Larsen, B. S., Yergey, J. A., & Cotter, R. J. (1985) *Biomed. Mass Spectrom. 12*, 586-587.
Lepage, P., Roecklin, D., Acker, M., Roitsch, C., Bitsch, F., Green, B., & Van Dorsselaer, A. (1989) in *Advances in Mass Spectrometry 1988, Bordeaux* (Longevialle, P., Ed.) Heyden and Son Ltd., London (in press).
Loison, G., Findeli, A., Bernard, S., Nguyen-Juilleret, M., Marquet, M., Riehl-Bellon, N., Carvallo, D., Guerra-Santos, L., Brown, S. W., Courtney, M., Roitsch, C., & Lemoine, Y. (1988) *Biotechnology 6*, 72-77.
Markwardt, F. (1985) *Biomed. Biochem. Acta 44*, 1007-1013.
Markwardt, F., Hauptmann, J., Nowak, G., Klessen, Ch., & Walsmann, P. (1982) *Thromb. Haemostasis 47*, 226-229.
McEwen, C. N. (1983) *Anal. Chem. 55*, 967-968.
Naylor, S., Findeis, F., Gibson, B. W., & Williams, D. H. (1986) *J. Am. Chem. Soc. 108*, 6359-6363.
Richter, W. J., Raschdorf, F., & Maerki, W. (1985) *Mass Spectrom. Health Life Sci. 24*, 193-206.
Riehl-Bellon, N., Carvallo, D., Acker, M., Van Dorsselear, A., Marquet, M., Loison, G., Lemoine, Y., Brown, S. W., Courtney, M., & Roitsch, C. (1989) *Biochemistry* (preceding paper in this issue).
Roepstorff, P., & Fohlman, J. (1984) *Biomed. Mass Spectrom. 11*, 601.
Yergey, J. A., Heller, D., Hansen, G., Cotter, R. J., & Fenselau, C. (1983) *Anal. Chem. 55*, 353-356.
Yergey, J. A., Cotter, R. J., Heller, D., & Fenselau, C. (1984) *Anal. Chem. 56*, 2236-2264.

Preparation of Biologically Active Platelet-Derived Growth Factor Type BB from a Fusion Protein Expressed in *Escherichia coli*

J. Hoppe,* H. A. Weich, and W. Eichner

Department of Cytogenetics, GBF—Gesellschaft für Biotechnologische Forschung mbH, Mascheroder Weg 1, D-3300 Braunschweig, FRG

Received April 14, 1988; Revised Manuscript Received November 15, 1988

ABSTRACT: Preparations of the mitogen platelet-derived growth factor (PDGF) from human platelets contain two related polypeptides termed A chain and B chain. PDGF-B is highly homologous to a portion of $p28^{v\text{-}sis}$, the transforming protein of *simian sarcoma virus*. We have studied the mitogenic potential of a PDGF-BB-like homodimer by expressing the sequence coding for the mature part of PDGF-B in *Escherichia coli*. Expression was achieved as cro-β-gal–PDGF-B fusion protein which was exclusively found in the "inclusion bodies". A monomeric PDGF-B fragment shortened by 12 amino acid residues from the NH_2 terminus was excised from the fusion protein by CNBr cleavage. After protection of thiols by S-sulfonation, this fragment was purified by gel permeation chromatography and reversed-phase high-performance liquid chromatography. This monomeric protein was dimerized in the presence of a mixture of reduced and oxidized glutathione to yield biologically active rPDGF-BB with an overall yield of ≈0.7 mg of rPDGF-BB/L of culture. *Escherichia coli* rPDGF-BB stimulated [^{3}H]thymidine incorporation into AKR2B fibroblast at concentrations of about 1 ng/mL.

Platelet-derived growth factor (PDGF)[1] is a major mitogen in serum which promotes the proliferation of fibroblasts and smooth muscle cells in vitro (Heldin et al., 1984; Deuel et al., 1985; Ross et al., 1986). PDGF elicits its mitogenic effect at low concentrations (1 ng/mL) by binding with high affinity to a specific cell surface receptor. This single polypeptide of M_r 180 000 is stimulated to autophosphorylate tyrosine residues. So far, in vivo substrates for this cytoplasmic tyrosine-specific protein kinase have not been identified unequivocally.

In vivo PDGF is stored in the α-granules in platelet from which it is released following platelet activation. Purified PDGF is a cationic glycoprotein of M_r 30 000. It exhibits considerable size heterogeneity with species between M_r 27 000 and 31 000 which are thought to result from different extents of glycosylation, processing, and the presence of two forms, A and B. All these forms exhibit identical biological activities which are destroyed by chemical cleavage of disulfide bridges. Since it was not possible to resolve the different species into homodimers of A chains or B chains, it is believed that biologically active PDGF is built up of two different polypeptide chains of $\approx M_r$ 14 000 linked together by disulfide bridges (Johnson et al., 1982, 1984).

Amino acid sequencing data in combination with cDNA sequencing data have revealed that the A and B chains are partially homologous. Interestingly enough, the B chain of PDGF was highly homologous to a portion of the predicted amino acid sequence of $p28^{v\text{-}sis}$, the transforming product of the *simian sarcoma virus* (SSV) (Doolittle et al., 1983; Waterfield et al., 1983). The relationship between PDGF-B and $p28^{v\text{-}sis}$ has raised the possibility that $p28^{v\text{-}sis}$ might act as

* Address correspondence to this author at the Institute for Physiological Chemistry, University of Würzburg, Koellikerstrasse 2, 8700 Würzburg, FRG.

[1] Abbreviations: HBS, Hepes-buffered saline; PBS, phosphate-buffered saline; PDGF, platelet-derived growth factor; rPDGF-BB, recombinant platelet-derived growth factor type BB; $p28^{v\text{-}sis}$, transforming protein from simian sarcoma virus; cro-β-gal, fusion protein from cro repressor and β-galactosidase; DTNB, dithionitrobenzoic acid (Ellman's reagent); FCS, fetal calf serum.

0006-2960/89/0428-2956$01.50/0

a mitogen by binding to the PDGF receptor [for a review, see Heldin et al. (1984) and Deuel et al. (1985)]. Intriguingly, $p28^{v\text{-}sis}$ forms a homodimer which is further processed to a size similar to that of PDGF (Robbins et al., 1985). There are several reports presenting evidence that homodimers of type A–A and B–B express the full biological activity. First, osteosarcoma cell derived growth factor, which is a PDGF-like mitogen, is a homodimer of A chains (Heldin et al., 1986; Betsholtz et al., 1986). Second, PDGF from porcine platelets is probably a B–B homodimer (Stroobant & Waterfield, 1984). Third, active homodimers of B chains have been expressed in yeast (Kelly et al., 1985).

Previous attempts to express PDGF-B in *Escherichia coli* have not led to biologically active products. Apparently, folding of the polypeptide chain and closing of disulfide bridges do not occur correctly in *E. coli* (Devare et al., 1984; Wang & Williams, 1984). Here we report the expression of PDGF-B in a fusion protein expression vector system, the purification of thiol-protected monomeric rPDGF-B after cleavage with cyanogen bromide, and its renaturation into active dimers.

Materials and Methods

Materials. Plasmids pEx 1–3 and strain NF1 were obtained from K. Stanley. Purified PDGF was prepared from fresh platelets essentially as described (Johnson et al., 1982). The final step was high-performance liquid chromatography using a 4 mm × 250 mm column packed with RP-P (Synchropak) reversed-phase support. PDGF was eluted with a gradient of acetonitrile in 0.3% aqueous trifluoroacetic acid. Chemicals were of the highest available purity. Growth media and supplements were from Boehringer (Mannheim) or from Gibco. Radioactive compounds were from Amersham.

Bacterial Strains and DNA Methods. All transformations using expression vectors (Stanley & Luzio, 1984) pEx 1–3 were performed using strain NF1 (K-12 ΔDH1 Δtrp) which is a lac Z^-am strain carrying the defective λ prophage (λNam7Nam53cI 857 ΔDH1).

The c-sis-containing 2-kb *Bam*HI DNA fragment from the clone pMVW-2 (Weich et al., 1986) was subcloned in reverse orientation in M13 mp18 vector. the 3′-encoding region for the PDGF-B chain was eliminated by digestion with *Sma*I, and a translation stop linker (obtained from P-L Biochemicals) was inserted into this site. To eliminate the 5′-encoding region of the c-sis gene, the plasmid was digested with *Pst*I and *Sal*I to create 5′ and 3′ sticky ends for exonuclease III digestion as described (Henikoff, 1984). Second strand was eliminated by S_1 digestion, and the insert was made blunt-ended by filling in with DNA polymerase I Klenow fragment. After ligation and transformation, colonies containing the PDGF-B homologous sequence were selected, tested for size of insert, and subsequently sequenced to determine the extent of digestion by exonuclease III. A plasmid was isolated which contained an ATG start codon from the *Sph*I site of M13 mp18 multicloning site in frame with the PDGF-B sequence just at the beginning of the mature PDGF-B sequence, leading to a slightly modified NH_2 terminus (Met-Pro-Leu-Gly- instead of Ser-Leu-Gly-). PDGF-B-encoding sequences were excised by a partial *Sph*I/*Eco*RI digest and were ligated into the *Sph*I/*Eco*RI site in the polylinker in the plasmid of pJLA 504 to create a *Sal*I site at the 3′ end (Schauder et al., 1987). This plasmid was partially digested with *Sph*I. Protruding 3′ ends of the *Sph*I site were made blunt-ended by T4 polymerase treatment. A 390 bp fragment which contained the PDGF-B sequence was obtained by *Sal*I digestion and was ligated into the *Sal*I/*Eco*RV site of pEx-1 (Figure 1). The *Sal*I/*Eco*RV digestion of plasmid pEx-1 lead to a deletion of 1097 nucleotides coding for roughly two-thirds of the β-galactosidase. This deletion did not alter the high expression of a cro-β-gal fusion protein. The purification of the PDGF-B fragment after CNBr cleavage was greatly facilitated, and the yields were improved. Strains obtained after transformation of this plasmid into NF-1 cells were grown to a density of 0.3 ODU (440 nm) at 30 °C and then shifted to 42 °C for 3 h to induce the production of the cro-β-gal fusion protein. Inclusion bodies wee prepared from these cultures as described (Stanley & Luzio, 1984) and analyzed by SDS–PAGE (Hoppe et al., 1986). Some fusion proteins exhibited a molecular weight of about 65 000 instead of 48 000, indicating the expression of PDGF-B sequences. The plasmid was termed pE-pF14.

Biological Assays. Growth-promoting activity was determined as described (Shipley et al., 1984) using AKR 2B cells in the presence of 2 μg/mL insulin.

Analytical Methods. SDS–PAGE was done as described using 10% or 13.5% polyacrylamide gels (Hoppe et al., 1986). Amino acid analysis was performed as described using an LC 2000 amino acid analyzer (Biotronic).

Amino-terminal sequence analysis was done with a solid-phase sequenator from Sequemat as described after coupling the protein to diisothiocyanato-activated porous glass beads (Hoppe et al., 1986) or with a gas–liquid-phase sequenator (Applied Biosystems) with online detection.

Protein content was determined by amino acid analysis or by the methods of Bradford (1976) or Redinbaugh (1986) after calibration for the PDGF variants by amino acid analysis.

Growth of Cells and Preparation of Inclusion Bodies. *E. coli* cells were grown in LB medium containing 50 μg/mL ampicillin in 1-L cultures at 30 °C to a density of 0.2 ODU at 440 nm and then shifted to 42 °C for 3 h. Cells were pelleted by centrifugation at 5000*g* for 10 min and suspended in 20 mL of 20 mM Tris-HCl/0.5 mM EDTA, pH 7.8, per liter of culture. Cells were disrupted by two passages through a Ribi press at 20 000 psi. Inclusion bodies were harvested by centrifugation at 6000*g* for 10 min and were washed once with 20 mM Tris-HCl, 0.5 mM EDTA, and 2% Triton X-100.

Reduction and CNBr Cleavage. Inclusion bodies obtained from a 30-L culture were dissolved in 100 mL of 50 mM Tris-HCl, pH 7.8, 2% SDS, and 2% mercaptoethanol for 1 h at 37 °C. A small amount of unsoluble material was removed by centrifugation at 20000*g* for 30 min. To the supernatant were added 2 volumes of acetone. After 15 min at 0 °C, a voluminous precipitate was collected by centrifugation (10 min, 6000*g*). The pellet was dissolved in 80 mL of formic acid. After the addition of 20 mL of H_2O, insoluble material was removed by centrifugation at 200000*g* for 1 h. One gram of CNBr and 200 μL of mercaptoethanol were added, and the reaction mixture was left overnight at room temperature. The solution was dried down in vacuo. To the residue was added 80 mL of 6 M Gdn·HCl, and the pH was raised to 7.5 by the addition of concentrated NaOH solution.

Protection of Thiol Group by S-Sulfonation. To the above solution were added 1 g of Na_2SO_3 and 0.25 g of $Na_2S_4O_6$, and the mixture was left at room temperature for 5 h. Insoluble material was removed by centrifugation at 20000*g* for 1 h.

Purification of S-Sulfonated rPDGF-B. The above mixture was directly applied onto a column 5 cm × 100 cm packed with Sephacryl S200 equilibrated in 4 M Gdn·HCl/50 mM Tris-HCl, pH 7.4. The flow rate was 160 mL/h, and fractions of 15 mL were collected. Aliquots of the fractions were analyzed by SDS–PAGE, and those fractions containing peptides of about 14 kDa were pooled and dialyzed twice against 5 L

of H_2O. After dialysis, formic acid was added to a final concentration of 10%. Upon addition of the formic acid, part of the precipitate that was formed during dialysis was dissolved. The suspension was centrifuged at 20000*g* for 15 min, and the supernatant was adsorbed onto a HPLC column (2 cm × 25 cm) packed with Si 300 polyol butyl 5 μm (Serva) at a flow rate of 2.5 mL/min. The column was previously equilibrated in 10% formic acid. After application of the sample, the column was washed with 2 volumes of 10% formic acid in H_2O. rPDGF-B was eluted with a linear gradient from 10 formic acid in H_2O to 10% formic acid/25% 2-propanol in H_2O during 180 min at a flow rate of 2.5 mL/min. Fractions were collected, each 3 min. The protein eluted between 40 and 60 min. Fractions containing S-sulfonated rPDGF-B were dialyzed extensively against H_2O and then concentrated in vacuo.

Dimerization and Purification. S-Sulfonated monomeric rPDGF-B was adjusted to 0.4 mg/mL in 50 mM Tris-HCl, pH 7.8, containing 5 mM reduced glutathione and 0.5 mM oxidized glutathione. One molar urea had to be added before the adjustment of the pH to keep the protein soluble. The mixture was left at room temperature for 2 days. For purification, portions of 2 mg of protein were adsorbed onto a (0.4 cm × 25 cm) Vydac 214 TP column. Proteins were eluted by application of a 70-min gradient from 20% B to 60% B in A at a flow rate of 0.7 mL/min (A is 0.1% trifluoroacetic acid in H_2O; B is 0.1% trifluoroacetic acid and 70% acetonitrile in H_2O). Fractions of 0.7 mL were collected. The dimer eluted first at 40% B. Fractions were concentrated in vacuo and were stored at −20 °C.

For large-scale purification, the reaction mixture was passed through a column packed with S-Sepharose equilibrated with 20 mM Tris-HCl, pH 7.5. About 1 mL of S-Sepharose was sufficient for 20 mg of protein. Monomeric forms were not adsorbed or were washed out at low ionic strength. Dimeric proteins were eluted with 20 mM Tris-HCl containing 0.7 M NaCl. Pooled fractions were stored at −20 °C.

Results

Construction of PDGF-B Expression Plasmids in E. coli. In previous reports, two different expression systems have been described: (a) direct expression (Devare et al., 1984) and (b) expression as a fusion protein linked to a portion of the small t antigen (Wang & Williams, 1984). Whereas in (a) the sis protein content was about 10% of the total cellular protein, the yield in (b) was quite low, a portion of the protein being soluble, the other portion precipitated in the inclusion bodies. To overcome proteolytic breakdown of the sis protein in the *E. coli* cell and to facilitate purification, we chose the pEx system which promotes expression of a cro-β-gal fusion protein in amounts up to 30% of the total protein in *E. coli* (Stanley & Luzio, 1984) (Figure 1). To increase the yield of PDGF-B protein, a large portion of the β-galactosidase sequence was deleted by *Eco*RV/*Sal*I digestion. Identification of a clone expressing a cro-β-gal–PDGF-B fusion protein is facilitated by the fact that only one long-reading frame exists in the PDGF-B gene which will lead to a significant increase in the molecular weight of the cro-β-gal protein. Thus, several clones were readily isolated, showing a molecular weight increased by 14000.

Isolation of Thio-Protected Monomeric rPDGF-B. PDGF-B contains only one methionine residue, which is located at position 12 in the NH_2-terminal part of the protein. Cleavage of the fusion proteins after methionine residues with CNBr resulted in PDGF-B fragments which are devoid of the first 12 amino acids of the mature PDGF-B chain but contained the entire C-terminus (Figure 2). To prevent oxidation of thiols during purification, after CNBr fragmentation thiol residues were protected as *S*-sulfonates.

Small CNBr fragments derived from the *E. coli* β-galactosidase were removed by gel permeation chromatography using Sephacryl S200 in 4 M GDn·HCl/50 mM Tris-HCl, pH 7.4. Upon dialysis against water, a precipitate appeared which contained the peptides and a large amount of unidentified nonprotein material, probably cell wall fragments. Peptides were extracted from this precipitate with 10% formic acid which was also used for final HPLC chromatography. Due to the high polarity of S–sulfonated PDGF-B, only a low percentage of 2-propanol was needed to elute the protein from the reversed-phase support. The protein appeared to be pure as judged by SDS–PAGE (Figure 3), amino acid analysis, and NH_2-terminal sequence analysis (data not shown).

After dialysis against water, the peptide may be concentrated in vacuo. It remains soluble in water at slightly acidic pH even after complete drying. The yield at this step is 2 mg of peptide fragment from 1 L of culture.

Renaturation. The protein content was adjusted to 0.4 mg/mL in 50 mM Tris-HCl, pH 7.8, containing 1 M urea. For the formation of disulfide bridge, 5 mM reduced glutathione and 0.5 mM oxidized glutathione were added, and the mixture was incubated for 2 days. Dimerization occurred rather specifically and in high yields as indicated by the appearance of a band of M_r 24000 comprising about 20% of the protein mass (Figure 3). Little, if any, higher molecular weight forms appeared. The yield was 0.7–1 mg from 1 L of culture. rPDGF-BB stimulated [^{3}H]thymidine incorporation at concentrations in the range from 1 to 3 ng/mL (50% activation). PDGF purified from platelets was slightly less active (1.5–4 ng/mL). Typical results are given in Figure 4. Remarkably, both PDGF forms stimulated the [^{3}H]thymidine incorporation to the same maximum. rPDGF-BB is thus qualitatively comparable with PDGF from human platelets.

Discussion

PDGF is a dimer linked together by disulfide bridges which are essential for growth-promoting activity. Thus, great care has to be taken to ensure the proper folding of the polypeptide chain and the closing of S–S bonds. From previous attempts to express PDGF-B in *E. coli*, it was clear that dimerization does not spontaneously occur in *E. coli*. Rather, monomeric species appeared which in one case had some binding affinity to the PDGF receptor (Wang & Williams, 1984). To circumvent this shortcoming, two strategies are feasible. The first, described recently, is to use a eucaryotic host. By joining a yeast secretion signal to the sis protein, Kelly et al. (1984) were able to produce biologically active PDGF–B dimers from yeast cells. These cells were able to secrete 5–20 ng/mL PDGF into the medium, but this expression rate is quite low for mass production of recombinant PDGF.

In our strategy to express PDGF–B, we took advantage of the high expression and stability of the gene products from the family of pEx plasmids (Stanley & Luzio, 1984). Furthermore, the location of methionine residues in the amino acid sequence of mature PDGF-B permitted the cleavage by CNBr to yield a protein just devoid of a few residues at the NH_2 terminus. Care was taken to protect the thiols during purification. The method of choice appeared to be S–sulfonation whereas chromatography in the presence of mercaptoethanol or modification of thiols by DTNB failed. Dimerization occurred spontaneously when protection groups were removed by the addition of a mixture of reduced and oxidized glutathione, yielding biologically active rPDGF-BB.

The described PDGF-B variant is devoid of the first 12

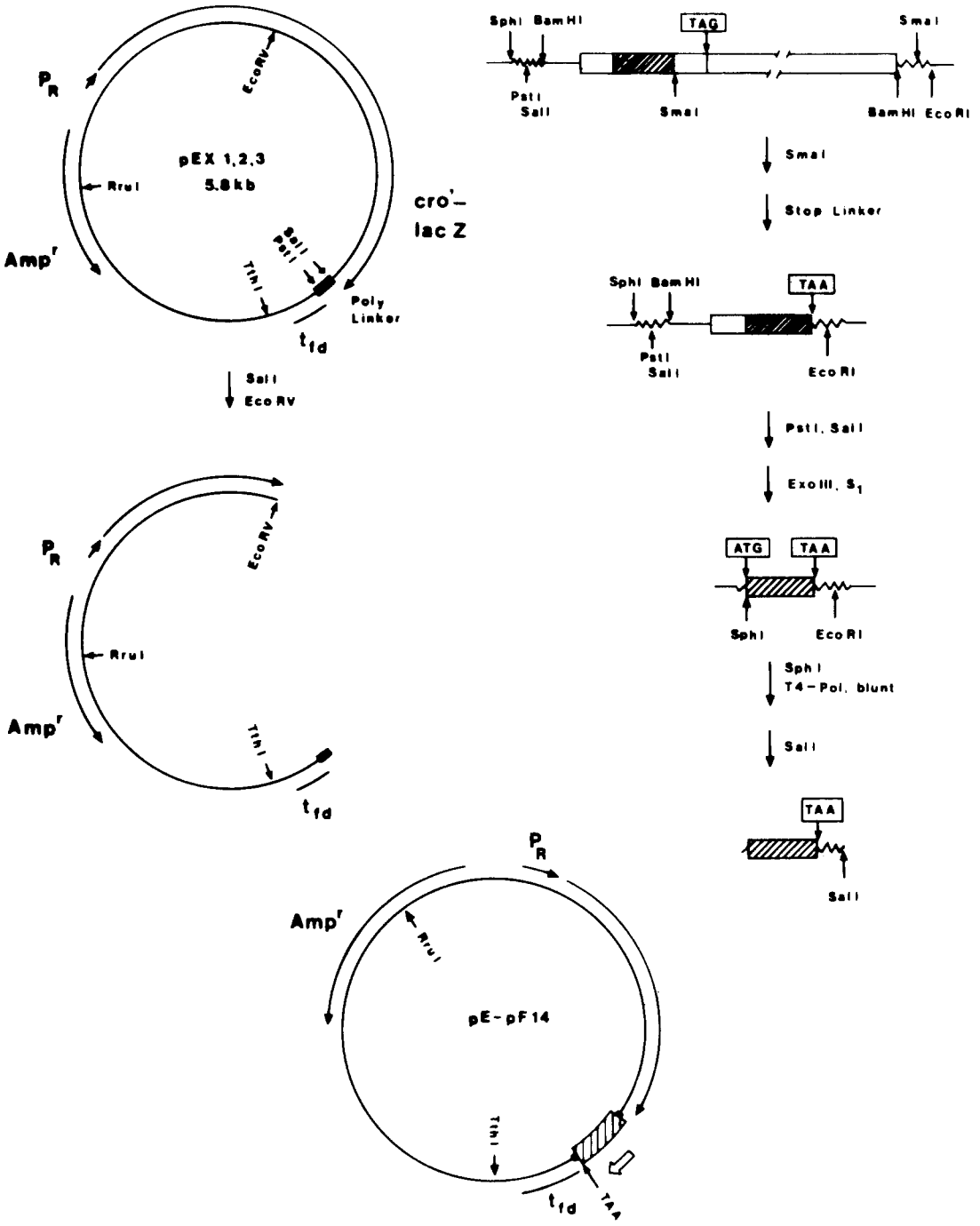

FIGURE 1: Strategy for the construction of the plasmid pE-pF14. A 2-kb *Bam*HI fragment from plasmid pMWV-2 was ligated in reverse direction into the M13 mp18 phage (open boxes, c-sis mRNA coding sequences; hatched boxes, region of mature PDGF-B). The long 3′-terminal part including the original stop codon TAG was deleted by *Sma*I digestion. A stop linker was ligated into this site, and 5′ sequences up to the region of mature PDGF were eliminated by exonuclease III/S1 digestion. After a passage through the vector J1A504 which generated a *Sal*I site at the 3′-end region, this plasmid was partially digested with *Sph*I. After treatment with T4 polymerase, the coding region was excised by cleavage with *Sal*I. This fragment was then ligated into pEx-1 treated with *Eco*RV/*Sal*I. P_R, bacteriophage λ promoter; cro′-lacZ, cro-β-galactosidase fusion protein; t_{fd}, phage terminator.

amino acid residues due to cleavage with CNBr. Apparently, these amino-terminal residues are not essential for activity and proper folding of the proteins in vitro. These findings are in perfect agreement with recent reports which identified the core region of the PDGF important for transforming activity in vivo. It was shown first by Richter-King et al. (1985) and Hannink and Donoghue (1986) that regions encoding amino-terminal prosequences of the sis gene may be deleted without affecting the ability of the protein to form dimers and to transform the host. A more detailed analysis (Sauer et al., 1986) showed that N-terminal residues could be deleted up to the first cysteine residue (Cys-16). The rPDGF-B described here has a C-terminal extension of five residues. Apparently, neither modification has an adversed effect on the mitogenic activity. rPDGF-BB stimulated [^{3}H]thymidine incorporation into AKR-2B fibroblasts at somewhat lower concentrations than PDGF from platelets. This difference might be due to the fact that PDGF from human platelets contains predominantly heterodimer (AB). Furthermore, platelet PDGF is partially nicked by proteases (Waterfield et al., 1983; Johnson et al., 1984).

In conclusion, we have been able to renature PDGF from a fusion protein expressed in high amounts in *E. coli*. This material which has a very high biological activity can be

```
          1        10        20        30        40        50        60
PDGF-B    SLGSLTIAEPAMIAECKTRTEVFEISRRLIDRTNANFLVWPPCVEVQRCSGCCNNRNVQC
rPDGF-B                 IAECKTRTEVFEISRRLIDRTNANFLVWPPCVEVQRCSGCCNNRNVQC

                  70        80        90       100       110
PDGF-B    RPTQVQLRPVQVRKIEIVRKKPIFKKATVTLEDHLACKCETVAAARPVT
rPDGF-B   RPTQVQLRPVQVRKIEIVRKKPIFKKATVTLEDHLACKCETVAAARPVTRSPLN
```

FIGURE 2: Amino acid sequences of PDGF-B and rPDGF-B. Note that in rPDGF-B the first 12 amino acid residues are deleted by CNBr cleavage at methionine-12. Furthermore, rPDGF-B contains a five amino acid extension of the C-terminus. The last three residues are not related to any PDGF sequences and originate from the stop linker fused at the *Sma*I site of the PDGF cDNA.

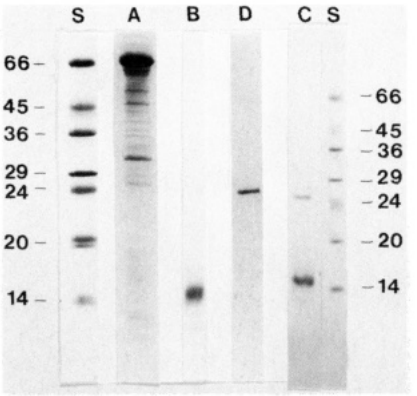

FIGURE 3: Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. (A) Lysate from whole *E. coli* cells; (B) monomeric S-sulfonated rPDGF-B; (C) rPDGF-B after renaturation; (D) purified rPDGF-BB; (S) standards (in kilodaltons).

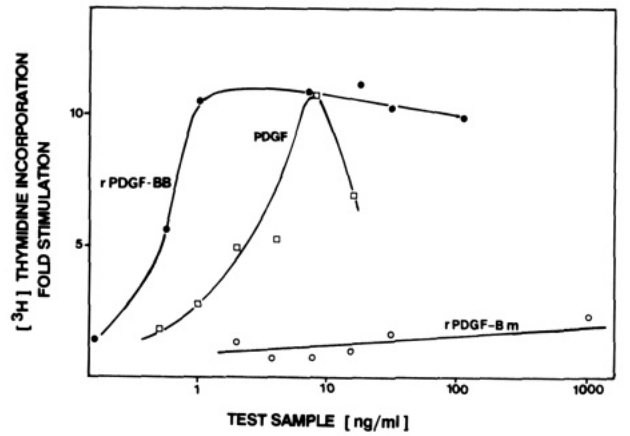

FIGURE 4: Stimulation of [^{3}H]thymidine incorporation into quiescent AKR-2B fibroblasts by PDGF from platelets and rPDGF-B. (□) PDGF from platelets; (○) monomer S-sulfonated rPDGF-B before renaturation; (●) rPDGF-BB after renaturation.

readily isolated in large amounts and may thus provide an alternative source for PDGF from human platelets.

ACKNOWLEDGMENTS

We thank Prof. W. Sebald for valuable discussions and C. Weigelt and L. Schumacher for expert technical assistance.

REFERENCES

Betsholtz, C., Johnsson, A., Heldin, C.-H., Westermark, B., Lind, P., Urdea, M. S., Eddy, R., Shows, T. B., Philpott, K., Mellor, P. L., Knott, T. J., & Scott, J. (1986) *Nature 320*, 695–699.

Bradford, M. B. (1976) *Anal. Biochem. 72*, 248–253.

Deuel, T. F., Tong, B. D., & Huang, J. S. (1985) *Curr. Top. Cell. Regul. 26*, 51–61.

Devare, S. G., Shatzman, A., Robbins, K. C., Rosenberg, M., & Aaronson, S. A. (1984) *Cell 36*, 43–49.

Doolittle, R. F., Hunkapiller, M. W., Hood, L. E., Devare, S. G., Aaronson, S. A., Robbins, K. C., & Antoniades, H. N. (1983) *Science 221*, 275–276.

Hannink, M., & Donoghue, D. J. (1986) *Mol. Cell. Biol. 6*, 1343–1348.

Heldin, C.-H., & Westermark, B. (1984) *Cell 37*, 9–20.

Heldin, C.-H., Johnsson, A., Wennergren, S., Wernstedt, C., Betsholtz, C., & Westermark, B. (1986) *Nature 319*, 511–514.

Henikoff, S. (1984) *Gene 28*, 351–359.

Hoppe, J., Gatti, D., Weber, H., & Sebald, W. (1986) *Eur. J. Biochem. 155*, 259–264.

Johnsson, A., Heldin, C.-H., Westermark, B., & Wasteson, A. (1982) *Biochem. Biophys. Res. Commun. 104*, 66–74.

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., & Waterfield, M. D. (1984) *EMBO J. 3*, 921–928.

Kelly, J. D., Raines, E. W., Ross, R., & Murray, M. J. (1985) *EMBO J. 4*, 3399–3405.

Redinbaugh, M. G., & Turley, R. B. (1986) *Anal. Biochem. 153*, 267–271.

Richter-King, C., Giese, N. A., Robbins, K. C., & Aaronson, S. A. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 5295–5299.

Robbins, K. C., Leal, F., Pierce, J. H., & Aaronson, S. A. (1985) *EMBO J. 4*, 1783–1792.

Ross, R., Raines, E. W., & Bowen-Pope, D. F. (1986) *Cell 46*, 155–169.

Sauer, M. K., Hannink, M., & Donoghue, D. J. (1986) *J. Virol. 59*, 292–300.

Schauder, B., Blöcker, H., Frank, R., & McCarthy, J. E. G. (1987) *Gene 52*, 279–283.

Shipley, G. D., Childs, C. B., Volkenant, M. E., & Moses, H. L. (1984) *Cancer Res. 44*, 710–716.

Stanley, K. K., & Luzio, J. P. (1984) *EMBO J. 3*, 1429–1434.

Stroobant, P., & Waterfield, M. D. (1984) *EMBO J. 3*, 2963–2967.

Wang, J. Y. J., & Williams, L. T. (1984) *J. Biol. Chem. 259*, 10645–10648.

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

Weich, H. A., Sebald, W., Schairer, H. U., & Hoppe, J. (1986) *FEBS Lett. 198*, 344–348.