Platelet-derived growth factor (PDGF) is a potent mitogen for cells of mesenchymal origin. Alternative exon splicing is responsible for two forms of the PDGF A-chain which differ at the carboxyl terminus by a highly basic region consisting of 18 amino acids. To clarify the function of the region, we synthesized an octadecapeptide corresponding to this extension (A194–211), incorporated a tyrosine residue at the amino terminus, and used the radiolabeled construct in binding studies with Balb/c3T3 cells and a variety of human cell lines. 125I-(Y)A194–211 bound specifically, reversibly, saturably, and with low affinity to a large population of binding sites on these cells. In addition, (Y)A194–211 markedly reduced the binding of its parent protein, 125I-PDGF-AA1, to its receptor. (Y)A194–211 also attenuated the binding of epidermal growth factor and several other isoforms of PDGF, but did not interfere with the binding of transferrin to its receptor. These observations were not due to competitive binding of peptide directly to known receptors for the respective growth factors, but was likely due to interaction of (Y)A194–211 with extracellular glycosaminoglycan. Thus, A194–211 may represent an additional heparin binding domain on mature PDGF-AA1, and as an isolate, is capable of modulating interactions between several potent growth factors and their respective receptors.

Platelet-derived growth factor (PDGF) is the major mitogen in serum for mesenchymal cells in culture. PDGF is thought to play a major role in the pathology of the atherosclerotic plaque (Ross, 1985), and the establishment of malignancies (Poggi et al., 1988). Several forms of PDGF have been identified. The form of PDGF secreted by platelets is a heterodimeric molecule with a molecular mass of 30 kDa. It consists of two similar but nonidentical polypeptide chains designated A and B (Heldin et al., 1988a) that are encoded by different genes. Different isoforms of PDGF (-AA, -AB, -BB) are expressed by a number of other normal and tumor cells in culture, each form having characteristic biological properties (Bywater et al., 1988; Sturani et al., 1989). Two types of receptors for PDGF have been identified. The PDGF-AB and -BB dimers bind to both Type A and Type B receptors, whereas the PDGF-AA homodimer binds only to the Type A receptor (Hart et al., 1988; Heldin et al., 1988b). Alternative splicing of A-chain RNA exon 6 has been demonstrated to yield two distinct forms. The Aα-chain consists of 196 amino acid residues, whereas the Aβ-chain begins with the same 193 residues but continues with a unique 18-amino acid extension at the carboxyl terminus (A194–211) (Collins et al., 1987). The precise function of the octadecapeptide extension has not been elucidated fully (Matoskova et al., 1989; Rorsman et al., 1988). The region is thought to act as a nuclear transport signal as it shares close structural homology with the signal peptide of the SV40-T antigen (Deuel, 1987; Tong et al., 1987), and PDGF itself has been observed to translocate to the nucleus after binding to the membrane surface (Rakowicz-Szulczyska et al., 1986; Yeh et al., 1987). Other investigators have suggested that A194–211 increases the biological potency of PDGF-AA (Collins et al., 1987), and improves the efficiency of homodimer formation (Bonthron et al., 1988). However, it is not clear from these studies precisely how the A194–211 extension interacts with cells to augment or modify the biological activity of the parent molecule.

This study was designed to characterize directly interactions between the isolated tyrosinated A194–211 peptide and cells known to be responsive to PDGF. We demonstrate that 125I-(Y)A194–211 binds specifically, reversibly, and saturably to a large population of cell-surface binding sites. These sites are distinct from the PDGF receptor. Moreover, peptide binding markedly influences interactions not only between the dimeric forms of PDGF-A and its receptor but also between selected other growth factors and their respective receptors. Peptide inhibition of growth factor binding was not due to competition for occupancy of a common receptor, but likely due to interaction with glycosaminoglycan at the cell surface. These findings suggest that (Y)A194–211 binding to cells can modify cellular responsiveness to several growth factors.

**Experimental Procedures**

**Materials**—The human osteosarcoma cell line, U2OS, was a generous gift of Drs. A. Wasteson and B. Westermark, Uppsala University, Uppsala, Sweden. The human squamous cell carcinoma line, A431, was a gift of Prof R. Sutherland, Garvan Institute of Medical Research, Sydney. Human platelet PDGF-AB (approximately 70% AB and 30% BB) (Hart et al., 1990) was purchased from British
PDGF-A Peptide Binds Cells and Modulates Growth Factor Binding

Biotechnology, Oxford, England. Bovine serum albumin (BSA, Cohn's Fraction V), l-lysyl-l-lysyl-glycyl-glutamine (KKGG, l-lysotropin fragment, 88–91), t-arginine, l-lysine, murine epidermal growth factor (EGF), choline-T, sodium metabolizable, Trypsin blue, protease-K, and proteinase-K were obtained from Sigma. Sodium-221 was purchased at 0.06% trypsin, 0.05% EDTA solution was purchased from ICN-Flow Laboratories, North Ryde, Australia; glyceral was obtained from M & B, West Footscray, Australia; EDTA, PMSF, sucrose, and Triton X-100 were purchased from BDH Laboratories, Melbourne. Transferrin was obtained from the Commonwealth Serum Laboratories, Melbourne, and sodium heparin was purchased from David Bull Laboratories.

Synthesis of Peptides—The amino acid sequence of peptides of PDGF was derived from the cDNA sequence of the A-chain expressed by the human glioma U343 cell line (Betzolz et al., 1986). Tyrosinase peptide A194–211 (YGRPRGGKRRKKRRKLPRT), untyro- sininated A194–211 (GRPRGGKRRKKRRKLPRT), and two control peptides, A184–200 (LNPDYREEDTGPRRESG) and MR361–380 (YSPDTQEGKQAEGVFPFPKTEV), were prepared using an automated peptide synthesizer (Applied Biosystems, 4304) and the t-Boc protocol. The tyrosine residue introduced at the amino terminus of (Y)A194–211 allowed radioiodination of the peptide. The presence of this residue did not alter peptide binding (reported in detail under "Results"). MR361–380 represents a segment of the unrelated mineralocorticoid receptor sequence. Seven of the 17 residues in A184–200 overlap with the residues in untyrosinylated A194–211. Purification of the peptides was carried out by HPLC on a reversed-phase C18 column (Waters, Milford, MA) using a gradient of 0–50% acetonitrile/0.1% trifluoroacetic acid. Amino acid analysis was used to estimate the mass of peptide and to ensure correct residue composition. The chloramine-T method was used for the radioiodination of peptides and proteins (Greenwood et al., 1963).

Cell Culture—Murine Balb/c3T3 fibroblasts, human foreskin fibroblasts, and the A431 and U20S cell lines were grown in Dulbecco's medium. All media contained 50 μg/ml streptomycin and 50 IU/ml penicillin, and all cultures were maintained at 37°C in a humidified atmosphere of 5% CO2/air.

Treatment of Monolayers with Pronase-E and Proteinase-K—125I-(Y)A194–211 was allowed to bind to confluent 3T3 cells. The cells had been washed previously once with binding buffer and once more with saline. Following digestion, the cells were washed again with saline, and proteolytic activity neutralized by the addition of 1 mM PMSF in saline. The cells were resuspended with binding buffer and incubated in the buffer for 8 min at 4°C. 125I-(Y)A194–211 was incubated with the cells for 1.5 h at 4°C in the absence and presence of 10 μM unlabeled peptide. Ligand binding was terminated by resuspending the cells and centrifuging the suspension at 200 x g for 15 min at 4°C. Bound radioactivity was assessed by γ-counting the cell pellet.

RESULTS

Binding of 125I-(Y)A194–211 to 3T3 Fibroblasts—To determine whether there was a specific interaction between the (Y)A194–211 peptide and cells known to express the PDGF receptor, 3T3 fibroblasts were tested for their capacity to bind 125I-(Y)A194–211. 125I-(Y)A194–211 bound to 3T3 fibroblasts at 4°C (Fig. 1). The binding was time-dependent and maximal after 80 min. Non specific binding was determined in the presence of a large excess concentration (14 μM) of unlabeled peptide. Approximately 15% of total added 125I-(Y)A194–211 was bound specifically.

In separate experiments, incubation of 125I-(Y)A194–211 with increasing concentrations of unlabeled (Y)A194–211 under equilibrium conditions resulted in dose-dependent inhibition of tracer binding (not shown). The results confirmed that ligand binding was both specific and saturable. A Scatchard plot (Scatchard, 1949) constructed using this data predicted a Kd of 6.4 (±1.9 S.E.) x 10^-7 M and 16.0 (±1.4) x 10^6 sites per cell.

Reversibility of 125I-A194–211 Binding—To determine whether binding was reversible, the capacity of excess unlabeled (Y)A194–211 that could displace prebound tracer was evaluated (Fig. 2). 125I-(Y)A194–211 was incubated with 3T3 cells for 100 min at 4°C to ensure that binding had reached

![Fig. 1. Time course binding of 125I-(Y)A194–211.](image-url)
within 15 min (Fig. 2). In contrast, when buffer without unlabeled peptide was added to the cells, only a slight decline in binding was quantitated in the presence of several unlabeled competitors. The ordinate encompasses the range 40-100% of total bound 125I-(Y)A194-211. The symbols represent means and standard errors of three experiments.

Specificity of 125I-(Y)A194-211 binding. Increasing concentrations of unlabeled synthetic peptides (Y)A194-211 ( ), untyrosinated A194-211 (O), A184-200 ( ), and MR361-380 ( ), and KKG ( ) were combined with 125I-(Y)A194-211 (100,000 cpm) and incubated with confluent 3T3 cells for 2.5 h at 4°C. Cell-bound radioactivity was quantitated as described under “Experimental Procedures.” The ordinate encompasses the range 40-100% of total bound 125I-(Y)A194-211. The symbols represent means and standard errors of three experiments.

Effect of (Y)A194-211 on Cellular Binding of Other Growth Factors—Because A194-211 is structurally identical to the carboxyl terminus of the other PDGF A-chain and because it profoundly influences full length PDGF binding, we speculated that its effects probably were mediated through the PDGF receptor. Both forms of the A-chain bind to the PDGF Type A receptor. Accordingly, binding of other structurally unrelated growth factors to their respective distinct receptors were expected to be unaffected by the presence of (Y)A194-211. Receptors for each of these ligands are known to be present on these cells (Brooks et al., 1990; Staddon et al., 1990; Davis and Meisner, 1987; Tanner and Lienhard, 1987). For control purposes, 125I-EGF was incubated with 3T3 cells in the presence of increasing concentrations of unlabeled EGF or (Y)A194-211. As expected, EGF competed for binding of 125I-EGF. However, (Y)A194-211 also inhibited 125I-EGF binding albeit at higher concentrations than EGF. The concentrations of (Y)A194-211 and EGF required to effect displacement of 125I-EGF binding by 50% were 100 μM and 0.5 nM, respectively (Fig. 5B). With 125I-transferrin, binding to 3T3 cells was observed and was inhibited by unlabeled transferrin in a dose-dependent fashion (Fig. 5C). However, presence of 100 μM unlabeled (Y)A194-211 failed to attenuate 125I-transferrin binding (Fig. 5C). In other experiments, unlabeled PDGF-AAl, 125I-PDGFAAs, and 125I-PDGF-AB were combined separately with 125I-PDGF-AAl and 125I-PDGFAAs were 20 and 10 μM, respectively (Fig. 4A), and 50 and 1 μM for 125I-PDGF-AB and 125I-(Y)A194-211, respectively (Fig. 4B). When 125I-PDGF-AB was coincubated with increasing concentrations of unlabeled PDGF-AB and (Y)A194-211, the concentrations of these competitors required to effect a 50% displacement of 125I-PDGF-AB were 0.5 nM and 5 μM, respectively (Fig. 5A). These data suggest that both PDGF and (Y)A194-211 bound to a common site. Moreover, that greater concentrations of peptide were required for the displacement of PDGF indicates that PDGF binds to 3T3 cells with affinity greater than (Y)A194-211.

FIG. 4. Competition of binding of 125I-PDGF-AAl, 125I-PDGFAAs, 125I-PDGF-AB, and 125I-(Y)A194-211 using unlabeled (Y)A194-211. Increasing concentrations of unlabeled (Y)A194-211 were combined separately with 125I-PDGF-AAl, 125I-PDGFAAs ( ), 500,000 cpm) and 125I-PDGF-AB (C, 500,000 cpm) (A) or 125I-PDGF-AB (C, 100,000 cpm) (B) and incubated with confluent 3T3 cells for 2.5 h at 4°C. Cell-bound radioactivity was quantified as described under “Experimental Procedures.” The symbols represent means and standard errors of two experiments.
labeled transferrin (5 μM) did not compete with the binding of 125I-(Y)A194–211 (not shown). Collectively, these data suggest that (Y)A194–211 can inhibit the binding of PDGF and EGF, but not transferrin. Thus, (Y)A194–211 binds to sites related to, or that interact with, those for PDGF and EGF. These sites appear to be independent of those for transferrin.

A series of Scatchard plots were constructed using data from competition of 125I-EGF binding by various concentrations of EGF in the presence and absence of 120 μM (Y)A194–211 (not shown). The peptide reduced the population of EGF binding sites by 25% without attenuating the affinity with which the growth factor bound. These experiments suggest that (Y)A194–211 did not inhibit the binding of growth factors by competitive inhibition for the receptor. Hence, another mechanism was responsible for the inhibition observed.

**Binding of 125I-(Y)A194–211 and 125I-PDGF to Other Cell Lines**—To determine whether interaction between (Y)A194–211 and PDGF binding was confined to 3T3 cells, 125I-(Y)A194–211 and 125I-PDGF-AB binding were compared in a variety of human normal and tumor cells in the absence or presence of increasing concentrations of unlabeled (Y)A194–211. All cell lines tested bound 125I-(Y)A194–211 (Table I). In contrast, 125I-PDGF-AB bound to foreskin fibroblasts but did not bind to HUVEC, U2OS, or A431 cells. Scatchard analysis of binding data revealed that 125I-(Y)A194–211 binding affinities with these cells were similar to that observed with 3T3 cells (not shown). In experiments carried out on cells in suspension, 125I-(Y)A194–211 and 125I-PDGF-AB were not bound by human peripheral blood lymphocytes (not shown). 125I-PDGF-AB was found to bind only to cells known to express significant levels of its receptor. However, 125I-PDGF-AB was inhibited from binding when coincubated with increasing concentrations of unlabeled (Y)A194–211 (Table I). These experiments indicate that (Y)A194–211 binding is not confined to 3T3 cells and that the site to which it binds is apparently not the PDGF receptor per se. In other experiments, gel filtration of 125I-(Y)A194–211 preincubated with and without unlabeled PDGF demonstrates that growth factor is not bound by peptide in solution (not shown). Thus, (Y)A194–211 interferes with the binding of this growth factor through another mechanism.

**Effect of Pronase-E and Proteinase-K Treatment of Monolayers—**Pronase-E treatment of 3T3 cells after 125I-(Y)A194–211 binding resulted in the release of radioactivity 3.5-fold greater than the saline control. Similar results were obtained with 125I-PDGF-AB, where the release of radioactivity was 2.5-fold greater than the control. Treatment of 3T3 cells with proteinase-K prior to the addition of 125I-(Y)A194–211 abolished completely the specific binding of the tracer. In contrast, cells preincubated with saline without enzyme retained the ability to bind 125I-(Y)A194–211. These data indicate that 125I-(Y)A194–211 binding is extracellular, and requires presence of cell surface proteins. Chemical cross-linking with DSS failed to identify the specific moiety to which (Y)A194–211 binds.

**Role of Heparin-like Glycosaminoglycans on (Y)A194–211 Binding**—Heparin-like glycosaminoglycans such as heparan sulfate have been found to mediate the cellular binding of synthetic peptides of FGF and peptide inhibition of binding of the parent protein (Baird et al., 1986; Moscatelli, 1987). Heparan sulfate is bound to collagen proteins at the extracellular matrix of most cell membranes. To delineate whether heparin-like glycosaminoglycans also played a role in the binding of (Y)A194–211, 125I-(Y)A194–211 was allowed to bind to 3T3 cells in the presence of varying concentrations of unlabeled (Y)A194–211, KGGE, and heparin. As expected, 125I-(Y)A194–211 binding was inhibited in a dose-dependent fashion in the presence of the unlabeled peptide, whereas KGGE had no...
PDGF-A Peptide Binds Cells and Modulates Growth Factor Binding

**Experimental Procedures.** The assumed molecular mass of unlabeled (Y)A194-211 absorbed previously to nitrocellulose discs. The peptide bound to 125I-heparin in a dose-dependent manner (Fig. 7B). (Y)A194-211 bound approximately 30% of the total 125I-heparin added in the absence of competitor. The amphiphilicity of (Y)A194-211 was unlikely to account for our observations as KKGE was not bound by 125I-heparin (Fig. 7B). To determine whether (Y)A194-211 bound to heparin-like glycosaminoglycans at the cell-surface, monolayers were treated with heparinase (12.5 units/ml) for 1 h at 37 °C prior to addition of 125I-(Y)A194-211. This resulted in attenuation of tracer binding by 17%. These results suggest interaction of (Y)A194-211 with extracellular glycosaminoglycan.

In other experiments, a standard displacement of 125I-(Y)A194-211 binding by 10 μM unlabeled peptide was carried out in the absence and presence of 4 μM (50 ng/ml) heparin. This concentration did not attenuate 125I-(Y)A194-211 binding to the cells (Fig. 6). Heparin reduced the degree with which unlabeled (Y)A194-211 could displace tracer from 70% in the absence of heparin, to 26% with heparin present (not shown). These data indicate that heparin increased the affinity with which the peptide bound to the cells. Thus, heparin-like glycosaminoglycans appear to modulate binding of (Y)A194-211.

**Discussion**

The precise function of the carboxyl terminal region of the alternative form of the PDGF A-chain is unclear. The present study has used the synthetic peptide approach to address this issue. Our results demonstrate that 125I-(Y)A194-211 binds to a variety of cultured cells including 3T3 cells (Table I). The binding of 125I-(Y)A194-211 was time-dependent (Fig. 1), and addition of excess unlabeled (Y)A194-211 to cells prebound with 125I-(Y)A194-211 resulted in significant displacement of tracer (Fig. 2). 125I-(Y)A194-211 binding was inhibited in a dose-dependent manner by its unlabeled counterpart, but not by peptides of similar length and different sequence. One of these peptides overlaps with A194-211 by 7 of its 17 residues (Fig. 3). Untyrosinated A194-211 competed with 125I-(Y)A194-211 with concentration dependence similar to that of the unlabeled tyrosinated peptide indicating that the presence of a tyrosine residue at the amino terminus did not alter the affinity of peptide binding (Fig. 3). Binding was saturable, and Scatchard analysis at equilibrium revealed only one class of low affinity binding sites.

PDGF is known to bind to 3T3 cells via high affinity receptors on the membrane surface (Yarden et al., 1986; Heldin et al., 1981). Experiments were designed to ascertain whether the binding of (Y)A194-211 was mediated by the PDGF receptor. The comparative affinities of PDGF-AB and (Y)A194-211 as 10^-10 and 10^-7 M, respectively suggest that (Y)A194-211 binds to its sites with relatively weaker affinity than PDGF binds to its sites. The competition studies involving 125I-PDFG-AA, 125I-PDGFAA, 125I-PDGFA-AB, and 125I-(Y)A194-211 revealed that (Y)A194-211 inhibits the binding of not just PDGF-AA, but all dimeric forms of PDGF A-chain (Figs. 4 and 5B). That greater concentrations of unlabeled (Y)A194-211 than unlabeled PDGF-AB were required to displace 125I-PDGF-AB support the relative dissociation constants (Fig. 4). Because binding of each form of PDGF was affected by the presence of the peptide and all dimeric forms of the PDGF A-chain bind to the Type A receptor (Hart et al., 1988), the respective binding sites may either be identical or be in such close proximity that occupancy of one site affects the function of the other. However, as population of sites bound by (Y)A194-211 greatly exceeds the known number of receptors for PDGF, peptide binding stoichiometry is not 1:1 with respect to the known PDGF receptors. Subsequent experiments demonstrated that (Y)A194-211 interfered with the interaction of another serum growth factor, 125I-EGF, with its high affinity receptor (Fig. 5B). It appears therefore that (Y)A194-211 inhibits the receptor binding of PDGF, EGF, and possibly other growth factors not yet investigated which could account for the large population of binding sites for (Y)A194-211. (Y)A194-211 inhibition, however, does not extend to interfering with the interaction between transferrin and its receptor (Fig. 5C).

Several approaches were used to delineate the mechanism by which (Y)A194-211 binds to cells and inhibits the binding of several growth factors to high affinity receptors. First, to
PDGF-A Peptide Binds Cells and Modulates Growth Factor Binding

Determine whether this was due to competition for occupancy of a common receptor, Scatchard plots were constructed using data from EGF binding in the absence and presence of (Y)A194–211. These data revealed that (Y)A194–211 reduced the population of binding sites for EGF without altering the affinity with which the growth factor bound. Accordingly, competition by (Y)A194–211 was unlikely to be due to its binding to the growth factor receptor per se. Furthermore, the population of sites bound by (Y)A194–211 was constant irrespective of the presence of the PDGF receptor suggests also that a site other than the receptor was bound (Table I). Second, gel filtration studies demonstrated that (Y)A194–211 did not bind to growth factor in solution which could have resulted in steric interference of the region on the growth factor responsible for receptor binding. Third, simple Coulombic forces were not responsible for mediating the interaction between 125I-(Y)A194–211 and its binding site. A highly basic tetrapeptide (KKGE) failed to compete with 125I-(Y)A194–211 binding (Fig. 3). Similarly, inclusion of 100 mM L-arginine or L-lysolecithin in the binding buffer did not result in significant reduction of 125I-(Y)A194–211 binding. We have used competitive radioimmunoassay (not shown) to demonstrate that (Y)A194–211 does not bind to a preparation of phospholipid in a cell-free system which reflects the proportionate ratio of phospholipids in biological membranes (Pengo et al., 1987).

The likely mechanism of (Y)A194–211 binding and inhibition is by interaction with heparin-like glycosaminoglycans at the cell surface. Synthetic analogues have been used by other investigators for the identification of the heparin binding domains of various growth factors on the basis of their ability to bind to heparin and inhibit the cellular binding of parent proteins (Baird et al., 1988; Moscatelli, 1987). Heparin interfered with the binding of 125I-(Y)A194–211 to the cells (Fig. 6). Using a cell-free system, (Y)A194–211 bound to heparin specifically and in a dose-dependent manner (Fig. 7). Heparinase treatment of cells reduced the ability of 125I-(Y)A194–211 to bind by 17%. 125I-(Y)A194–211 bound to cells with greater affinity in the presence of heparin which supports the findings of others that heparin-like glycosaminoglycans augment the capacity of certain growth factors to bind to cells (Adler and Eng, 1990; Schreiber et al., 1985; Thornton et al., 1985). Experiments using pronase-E and proteinase-K demonstrate that the binding site of 125I-(Y)A194–211 is extracellular and requires the presence of cell-surface proteins. Indeed, cell-associated heparan sulfate proteoglycans are sensitive to digestion by wide-spectrum serine proteases (Saksela and Rifkin, 1990). The large population of sites bound with low affinity, the involvement of extracellular protein, and the failure to chemically cross-link 125I-(Y)A194–211 to its binding site are all consistent with binding to heparin-like proteoglycan. Peptide bound to these sites could result in steric interference of growth factor binding which down-regulates the number of available growth factor receptors.

The phenomenon described in this paper is superficially similar to the behavior of suramin and other synthetic peptides with respect to growth factor binding. Suramin inhibits the cellular binding of several growth factors including PDGF, but does not itself bind to the cell. The mechanism by which this polyamionic, antiparasitic drug function is uncertain (Guo et al., 1990), although there is evidence that it could act by binding to growth factors causing conformational changes in the molecules preventing efficient binding of growth factor to receptor (Sjoland and Thyberg, 1999; Hosang, 1985). (Y)A194–211 prevents the high affinity binding of radiolabeled growth factor to cells and inhibits growth-stimulated DNA synthesis and mitosis, but (Y)A194–211, unlike suramin, can bind to cells as a ligand in its own right. Moreover, synthetic peptides have been used to study the interactions between 125I-labeled growth factors such as EGF (Katsuraya and Tanaka, 1989) and fibroblast growth factor (Baird et al., 1988), and clotting factors such as fibrinogen (Kloczewiak et al., 1989) and Factor IX (Ryan et al., 1989) with cultured cells. However, (Y)A194–211 contrasts with the peptide competitors used in these studies as the primary sequence of (Y)A194–211 does not appear in the structure of the agonists with which it competes.

Thus, these investigations reveal a previously unreported phenomenon, whereby a synthetic peptide derived from a sequence of a native growth factor can act not just bind to cells independently of its parent molecule, but has the ability to modulate the binding of more than one serum growth factor to their own cell-surface receptors. The proteoglycan binding capacity of the carboxyl terminal sequence unique to PDGF-AA, may provide this form of PDGF with another cellular binding site that is independent of the native receptor particularly as it is expected to occupy a surface location due to its relative hydrophilicity (Kyte and Doolittle, 1982). Indeed, the cationic amino acids which feature prominently in (Y)A194–211 are thought to mediate the interaction between heparin-binding growth factors and heparin (Sudhalter et al., 1989; Lobb et al., 1986). Accordingly, A194–211 could account for the reported greater biological potency of this dimeric form of the A-chain (Collins et al., 1987). Synthetic peptides such as the one described in this report have potential for modulating cellular responsiveness to highly potent growth factors.

Acknowledgments—We thank Dr. C.-H. Heldin (Ludwig Institute for Cancer Research, Uppsala, Sweden) for providing us with recombinant PDGF-AA, and PDGF-AAs. We are grateful to Dr. Joan Dawes (Heart Research Institute, Sydney) for the supply of 125I-heparin, and Dr. Genevieve Evin (Mental Health Research Institute, Melbourne) and Dr. Albert Tseng (Pacific Biotechnology, Sydney) for peptide synthesis and assistance with amino acid analysis. We are thankful to Prof. Rob Sutherland and Dr. Christine Clarke (Garvan Institute of Medical Research, Sydney) for valuable discussions.

REFERENCES

Adler, S., and Eng, B. (1990) Am. J. Pathol. 136, 557–563
Baird, A., Schubert, D., Ling, N., and Guillemin, R. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 2234–2238
Betsholtz, C., Johnson, A., Heldin, C.-H., Westermark, B., Lind, P., Urdea, M. S., Eddy, R., Shows, T. B., Philpott, K., Mellor, A. L., Knott, T. J., and Scott, J. (1986) Nature 320, 695–699
Bonthron, D. T., Morton, C. C., Orkin, S. H., and Collins, T. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 1495–1496
Bowen-Pope, D. F., and Ross, R. (1982) J. Biol. Chem. 257, 5161–5171
Brooks, G., Goss, M. W., and Hart, I. R. (1990) Carcinogenesis 11, 1223–1227
Bywater, M., Rorsman, F., Bongcam-Rudloff, E., Mark, G., Hammacher, A., Heldin, C.-H., Westermark, B., and Betsholtz, C. (1988) Mol. Cell. Biol. 8, 2753–2762
Collins, T., Bonthron, D. T., and Orkin, S. H. (1987) Nature 328, 621–624
Davis, R. J., and Meisner, H. (1987) J. Biol. Chem. 262, 16041–16047
Deuel, T. F. (1987) Annu. Rev. Cell Biol. 3, 443–492
Greenwood, F. C., Hunter, W. M., and Glover, J. S. (1963) Biochem. J. 89, 114–123
Guo, X.-J., Fantini, J., Roubin, R., Marvaldi, J., and Rougon, G. (1990) Cancer Res. 50, 5164–5170
Hart, C. E., Bailey, M., Curtis, D. A., Osborn, S., Raines, R., Ross, R., and Forstrom, J. W. (1990) Biochemistry 29, 166–172
Hart, C. E., Forstrom, J. W., Kelly, J. D., Seifert, R. A., Smith, R. A., Ross, R., Murray, M. J., and Bowen-Pope, D. F. (1988) Science 240, 1529–1531

2 L. M. Khachigian and C. N. Chesterman, unpublished data.
PDGF-A Peptide Binds Cells and Modulates Growth Factor Binding

Heldin, C.-H., Hammacher, A., Nister, M., and Westermark, B. (1988a) Br. J. Cancer 57, 591–593
Heldin, C.-H., Backstrom, G., Ostman, A., Hammacher, A., Ronnstrand, L., Rubin, K., Nister, M., and Westermark, B. (1988b) EMBO J. 7, 1387–1394
Heldin, C.-H., and Westermark, B. (1987) J. Cell Physiol. 5, (suppl.) 31–34
Heldin, C.-H., Westermark, B., and Wasteson, A. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 3664–3668
Hosang, M. (1985) J. Cell. Biochem. 29, 265–273
Jaffe, E. A., Nachman, R. L., Becker, C. G., and Minick, C. R. (1973) J. Clin. Invest. 52, 2745–2756
Katsau, M., and Tanaka, S. (1989) J. Biochem. (Tokyo) 106, 87–92
Kloczewiak, M., Timmons, S., Bednarek, M. A., Sakon, M., and Hewiger, J. (1988) Biochemistry 28, 2915–2919
Kyte, J., and Doolittle, R. F. (1982) J. Mol. Biol. 157, 105–132
Lobb, R., Sasse, J., Sullivan, R., Shing, Y., D’Amore, P., Jacobs, J., and Klagsbrun, M. (1986) J. Biol. Chem. 261, 1924–1928
Maciag, T., Cerundolo, J., Ilsley, S., Kelley, P. R., and Forand, R. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 5674–5678
Matoskova, B., Rorsman, F., Svensson, V., and Betsholtz, C. (1989) Mol. Cell. Biol. 9, 2251–2253
Moscatelli, D. (1987) J. Cell Physiol. 131, 123–130
Nister, M., Libermann, T. A., Betsholtz, C., Pettersson, M., Claesson-Welsh, L., Heldin, C.-H., Schlessinger, J., and Westermark, B. (1988) Cancer Res. 48, 3910–3918
Pengo, V., Tjagjarjan, P., Shapiro, S., and Heine, M. J. (1987) Blood 70, 69–76
Poggi, A., Vicenzi, E., Cioc, V., and Wasteson, A. (1988) Haematologica 18, 18–28
Rakowicz-Szulcynska, E. M., Rodeck, U., Herlyn, M., and Koprowski, H. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 3728–3732
Rorsman, F., Bywater, M., Knott, T. J., Scott, J., and Betsholtz, C. (1988) Mol. Cell. Biol. 8, 571–577
Rosa, R. (1985) Annu. Rev. Med. 38, 71–79
Ryan, J., Wolitzky, B., Heimer, E., Lambroso, T., Felix, A., Tam, J. P., Huang, L. H., Nawroth, P., Wilmer, G., Kisiel, W., Nelsestuen, G. L., and Stern, D. M. (1989) J. Biol. Chem. 264, 20283–20287
Saksela, O., and Rifkin, D. B. (1990) J. Cell. Biol. 110, 767–775
Sariban, E., Sitaras, N. M., Antoniades, H. N., Kufe, D. W., and Pantazis, P. (1988) J. Clin. Invest. 82, 1157–1164
Scatchard, G. (1949) Ann. N. Y. Acad. Sci. 51, 660–672
Schreiber, A. B., Kenney, J., Kowalski, W. J., Friesel, R., Mehlman, J., and Maciag, T. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 6138–6142
Sjolund, M., and Thyberg, J. (1989) Cell Tissue Res. 256, 35–43
Staddon, J., and Chanter, N., Lax, A. J., Higgins, T. E., and Hawiger, J. (1985) Proc. Natl. Acad. Sci. U. S. A. 76, 5674–5678
Tanner, L. I., and Leinhard, G. E. (1987) J. Biol. Chem. 262, 8975–8980
Thornton, S. C., Mueller, S. N., and Levine, E. M. (1983) Science 222, 623–625
Tong, B. D., Auer, D. E., Jaye, M., Kaplow, J. M., Ricca, G., McCaugherty, E., Drohan, W., and Deuel, T. F. (1987) Nature 328, 619–621
Yarden, Y., Escobedo, J. A., Huang, W.-J., Yang-Feng, T. L., Daniel, T. O., Tremble, P. M., Chen, E. Y., Ando, M. E., Harksen, R. N., Francke, U., Fried, V. A., Ullrich, A., and Williams, L. T. (1986) Nature 323, 226–232
Yeh, H.-J., Pierce, G. F., and Deuel, T. F. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 2317–2321