as label as described under "Materials and Methods." As a control, a CM-cellulose fraction of GGF was titered against the same batch of Schwann cells tested for PDGF response. Note that this is a relatively impure fraction of GGF. •—•, PDGF:Schwann cells; ○—○, PDGF:Schwann cells; ▼—▼, PDGF:3T3 cells.
Although there is currently considerable interest in the mechanism of action of mitogenic growth factors at a molecular level, almost nothing is known about their significance in vivo. There is little information about their “endocrinology” in the sense of where they are made and released, or of the identity of their targets under circumstances of cell division in development and regeneration. The location of GGF in the nervous system and pituitary raises some possibilities as to its function. The mechanism of action of mitogenic growth factors at a molecular level, almost nothing is known about their "endocrinology" in the sense of where they are made and released, or of the identity of their targets under circumstances of cell division in development and regeneration. The location of GGF in the nervous system and pituitary raises some possibilities as to its function.

References


