Predimerization of Recombinant Platelet-Derived Growth Factor Receptor Extracellular Domains Increases Antagonistic Potency†

Olli Leppänen,‡ Keiji Miyazawa,‡,§ Gudrun Bäckström, Kristian Pietras, Tobias Sjöblom, Carl-Henrik Heldin, and Arne Östman*

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

Received August 16, 1999; Revised Manuscript Received December 8, 1999

ABSTRACT: Platelet-derived growth factor (PDGF) is a dimeric growth factor acting through tyrosine kinase α- and β-receptors. In both receptors, the extracellular parts are composed of five Ig-like domains. Functional mapping of the extracellular part of the receptors have shown that ligand-binding occurs to Ig-like domains 2 and 3 and that Ig-like domain 4 is involved in receptor−receptor interactions. Recombinant GST-fusion proteins of PDGF α-receptor Ig-like domains 1−4 and β-receptor Ig-like domains 1−3 (αRD1−4-GST and βRD1−3-GST) were generated and compared with their cleaved counterparts (αRD1−4 and βRD1−3) with regard to their ability to block PDGF binding to cell surface receptors. In the case of both the α- and the β-receptors, 100−1000-fold lower concentrations of the GST-fusion proteins were required, as compared to the cleaved forms, for inhibition of PDGF binding to cell surface receptors. αRD1−4-GST and βRD1−3-GST, in contrast to αRD1−4 and βRD1−3, were shown to occur as ligand independent dimers. Covalently cross-linked αRD1−4 dimers displayed a 50-fold increased potency as compared to αRD1−4. We thus conclude that the dimeric nature of αRD1−4-GST and βRD1−3-GST is responsible for the high antagonistic potency of the fusion proteins.

Platelet-derived growth factor (PDGF)[1] is a family of disulfide-bonded dimeric isoforms of A- and B-chains acting on connective tissue cells, glia cells, and endothelial cells (*1*). The biological effects of PDGF are mediated through α- and β-tyrosine kinase receptors (*2*). The PDGF A-chain binds only α-receptors, whereas the B-chain binds both PDGF α- and β-receptors. PDGF receptor driven proliferation has been implicated in a number of diseases, including malignancies, chronic inflammatory diseases, atherosclerosis, and restenosis (*3, 4*). Potent and specific PDGF antagonists are, therefore, highly warranted to promote studies exploring the possibility to interfere with disease processes through blockage of PDGF receptor signaling.

The antiparallel dimeric PDGF molecule displays two receptor binding regions, each one made up by epitopes derived from both subunits (*5*−*8*). One PDGF dimer thus binds two receptors, thereby causing receptor dimerization and activation (*9, 10*). The extracellular parts of PDGF α- and β-receptors are made up of five Ig-like domains. Mapping of the functional roles of the different Ig-like domains has revealed that ligand binding occurs predominantly to Ig-like domain 2 in the case of PDGF-BB binding to PDGF α- and β-receptors, whereas PDGF-AA binding to PDGF α-receptors occur to Ig-like domain 2 and 3 of the PDGF α-receptor (*11*−*14*). In PDGF α- as well as β-receptor, Ig-like domain 4 contributes to receptor dimerization through receptor−receptor interactions (*12, 15, 16*).

Recombinant soluble forms of extracellular domains of growth factor receptors represent a class of growth factor antagonists that has been generated for many growth factors. In the case of PDGF receptors, recombinant forms of PDGF α- and β-receptor extracellular domains encompassing either all five Ig-like domains or Ig-like domains 1−4 or 1−3 have been described and shown to block PDGF binding to cell surface receptors with IC_{50} values between 20 and 200 nM (*13, 17, 18*).

In this paper, we demonstrate the expression and purification of GST-fusion proteins of human PDGF α-receptor Ig-like domains 1−4 and β-receptor Ig-like domains 1−3 (αRD1−4-GST and βRD1−3-GST), which act as PDGF antagonists at 100−1000-fold lower concentrations as compared with the corresponding proteins without GST domains (αRD1−4 and βRD1−3). We also show that in the absence of ligand αRD1−4-GST and βRD1−3-GST, in contrast to αRD1−4 and βRD1−3, occur as noncovalent dimers and that covalent predimerization of αRD1−4 dramatically increases its antagonistic potency. From these findings, we conclude that the differences between the GST-fusion proteins and their cleaved counterparts are explained by avidity effects.

† Olli Leppänen and Keiji Miyazawa were partly supported by the Swedish Society of Medicine and by the Uehara Memorial Life Science Foundation, respectively.

‡ These authors contributed equally to this work.

§ Present address: Department of Life Science, Faculty of Bioscience and Biotechnology, Tokyo Institute of Technology, 4259 Nagatsuta, Midori-Ku, Yokohama 226, Japan.

* To whom correspondence should be addressed. Fax: +46-18-160420. Telephone: +46-18-160414. E-mail: Arne.Ostman@LICR.uu.se.

[1] The abbreviations used are: BS^3, bis(sulfosuccinimidyl)suberate; CHO cells, chinese hamster ovary cells; FCS, fetal calf serum; GST, glutathione S-transferase; Ig, immunoglobulin; PAE cells, porcine aortic endothelial cells; PDGF, platelet-derived growth factor; VEGF, vascular endothelial cell growth factor.

10.1021/bi9919192 CCC: $19.00
Published on Web 02/12/2000

EXPERIMENTAL PROCEDURES

Establishment of a CHO Cell Line Expressing βRD1−3-GST. A PCR product spanning the sequences encoding amino acids 1−317 of the human PDGF β-receptor, flanked by 5′ *Pst*I and 3′ *Kpn*I sites, was generated. The fragment was cloned into the pCR-Script SK(+) vector (Stratagene) and sequenced by the dideoxy chain termination method. The fragment was excised by *Pst*I and *Kpn*I and cloned into the corresponding sites of the pMT2SM-GST vector (a gift from Dr. M. Gebbink, Netherlands Cancer Institute) to generate the vector pMT2SM-PDGFβRD1−3-GST. CHO DG44(dhfr$^-$) cells (a gift from Dr. L. Chasin, Columbia University, New York, NY) were cotransfected with pMT2SM-PDGFβRD1−3-GST and the G418-resistance carrying plasmid RD2 at a 20:1 ratio, using the calcium phosphate method. After transfection, cells were grown in Ham F-12 medium containing 10% FCS and 0.4 mg/mL G418. G418 resistant clones were screened for secretion of βRD1−3-GST by an immunoprecipitation-based assay using ^{125}I-labeled PDGF-BB and GST anti-serum. After the first screening, the best cell line (clone 6-3) was maintained in DMEM containing 10% dialyzed FCS (Hyclone) and increasing concentrations of methotrexate (ICN Biomedicals Inc.) to obtain clones with higher expression of the recombinant protein. Clone 5/2-2, which was obtained after selection under 5 μM methotrexate, was used for large scale expression. This clone secreted approximately 1 μg/mL of βRD1−3-GST when cultured for 24 h in roller bottles with 150 mL serum-free medium.

Production and Purification of βRD1−3-GST, βRD1−3, αRD1−4-GST, and αRD1−4. For the purification of βRD1−3-GST and βRD1−3, CHO clone 5/2-2 was expanded in 15 roller bottles in the presence of 5 μM methotrexate. After the cells reached confluency, they were cultured in RDF medium (RPMI 1640/ Dulbecco's modified minimum essential medium/ Ham F-12 (2:1:1)), supplemented with 990 mg/L glutamine, 10 mM HEPES, pH 7.4, 200 mg/L proline, 100 000 units/L penicillin, 100 mg/L streptomycin, and 50 mg/L gentamycin. Cultures were grown in 5 day cycles composed of 2 days in the presence of 10% FCS and 3 days in the absence of serum.

For purification of βRD1−3-GST, serum-free conditioned medium (3 liter) was filtered through a mesh to remove cell debris and applied onto a 2.5 × 6 cm Q-Sepharose column (Amersham Pharmacia Biotech). After the column was washed with 600 mL of PBS, the bound protein was eluted in 0.35 M NaCl, 10 mM sodium phosphate, pH 7.4. The crude protein preparation thus obtained was incubated with 750 μL of gelatine-Sepharose (Amersham Pharmacia Biotech) for 16 h at 4 °C. The gel was washed with 7.5 mL of the Q-Sepharose elution buffer. The flow-through fraction and the wash fraction was then incubated with 750 μL glutathione-Sepharose (Amersham Pharmacia Biotech) for 16 h at 4 °C. The gel was then washed with 7.5 mL of the buffer, and the bound protein eluted in 10 mM reduced glutathione, 50 mM Tris-HCl, pH 8.0. The protein was concentrated by Centricon 50 (Amicon) to 200 μL and applied to a Superdex 200 column (10/30) (Amersham Pharmacia Biotech) in 0.5 M NaCl, 20 mM Tris-HCl, pH 7.5. After analysis of eluted fractions by SDS-gel electrophoresis, fractions that contained βRD1−3-GST (eluted from 12 to 13 mL) were pooled and purified protein concentrated to approximately 20 mg/mL.

To obtain βRD1−3, 1.5 mg of βRD1−3-GST at a concentration of 3 mg/mL was incubated with 14 units of bovine thrombin (Sigma) for 2 h at 37 °C. After concentration, the material was subsequently applied to a Superdex 200 column (16/60) and pure βRD1−3 was obtained by pooling the fractions eluting between 70.5 and 75.5 ml.

αRD1−4-GST was purified from serum-free conditioned medium from the CHO cell clone C5-2, obtained as described (*13*). After Q-Sepharose chromatography of two liters of conditioned medium, performed as described above, the eluate was concentrated by Centricon 50 to 800 μL and subjected to gel permeation chromatography on a Superdex 200 column (16/60) in 0.5 M NaCl, 20 mM Tris-HCl, pH 7.5. After analysis of fractions by SDS-gel electrophoresis, the eluate between 56 and 59 mL was pooled. αRD1−4 was purified as described (*13*). Purity of the proteins was assessed by SDS-gel electrophoresis and the concentration of the purified proteins was determined by amino acid analysis.

Cross-linking of αRD1−4, αRD1−4-GST, βRD1−3-GST, and βRD1−3. Recombinant receptor extracellular domains at 4 μM concentrations were incubated in the absence or presence of 1 mM bis(sulfosuccinimidyl)suberate (BS^3) for 1 h at room temperature after which methylammonium chloride was added to a final concentration of 30 mM. Samples were analyzed by SDS-gel electrophoresis using 7% polyacrylamide gel under reducing conditions and proteins visualized by Coomassie staining.

Generation and Purification of a Covalent αRD1−4 Dimer. A 4 mg sample of αRD1−4-GST was cross-linked as described above and subsequently incubated with 50 units of bovine thrombin (Sigma) at 37 °C for 5 h. After concentration and buffer exchange to 20 mM Tris-HCl, pH 7.5, 0.15 M NaCl by Centricon 50, the sample was subjected to gel permeation chromatography on a Superdex 200 column (16/60) in 20 mM Tris-HCl, pH 7.5, 0.15 M NaCl; 1 ml fractions eluted from 56 to 68 mL were concentrated 5-fold by Centricon 50 and analyzed by SDS-gel electrophoresis. The fractions eluting at 60−61 mL in which αRD1−4 dimers were most enriched were pooled and used for further experiments. Concentration of the αRD1−4 dimer was determined by amino acid analysis.

Analysis of Effects of Soluble Receptors on PDGF Binding to Cell Surface PDGF Receptors. Binding assays were performed as described previously (*19*), using porcine aortic endothelial cells stably expressing the PDGF α-receptor (PAE-αR cells) (*20*). ^{125}I-labeled PDGF-AA, -AB, or -BB at concentrations of 0.16 nM were preincubated with various concentrations of αRD1−4, αRD1−4-GST, βRD1−3, or βRD1−3-GST in binding buffer for 15−30 min before addition to cell cultures. Samples were incubated at 4 °C for 2 h and after washes, cell-associated radioactivity was recovered and quantified in a γ-counter.

Analysis of Effects of Soluble Receptors on PDGF-induced PDGF α-Receptor Tyrosine Phosphorylation. PDGF-BB at concentrations of 0.08 nM were preincubated with various concentrations of βRD1−3 or βRD1−3-GST in ice-cold binding buffer for 15−30 min before addition to PAE-αR cells. Samples were incubated with cells at 4 °C for 2 h and after washes, cells were lysed by incubation at 4 °C for 15 min with lysis buffer (0.5% Triton X-100, 0.5% deoxycho-

late, 0.15 M NaCl, 20 mM Tris-HCl, pH 7.5, 10 mM EDTA, 1% Trasylol, 1 mM phenylmethylsulfonyl fluoride, and 0.25 mM Na-orthovanadate). The cell lysates were cleared by centrifugation at 13000*g* for 15 min and subjected to PDGF α-receptor immunoprecipitation using a antiserum raised against a peptide corresponding to the carboxy-terminal part of the human PDGF α-receptor (*20*). Immune complexes were recovered with Protein-A-Sepharose (Amersham-Pharmacia Biotech) and washed three times in lysis buffer and once in 20 mM Tris-HCl, pH 7.5. After SDS-gel electrophoresis using 7% polyacrylamide gel, proteins were transferred to Hybond C-extra nitrocellulose membranes and subjected to immunoblotting analysis with PY20 phosphotyrosine antibodies (Transduction Laboratories) and subsequently with PDGF α-receptor antiserum. For detection, horseradish peroxidase-conjugated anti-mouse and anti-rabbit antibodies (Amersham Life Science) were used together with enhanced chemiluminescence (Amersham Pharmacia Biotech).

[^{3}H]-Thymidine Incorporation Assay. The incorporation of [^{3}H]-thymidine into PAE cells expressing the PDGF α-receptor in response to 10 ng/mL of PDGF-AA, -AB, or -BB, or 10% FCS in the absence or presence of various concentrations of αRD14-GST or *β*RD1−3-GST, was performed as described (*21*).

RESULTS

Expression and Purification of αRD1−4, αRD1−4-GST, βRD1−3, and βRD1−3-GST. The expression vector encoding αRD1−4-GST (Figure 1A), the establishment of a stable CHO cell line secreting αRD1−4-GST, and the purification of αRD1−4 has been described previously (*13*). For the production of *β*RD1−3-GST (Figure 1A), an expression vector encoding a fusion protein of PDGF *β*-receptor Ig-like domains 1−3 and GST was generated. After transfection into CHO(dhfr$^-$) cells and selection in increasing concentration of methotrexate, a clone that secreted approximately 1 *μ*g of *β*RD1−3-GST per mL and 24 h was identified. The two CHO(dhfr$^-$) clones producing αRD1−4-GST and *β*RD1−3-GST, respectively, were expanded and grown in cycles consisting of 2 days of culture in the presence of serum followed by 3 days of culture in serum-free medium. Serum-free conditioned medium was used for purification of recombinant proteins.

αRD1−4-GST, *β*RD1−3, and *β*RD1−3-GST were purified through the use of ion-exchange chromatography, glutathione affinity purification, and gel permeation chromatography (for details see Experimental Procedures). Preparations of more than 90% purity of the four proteins were obtained as determined by SDS-gel electrophoresis and Coomassie staining (Figure 1B). The yield of the four recombinant proteins ranged between 4 mg (αRD1−4-GST) and 0.5 mg (*β*RD1−3) per liter of conditioned medium.

Interference with PDGF Receptor Binding and Receptor Activation by Soluble PDGF Receptor Extracellular Domains. To determine the PDGF antagonistic potency, the soluble proteins were analyzed for their abilities to block binding of iodinated PDGF-AA, -AB, and -BB to cell surface PDGF α-receptors (Figure 2A). αRD1−4 reduced the binding of iodinated PDGF-AA and -BB to 50% at 300−1000 nM (Figure 2A, top panel). In contrast, αRD1−4-GST

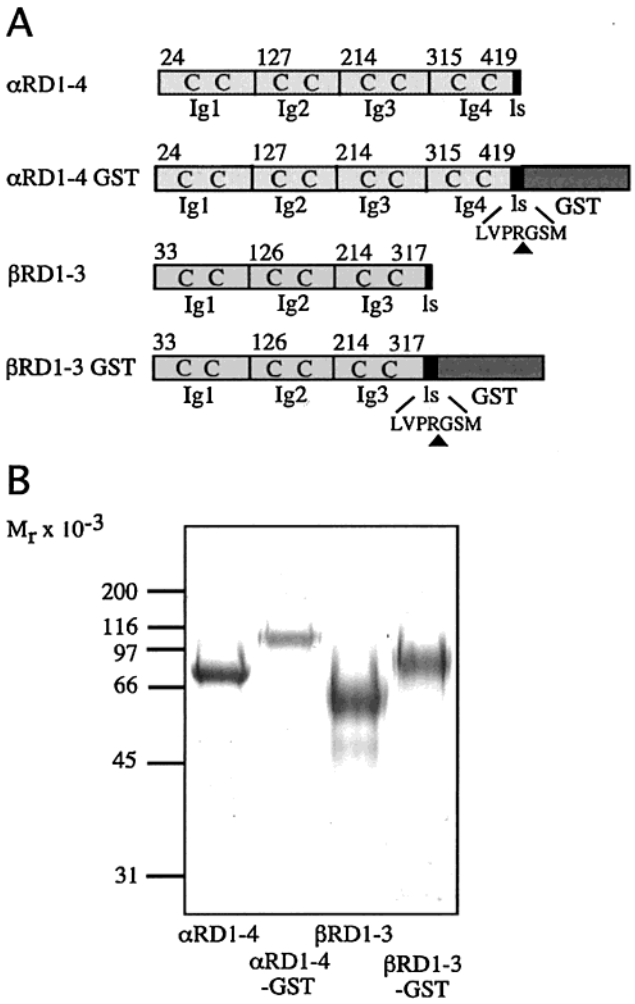

FIGURE 1: Purification of αRD1−4-GST, αRD1−4, *β*RD1−3-GST, and *β*RD1−3. (A) αRD1−4-GST and *β*RD1−3-GST are composed of Ig-like domain 1−4 and 1−3 of the PDGF α- and *β*-receptors, respectively, and a carboxy-terminal GST domain as indicated. By cleavage with thrombin at cleavage sites present in the linker sequence (ls), αRD1−4 and *β*RD1−3 were obtained. (B) Analysis by 10% SDS-gel electrophoresis under reducing conditions of purified recombinant soluble PDGF receptors. Proteins were visualized by Coomassie staining. Positions of marker proteins are indicated to the left.

gave a 50% reduction of binding at 1−10 nM (Figure 2A, top panel). Similar results were obtained with αRD1−4 and αRD1−4-GST using iodinated PDGF-AB (data not shown). In the case of *β*RD1−3-GST and *β*RD1−3, the presence of the GST domain also increased the PDGF-BB neutralizing activity more than 100-fold (Figure 2A, bottom panel). However, in the analyzed concentration ranges, neither *β*RD1−3-GST nor *β*RD1−3 affected PDGF-AA or -AB binding to cell surface receptors (Figure 2A, bottom panel).

*β*RD1−3 and *β*RD1−3-GST were also analyzed with regard to their ability to block ligand-induced PDGF α-receptor tyrosine phosphorylation. As shown in Figure 2B, preincubation of 0.08 nM of PDGF-BB with 1 nM of *β*RD1−3-GST completely reduced PDGF-BB-induced PDGF α-receptor tyrosine phosphorylation, whereas preincubation with 500 nM of *β*RD1−3 only partially reduced PDGF-BB-induced receptor tyrosine phosphorylation.

Interference with PDGF-induced [^{3}H]-Thymidine Incorporation by αRD1−4-GST and βRD1−3-GST. Porcine aortic endothelial (PAE) cells, stably transfected with the PDGFα-

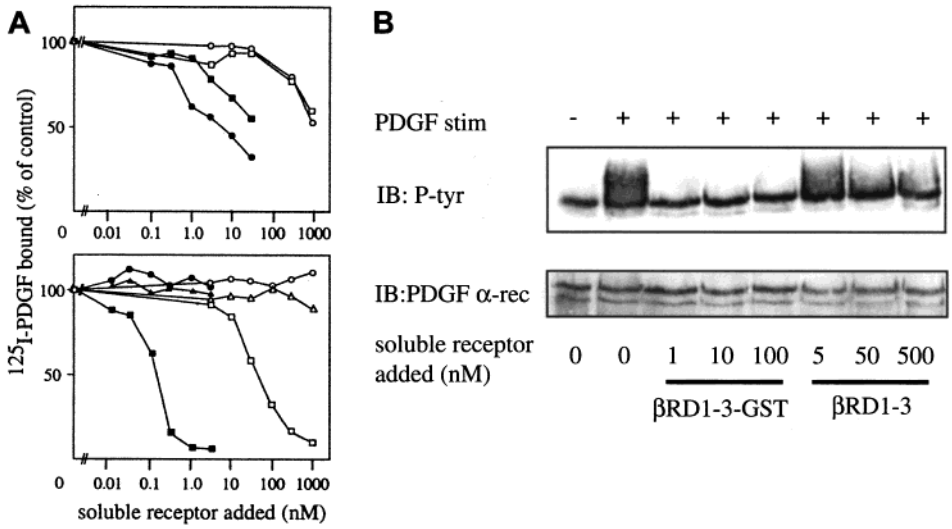

FIGURE 2: Inhibitory effects on PDGF-receptor binding and receptor activation of soluble recombinant PDGF receptors. (A) Neutralization by soluble PDGF α-receptors (top panel) and PDGF *β*-receptors (bottom panel) of PDGF binding to the cell surface PDGF α-receptor. PAE cells expressing PDGF α-receptor were incubated for 2 h at 4 °C with indicated concentrations of αRD1−4-GST or *β*RD1−3-GST (filled symbols), αRD1−4 or *β*RD1−3 (open symbols) together with 0.16 nM of ^{125}I-PDGF-AA (circles), ^{125}I-PDGF-AB (triangles), or ^{125}I-PDGF−BB (squares). (B) Neutralization by soluble PDGF receptors of ligand-induced receptor tyrosine phosphorylation. PAE cells expressing PDGF α-receptor were incubated for 2 h at 4 °C with or without 0.08 nM PDGF-BB that had been preincubated with indicated concentrations of soluble PDGF receptors. PDGF α-receptors were immunoprecipitated and subsequently analyzed by phosphotyrosine (P-tyr) and PDGF α-receptor (PDGF α-rec) immunoblotting.

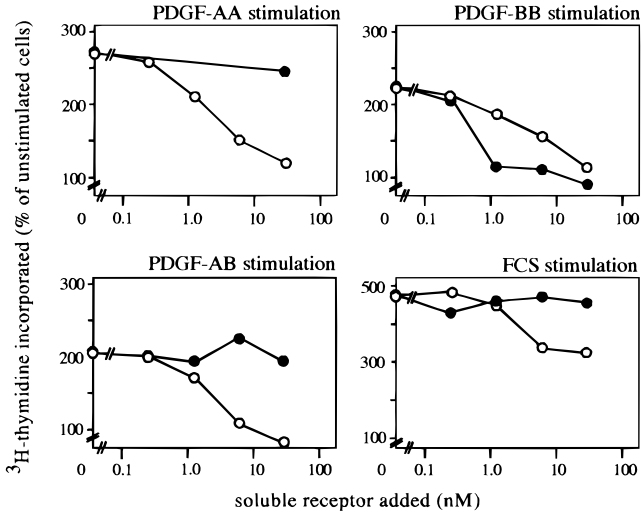

FIGURE 3: Neutralization by αRD1−4-GST and *β*RD1−3-GST of PDGF and FCS-stimulated [^{3}H]-thymidine incorporation. Serum-starved PAE cells expressing the PDGF α-receptor were incubated for 24 h at 37 °C with 0.6 nM of PDGF-AA, -AB, -BB, or 10% FCS together with indicated concentrations of αRD1−4-GST (open circles) or *β*RD1−3-GST (closed circles).

receptor, were left unstimulated, stimulated with 10 ng/mL of PDGF-AA, -AB, or -BB, or 10% FCS in the absence or presence of αRD1−4-GST and *β*RD1−3-GST (Figure 3). A 50% reduction of [^{3}H]-thymidine incorporation induced by PDGF-AA, -AB, and -BB was observed at 1−10 nM of αRD1−4-GST (Figure 3, upper and lower left and upper right panels). Whereas *β*RD1−3-GST reduced PDGF-BB-induced [^{3}H]-thymidine incorporation to 50% at 0.5−1 nM (Figure 3, upper right panel), no effects on PDGF-AA or -AB stimulation was observed at concentrations up to 100 nM of *β*RD1−3-GST (Figure 3, upper and lower left panels). Finally, some reduction in 10% FCS-induced [^{3}H]-thymidine incorporation was observed with αRD1−4-GST, but not with *β*RD1−3-GST (Figure 3, lower right panel). When αRD1−4 was tested for PDGF-AA neutralizing activity in this assay, inhibitory effects were only observed at concentrations of αRD1−4 exceeding 100 nM (data not shown).

The experiments in Figures 2 and 3, thus together, demonstrate a striking increase in antagonistic potency of the soluble extracellular domains of the PDGF receptors when they occur as fusion proteins with GST. Subsequent experiments were performed to investigate the mechanism(s) underlying this effect.

Presence of GST Domain Leads to Formation of Ligand-independent Dimers of Receptor Extracellular Domains. GST is normally occurring as a dimer (*22*, *23*), and GST-induced dimerization of fusion proteins has been demonstrated previously (*24*). To investigate if αRD1−4-GST and *β*RD1−3-GST occurred as dimers, cross-linking experiments were performed in the absence of ligand. αRD1−4GST and *β*RD1−3-GST, and the corresponding proteins lacking the GST domain, were incubated with or without the cross-linker BS^{3} and subjected to analysis by SDS-gel electrophoresis. Incubation of αRD1−4-GST and *β*RD1−3-GST with cross-linker led to the appearance of novel components of approximately 220 and 180 kDa, respectively (Figure 4A, lanes 3, 4, 7, and 8). Since the monomeric forms of αRD1−4-GST and *β*RD1−3-GST migrate as 110 kDa and 80 kDa proteins, respectively, the novel components most likely represent covalently linked dimeric forms. No ligand-independent dimers were observed after incubation of αRD1−4 or *β*RD1−3 with the cross-linker (Figure 4A, lanes 1, 2, 5, and 6).

We thus conclude that αRD1−4-GST and *β*RD1−3-GST occur as ligand-independent dimers. To investigate if this property mediates the high antagonistic potency of αRD1−4-GST and *β*RD1−3-GST, we attempted to generate covalently linked forms of αRD1−4 to explore if such a species would display inhibitory effects at lower concentrations as compared to αRD1−4.

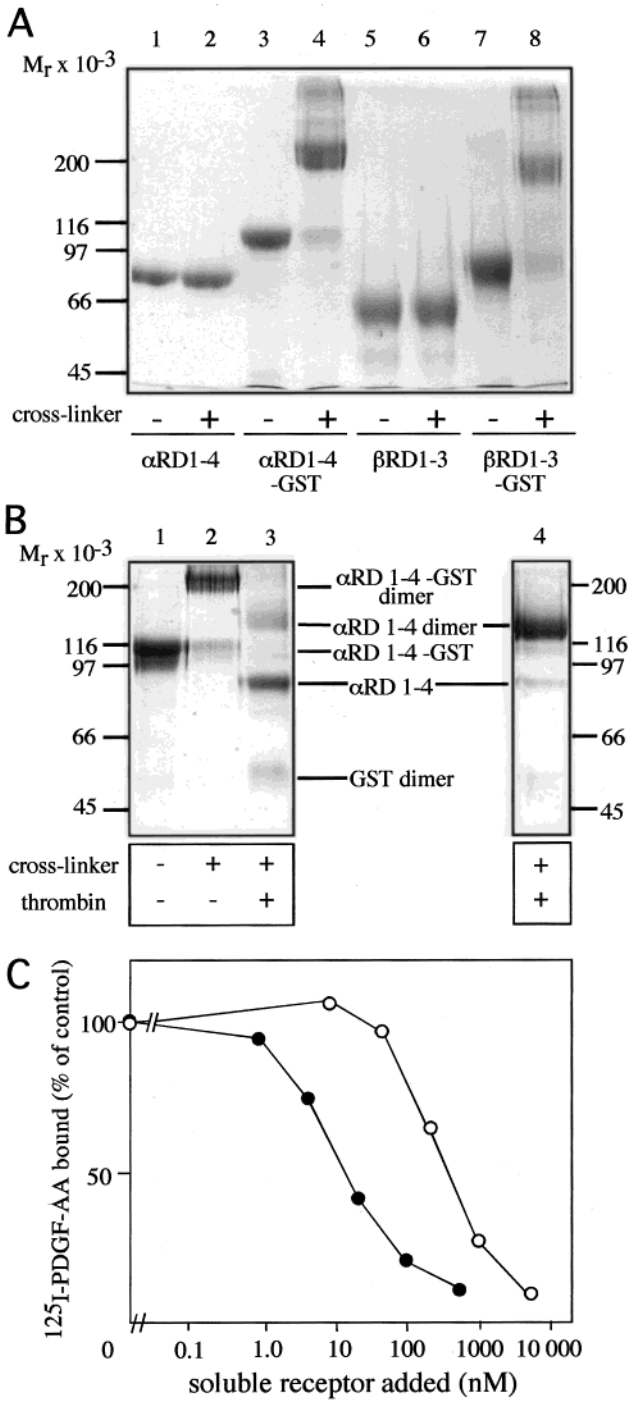

FIGURE 4: Correlation between predimerization of receptors and high inhibitory potency. (A) Demonstration of ligand-independent αRD1−4-GST and *β*RD1−3-GST dimers. Recombinant soluble PDGF receptors were incubated in the absence or presence of the cross-linker BS^3 and subsequently analyzed by 7% SDS-gel electrophoresis under reducing conditions. Proteins were visualized by Coomassie staining. Positions of marker proteins are indicated to the left. (B) Purification of a covalently cross-linked αRD1−4 dimer. αRD1−4-GST (lane 1) was cross-linked with BS^3 to obtain αRD1−4-GST dimers (lane 2) that were subsequently cleaved with thrombin to generate αRD1−4 dimer (lane 3). After gel permeation chromatography of the thrombin cleaved material, a fraction enriched for αRD1−4 dimer was obtained (lane 4). Proteins were visualized by Coomassie staining. Positions of marker proteins are indicated. (C) Neutralization by αRD1−4 and αRD1−4 dimer of PDGF binding to cell surface PDGF α-receptor. PAE cells expressing the PDGF α-receptor were incubated for 2 h at 4 °C with 0.16 nM of ^{125}I-PDGF-AA together with various concentrations of αRD1−4 (open circles) or αRD1−4 dimers (closed circles).

Generation and Functional Characterization of αRD1−4 Dimers. Cross-linking of 5 mg of αRD1−4-GST was performed as described above. After subsequent cleavage with thrombin, the sample was analyzed by SDS-gel electrophoresis (Figure 4B, lanes 1−3). After cross-linking and cleavage with thrombin, novel components of 130 kDa and 54 kDa were observed in addition to the 80 kDa αRD1−4 and 110 kDa αRD1−4-GST (Figure 4B, lane 3). On the basis of its size and mass spectometrical identification of tryptic peptides, the 54 kDa was identified as a cross-linked GST dimer (data not shown). Mass spectometrical identification of peptides derived from the 130 kDa form demonstrated that it was composed of αRD1−4, which, considering its size, is likely to be a covalent dimer. By gel permeation chromatography a fraction enriched for the αRD1−4-dimer was obtained (Figure 4B, lane 4).

To investigate the functional consequences of covalent cross-linking of αRD1−4, αRD1−4 dimer was compared with αRD1−4 for its ability to block PDGF-AA binding to cell surface receptors. As shown in Figure 4C, cross-linking of αRD1−4 dramatically improved the antagonistic potency and shifted the IC_{50} concentration to 3−10 nM.

DISCUSSION

In this paper, we describe the characterization of four recombinant forms of PDGF α- and *β*-receptor extracellular domains. αRD1−4-GST and *β*RD1−3-GST acts as PDGF antagonists of high potency and with different isoform specificity; αRD1−4-GST blocks all isoforms of PDGF, and *β*RD1−3-GST is specific for PDGF-BB (Figures 2 and 3). We conclude that their inhibitory potency at low concentrations, as compared to αRD1−4 and *β*RD1−3, is explained by their property of existing as predimerized forms (Figure 4).

Although αRD1−4-GST and the covalently linked αRD1−4 dimer both were much more potent than αRD1−4, neither protein was as efficient as *β*RD1−3-GST in blocking PDGF-BB binding to cell surface receptors (Figure 2A) or PDGF-BB-induced DNA synthesis (Figure 3). The reason for this remains unclear since α- and *β*-receptors bind PDGF-BB with equal affinity (*25*). However, it cannot be excluded that GST-induced dimerization of *β*RD1−3-GST puts the ligand-binding regions of Ig-like domains 1−3 in a relative position that is more favorable for binding of the dimeric PDGF-BB molecule than in the case of αRD1−4-GST. It is also possible that the cross-linking procedure used to generate αRD1−4 dimers, modified residues directly involved in ligand binding.

Since PDGF is a dimeric growth factor containing two receptor binding sites, the positive effect of predimerization on the ability to bind PDGF is most likely explained by avidity effects. The concentrations of αRD1−4 and *β*RD1−3 required to obtain blocking of binding of PDGF to cell surface receptors are similar to those previously reported for soluble PDGF extracellular domains encompassing all five Ig-like domains or Ig-like domains 1−3 (*17*, *18*, *26*). The only previously described soluble form of PDGF receptor that blocks PDGF signaling at concentrations as low as those observed for *β*RD1−3-GST is a PDGF *β*-receptor Ig-chimera where the PDGF extracellular domain is also presented as a predimerized protein (*27*).

In the case of the structurally related dimeric growth factor VEGF and its binding to VEGF-receptor 2 (KDR), a strong avidity effect by dimerization of receptors was also recently described (*28*). Whereas VEGF bound to a soluble dimeric VEGF-receptor 2, made as an Ig-fusion protein, demonstrated a K_d of 50 pM, binding to soluble monomeric VEGF-receptor 2 occurred with a K_d of 5 nM.

Whether the proteins described in this paper will be useful to investigate the role of PDGF in physiological and pathological settings remain at present unknown and will require further studies. However, we conclude that, in general, antagonists targeting PDGF and other bivalent ligands will act at lower concentrations if presented as predimerized molecules.

ACKNOWLEDGMENT

We thank Dr. Ulf Hellman for mass spectrometrical analysis of recombinant proteins and Ingegärd Schiller for secretarial assistance.

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BI9919192