The EMBO Journal no.3 vol.12 pp.2963–2967, 1984

Purification and properties of porcine platelet-derived growth factor

P.Stroobant and M.D.Waterfield

Imperial Cancer Research Fund Laboratories, Lincoln's Inn Fields, London WC2A 3PX, UK

Communicated by B.Griffin

The purification to homogeneity of a potent growth factor from porcine platelets is described. This cationic mitogen is named porcine platelet-derived growth factor (PDGF) on the basis of close structural, functional and immunological similarities to human PDGF. Porcine PDGF, like its human homologue, is a hydrophobic, disulphide cross-linked protein, which is stable to heat, acid, sodium dodecyl sulphate (SDS), and guanidine. The purified protein has an apparent mol. wt. on SDS-polyacrylamide gels of 38 000, similar to those reported for human PDGF (27 500–35 000). Amino terminal sequence analysis of native porcine PDGF gave a single 15 amino acid residue sequence, of which 11 residues were identical to the amino terminal sequence of the B chain of human PDGF. Gel permeation h.p.l.c. in guanidine solutions of the reduced protein revealed a single species of mol. wt. 17 000 suggesting that native porcine PDGF may be a homodimer of a 17 000 mol. wt. chain. Since porcine PDGF can be purified at low cost from large quantities of fresh platelets, it provides an alternative source of PDGF for structural and functional studies, and could be of use in preparing defined media for cell culture.

Key words: porcine/PDGF/growth factor/purification/DNA synthesis

Introduction

The purification of human platelet-derived growth factor (PDGF) to homogeneity has recently been reported by four laboratories (Heldin *et al.*, 1981; Deuel *et al.*, 1981; Raines and Ross, 1982; Antoniades, 1981). Human PDGF is a strongly cationic and hydrophobic protein for which mol. wt. forms in the range 27 500–35 000 have been reported. It is remarkably resistant to heat and chemical denaturants, perhaps due to stabilisation of the protein structure by many disulphide bonds. Studies over the past 13 years have established PDGF as a growth factor of major interest, which is thought to play a role in normal tissue repair processes after being released from platelets when blood vessels are injured (Westermark *et al.*, 1983; Deuel and Huang, 1983). In addition, PDGF has been implicated in the pathogenesis of atherosclerosis (Ross, 1983), in bone resorption following abnormal platelet release (Tashjian *et al.*, 1982; Key *et al.*, 1983), and in the growth of tumour cells (Heldin *et al.*, 1980; Dicker *et al.*, 1981; Nister *et al.*, 1982; Katoh and Takayama, 1982). More recent evidence for the direct involvement of PDGF in neoplasia has come from the discoveries that the *sis* oncogene of Simian sarcoma virus codes for a protein which is almost identical in amino acid sequence to one of the two homologous polypeptide chains (the B chain) found in human PDGF (Waterfield *et al.*, 1983; Doolittle *et al.*, 1983; Johnsson *et al.*, 1984). Further evidence for structural, functional and immunological relatedness of this protein to PDGF has been subsequently presented (Deuel *et al.*, 1983; Robbins *et al.*, 1983).

Despite considerable interest in the properties of human PDGF including in particular its activities as a potent mitogen and chemoattractant for mesenchymal and other cells (Westermark *et al.*, 1983; Deuel and Huang, 1983), the difficulties in obtaining the large quantities of human platelets required for its isolation have severely limited the availability of pure factor for structural and functional studies. In addition, human PDGF is often extensively proteolysed, probably due to premature release from platelets during storage in outdated platelet-rich plasma concentrates from blood banks, the most commonly used starting material for PDGF purification. In an attempt to avoid these problems, we have purified PDGF from fresh porcine platelets, which can be collected on a large scale at low cost.

We now report the purification to homogeneity of a potent mitogen from porcine platelets which on the basis of close physical and biological similarities to human PDGF we term porcine PDGF.

Results

Purification of porcine platelet cationic growth factor

Initial studies (data not shown) indicated that freshly isolated porcine platelets treated with thrombin or adrenaline, or disrupted by heating or freeze-thawing released growth factor activity. A cationic growth factor was detected in freeze-thawed platelet extracts, suggesting that porcine platelets might contain a protein resembling human PDGF, which has a pI of ~10. The cationic factor was therefore purified to homogeneity, beginning with three protein purification steps previously used for human PDGF (Deuel *et al.*, 1981), followed by additional gel permeation and reverse-phase h.p.l.c. procedures developed specifically for the porcine factor. Growth factor activity was monitored throughout the purification with a DNA synthesis assay using Swiss 3T3 cells.

Briefly, the purification procedure (fully described in Materials and methods) began with the isolation of platelets from fresh anticoagulated porcine blood using continuous flow centrifugation. After storage at −20°C, platelets (5 kg from 980 l blood) were rapidly thawed in a microwave oven and then boiled in buffer to release growth factor activity and inactive proteolytic enzymes. The platelet lysate was batch-mixed overnight with sulfadex (a strong cation exchanger) and the cationic growth factor eluted with 1.5 M NaCl/10 mM sodium phosphate, pH 7.4. The active peak was batch-mixed overnight with with Blue Sepharose under conditions favouring hydrophobic interactions, and the cationic and hydrophobic growth factor eluted using 50% (v/v) ethanediol. The active peak was dialysed against 1 N acetic

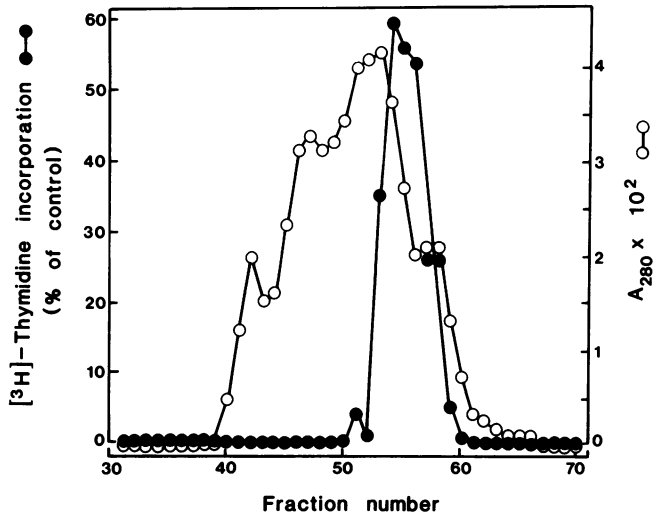

Fig. 1. Gel permeation h.p.l.c. of porcine platelet cationic growth factor. Conditions as described in Materials and methods.

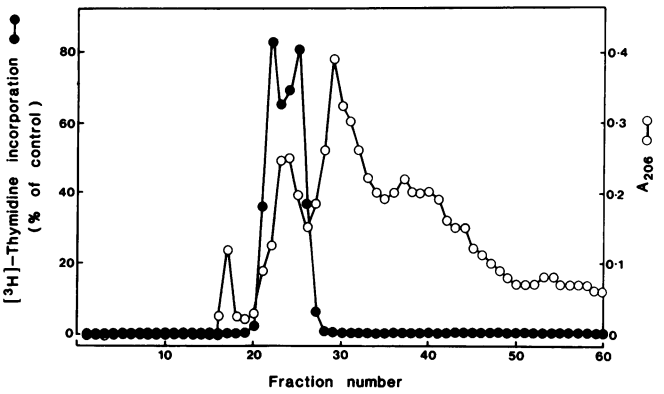

Fig. 2. Reverse-phase h.p.l.c. of porcine platelet cationic growth factor. Conditions as described in Materials and methods.

Table I. Purification summary for porcine platelet cationic growth factor

Fraction	Total protein (mg)	Total volume (ml)	Total activity (U^c)	Specific activity (U/mg)	Cumulative yield (%)
Platelet lysate[a]	17 064	5400	169 020	9.9	100
Sulfadex	941	2240	134 550	143	80
Blue Sepharose	92.8	200	117 640	1268	70
Bio-Gel	21.9	96	45 715	2087	27
Gel permeation h.p.l.c.	5.0	25	14 967	2993	9
Reverse phase h.p.l.c.	0.266[b]	57	9429	35 447	6

[a]Prepared using 5.0 kg platelets from 980 l porcine blood.
[b]Determined by amino acid analysis.
[c]One unit of DNA synthesis activity gives half maximal stimulation in a 2 ml culture volume under serum-free conditions.

acid, lyophilised, re-suspended in 6 M guanidine/0.1 M potassium phosphate, pH 4.5, and run on a Bio-Gel P-150 column in 1 N acetic acid. The active peak was then lyophilised, re-suspended in 6 M guanidine/0.1 M potassium phosphate, pH 4.5 and run on a gel permeation h.p.l.c. column in the same solvent (Figure 1). A single peak of growth factor activity was obtained. The active peak was finally run on a reverse-phase h.p.l.c. column in 0.1% trifluoroacetic acid, using a 1–60% linear gradient of acetonitrile (Figure 2). A single

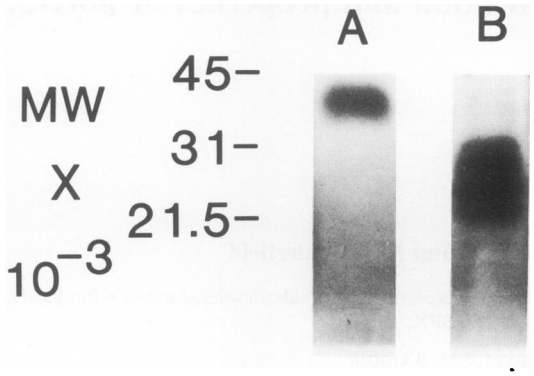

Fig. 3. SDS-polyacrylamide gel electrophoresis of porcine platelet cationic growth factor. **Track A**, non-reduced; **track B**, reduced. Mol. wt. standards (ovalbumin, 45 000; carbonic anhydrase, 31 000; soybean trypsin inhibitor, 21 500) are indicated.

peak of growth factor activity was obtained, eluted at an acetonitrile concentration of 42%. The purification summary for a typical preparation of the porcine platelet cationic growth factor (Table I) indicates a 3.6 x 10^3-fold purification with a 6% yield. The actual value may be higher, since platelets also contain non-cationic growth factors which would distort the estimates of growth factor activity in the starting material (Westermark *et al.*, 1983).

Properties of porcine platelet cationic growth factor

The protein composition of the purified porcine platelet growth factor peak fraction from the reverse-phase h.p.l.c. column step was analysed using SDS-polyacrylamide gel electrophoresis. A single broad silver-staining band with an apparent mol. wt. of 38 000 was seen under non-reducing conditions (Figure 3A). When a similar gel using larger amounts of material was run, subsequently sliced, and the gel pieces eluted and assayed, DNA synthesis activity was found only in a region of the gel which corresponded exactly to the single mol. wt. 38 000 band observed either with the silver stain or with a Coomassie stain (data not shown). One unit of DNA synthesis activity (the amount required for half-maximal stimulation under serum-free conditions) was equivalent to 14 ng/ml of the purified porcine growth factor compared with 44 ng/ml for human PDGF under identical conditions. This suggests that the purified porcine platelet cationic protein fraction is a protein of apparent mol. wt. 38 000 which is a potent mitogen for Swiss 3T3 fibroblasts.

When the porcine growth factor was run on an SDS-polyacrylamide gel under reducing conditions, four silver-staining bands with apparent mol. wts. of 31 500, 28 700, 25 100 and 22 400 were visible (Figure 3B), suggesting that it is a disulphide cross-linked protein, possibly consisting of four distinct chains. However, when the growth factor was fully reduced and alkylated with [^{14}C]iodoacetamide, and then run on gel permeation h.p.l.c. in a guanidine solution (Waterfield *et al.*, 1984), a single radioactive species of mol. wt. 17 000 was observed (data not shown), suggesting that the apparent heterogeneity on SDS gels was not due to real differences in mol. wt. between the four subspecies but to problems in reduction of cystine residues or in SDS binding.

To determine if the porcine platelet growth factor was antigenically related to human PDGF, a Western blot of the two growth factors was probed with antiserum against human PDGF. Control human PDGF (Figure 4A) and the porcine growth factor (Figure 4B) each gave a strong positive reac-

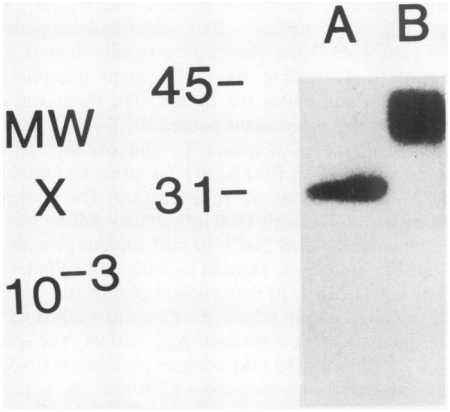

Fig. 4. Western blot of porcine platelet cationic growth factor and human PDGF, probed with anti-human PDGF antiserum. **Track A**, human PDGF (200 ng, supplied by C.-H.Heldin); **track B**, porcine platelet cationic growth factor (200 ng); mol. wt. standards (ovalbumin, 45 000; carbonic anhydrase, 31 000) are indicated.

Table II. Amino acid composition of porcine platelet cationic protein

Amino acid	Residues/mol[a]
Aspartic acid	22
Threonine	21
Serine	18
Glutamic acid	34
Proline	56
Glycine	28
Alanine	16
Cysteine	15
Valine	26
Methionine	0
Isoleucine	9
Leucine	14
Tyrosine	1
Phenylalanine	8
Histidine	32
Lysine	21
Arginine	34
Tryptophan	NE[b]

[a]Based on mol. wt. 38 000; total residues = 355. The nearest integer from the average of two determinations is shown.
[b]NE = not estimated.

tion, indicating that the two growth factors bear antigenic determinants in common.

An amino acid analysis of the porcine platelet cationic protein is shown in Table II. The composition is similar to that of human PDGF (Deuel *et al.*, 1981), with a high number of acidic, basic and cysteine residues. Since extensive data is available on the amino acid sequence of human PDGF (Johnsson *et al.*, 1984) an amino terminal sequence of the non-reduced porcine platelet growth factor was determined, the data for which is shown in Figure 5. A total of 15 amino acid residues could be unambiguously assigned. Comparison of this sequence with the amino terminal sequence of the B chain of human PDGF (Johnsson *et al.*, 1984) (Figure 6) showed that the two amino termini could be exactly aligned, showing 11 out of 15 identical residues, with three conservative substitutions. No sequence similar to the human PDGF A chain was detected.

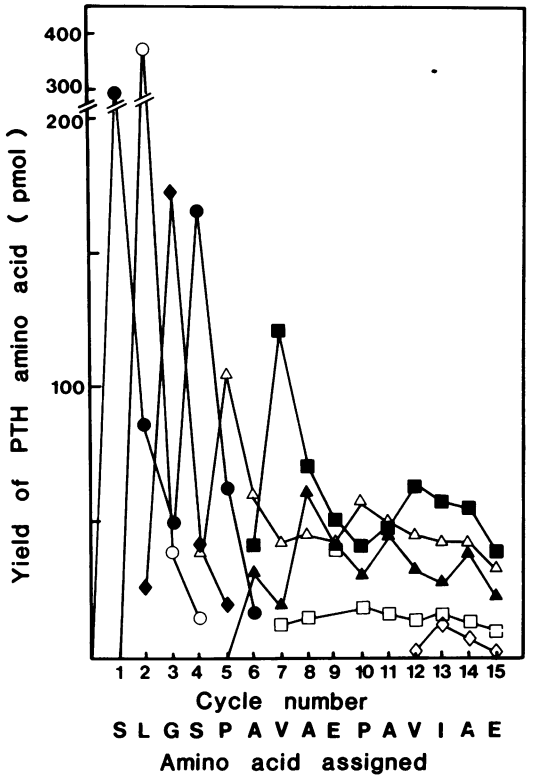

Fig. 5. Analysis of the amino acid sequence of non-reduced porcine platelet cationic growth factor. The yield of the PTH amino acids liberated at each step of Edman degradation is plotted against the cycle number. The techniques used are as described (Waterfield *et al.*, 1984). Data from one of two runs using ~0.2 nmol of material are shown.

Porcine PDGF	S	L	G	S	P	A	V	A	E	P	A	V	I	A	E	X
Human PDGF, B-chain	S	L	G	S	L	T	I	A	E	P	A	M	I	A	E	C

Fig. 6. Comparison of the amino terminal amino acid sequences of porcine PDGF and the B chain of human PDGF. X denotes unidentified amino acid residue. Residues in common are enclosed in boxes.

Discussion

The results presented describe the purification to apparent homogeneity of a cationic growth factor from porcine platelets, using conventional column separation techniques combined with gel permeation and reverse-phase h.p.l.c. If the physical properties of the porcine cationic growth factor established here, together with the biological activities of our partially and highly purified preparations of the same factor described elsewhere are compared with the corresponding properties of human PDGF (Table III), a clear and striking similarity between the two growth factors is apparent. On the basis of this close similarity we conclude that the porcine cationic growth factor is very similar in structure and function to human PDGF, and propose that it be named porcine platelet-derived growth factor. Evidence for PDGF related molecules in a variety of organisms has been presented (Singh *et al.*, 1982).

Porcine PDGF is clearly different in some respects from human PDGF. The apparent mol. wt. on SDS gels for different preparations of the porcine molecule is consistently 38 000, compared with a range of values centered around

Table III. Similarities between porcine and human PDGF

Properties	Reference	
	Porcine	Human
Physical properties		
Cationic	a	Westermark *et al.* 1983
Hydrophobic	a	Westermark *et al.* 1983
Stable to heat, acid, SDS, 6 M guanidine	a	Westermark *et al.* 1983
Unstable to reduction	a	Westermark *et al.* 1983
Immunologically related	a	a
NH_2-terminal sequence homology	a	a
Biological properties		
Potent mitogen for fibroblasts which synergizes with insulin	Lopez-Rivas *et al.* 1984	Dicker *et al.* 1981
Transmodulates EGF receptor	Collins *et al.* 1983	Collins *et al.* 1983
Stimulates production of prostaglandins and cAMP	Rozengurt *et al.* 1983	Rozengurt *et al.* 1983
Stimulates ionic fluxes	Lopez-Rivas *et al.* 1984	Cassel *et al.* 1983

[a]This report.

30 000 for human PDGF. The observations that reduced porcine PDGF runs on gel permeation h.p.l.c. in guanidine solutions as a single peak with a mol. wt. of 17 000, whereas human PDGF under the same conditions gives a series of peaks of mol. wt. 13 000, 8840, 5320 and 3454 (Waterfield *et al.*, 1983), suggest that human PDGF is proteolytically degraded, a process which probably occurs during storage in the outdated clinical platelet packs commonly used for purification. The use of freshly isolated porcine platelets probably avoids such degradation. Because only a single amino terminal amino acid sequence was determined from non-reduced porcine PDGF, it seems possible that the molecule is composed of a 17 000 mol. wt. homodimer, although further studies are needed to resolve the question of whether a second blocked amino terminal sequence from a second chain of the A type found in human PDGF is present. The apparent mol. wts. of the non-reduced molecule and the reduced subspecies on SDS gels may be misleading, since both cationic and carbohydrate-containing proteins and both the A and B chains of human PDGF are known to run anomalously on SDS gels (Johnsson *et al.*, 1984).

Since porcine PDGF is functionally and structurally related to human PDGF and can be isolated readily on a large scale, it provides an alternative source of PDGF for a wide variety of studies (Westermark *et al.*, 1983; Deuel and Huang, 1983). In addition the relatively low cost of producing porcine PDGF may enable its use in synthetic growth media for large-scale cell culture and where defined growth conditions are important.

Materials and methods

Materials

Foetal bovine serum was obtained from Gibco Europe (UK), [^{3}H]thymidine from the Radiochemical Centre (Amersham, UK). Blue Sepharose from Pharmacia, and guanidine from Sigma. Other reagents were of the highest purity commercially available.

Purification of porcine PDGF

The details for a typical preparation of porcine PDGF are given. Porcine platelets were isolated by differential centrifugation of porcine blood anti-coagulated with one-fifth volume of 40 mM Na_2 EDTA/145 mM NaCl/5 mM Tris, pH 6.6, and stored at −20°C. Accumulated platelets (5 kg batches from 980 l anti-coagulated blood) were rapidly thawed in a microwave oven, and added to 4 l boiling 100 mM sodium phosphate/10 mM Na_2 EDTA, pH 7.4, and then boiled for 10 min. The lysate was centrifuged at 12 000 *g* for 30 min, the supernatant poured off through a gauze filter, and the pellet washed with two successive 1 l aliquots of 1 M NaCl/10 mM sodium phosphate/5 mM Na_2 EDTA, pH 7.4, using an Omnimixer (Sorvall) at setting 3 with 3 x 10 s bursts for re-suspension. The pooled supernatants were stirred overnight at 4°C with 1800 ml sulfadex (Miletich *et al.*, 1980) previously equilibrated with 0.1 M NaCl/10 mM sodium phosphate/5 mM Na_2 EDTA, pH 7.4. The slurry was allowed to settle, the sulfadex batch-washed with 10 x 1 l of 0.5 M NaCl/10 mM sodium phosphate/5 mM Na_2 EDTA, pH 7.4, and a sulfadex column prepared which was washed with a further 3 l of wash buffer so that the flow-through A_{280} = 0.04. The column was then eluted with 3 l 1.5 M NaCl/10 mM sodium phosphate/5 mM Na_2 EDTA, pH 7.4. The active fractions were pooled (2240 ml) and stirred overnight at 4°C with 75 g Blue Sepharose previously equilibrated with sulfadex elution buffer. A column was poured with the settled slurry and was eluted with 1 M NaCl/10 mM sodium phosphate, pH 7.4, containing 50% (v/v) ethanediol. The active fractions were pooled (200 ml), dialysed against 1 N acetic acid, lyophilised, re-suspended in 2 ml 6 M guanidine/0.1 M potassium phosphate, pH 4.5, and run in 50 mg aliquots on a Bio-Gel P-150 column (2 cm x 180 cm) in 1 N acetic acid. The active peaks were lyophilised, re-suspended in 6 M guanidine/0.1 M potassium phosphate, pH 7.5, and run in 3 mg aliquots on a gel permeation h.p.l.c. column (TSK SW 3000, LKB) in the same buffer at 0.5 ml/min, collecting 0.5 ml fractions. The active peak was finally run in 0.7 mg aliquots on a reverse-phase h.p.l.c. column (Synchropac RPP; Synchrom, Indiana) using 0.1% trifluoroacetic acid with a 1−60% linear gradient of acetonitrile at 1 ml/min, collecting 0.5 ml fractions.

Cell culture

Cultures of Swiss 3T3 cells (Todaro and Green, 1963) were grown at 37°C in humidified 10% CO_2/90% air in Dulbecco's modified Eagle's medium (DME) containing 10% foetal bovine serum, penicillin (100 units/ml) and streptomycin (100 μg/ml). For experiments, 10^5 cells were subcultured into 3 mm Nunc Petri dishes with medium containing 10% foetal bovine serum. Such cultures were used 6−8 days later at a time when the cells were confluent and quiescent as judged by cytofluorometric and autoradiographic analysis (Dicker and Rozengurt, 1980).

DNA synthesis

All determinations of DNA synthesis were carried out in DME/Weymouth's medium 1:1 (v/v). The cultures were washed twice with DME medium to remove residual serum immediately prior to assay. DNA synthesis was assessed by incorporation of [^{3}H]thymidine into acid-insoluble material after a 40 h incubation with 2 ml of medium containing 0.9−1.25 μM thymidine (Rozengurt and Heppel, 1975), using 10% foetal calf serum as the control for maximal stimulation.

Western blot

Samples were run on SDS-polyacrylamide gels in the absence of reducing agent. Gels were transferred onto diazotized paper (Western blot) at 0.5 A and 20 V for 3 h on an Electroblot apparatus (Bio-Rad) essentially as described by Symington *et al.* (1981). The buffer was 25 mM sodium phosphate, pH 6.5 at 23°C. Diazotized paper was prepared and activated as described by Seed (1982). After transfer, excess reactive groups were inactivated by incubating the paper in 100 ml 0.1 M Tris-HCl, pH 9.0, 10% (v/v) ethanolamine, and 0.25% (w/v) gelatin for 2 h at 23°C. The paper was incubated for 18 h at 23°C with 5 ml of 50 mM Tris-HCl buffer, pH 7.4, containing 150 mM NaCl, 5 mM EDTA, 0.25% (w/v) gelatin and 0.1% (v/v) Triton X-100 (buffer A) containing 25 μl of anti-human PDGF antiserum (supplied by T.F. Deuel). The paper was then washed with 2 x 100 ml of buffer A for 30 min at 23°C to remove unbound antibodies and then incubated for 1 h in 5 ml of this buffer containing 2 x 10^6 c.p.m./ml ^{125}I-labelled goat anti-rabbit IgG (specific activity ~3 x 10^{18} c.p.m./mol). The unbound antibodies were removed by washing the paper in 2 x 100 ml of buffer A. The paper was then wrapped in cellophane and autoradiographed for 18 h at −70°C using X ARS film (Kodak) and an intensifying screen.

Protein analysis

Amino acid analyses were made following hydrolysis in 6 N HCl *in vacuo* using a Beckman 6300 analyser. Cysteine residues were determined following performic acid oxidation (Hirs, 1967) and hydrolysis. Amino terminal sequences were determined using a gas phase sequencer constructed and operated as described by Hewick *et al.* (1981), and phenylthiohydantoin amino acids were analysed as described by Waterfield *et al.* (1983).

Protein gels

SDS-polyacrylamide gel analysis was carried out using 12−22% gradient gels,

as described by Laemmli, 1970. Gels were silver-stained using the method of Sammons *et al.* (1981).

Acknowledgements

We thank D.J.Cheng for excellent technical assistance, W.Gullick for performing the Western blot analysis, N.F.Totty for sequence data, R.Philp for amino acid analysis, E.Rozengurt for helpful discussions, T.F.Deuel and C.-H.Heldin for kindly providing human PDGF antiserum and human PDGF, respectively, and Speywood Laboratories Limited (Wrexham, Clwyd, UK) for supplying porcine platelets.

References

Antoniades,H.N. (1981) *Proc. Natl. Acad. Sci. USA,* **78**, 7314-7317.

Cassel,D., Rothenberg,P., Zhuang,Y.-X., Deuel,T.F. and Glaser,L. (1983) *Proc. Natl. Acad. Sci. USA,* **80**, 6224-6228.

Collins,M.K.L., Sinnett-Smith,J.W. and Rozengurt,E. (1983) *J. Biol. Chem.,* **258**, 11689-11693.

Deuel,T.F. and Huang,J.S. (1983) *Prog. Hematol.,* **13**, 202-221.

Deuel,T.F., Huang,J.S., Proffitt,R.T., Baenziger,J.U., Chang,D. and Kennedy,B.B. (1981) *J. Biol. Chem.,* **256**, 8896-8899.

Deuel,T.F., Huang,J.S., Huang,S.S., Stroobant,P. and Waterfield,M.D. (1983) *Science (Wash.),* **221**, 1348-1350.

Dicker,P. and Rozengurt,E. (1980) *Nature,* **287**, 607-612.

Dicker,P., Pohjanpelto,P., Pettican,P. and Rozengurt,E. (1981) *Exp. Cell Res.,* **135**, 221-227.

Doolittle,R.F., Hunkapiller,M.W., Hood,L., Devare,S.G., Robbins,K.C., Aaronson,S.A. and Antoniades,H.N. (1983) *Science (Wash.),* **221**, 275-277.

Heldin,C.-H., Westermark,B. and Wasteson,A. (1980) *J. Cell Physiol.,* **105**, 235-246.

Heldin,C.-H., Westermark,B. and Wasteson,A. (1981) *Biochem. J.,* **193**, 907-913.

Hewick,R.M., Hunkapiller,M.W., Hood,L.E. and Dreyer,W.J. (1981) *J. Biol. Chem.,* **256**, 7990-7997.

Hirs,C.H.W. (1967) *Method Enzymol.,* **11**, 59-62.

Johnsson,A., Heldin,C.-H., Wasteson,A., Westermark,B., Deuel,T.F., Huang,J.S., Seeburg,P.H., Gray,A., Ullrich,A., Scrace,G., Stroobant,P. and Waterfield,M.D. (1984) *EMBO J.,* **3**, 921-928.

Katoh,Y. and Takayama,S. (1982) *Carcinogenesis,* **3**, 867-873.

Key,L.L., Carnes,D.L., Weichselbaum,R. and Anast,C.S. (1983) *Endocrinology,* **112**, 761-762.

Laemmli,U.K. (1970) *Nature,* **227**, 680-685.

Lopez-Rivas,A., Stroobant,P., Waterfield,M.D. and Rozengurt,E. (1984) *EMBO J.,* **3**, 939-944.

Miletich,J.P., Broze,G.J. and Majerus,P.W. (1980) *Anal. Biochem.,* **105**, 304-310.

Nister,M., Heldin,C.-H., Wasteson,A. and Westermark,B. (1982) *Ann. NY Acad. Sci.,* **397**, 25-33.

Raines,E.W. and Ross,R. (1982) *J. Biol. Chem.,* **257**, 5154-5160.

Robbins,K.C., Antoniades,H.N., Devare,S.G., Hunkapiller,M.W. and Aaronson,S.A. (1983) *Nature,* **305**, 605-608.

Ross,R. (1983) in *Development of the Vascular System,* Ciba Foundation Symposium **100**, pp. 198-206.

Rozengurt,E. and Heppel,L.A. (1975) *Proc. Natl. Acad. Sci. USA,* **72**, 4492-4495.

Rozengurt,E., Stroobant,P., Waterfield,M.D., Deuel,T.F. and Keehan,M. (1983) *Cell,* **34**, 265-272.

Sammons,D.W., Adams,L.D. and Nishizawa,E.E. (1981) *Electrophoresis,* **2**, 135-141.

Seed,B. (1982) *Nucleic Acids Res.,* **10**, 1799-1810.

Singh,J.P., Chaikin,M.A. and Stiles,C.D. (1982) *J. Cell Biol.,* **95**, 667-671.

Symington,J., Green,M. and Brackmann,K. (1981) *Proc. Natl. Acad. Sci. USA,* **78**, 177-181.

Tashjian,A.H., Hohmann,E.L., Antoniades,H.N. and Levine,L. (1982) *Endocrinology,* **111**, 118-124.

Todaro,G.J. and Green,H. (1963) *J. Cell Biol.,* **17**, 299-313.

Waterfield,M.D., Scrace,G.T., Whittle,N., Stroobant,P., Johnsson,A., Wasteson,A., Westermark,B., Heldin,C.-H., Huang,J.S. and Deuel,T.F. (1983) *Nature,* **304**, 35-39.

Waterfield,M.D., Scrace,G.T., Whittle,N., Stockwell,P.A., Stroobant,P., Johnsson,A., Wasteson,A., Westermark,B., Heldin,C.-H., Huang,J.S. and Deuel,T.F. (1984) in *Cancer Cells (1) The Transformed Phenotype,* Cold Spring Harbor Laboratory Press, NY, pp. 25-33.

Westermark,B., Heldin,C.-H., Ek,B., Johnsson,A., Mellstrom,K. and Wasteson,A. (1983) in Guroff,G. (ed.), *Growth and Maturation Factors,* Wiley and Sons, Vol. **1**, pp. 73-115.

Received on 6 August 1984