Identification of three amino acid residues in the B-chain of platelet-derived growth factor with different importance for binding to PDGF α- and β-receptors

Joachim Kreysing**, Arne Östman*, Monique van de Poll***, Gudrun Bäckström, Carl-Henrik Heldin

Ludwig Institute for Cancer Research, Box 595, Biomedical Center, S-751 24 Uppsala, Sweden

Received 5 March 1996

Abstract The B-chain homodimer isoform of platelet-derived growth factor (PDGF) binds with high affinity both to α- and to β-receptors. In order to localize amino acid residues in PDGF-BB of differential importance for the binding to the two receptors, PDGF-BB mutants were analyzed in which single amino acid residues were changed to alanine residues. We found that Phe-118 in loop 1 of the PDGF B-chain is crucial for binding to both receptors, and that the surrounding amino acids, Asn-117 and Leu-119, appear to be important primarily for binding to the β-receptor. In contrast, Lys-161 in loop 3 was found to be more important for binding to α-receptors than β-receptors. Previous studies have shown that the receptor binding epitope of PDGF-BB is composed mainly of loops 1 and 3; the findings of the present study show that the α- and β-receptors interact with different amino acid residues in these regions.

Key words: Platelet-derived growth factor; Receptor binding

1. Introduction

Platelet-derived growth factor (PDGF) occurs as homo- or heterodimeric isoforms of A- and B-polypeptide chains [1]. The three-dimensional structure of PDGF-BB has recently been determined [2]; the two disulfide-bonded subunits are arranged in an antiparallel manner, each of which contains three intramolecular disulfide bonds arranged in a tight knot-like structure and two twisted β-sheets. Thus, each subunit consists of two loops (loops 1 and 3) pointing in one direction and another (loop 2) pointing in the other direction. Mutational analyses have revealed that amino acid residues in loops 1 and 3 are important for receptor binding [3-6]. Also loop 2, which is located close to loops 1 and 3 in the other subunit in the dimer, is important for receptor binding, in particular for binding to the β-receptor [4,7].

PDGF exerts its cellular effects by binding to two structurally similar protein tyrosine kinase receptors [8,9]. The α-receptor binds both PDGF A- and B-chains, whereas the β-receptor binds only the B-chain with high affinity. Ligand binding induces receptor dimerization. Activation of either α- or β-receptors leads to stimulation of cell growth. However, on human fibroblasts and smooth muscle cells only the β-receptor mediates a stimulatory effect on chemotaxis; activation of the α-receptor in fact inhibits chemotaxis of these cells [10-13].

The stimulatory effect of PDGF-BB and PDGF-AB on chemotaxis of fibroblasts and smooth muscle cells, mediated via the PDGF β-receptor, will thus be modulated by inhibitory signals via the α-receptor present on the same cells [14]. A PDGF B-chain mutant which has lost the ability to bind to the α-receptor but retains the β-receptor binding activity would thus be expected to have an increased chemotactic activity. The present study was undertaken with the aim of identifying amino acid residues in PDGF-BB with a differential role in binding to the two PDGF receptors.

2. Materials and methods

2.1. Construction of cDNAs encoding PDGF mutants

cDNA encoding the PDGF-Bstop mutant in which codon 191 in the PDGF B-chain was mutated to a stop codon have been described [15]. Single amino acid residue mutants of PDGF-Bstop with amino acid residues N117, F118, L119, V159, R160, K161, K162 changed to alanine residues were carried out using the Altered Sites in vitro mutagenesis system (Promega).

2.2. Expression of recombinant proteins

The DNA constructions encoding wild-type or mutant PDGF chains were cloned into the pSV7d expression vector and transfected into COS cells as described [15], using 15 μg of plasmid DNA and $0.5–1 \times 10^6$ cells in 60 mm culture dishes. Serum-free conditioned media were collected 3 days after transfection.

2.3. Receptor binding analysis of recombinant proteins

Binding analysis was performed as described by Andersson et al. [7]. Briefly, conditioned media from transfected COS cells were desalted and concentrated 10-fold using a reversed-phase C4 column. The amount of PDGF recombinant proteins was determined by a radioimmunoassay using a rabbit antiserum against PDGF-BB [16]. The amount of PDGF β-receptor binding activity was determined by analyzing serial dilutions of conditioned media with regard to their ability to compete with ^{125}I-PDGF-BB for binding to human foreskin fibroblasts (AG 1518) on which the α-receptor had been downregulated by prior incubation of the cells with 50 ng/ml of PDGF-AA for 30 min at 37°C [17]. The α-receptor binding capacity was analyzed according to the ability to compete with ^{125}I-PDGF-AA for binding to human fibroblasts.

3. Results

Previous studies have shown that Phe-118 in loop 1 is important for receptor binding [18], and that the amino acid residues in the A-chain corresponding to the conserved Lys-161 and Lys-162 are important for binding to the PDGF α-receptor [19]. In our attempts to identify amino acid residues

*Corresponding author.

***Present address:* Institut für Biochemie, Universität Kiel, Olshaussenstr. 40, D-24098 Kiel, Germany.

****Present address:* Department of Cell Biology, University of Nijmegen, Toernooiveld, 6525 ED Nijmegen, The Netherlands.

PII S0014-5793(96)00349-3

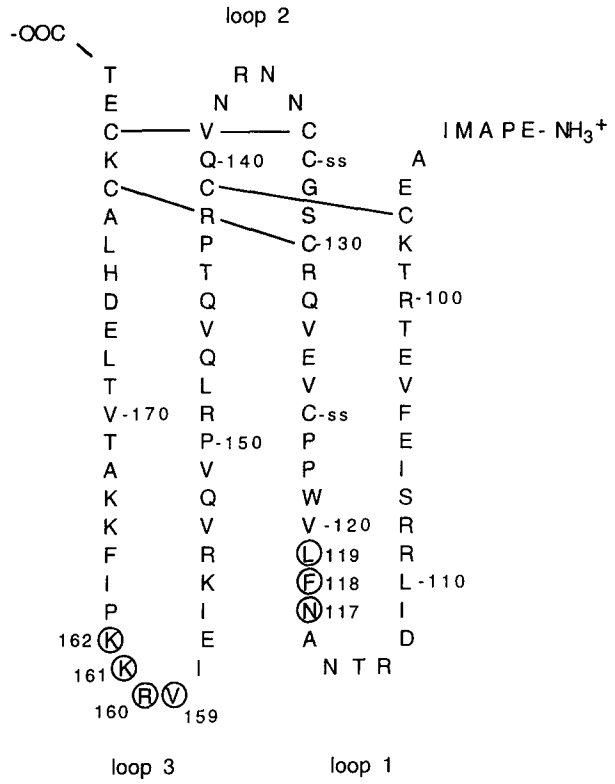

Fig. 1. Schematic illustration of the PDGF B-chain structure. The amino acid residues in the loop 1 and 3 regions that were replaced in the PDGF B-chain mutants are marked with circles.

with different importance for binding to α- and β-receptors, we therefore focused on conserved amino acid residues in the regions around these amino acid residues.

A series of expression vectors coding for wild-type PDGF B-chain and B-chain mutants (schematically shown in Fig. 1) was prepared and used for transient expression in COS cells. The abilities of the mutant PDGF molecules to compete for binding of ^{125}I-PDGF-AA and ^{125}I-PDGF-BB to α- and β-receptors, respectively, were determined and compared to that of wild-type PDGF-BB. In order to achieve efficient secretion of wild-type PDGF-BB and PDGF-BB mutants, vector constructs were used coding for truncated molecules lacking the basic motif in the C-terminus of the PDGF B-chain which mediates retention intracellularly and to the extracellular matrix [15,20,21].

A mutant with Phe-118 replaced with an alanine residue was found to have a more than 10-fold lower ability to bind to α- as well as to β-receptors (Fig. 2). Mutation of the neighbouring Asn-117 to an alanine residue had no effect on binding to the α-receptor but decreased the binding to β-receptors about 4-fold. The L119A mutant showed 2-fold lower binding to the α-receptor and 4-fold lower binding to the β-receptor. In conclusion, these mutations in loop 1 establish an important role for Phe-118 in the interaction with both α- and β-receptors, and show that the neighbouring residues Leu-119 and Asn-117 are important primarily for binding to the β-receptor.

In the loop 3 region, mutation of Val-159, Arg-160 or Lys-162 to alanine residues had no effect on binding to either α- or β-receptors. Interestingly, the K161A mutant in contrast

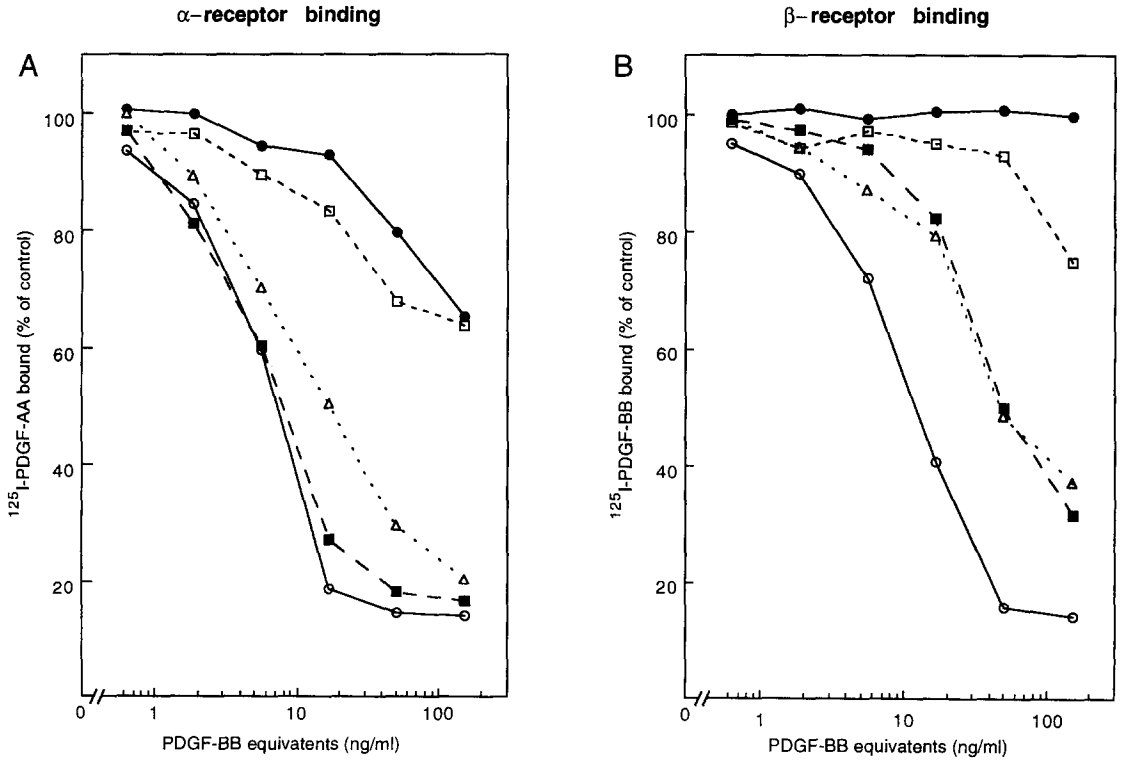

Fig. 2. PDGF receptor competing activities of PDGF-BB loop 1 mutants. Concentrated conditioned media from mock transfected COS cells (●), or COS cells transfected with wild-type PDGF-B (○), or N117A (■), F118A (□), or L119A (△) PDGF-B mutants, were analysed for their abilities to compete with the binding of ^{125}I-PDGF-AA to PDGF α-receptors (A), and with the binding of ^{125}I-PDGF-BB to PDGF β-receptors (B). The concentrations of PDGF-BB mutants in the conditioned media were determined by a radioimmunoassay, and are expressed as PDGF-BB equivalents.

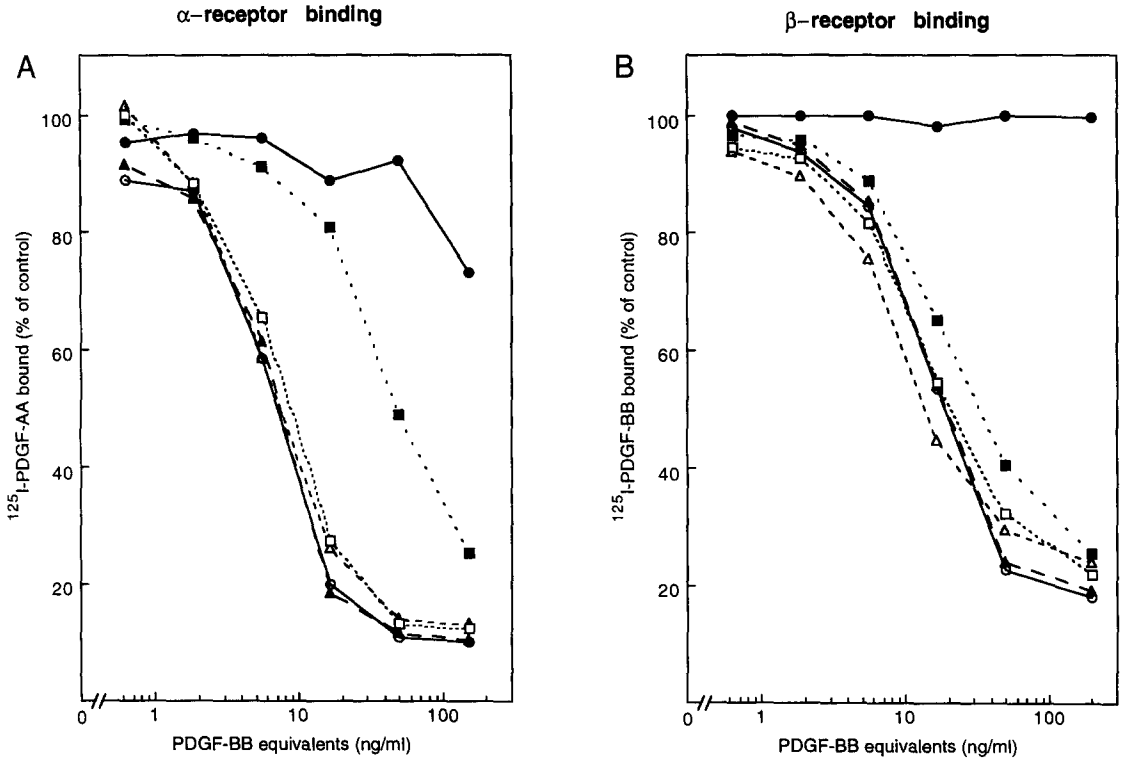

Fig. 3. PDGF receptor competing activities of PDGF-BB loop 3 mutants. Concentrated conditioned media from mock transfected COS cells (●), or COS cells transfected with wild-type PDGF-B (○), or V159A (▲), R160A (△), K161A (■), or K162A (□) PDGF-B mutants, were analysed for their abilities to compete with the binding of ^{125}I-PDGF-AA to PDGF α-receptors (A), and with the binding of ^{125}I-PDGF-BB to PDGF β-receptors (B). The concentrations of PDGF-BB mutants were determined as described in the legend to Fig. 2.

showed about 2-fold lower β-receptor binding activity and about 5-fold lower α-receptor binding activity (Fig. 3). Attempts were made to find other mutants with an even larger difference in their abilities to interact with α- and β-receptors. However, a double mutant K160A/K161A was found to have the same characteristics as the K161A mutant, whereas a K161D mutant showed more than 10-fold reduction, as compared to wild type, in binding to both α- and β-receptors (data not shown).

4. Discussion

We have shown in this communication that a region around Phe-118 in loop 1 of the PDGF B-chain is important for binding to the PDGF α-receptor, and to an even larger extent to the β-receptor. In contrast, Lys-161 in loop 3 is more important for binding to α-receptors than to β-receptors. Our results thus demonstrate a differential importance of certain amino acid residues in PDGF-BB for binding to the two PDGF receptors. The receptor binding properties of the PDGF-BB mutants studied are summarized in Table 1.

Table 1. Summary of binding characteristics of PDGF B-chain mutants

	α-Receptor binding	β-Receptor binding
wt PDGF B-chain	+++	+++
N117A mutant	+++	++
F118A mutant	–	–
L119A mutant	(+)++	++
V159A mutant	+++	+++
R160A mutant	+++	+++
K161A mutant	+	++
K162A mutant	+++	+++

The abilities of wild-type, as well as mutant forms of PDGF B-chain to inhibit the binding of ^{125}I-PDGF-AA and ^{125}I-PDGF-BB to PDGF α- and β-receptors, were determined. +++, less than 2-fold decreased receptor binding compared to wild-type B-chain; ++, 2–4-fold decreased receptor binding; +, 4–10-fold decreased receptor binding; –, more than 10-fold decreased receptor binding.

PDGF-AA binds exclusively to α-receptors and does not activate β-receptors. However, there is no known ligand which activates β-receptors without also activating α-receptors. In view of the inhibitory effect of α-receptors on chemotaxis of fibroblasts and smooth muscle cells, a mutated PDGF molecule activating β-receptors but not α-receptors could have a greater stimulatory effect than wild-type PDGF, e.g. on wound healing which involves stimulation of cell growth, matrix production as well as chemotaxis. The finding in the present work that Lys-161 is of greater importance for binding to α-receptors than to β-receptors provides a first step towards the design of PDGF B-chain mutants which exclusively bind to the β-receptor of PDGF.

Acknowledgements: We thank Maria Andersson for helpful discussions, Christer Wernstedt for oligonucleotides and Ingegärd Schiller for help in the preparation of this manuscript.

References

[1] Heldin, C.-H., Östman, A. and Westermark, B. (1993) Growth Factors 8, 245–252.
[2] Oefner, C., D'Arcy, A., Winkler, F.K., Eggimann, B. and Hosang, M. (1992) EMBO J. 11, 3921–3926.

[3] Östman, A., Andersson, M., Hellman, U. and Heldin, C.-H. (1991) J. Biol. Chem. 266, 10073–10077.
[4] LaRochelle, W.J., Pierce, J.H., May-Siroff, M., Giese, N. and Aaronson, S.A. (1992) J. Biol. Chem. 267, 17074–17077.
[5] Jaumann, M., Tatje, D. and Hoppe, J. (1992) FEBS Lett. 302, 265–268.
[6] Clements, J.M. et al. (1991) EMBO J. 10, 4113–4120.
[7] Andersson, M., Östman, A., Kreysing, J., Bäckström, G., van de Poll, M. and Heldin, C.-H. (1995) Growth Factors 12, 159–164.
[8] Heldin, C.-H. and Westermark, B. (1990) Cell Regulation 1, 555–566.
[9] Raines, E.W., Bowen-Pope, D.F. and Ross, R. (1990) in: Handbook of Experimental Pharmacology, Peptide Growth Factors and their Receptors, vol. 95, part I (Sporn, M.B. and Roberts, A.B. eds.) pp. 173–262, Springer, Heidelberg.
[10] Siegbahn, A., Hammacher, A., Westermark, B. and Heldin, C.-H. (1990) J. Clin. Invest. 85, 916–920.
[11] Eriksson, A., Siegbahn, A., Westermark, B., Heldin, C.-H. and Claesson-Welsh, L. (1992) EMBO J. 11, 543–550.
[12] Koyama, N., Morisaki, N., Saito, Y. and Yoshida, S. (1992) J. Biol. Chem. 267, 22806–22812.
[13] Koyama, N., Hart, C.E. and Clowes, A.W. (1994) Circ. Res. 75, 682–691.
[14] Yokote, K., Margolis, B., Heldin, C.-H. and Claesson-Welsh, L. (1996) J. Biol. Chem., in press.
[15] Östman, A., Andersson, M., Betsholtz, C., Westermark, B. and Heldin, C.-H. (1991) Cell Regul. 2, 503–512.
[16] Thyberg, J., Östman, A., Bäckström, G., Westermark, B. and Heldin, C.-H. (1990) J. Cell Sci. 97, 219–229.
[17] Östman, A. et al. (1989) Growth Factors 1, 271–281.
[18] Maher, D.W., Strawn, L.M. and Donoghue, D.J. (1993) Oncogene 8, 533–541.
[19] Fenstermaker, R.A. et al. (1993) J. Biol. Chem. 268, 10482–10489.
[20] LaRochelle, W.J., May-Siroff, M., Robbins, K.C. and Aaronson, S.A. (1991) Genes Dev. 5, 1191–1199.
[21] Raines, E.W. and Ross, R. (1992) J. Cell Biol. 116, 533–543.