

Purification and Properties of Epithelial Growth Inhibitor (EGI) from Human Platelets: Its Separation from Type β Transforming Growth Factor (TGF- β)¹

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We previously reported that sera from various kinds of animals contain a protein(s) capable of inhibiting the growth of the non-malignant epithelial cell line derived from Buffalo rat liver (BRL). In the present study, a similar epithelial cell-specific growth inhibitor (EGI) was purified to homogeneity from an acid-ethanol extract of human platelets. During purification, EGI was separated from the major component of type β transforming growth factor (TGF- β), which can stimulate the colony formation of the non-malignant fibroblastic cell line derived from rat kidney (NRK) in soft agar in the presence of epidermal growth factor (EGF). The purified EGI had an M_r of 27,000, and was composed of two subunits identical in M_r . It significantly inhibited the growth in monolayer cultures of three non-malignant epithelial cell lines, BRL, MDCK (from Madin-Darby canine kidney) and BSC-1 (from African green monkey kidney), at doses lower than 40 pg/ml in medium containing 10% fetal calf serum. Its inhibitory activity was stable against heating at 90°C for 3 min, but not against treatment with 50 mM dithiothreitol. In addition, TGF- β was also partially purified from the same extract. The purified TGF- β did not show any inhibitory activity toward the growth of BRL, MDCK, BSC-1, or NRK.

Many studies have suggested that mammalian tissues contain a class of proteins called chalone, which inhibit the growth of cells *in vivo*, which led to the hypothesis that cell division is controlled

by a negative feedback mechanism (1–8). However, this possibility is not widely accepted yet because of difficulties encountered in the separation and purification of such growth inhibitory

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Abbreviations: EGF, epidermal growth factor; EGI, epithelial cell-specific growth inhibitor; HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; HPLC, high performance liquid chromatography; PBS, phosphate-buffered saline (pH 7.4) composed of 8.0 mM Na₂HPO₄, 1.5 mM KH₂PO₄, 137 mM NaCl, and 2.7 mM KCl; PMSF, phenylmethylsulfonyl fluoride; SDS, sodium dodecyl sulfate; TFA, trifluoroacetic acid; TGF- β , type β transforming growth factor.

proteins from non-specific cytotoxic substances. It appears, therefore, that the purification and characterization of these proteins are vitally important as the first step for the clarification of their biological significance.

It is known that type β transforming growth factor (TGF- β) stimulates the growth in soft agar medium (anchorage-independent growth) of several non-malignant fibroblastic cell lines (9, 10). For the growth in soft agar of the cell line derived from normal rat kidney (NRK), in addition to TGF- β EGF has to be added to the medium (10). TGF- β exists in various kinds of normal and neoplastic tissues (11, 12), and has been isolated from human platelets (9, 10, 13), human placenta (14), bovine kidney (15), and human tumor cells (16). It is a protein with an M_r of 25,000, and is composed of two apparently identical subunits cross-linked with disulfide bonds. Derynck *et al.* (17) recently determined its complete DNA sequence. Tucker *et al.* (18) reported that TGF- β purified from human platelets inhibits the growth in monolayer cultures of several kinds of cell lines, and that it is similar or identical to the growth inhibitor that was purified from the conditioned medium of the cell line derived from African green monkey kidney (BSC-1).

We previously reported that rat serum contains a growth inhibitor with an M_r of about 220,000 and a pI of 5.2 (19, 20). It inhibits the growth in monolayer culture of the cell line derived from normal Buffalo rat liver (BRL), but hardly at all that of BRL transformed with Rous sarcoma virus (RSV-BRL). We recently found that the growth inhibitor described above liberates an active protein with an M_r of about 30,000 when treated with urea or guanidine chloride, and that the growth inhibitory activity toward BRL is significantly lower with the serum prepared from platelet-poor plasma than with that from the whole blood. This suggests that at least a part of the growth inhibitory activity of the serum is due to a substance liberated from platelets during coagulation of blood (to be published).

The present paper reports the separation and purification of a growth inhibitory protein (epithelial cell-specific growth inhibitor; EGI) and TGF- β from human platelets. The properties of these two proteins are also described.

MATERIALS AND METHODS

Cell Lines and Cell Cultures—The cell lines, BRL, NRK (from normal rat kidney), A431 (from human epidermoid carcinoma of the vulva), Balb 3T3 (from Balb mouse embryo), and Swiss 3T3 (from Swiss mouse embryo), were kind gifts from Prof. Katsuzo Nishikawa, Kanazawa Medical University, Kanazawa. BRL was originally established from liver cells of a Buffalo rat by Coon in 1968 (21). RSV-BRL was established by transforming BRL with Rous sarcoma virus (19). The NRK was recloned to eliminate spontaneously transformed cells, and the clone thus obtained (NRK-C2) was used. The cell lines, MDCK (from normal Madin-Darby canine kidney), BSC-1 (from normal African green monkey kidney), FL (from normal human amniotic membrane), and HeLa-S3 (from human uterine carcinoma), were provided by the Japanese Cancer Research Resource Bank. The fibroblastic diploid cell strain (YH-1) (22), which had been established from human diploid embryo skin tissues, and its SV40-transformant (B-32) (22) were kind gifts from Assoc. Prof. Sadao Gotoh, School of Medicine, University of Occupational and Environmental Health, Kita-kyushu. All the cell lines were cultured at 37°C under a humidified atmosphere of 5% CO₂ and 95% air. The culture medium used was a basal medium supplemented with 10% (v/v) of fetal calf serum (GIBCO, Grand Island, N.Y.). The basal medium (DEM/F12) consisted of a 1 to 1 mixture of Dulbecco's modified Eagle's medium (GIBCO) and Ham's F12 medium (GIBCO), which was supplemented with 15 mM HEPES, 1.2 mg/ml of NaHCO₃, 100 U/ml of penicillin G, and 0.1 mg/ml of streptomycin sulfate.

Assay of Growth Inhibitory Activity—Growth inhibitory activity in monolayer cultures was measured by the method of Miyazaki *et al.* (19). For the assays, BRL was routinely used as the indicator. The cells (1×10^4 /dish) were seeded in 35 $\times$ 10 mm Falcon tissue culture dishes (Becton, Dickinson Labware, Oxnard, Calif.) containing 2 ml of DME/F12 with 10% of fetal calf serum. When fractions obtained on chromatographies were assayed, 1 μ l of each fraction was directly added to the culture medium. For the controls, the elution buffers used in the chromatographies were added.

It was determined that the acetic acid, trifluoroacetic acid (TFA), acetonitrile or urea, which had been added to the elution buffers, did not affect the cell growth. After four-days culture, the grown cells were counted with a microcellcounter CC-108 (Sysmex, Kakogawa, Hyogo). One unit of growth inhibitory activity is defined as the amount of a growth inhibitor required to inhibit the cell growth by 50%.

Assay of TGF- β Activity—The activity of TGF- β was assayed by the method of Roberts *et al.* (13) with some modifications. For the assays, NRK cells were grown in 35 $\times$ 10 mm tissue culture dishes containing two layers of soft agar. The bottom layer (1.0 ml) contained 0.5% (w/v) of agar (Special agar-noble, Difco Lab., Detroit) and 5% of fetal calf serum in DME/F12. Whereas the top layer (0.7 ml) contained 6.3×10^5 cells, 0.4% (w/v) of agar, 5% (v/v) of fetal calf serum and 7 ng of EGF (Collaborative Research, Lexington, Mass.) in DME/F12. The samples to be assayed were dissolved in 0.1 ml of PBS and then applied onto the top layer. After 7-days culture, colonies larger than 60 μ m in diameter formed in the top layer were counted under a microscope. The activity of TGF- β was expressed as the increase in the number of colonies.

Preparation of a Human Platelet Extract—Outdated human blood was kindly provided by the National Cardiovascular Center, Suita. The blood was centrifuged at $100 \times g$ for 30 min to precipitate erythrocytes. The resultant supernatant was centrifuged at $4,000 \times g$ for 15 min. The resultant precipitate, rich in platelets, was suspended in PBS, followed by centrifugation in the same manner as above. The precipitate thus obtained is hereafter called platelets. It was stored at -20°C until use. About 1.0–1.5 g wet weight of platelets was obtained per liter of the blood.

Extraction from the platelets was carried out by essentially the same method as that used for the extraction of TGF- β by Roberts *et al.* (11, 13). The frozen platelets were immersed in 4 volumes (4 ml/g of frozen platelets) of a mixture of 792 ml of 95% ethanol, 192 ml of water and 16 ml of concentrated HCl, which contained 53 mg of PMSF and 3 mg of pepstatin A as protease inhibitors (acid-ethanol). The resultant suspension was homogenized in a Potter-Elvehjem Teflon homogenizer by 15 up-down strokes, stirred for 3 h in an

ice-water bath, and then centrifuged at $11,000 \times g$ for 15 min. The resultant supernatant was mixed with 4 volumes of ether and 2 volumes of ethanol, previously cooled. The mixture was allowed to stand overnight, followed by centrifugation at $10,000 \times g$ for 15 min. The resultant precipitate was dried under vacuum and then dissolved in 1 M acetic acid. The solution thus obtained is hereafter called the platelet extract. All the procedures were performed at 4°C , unless otherwise designated.

Chromatographies—Molecular-sieve chromatography was performed on a Toyopearl HW50 (Toyosoda Co., Ltd., Tokyo) column (1.6×100 cm), previously equilibrated with 1 M acetic acid. The platelet extract was applied to the column, and then the charged column was developed with 1 M acetic acid with an upward flow at a rate of 40 ml/h. The eluate was collected in 5-ml fractions.

Cation-exchange HPLC was carried out on a TSKgel CM-3SW column (4.6×150 mm) (Toyosoda), using a HPLC System (Gilson, Middleton, Wis.), a model C-R3A recorder (Shimadzu Co., Ltd., Kyoto) and a system controller NEC PC-8801mkII (Nippon Electronics Co., Ltd., Tokyo). Samples were dialyzed against 20 mM sodium acetate buffer containing 4 M urea (pH 4.5) (AU buffer) and then applied to the column, previously equilibrated with AU buffer. The charged column was washed with 30 ml of AU buffer and then eluted with a linear gradient of 0.0–1.0 M NaCl in AU buffer at a rate of 0.5 ml/min. The eluate was collected in 2-ml fractions.

Reverse-phase HPLC was carried out on a C18 column (4×120 mm, Fuji Photo Film Co., Ltd., Tokyo), previously equilibrated with 10% (v/v) acetonitrile–0.1% (v/v) TFA (pH 2). Samples were dialyzed against 0.1 M acetic acid. To the dialyzed samples were added TFA and acetonitrile to make final concentrations of 0.1% (v/v) and 10% (v/v), respectively. The resultant solutions were applied to the column. The charged column was eluted at a flow rate of 0.33 ml/min with a linear gradient of acetonitrile, from 25 to 45% (v/v), in 0.1% (v/v) TFA. The eluate was collected in 1-ml fractions.

SDS-Polyacrylamide Gel Electrophoresis—SDS-polyacrylamide gel electrophoresis was carried out on 12.5% (w/v) acrylamide slab gels ($90 \times 90 \times 1$ mm) by the method of Laemmli *et al.* (23).

Samples were dissolved in 62.5 mM Tris-HCl (pH 6.8) containing 2% (w/v) of sodium dodecyl sulfate (SDS) and 7% (v/v) of glycerol with or without 5% (v/v) of 2-mercaptoethanol. After electrophoresis at 20 mA for 1 h, the gels were stained with 0.25% (w/v) Coomassie brilliant blue R-250 in 45% (v/v) ethanol–8.2% (v/v) acetic acid. The stained gels were destained with 25% ethanol–8.2% acetic acid, and then with 5% (v/v) methanol–7.5% (v/v) acetic acid. The molecular weight markers used were phosphorylase *a* ($M_r=94,000$), bovine serum albumin ($M_r=68,000$), ovalbumin ($M_r=45,000$), carbonic anhydrase ($M_r=30,000$), soybean trypsin inhibitor ($M_r=21,500$), and lysozyme ($M_r=14,300$).

Reagents Used—Acetonitrile (special grade for HPLC use) was purchased from Kishida Chemical Co., Ltd., Osaka. TFA was obtained from Merck, Darmstadt.

RESULTS

Purification of EGI from Human Platelets—

Human platelets were extracted with acid-ethanol. The platelet extract inhibited the growth in monolayer culture of BRL in the basal medium containing 10% of fetal calf serum, whereas it stimulated

the colony formation of NRK cells in soft agar containing EGF (Fig. 1). The colony-forming activity of the extract for NRK cells was hardly observed in soft agar without EGF, indicating that TGF- β present in the extract was responsible for the activity (data not shown).

The platelet extract was subjected to molecular-sieve chromatography on a Toyopearl HW50 column (Fig. 2). The growth inhibitory activity

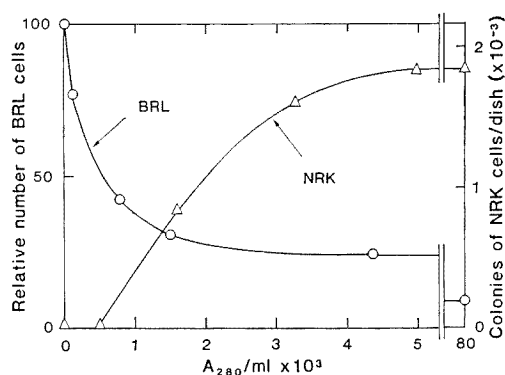


Fig. 1. Effects of the platelet extract on the growth in monolayer culture of BRL (○) and on the colony formation in soft agar of NRK (△). Each point represents the average for duplicate experiments. Other experimental conditions are given in the text.

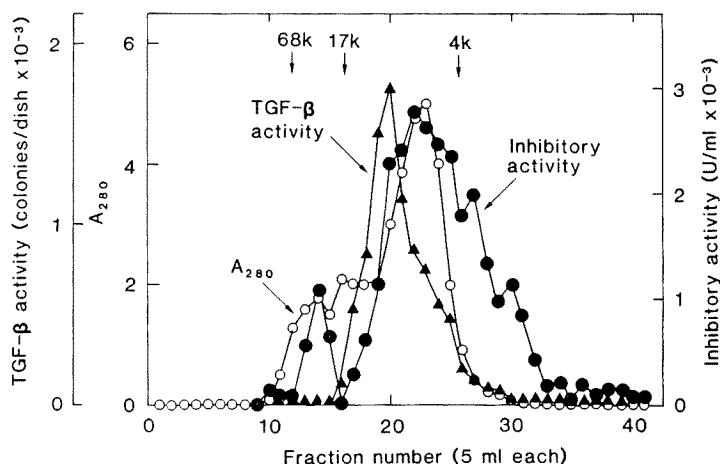


Fig. 2. Molecular-sieve chromatography of the acid-ethanol extract on a Toyopearl HW50 column. About 10 ml of the acid-ethanol extract ($A_{280}/\text{ml}=15$) was applied to a 200-ml column of Toyopearl HW50, previously equilibrated in 1 M acetic acid. The elution positions of bovine serum albumin (M_r 68,000), myoglobin (M_r 16,800), and insulin β chain (M_r 3,700) are indicated by arrows. Growth inhibitory activity (●), TGF- β activity (▲), A_{280} (○). Fraction Nos. 18–22 (part I) and Nos. 23–28 (part II) were collected and used for further purification.

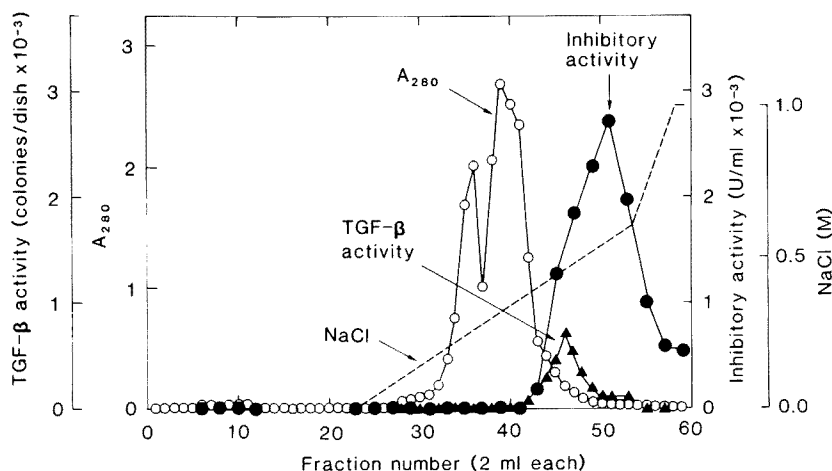


Fig. 3. Cation-exchange HPLC of part II obtained on molecular-sieve chromatography. Part II (Fig. 2) was used as the sample. Growth inhibitory activity (●), TGF- β activity (▲), A_{280} (○). Fraction Nos. 47–53 were collected and used for further purification of EGI.

was separated into a small peak, fraction Nos. 13–15, and a large broad peak, fraction Nos. 18–31. The elution pattern was reproducible as a whole. However, it was not appropriate to estimate the M_r values, because the elution of proteins from the column was retarded under the acidic conditions. TGF- β activity was eluted between the small and large peaks of the growth inhibitory activity. The large peak of the growth inhibitory activity was divided into the two parts, part I (fraction Nos. 18–22) and part II (fraction Nos. 23–28).

Part II was subjected to cation-exchange HPLC on a TSKgel CM-3SW column (Fig. 3). The growth inhibitory activity was effectively separated from contaminating proteins with an about 30-fold increase in specific activity. The TGF- β activity was eluted as a small peak at a NaCl concentration slightly lower than that for the elution of the growth inhibitory activity. The addition of 4 M urea was indispensable, because most of the growth inhibitory activity and TGF- β activity were not eluted even at 0.8 M NaCl in the absence of urea. The resultant fraction Nos. 47–53 were mixed.

The mixture described above was subjected to reverse-phase HPLC on a C18 column (Fig. 4). The growth inhibitory activity was eluted at about 38% acetonitrile, forming a sharp peak which coincided with an A_{280} peak and accompanied by

a trace of TGF- β activity. The resultant fractions, from 41 to 43 ml, were mixed and used as the purified EGI.

Table I summarizes the purification of the growth inhibitor.

By the simple three-step procedure, EGI was purified with an A_{280} of 0.03 from 15 liters of the blood. The specific activity increased about 5,000-fold with a yield of 29% from the platelet extract.

On SDS-polyacrylamide gel electrophoresis in the absence of 2-mercaptoethanol, the purified EGI formed a single protein band with an M_r of about 27,000 (Fig. 5). On electrophoresis in the presence of 2-mercaptoethanol, the purified EGI again formed a single band, but with an M_r of about 13,500, suggesting that it consisted of two subunits identical in M_r .

Purification of TGF- β —Part I (TGF- β -rich fractions) obtained on molecular-sieve chromatography was subjected to cation-exchange HPLC (Fig. 6). The peak fractions of the TGF- β activity (fraction Nos. 42–46) were pooled and then subjected to reverse-phase HPLC (Fig. 7). The major TGF- β activity was eluted at 31% acetonitrile, being separated from the growth inhibitory activity. The resultant fractions, from 14 to 25 ml, were mixed and used as the purified TGF- β . The purified TGF- β showed an about 2,000-fold increase in specific activity with a yield of about 9% from the platelet extract. On SDS-polyacryl-

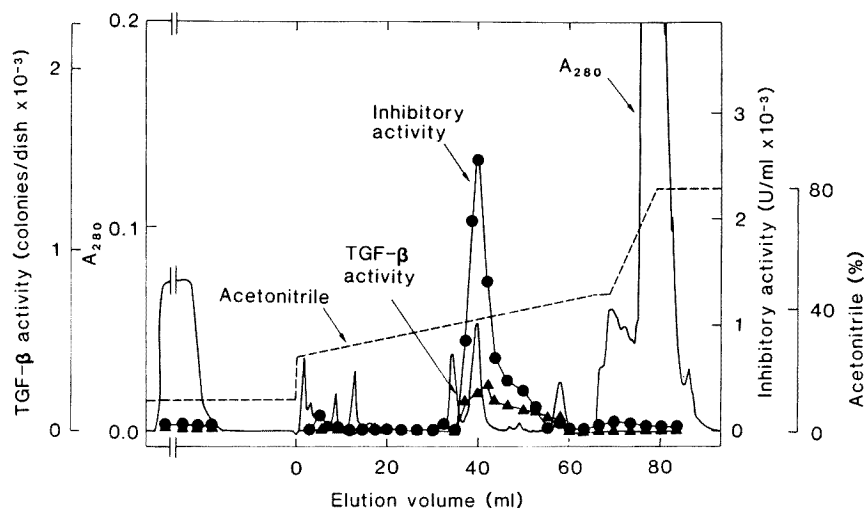


Fig. 4. Reverse-phase HPLC on a C18 column of EGI. The mixture of fraction Nos. 47–53 (Fig. 3) was used as the sample. Growth inhibitory activity (●), TGF- β activity (▲), A_{280} (—), acetonitrile (---).

TABLE I. Summary of the purification of the growth inhibitor. The extract was prepared from 15 liters of human blood.

Step	Total protein (A_{280})	Total activity (U)	Specific activity (U/ A_{280})	Yield (%)	Purification fold
Extract	500	6.0×10^5	1.2×10^3	(100)	(1)
Molecular-sieve chromatography	200	3.0×10^5	1.5×10^3	50	1.3
Cation-exchange HPLC	5.0	2.3×10^5	4.5×10^4	36	40
Reverse-phase HPLC	0.03	1.8×10^5	6.0×10^7	29	5,000

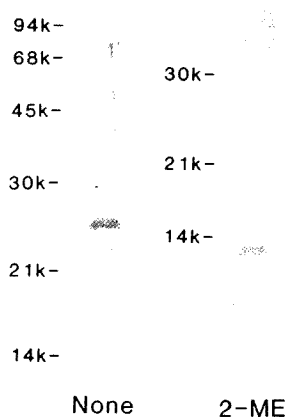


Fig. 5. SDS-polyacrylamide gel electrophoresis of the purified EGI. A portion ($A_{280} = 1.5 \times 10^{-6}$) of the purified EGI was lyophilized, dissolved in $10 \mu\text{l}$ of the sample buffer with (B) and without (A) 70 mM 2-mercaptoethanol. Other experimental conditions are given in the text.

amide gel electrophoresis in the absence of 2-mercaptoethanol, the purified TGF- β still showed several protein bands; the band of TGF- β could not be identified.

Biological Properties of Purified EGI and TGF- β —The purified EGI inhibited the growth of BRL in monolayer culture in the basal medium containing 10% fetal calf serum at low concentrations (Fig. 8A). The dose required for 50% growth inhibition (ED_{50}) was 8×10^{-8} A_{280}/ml . When the protein concentration of the purified EGI was determined by means of staining with Coomassie brilliant blue R-250 on a SDS gel using known amounts of bovine serum albumin as the standard, the ED_{50} was estimated to be about 20 pg/ml (0.7 pM). The purified EGI did not stimulate the colony formation of NRK cells in soft agar at a concentration of $A_{280}/\text{ml} = 1.0 \times 10^{-6}$ or lower. On the other hand, the purified TGF- β

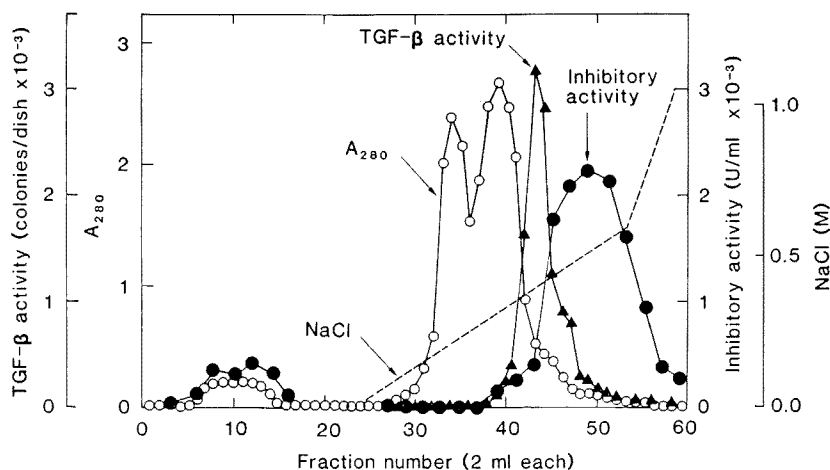


Fig. 6. Cation-exchange HPLC of part I obtained on molecular-sieve chromatography. Part I (Fig. 2) was used as the sample. Growth inhibitory activity (●), TGF- β activity (▲), A_{280} (○). Fraction Nos. 42–46 were collected and used for further purification.

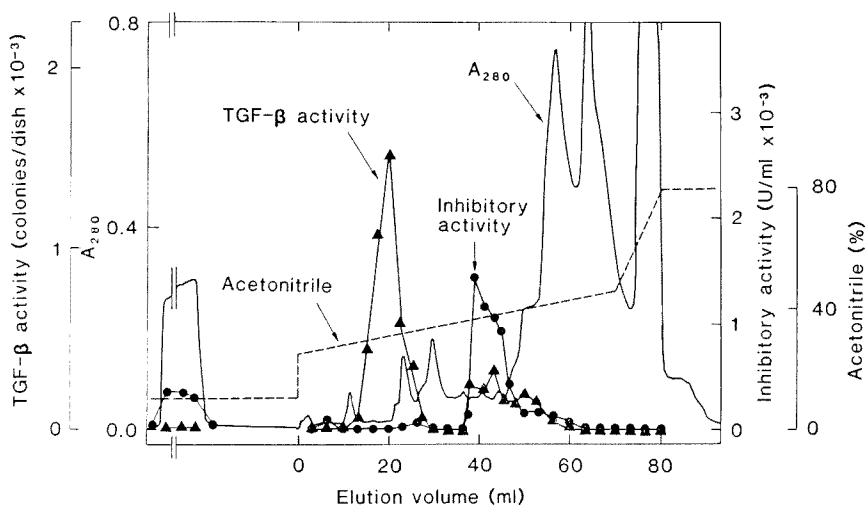


Fig. 7. Reverse-phase HPLC on a C18 column of TGF- β . The mixture of fraction Nos. 42–46 (Fig. 6) was used as the sample. Growth inhibitory activity (●), TGF- β activity (▲), A_{280} (—), acetonitrile (---).

stimulated the colony formation of NRK with an ED_{50} of $A_{280}/\text{ml} = 1.2 \times 10^{-5}$, whereas it showed no growth inhibitory activity toward BRL even at $A_{280}/\text{ml} = 8 \times 10^{-5}$ (Fig. 8B). The purified TGF- β hardly affected the colony formation of NRK cells in the absence of EGF or the growth of NRK, MDCK, and BSC-1 in monolayer cultures (data not shown).

Table II summarizes the effects of the purified EGI on the growth of various cell lines in monolayer cultures.

Of the four non-malignant epithelial cell lines tested (BRL, MDCK, BSC-1, and FL), all other than FL were significantly sensitive to the purified EGI. FL was originally obtained from normal human amniotic membranes. However, the FL

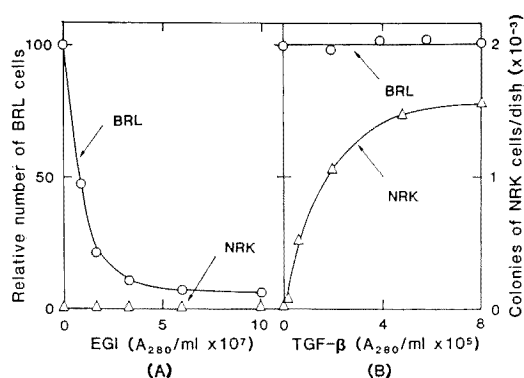


Fig. 8. Effects of the purified EGI (A) and TGF- β (B) on the growth in monolayer culture of BRL ($\circ$) and on the colony formation in soft agar of NRK (Δ). Each point represents the average for duplicate experiments. Other experimental conditions are given in the text.

TABLE II. Effect of the purified growth inhibitor on various cell lines. Cells were seeded at a density of 1×10^4 /dish in 10% fetal calf serum-containing medium with and without the purified growth inhibitor, and incubated for 4 days, except for BSC-1 (2×10^4 cells, 5 days), FL (1×10^4 cells, 5 days), A431 (2×10^4 cells, 4 days), MDCK (1×10^4 cells, 5 days), and YH-1 and B-32 (5×10^4 cells, 4 days). Each value represents the average for triplicate cultures.

Cell line	Cell number (% of control)	
	1.6×10^{-7} A_{280}/ml	8.0×10^{-7} A_{280}/ml
Epithelial		
Non-malignant: BRL	25	8
BSC-1	35	30
FL ^a	110	125
MDCK	25	10
Malignant: A431	55	50
HeLa-S3	105	115
RSV-BRL	85	75
Fibroblastic		
Non-malignant: Balb 3T3	106	114
NRK	86	78
Swiss 3T3	108	102
YH-1	90	80
Malignant: B-32	108	100

^a It formed colonies in soft agar.

TABLE III. Stability of the purified EGI. The purified EGI in PBS was incubated under the indicated conditions. The dithiothreitol was removed by dialysis at 4°C overnight after the incubation.

Treatment	Activity (%)
Non-treated	(100)
Heated at	
56°C for 30 min	100
90°C for 3 min	100
Incubated with	
50 mM dithiothreitol at 25°C for 1 h	5

used in the present study formed colonies in soft agar medium, indicating that it had been spontaneously transformed. All the five fibroblastic cell lines tested, whether malignant and non-malignant, were not sensitive to the purified EGI. These results suggest that the purified EGI was specific for non-malignant epithelial cells, other than A431. The purified EGI retained the full growth inhibitory activity after incubation at 56°C for 30 min or 90°C for 3 min, whereas it lost the activity almost completely on treatment with 50 mM dithiothreitol (Table III).

DISCUSSION

It was reported that platelets contain type β transforming growth factor (TGF- β) (9, 10), platelet-derived growth factor (PDGF) (24, 25), epidermal growth factor (EGF) (26) and some other growth regulatory factors (27–30). In the present study, a growth inhibitory protein was purified from human platelets to homogeneity. Since the purified protein inhibited the growth of several non-malignant epithelial cell lines, it is called epithelial cell-specific growth inhibitor (EGI). The purified EGI had an M_r of about 27,000, as determined on SDS-polyacrylamide gel electrophoresis under non-reducing conditions. In addition to EGI, TGF- β was partially purified from the same source.

TGF- β is a unique growth regulatory factor, which shows diverse growth-modulating activities depending upon the cell type and experimental conditions. It stimulates the colony formation in soft agar (anchorage-independent growth) of NRK

in the presence of EGF or type α transforming growth factor (TGF- α) (10, 12), whereas it stimulates that of mouse embryo AKR-2B in the absence of EGF or TGF- α (9). Recently, Tucker *et al.* (18) demonstrated that TGF- β is closely related to the growth inhibitor which has been purified from the conditioned medium of BSC-1 by Holley *et al.* (31, 32). As well as TGF- β , this inhibitor stimulates the anchorage-independent growth of AKR-2B, but inhibits the DNA synthesis of AKR-2B, BSC-1, and CCL-64 (mink lung) in monolayer cultures in serum-free media. On the other hand, Roberts *et al.* (33) reported that TGF- β inhibits the growth in monolayer culture of NRK in the presence of 2–7% calf serum, and that of human lung carcinoma A-549 in the presence of 2% fetal calf serum. However, the TGF- β purified in the present study did not show growth inhibitory activity toward monolayer cultures of BRL, BSC-1, and NRK cells in medium containing 10% fetal calf serum.

The purified EGI was specific for epithelial cells: it significantly inhibited the growth in monolayer culture of three non-malignant cell lines (BRL, MDCK, and BSC-1) in the presence of 10% fetal calf serum at a concentration of $A_{280} = 1.6 \times 10^{-7}$ (about 40 pg/ml). The malignant epithelial cell lines (RSV-BRL and HeLa-S3), the spontaneously transformed epithelial cell line (FL), and the five non-transformed and transformed fibroblastic cell lines (NRK, Balb 3T3, Swiss 3T3, YH-1, and B-32) were not sensitive, except for the human carcinoma cell line, A431. The purified EGI did not show TGF- β activity at low concentrations. However, when the concentration of EGI was increased to $A_{280/ml} = 2.5 \times 10^{-6}$ (about 0.6 ng/ml) or higher, it stimulated the colony formation of NRK cells in soft agar (data not shown). The effective dose for the activity was about 30-times higher than that for the growth inhibitory activity toward BRL cells. In addition, the maximum stimulation of the colony formation of NRK cells by EGI constituted about 20% of that in the case of the purified TGF- β . It remains to be determined whether the weak TGF- β -like activity in the EGI preparation is due to EGI itself or to a trace of a TGF- β -like contaminant.

The purified EGI was stable against heating at 90°C for 3 min, treatment with 1 M acetic acid and treatment with 4 M urea, but not against

treatment with 50 mM dithiothreitol. These physical and chemical properties of EGI are similar to those of TGF- β reported by other groups (9, 10). Preliminary analysis has shown that the two N-terminal amino acids of EGI are Ala-Leu, that is, consistent with those of TGF- β . Possibly, EGI is structurally related to TGF- β .

Recently, growth inhibitors have been highly purified from human platelets (9, 10), BSC-1 cells (31, 32), rat liver (34), human cancer cells (35, 36), and Swiss 3T3 cells (37). We previously reported that at least two types of growth inhibitors exist in sera: sera from rats and mice show much higher growth inhibitory activities toward BRL than RSV-BRL, whereas rabbit serum shows higher growth inhibitory activity toward RSV-BRL rather than BRL (19). In addition, it was also reported that the sensitivities of several clones of RSV-BRL to the growth inhibitor from rat serum were inversely correlated with their tumorigenicities (20). Together with these results, the present findings support the hypothesis that growth inhibitors, as well as growth factors, may play important roles in the control of cell proliferation *in vivo*.

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