Platelet-derived growth factor-BB and Basic Fibroblast Growth Factor Directly Interact in Vitro with High Affinity*

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Platelet-derived growth factor-BB (PDGF-BB) and basic fibroblast growth factor (bFGF) are potent growth factors active on many cell types. The present study indicates that they directly interact in vitro. The interaction was investigated with overlay experiments, surface plasmon resonance experiments, and solid-phase immunoassays by immobilizing one factor or the other and by steady-state fluorescence analysis. The interaction observed was specific, dose-dependent, and saturable, and the bFGF/PDGF-BB binding stoichiometry was found to be 2:1. $K_D$ values were found to be in the nanomolar and in the picomolar range, respectively. Basic FGF/PDGF-BB interaction was strongly reduced as a function of time of PDGF-BB proteolysis. Furthermore, docking analysis suggested that the PDGF-BB region interacting with bFGF may overlap, at least in part, with the PDGF-BB receptor-binding site. This hypothesis was supported by surface plasmon resonance experiments showing that an anti-PDGF-BB antibody, known to inhibit PDGF-BB binding with its receptor, strongly reduced bFGF/PDGF-BB interaction, whereas a control antibody was ineffective. According to these data, the observed bFGF/PDGF-BB complex formation might explain, at least in part, previous observations showing that PDGF-BB chemotactic and mitogenic activity on smooth muscle cells are strongly inhibited in the presence of bFGF.

Platelet-derived growth factor (PDGF)1 and basic fibroblast growth factor (bFGF) play a key role in development, wound repair, cancer growth (1, 2), and in vascular wall diseases including atherosclerosis and neointima accumulation at vascular injury sites (3–7).

Basic FGF is produced by fibroblasts and endothelial (EC), glial, and smooth muscle cells. PDGF is produced by platelets, monocytes, EC, and vascular smooth muscle cells (VSMC). Both factors act on different cells including EC, VSMC, fibroblasts, and other cells of mesenchymal origin. Both bFGF and PDGF are reported to be increased in a variety of conditions including tumor growth (8), thyroiditis (9), and brain abscess (10) and to localize at the nuclear, extracellular, and cytoplasmic (polyribosomes, endoplasmic reticulum, Golgi apparatus, and cytoplasmic surface of plasma membrane) compartments (11–13).

PDGF is a disulfide-linked dimer (14) consisting of two polypeptides, designated as A- and B-chain, sharing about 60% sequence homology (15). Three dimeric forms, AA and BB homodimers and AB heterodimer, have been described (16–18). Recently, PDGF-C and PDGF-D isoforms were also identified (19, 20).

PDGF-BB and PDGF-AA exhibit different functional effects, depending on the binding with two distinct cell surface tyrosine kinase receptors, $\alpha$ and $\beta$. The $\alpha$ receptors bind PDGF-AA, -AB, -BB, and -CC with high affinity, whereas $\beta$ receptors bind only BB and DD dimers with high affinity (19–22). PDGF-BB crystallographic analysis revealed that the two chains are arranged in an antiparallel manner. Each subunit consists of a tight cystine knot motif with two loops, loops 1 and 3, pointing in one direction and one loop, i.e. loop 2, pointing in the other directions. Due to this antiparallel arrangement, loops 1 and 3 of one subunit are juxtaposed to loop 2 of the other. Mutational analyses mapped the receptor-binding sites mainly within loops 1 and 3, and within loop 2 to some extent (23–27). The dimeric PDGF molecule thus displays two distinct receptor-binding regions, each one formed by epitopes derived from both subunits.

Basic FGF belongs to a family of at least 22 polypeptides (28–31). Basic FGF crystallographic analyses showed a “trefoil” fold consisting of three copies of a basic four-stranded antiparallel $\beta$-sheet (32, 33). This growth factor acts through high affinity tyrosine kinase receptors (34) and through low affinity heparan sulfate proteoglycan receptors (35–37). FGF receptors consist of four related molecules with alternatively spliced isoforms, each containing a highly conserved tyrosine kinase domain (38–40). Intracellular signaling is initiated by receptor dimerization and receptor transphosphorylation (41–43). Furthermore, it has been suggested that bFGF activates multiple signaling pathways by also utilizing FGF receptor monomers or multimers (44).

PDGF and bFGF may be released simultaneously and at the same site both in vitro and in vivo (45–50). We have shown previously that bFGF reduces in vitro the chemotactic and mitogenic activity of PDGF-BB on VSMC (51) and that bFGF angiogenic properties are markedly reduced by PDGF-BB (82).
We therefore tested in this study whether a direct interaction between the two factors occurs, which may underlie the observed functional inhibition. Fluorescence titration, overlay assays, surface plasmon resonance (SPR) measurements, and solid-phase immunoassays were carried out to evaluate and characterize the interaction between bFGF and PDGF-BB.

**EXPERIMENTAL PROCEDURES**

**Reagents**—The BIACore instrument (BIACoreX), sensor chips CM5, surfactant P20, the amine coupling kit containing N-hydroxysuccinimide, N-ethyl-N’-(3-diethylaminopropyl)carbodiimide, and ethanolamine hydrochloride were from Amersham Biosciences AB. Recombinant human PDGF-BB and acidic FGF (aFGF) were purchased from R & D Systems (Abingdon, UK); recombinant human bFGF, fibronectin, and vitronectin were purchased from Invitrogen; elastase was purchased from Worthington; bovine serum albumin fraction V (BSA) was purchased from Sigma; blotting grade blocker nonfat dry milk was from Bio-Rad; bFGF and Hybond ECL membranes were purchased from Amersham Biosciences AB. Antibodies used in this study include purified mouse anti-PDG-FBB/BB monoclonal antibody (clone 9J1), PharMingen, San Diego, CA, goat anti-human bFGF, and anti-human PDGF-BB antibodies (R & D Systems), rabbit anti-goat IgG (H + L), horseradish peroxidase-conjugated and rabbit anti-goat IgG (H + L) alkaline phosphate-conjugated antibodies (Pierce). Phenylnethylsulfon- nyl fluoride (PMSF), leupeptin, DTT, and trypsin were purchased from Sigma.

**Steady-state Fluorescence**—Steady-state fluorescence titration was performed on a PerkinElmer Life Sciences 500 fluorimeter. The excitation wavelength was set at 280 nm, and the emission originated by both tyrosyl and tryptophyl fluorophores was followed in the 300–360 nm range at 20 °C. In all experiments equal bandwidths were used for both excitation and emission, and the integration time was 1 s. To obtain information on binding constant and stoichiometry, experiments were carried out according to the Continuous Variation Method (52, 53). Variable amounts of the two proteins were mixed so that the sum of their concentrations was held constant at 2 μM throughout the experiment. The individual contribution of the two species, which is linear with the concentration and was estimated by distinct dilution experiments, was always subtracted from the total fluorescence signal. Under these conditions, the minimum fluorescence signal is recorded at the molar ratio corresponding with the complex stoichiometric ratio (52, 53). Two types of experiments were carried out, with protein concentra- tions falling within the fluorescence linearity. In the first set of experiments small aliquots of 2 μM bFGF dissolved in phosphate-buffer solution (PBS) without Ca2+ and without Mg2+, pH 7.2, were added to 2 μM PDGF-BB dissolved in the same buffer. In the range of molar ratios explored, i.e., [bFGF]/[PDGF-BB] from 0 to 1.7, the trend of the fluorescence signal was monotonic. Then a second set of experiments was carried out by adding small aliquots of 2 μM PDGF-BB dissolved in PBS without Ca2+ and without Mg2+, pH 7.2, to 2 μM bFGF dissolved in the same buffer with [bFGF]/[PDGF-BB] molar ratio from 0 to 1.8. A fluorescence minimum was observed at a PDGF-BB/bFGF molar ratio of about 0.5, suggesting a 2:1 bFGF/PDGF-BB binding stoichiometry. Fluorescence data were then smoothed by means of the Savitzky-Golay filter implemented in the software package Scientist by MicroMath (Salt Lake City, UT), and nonlinear least squares analysis was performed according to the one-step equilibrium (Reaction 1).

**PDGF-BB + 2bFGF ⇔ PDGF-BB(bFGF)2, Kd**

**Reaction 1**

**Overlay Experiments**—Three hundred fifty nanograms of either bFGF, PDGF-BB, aFGF, fibronectin, vitronectin, elastase, anti-bFGF, or anti-PDGF-BB antibodies were spotted onto a nitrocellulose membrane, which was then blocked with 5% milk in TPBS (0.1% Tween 20 in PBS). After washing extensively with TPBS, the membrane was incubated with 150 μl/bFGF (1 μCi, 2 μl, 10 ng/ml) for 4 h at room temperature and was washed extensively with TPBS. Nitrocellulose was exposed to Kodak X-OMat AR film (Eastman Kodak Co.) and subjected to densitometric analysis on GS 710 calibrated Imaging Densitometer (Bio-Rad).

**SPR Experiments**—SPR assays were performed on a BIACoreX instrument equipped with a two-flow cell sensor chip. Basic FGF immo- bilization was carried out on one flow cell, here referred to as "sample flow cell," and the second flow cell was used as a control cell. Basic FGF was covalently coupled to the CM5 sensor chip after activation of the carboxymethylated dextran surface by a mixture of 0.05 m N-hydroxysuccinimide and 0.2 μM N-ethyl-N’-(3-diethylaminopropyl)carbodiimide according to a published procedure (54). The coupling reaction was performed by injecting bFGF (80 μl, 1.25 μg/ml) diluted in 30 mM acetate buffer, pH 4.8. The residual activated groups were blocked with 1 μM ethanolamine hydrochloride, pH 8.5. Immobilized bFGF achieved about 500 resonance unit (RU) signals, corresponding approximately to a concentration of 0.5 ng/mm2 (55). The integrity of the immobilized growth factor was tested by injecting a polyclonal anti-bFGF antibody. All experiments were performed using HBS (10 mM Heps, 0.15 μM NaCl, 3 mM EDTA, 0.005% surfactant P20, pH 7.4) as running buffer and to dilute the injected factors. A flow rate of 30 μl/min was used throughout the experiments. Soluble ligands, namely PDGF-BB, aFGF, vitronectin, BSA, fibronectin, and PDGF-AA (30 μl), were injected for a 30-s association phase, followed by HBS flow for a 2-min dissociation phase. The response expressed as sensogram, in RU versus time, was monitored at 25 °C. In the current study, the RU response was always reported as the difference between signals arising from the sample and the reference cell. Therefore, bulk refractive index background and nonspecific bind- ing of the soluble ligands were always subtracted. Sensor chip regen- eration was successfully achieved by injecting 10 μl of 50 mM NaOH after each injection. All injections were carried out in triplicate. Experiments were carried out on two different sensor chips and similar results were obtained. A low immobilization level as well as a high flow rate (30 μl/min) and analyze concentrations suitable to limit the mass transport phenomenon were used to optimize the kinetic evaluation, according to published reports (55).

In additional experiments, PDGF-BB (10 μl, 500 ng) was injected for a 2-min association phase, both in the presence and in the absence of the following: 1 mM CaCl2, 1 mM MgCl2, 10 mg/ml polyethylene glycol (mol wt, 4000), 0.1 μM PMSF, or 0.1 mM DTT, followed by HBS flow for a 30-s dissociation phase. A flow rate of 5 μl/min was used throughout this experiment.

**Solid-phase Immunoassay**—Basic FGF binding to PDGF-BB was also evaluated by solid-phase immunoassays carried out as described (56) with some modifications. Briefly, microtiter plates (Costar) were coated by incubating 100 μl/well of PDGF-BB or heat-denatured PDGF-BB (100 °C for 20 min) or BSA (7 μg/ml in AC7.5 buffer (50 mM Tris-HCl, pH 7.5, 100 mM KCl, 3 mM MgCl2, 1 mM CaCl2) for 4 h at 4 °C. Then incubation with 3% BSA (300 μl/well) in AC7.5 buffer was carried out overnight at 4 °C. All subsequent operations were carried out at room temperature and by using 1 μl/well. After washing three times with AC7.5/BSA buffer (AC7.5 containing 0.1% Tween 20 and 1 mg/ml BSA) in the same buffer, wells were incubated with serial dilutions of bFGF or denatured PDGF-BB (100 °C for 20 min) for 4 h. They were washed four times as described above and incubated with anti-bFGF or anti-PDGF-BB antibo-odies for 1 h. Plates were then washed three times and incubated with rabbit anti-goat IgG (H + L) alkaline phosphatase-conjugated antibody (1:1000 dilution) for 1 h and washed once with AC7.5/BSA and twice with diethanolamine buffer (10 mM diethanolamine, 0.5 mM MgCl2). Plates were then stained with 1 mg/ml of p-nitrophenyl phosphate in diethanolamine buffer (100 μl/ml), and absorption at 405 nm (A405) was determined.

**Kinetic Data Analysis**—SPR sensogramms were analyzed by nonlinear least squares curve fitting using BIAevaluation software version 3.0 (Amersham Biosciences AB) and according to published procedures (57–59). Kinetic constants were determined from the association and dissociation curves from SPR experiments by fitting to a single site binding model (Langmuir model) (Reaction 2).

According to this model, a single exponential fit with a χ2 < 0.5 was computed. Equation 1 was used for the dissociation phase,

\[ R_t = R_0 \exp\left(-k_d(t-t_0)\right) \]  

where \( R_t \) is the amount of bound ligand expressed in RU at time \( t \), and \( t_0 \) is the beginning of dissociation phase. The final dissociation rate constant, \( k_d \), was calculated from the mean values obtained from injec- tions performed at least in triplicate. To analyze the association phase, Equation 2 was employed according to BIATechnology Handbook,

\[ R_t = R_\infty(1 - \exp(-k_s(t-t_0))) \]
where $R_0$ is the amount of bound ligand, expressed in RU, at equilibrium, and $t_0$ is the starting time of injection, as shown in Equation 3:

$$k_i = k_C + k_d$$  \hspace{1cm} (Eq. 3)

where $C$ is the concentration of analyte injected over the sensor chip surface. The association rate constant $k_C$ was determined as the slope of a $k_i$ versus $C$ plot. The apparent equilibrium dissociation constant, $K_{d,2}$, was determined as the ratio of these two kinetic constants ($k_d/k_C$).

Solid-phase immunoassay data were analyzed by nonlinear regression, by fitting to two independent, nonequivalent sites binding model, to evaluate the apparent equilibrium dissociation constants ($K_{d,1}$ and $K_{d,2}$) according to the following Reactions 3 and 4,

$$\text{PDGF-BB + bFGF} \rightleftharpoons \text{PDGF-BB(bFGF)}$$ \hspace{1cm} (REACTION 3)

$$\text{PDGF-BB(bFGF)} + \text{bFGF} \rightleftharpoons \text{PDGF-BB(bFGF)}_2$$ \hspace{1cm} (REACTION 4)

This choice was consistent with the biphasic appearance of double-reciprocal plots and was justified by the fact that a single immobilized PDGF-BB molecule shows two potential binding sites, one in each monomer.

**Basic FGF/PDGF-BB Docking—**PDGF-BB and bFGF crystallographic structures were from the Protein Data Bank (codes 1pdg and 2bbf, respectively). The 1pdg model consists of three chains, named A, B, and C. A and B form a noncrystallographic disulfide-linked BB dimer, whereas C is part of a crystallographic disulfide-linked dimer. The docking simulation was carried out by using the A and B chains. Both chains lack three short segments, not visible in the electron map. The GRAMM software was used (60) to search for the best putative six-dimensional search through the relative molecular translations and interactions between PDGF-BB and bFGF, by performing an exhaustive reciprocal plots and was justified by the fact that a single immobilized PDGF-BB was also subjected to SDS-PAGE (400 ng/lane) and transferred to a nitrocellulose membrane for Western blot analysis and revealed with a polyclonal anti-PDGF-BB antibody (R & D Systems).

**RESULTS**

**Fluorescence Analysis—**Increasing amounts of 2 $\mu$m stock solution of PDGF-BB were added to a fixed amount of 2 $\mu$m stock solution of bFGF, and the fluorescence was measured at 330 nm. Then the individual contribution of bFGF and PDGF-BB was subtracted from the total fluorescence signal, as described under “Experimental Procedures.” The resulting fluorescence was plotted against the concentration of the added species, showing a minimum at about 0.7 $\mu$m PDGF-BB (Fig. 1A). According to the Continuous Variation Method used to analyze these data (52, 53), the Job plot suggested that the formation of bFGF-PDGF-BB complex occurs with a 2:1 binding stoichiometry, implying that both disulfide-linked subunits of PDGF-BB are available to interact with monomeric bFGF. Similar results were obtained collecting the signal at other wavelengths (344 and 360 nm).

The minimum shown by Job plots does not substantially depend on the model chosen to treat binding data, provided the sum of concentrations of the two interacting species is large relative to the dissociation constant (53), as in the present case. In fact, the one-step dissociation constant ($K_{d,2}$) of the ternary complex was estimated in the picomolar range ($5.4 \times 10^{-12}$ M$^2$), and the total concentration of the two proteins was 2 $\mu$m throughout the experiment. These values were used to calculate the complex concentration as a function of the total concentration of PDGF-BB (Fig. 1B).

**Basic FGF/PDGF-BB Complex Formation Examined by Overlay Experiments—**To detect whether specific interaction exists between bFGF and PDGF-BB, several molecules, namely bFGF, PDGF-BB, aFGF, fibronectin, vitronectin, elastase, anti-bFGF, and anti-PDGF-BB antibodies, were immobilized onto a nitrocellulose membrane. The membrane was then overlaid with $^{125}$I-bFGF (1 $\mu$Ci; 10 ng/ml) and exposed to a Kodak X-Omat AR film. In these assays all species were under native conditions. Results shown in Fig. 2 indicate a marked interaction of labeled bFGF with immobilized bFGF, as well as with immobilized PDGF-BB and anti-bFGF antibody. In contrast, labeled bFGF did not show any interaction with aFGF, fibronectin, vitronectin, elastase, and anti-PDGF-BB antibody. Table I shows the mean densitometry values, expressed as percent versus bFGF, of four different experiments. These data
Fig. 2 Basic FGF binding with PDGF-BB, analyzed in overlay assays. Binding of ¹²⁵I-bFGF (1 μCi, 2 ml, 10 ng/ml) to 350 ng of immobilized bFGF, anti-bFGF antibody, PDGF-BB, elastase, fibronectin, vitronectin, and anti-PDGF-BB antibody. Proteins were spotted onto nitrocellulose filters, blocked in 5% milk, and incubated with the soluble ligand. Nitrocellulose was then exposed to a Kodak X-Omat AR film. The experiment was carried out 4 times with similar results. The reported data refer to a representative experiment.

Table I
Overlay assay, densitometric analysis for the interaction of labeled bFGF with different immobilized factors

| Immobilized Factors | % bFGF Densitometry/bFGF | Densitometry on bFGF |
|---------------------|---------------------------|----------------------|
| PDGF-BB             | 88.64 ± 22.92             |                      |
| Anti-bFGF           | 121.38 ± 18.00            |                      |
| Fibronectin         | 0.88 ± 0.07               |                      |
| Vitronectin         | 0.685 ± 0.02              |                      |
| aFGF                | 0.565 ± 0.17              |                      |
| Elastase            | 0.08 ± 0.01               |                      |
| Anti-PDGF-BB        | 0.53 ± 0.04               |                      |

show that under these conditions bFGF specifically interacts with PDGF-BB.

Basic FGF/PDGF-BB Interaction Examined by SPR Analysis—Kinetic experiments were performed on a BIAcoreX by immobilizing bFGF and subsequently injecting PDGF-BB as the analyte. Immobilization of bFGF yielded ~500 RU. Fig. 3A shows the response measured at the end of the association phase as a function of increasing PDGF-BB concentration. PDGF-BB bound the bFGF-coated chip in a concentration-dependent manner. Association and dissociation curves from a representative experiment performed with five increasing concentrations of PDGF-BB (12.5–150 nM) are shown in Fig. 3B. All sensorgrams report values obtained after subtraction of the signal on the control flow cell. The association phase lasted from time 0 to time 30 s and was analyzed by nonlinear least squares curve fitting as described under “Experimental Procedures” to yield $k_a$ values at each analyte concentration examined (57). A plot of $k_a$ versus PDGF-BB concentration produced a straight line (Fig. 3C) with a slope equal to the association rate constant ($k_a$). The $k_a$ value for PDGF-BB binding to immobilized bFGF was computed as $(1.27 ± 0.06) \times 10^6$ s$^{-1}$ m$^{-1}$.

The dissociation phase was recorded for 2 min after the end injection and was analyzed by nonlinear least squares curve fitting. The dissociation rate constant, $k_d$, was evaluated from traces obtained at ligand saturation (Fig. 3B) as we and others previously reported (57, 58). The $k_d$ value for PDGF-BB binding to immobilized bFGF was calculated as $(1.71 ± 0.06) \times 10^{-2}$ s$^{-1}$. The apparent equilibrium dissociation constant $K_D$ determined from the ratio of the two kinetic constants ($k_a/k_d$) was $(13.5 ± 8.0) \times 10^{-9}$ M.

SPR analysis was also performed to examine the interaction of aFGF, vitronectin, BSA, and fibronectin with bFGF-coated sensor chip. Fig. 4 shows the RU response observed by injecting 500 nM of each protein. Although PDGF-BB and anti-bFGF antibody bound bFGF with a response of about 200 and 400 RU, respectively, none of the other factors exhibited significant interaction with bFGF.

Basic FGF/PDGF-BB Interaction Examined in Solid-phase Immunoassay—Solid-phase immunoassays were carried out as an alternative approach to measure the interaction between bFGF and PDGF-BB. Different from the SPR analysis, in this experiment PDGF-BB was the immobilized species, and bFGF was added as free in solution. Basic FGF significantly bound PDGF-BB in a concentration-dependent manner (Fig. 5A). Nonlinear regression analysis was carried out according to a model involving binding to two independent nonequivalent sites, because of the biphasic appearance of the double-reciprocal plot. This procedure yielded apparent dissociation constants of $(5.2 ± 3.0) \times 10^{-9}$ M and $(6.67 ± 5.05) \times 10^{-7}$ M ($K_{D1}$ and $K_{D2}$, respectively). The $K_{D1}$ value computed from solid-phase immunoassay is close to the $K_{D1}$ value computed from
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...interaction in the presence of an alkylating agent, PMSF and DTT, respectively. Fig. 8 shows the RU responses observed in a representative experiment. PMSF did not affect bFGF/PDGF-BB interaction, whereas DTT significantly inhibited the interaction, up to 70%. These data suggest that PMSF-dependent covalent modification of serine residues does not influence the bFGF/PDGF-BB interaction, whereas the integrity of PDGF-BB disulfide-bridges is required for the binding to bFGF.

Interaction of Trypsinized PDGF-BB with bFGF Examined by SPR Analysis—To test further the specificity of the bFGF/PDGF-BB interaction, trypsinated PDGF-BB was injected onto immobilized bFGF, and the interaction was analyzed by SPR. Fig. 9A shows that, under these conditions, PDGF-BB lost the ability to be recognized by the specific antibody in Western blot, progressively as a function of time of trypsin treatment. Fig. 9B shows the RU response, measured 20 s after the end of association phase, observed by injecting PDGF-BB trypsinized at various times onto immobilized bFGF. Under these conditions trypsin alone did not show any interaction with immobilized bFGF (data not shown), whereas binding of PDGF-BB to bFGF strongly decreased as function of time of proteolysis, reaching a plateau after about 30 min.

DISCUSSION

In the present study we demonstrate for the first time that a direct interaction between human bFGF and human PDGF-BB occurs in vitro. The bFGF/PDGF-BB interaction has been observed in solid-phase-based assays, i.e. SPR, solid-phase immunoassay, and overlay experiments, by immobilizing either one factor or the other, as well as in a liquid-phase-based assay, i.e. in steady-state fluorescence experiments. The data obtained consistently show that the interaction is specific, dose-dependent, and saturable. Kinetic experiments carried out in SPR and solid-phase immunoassays consistently indicate a $K_D$ value, corresponding to the first step of the binding equilibrium, falling in the low nanomolar range (23.7 and 5.2 nM respectively), and fluorescence experiments indicate the $K_D$ value of the whole equilibrium in the picomolar range. The reported $K_D$ values for PDGF-BB and bFGF interaction with the corresponding receptors are in the low nanomolar ranges (66–69), suggesting that the bFGF/PDGF-BB complex formation, under certain conditions, may alter interaction with the corresponding receptors, thus modulating their activity. The hypothesis that the complex itself may alter binding with the bFGF and PDGF-BB receptors is currently under investigation. Findings of the present study should be discussed in light of our previous reported data (51) indicating that PDGF-BB chemotactc and mitogenic activity on primary smooth muscle cells are markedly inhibited in the presence of bFGF. We also collected data indicating that a reciprocal inhibitory effect is present, since bFGF activity on endothelial cells is markedly inhibited by PDGF-BB both in vitro and in vivo. These data demonstrate that the simultaneous presence of these molecules leads to a marked functional impairment of either factors. Basic FGF/PDGF-BB complex formation may represent the mechanism underlying, at least in part, the observed functional interference, although the direct link is still under evaluation. They also indicate a novel mechanism to modulate activity of these factors. As recently pointed out, protein-protein interactions are essential for almost all biological processes and homo- and hetero-dimerization may induce subtle changes in monomer protein concentration, therefore influencing protein activity (70).

Both bFGF and PDGF-BB are potent angiogenic factors (71) and are expressed in vivo under physiologic conditions (1). Despite this, angiogenesis is not observed under normal condi-
tions, and neo-angiogenesis processes are activated only under specific physiologic stimuli, such as the menstrual cycle, and in pathologic conditions such as wound healing, cancer growth, diabetic retinopathy, and ischemia. Mechanisms able to modulate these and/or other growth factors are under thorough investigation (72–74). Their activity is reported to be regulated by the expression level of the corresponding receptors (66, 75), by controlling their active folding (23) as well as heparin-binding features (76, 77). PDGF-BB activity is also modulated by its binding to /H92512-macroglobulin (78), and bFGF activity is also modulated by its binding with perlecan, sialoglycolipids, and thrombospondin-1 (79–81). An additional mechanism possibly playing a role to modulate the activity of PDGF-BB and bFGF, reported in the present study, is the direct interaction of these growth factors.

Experiments carried out in the presence of PMSF suggest that serine residues are not involved in the bFGF/PDGF-BB interaction. In contrast, reducing disulfide bonds of PDGF-BB with DTT almost completely abolished its interaction with immobilized bFGF (Fig. 6), suggesting that the integrity of disulfide bonds is required. This observation was further sup-
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