Heparin Amplifies Platelet-derived Growth Factor (PDGF)-BB-induced PDGF \(\alpha\)-Receptor but Not PDGF \(\beta\)-Receptor Tyrosine Phosphorylation in Heparan Sulfate-deficient Cells

EFFECTS ON SIGNAL TRANSDUCTION AND BIOLOGICAL RESPONSES

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Platelet-derived growth factor (PDGF) induces mitogenic and migratory responses in a wide variety of cells, by activating specific receptor tyrosine kinases denoted the PDGF \(\alpha\)- and \(\beta\)-receptors. Different PDGF isoforms bind in a distinct manner to glycosaminoglycans, particularly heparan sulfate. In the present study, we show potentiation by exogenous heparin of PDGF-BB-induced PDGF \(\alpha\)-receptor tyrosine phosphorylation in heparan sulfate-deficient Chinese hamster ovary (CHO) 677 cells. This effect was not seen for PDGF-AA treatment, and heparin lacked a potentiating effect on PDGF-BB stimulation of the PDGF \(\beta\)-receptor. Heparin did not affect the affinity of PDGF-BB binding for the PDGF receptors on CHO 677 cells. The PDGF-BB-stimulated PDGF \(\alpha\)-receptor phosphorylation was enhanced in a dose-dependent fashion by heparin at low concentration. The effect was modulated by 2-O- and 6-O-desulfation of the polysaccharide. Maximal induction of PDGF \(\alpha\)-receptor tyrosine phosphorylation (6-fold) in CHO 677 cells was achieved by treatment with a heparin decasaccharide, but shorter oligosaccharides consisting of four or more monosaccharide units were also able to augment PDGF \(\alpha\)-receptor phosphorylation, albeit at higher concentrations. Heparin potentiated PDGF-BB-induced activation of mitogen-activated protein kinase and protein kinase B (Akt) and allowed increased chemotaxis of the CHO 677 cells toward PDGF-BB. In conclusion, heparin modulates PDGF-BB-induced PDGF \(\alpha\)-receptor phosphorylation and downstream signaling, with consequences for cellular responsiveness to the growth factor.

Platelet-derived growth factor (PDGF)

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‡ The abbreviations used are: PDGF, platelet-derived growth factor; CHO, Chinese hamster ovary; CBS, chondroitin sulfate; FGF, fibroblast growth factor; FGF, FGF receptor; HS, heparan sulfate; MAPK, mitogen-activated protein kinase; PBS, phosphate-buffered saline; PKB, protein kinase B; SH, Src homology; BSA, bovine serum albumin; TBS-T, 0.2% Tween 20 in Tris-buffered saline.

capillary endothelial cells and neurons (reviewed in Refs. 1 and 2). Classically, PDGF is a dimeric molecule consisting of disulfide-bonded A and B polypeptides that assemble into homo- and heterodimers; i.e. PDGF-AA, PDGF-BB, and PDGF-AB (3). PDGF transduces cellular responses by binding to two related protein tyrosine kinase receptors, the PDGF \(\alpha\)- and \(\beta\)-receptors. PDGF-AB and PDGF-BB bind to both PDGF \(\alpha\)- and \(\beta\)-receptors with similar affinity (4), in contrast to PDGF-AA, which binds only to the PDGF \(\alpha\)-receptor (5–7). Recently, additional PDGF-related polypeptides were identified and denoted PDGF-C and -D. These novel isoforms do not appear to form heterodimers but exist as PDGF-CC and -DD (8–10), which bind to PDGF \(\alpha\)- and \(\beta\)-receptors, respectively.

Upon binding of PDGF, the receptors dimerize, leading to autophosphorylation of tyrosine residues in trans between two receptor molecules in the dimer. The phosphorylated tyrosine residues, in combination with surrounding amino acid residues, form binding sites for signaling proteins equipped with Src homology (SH) 2 domains. The PDGF receptors are known to associate with members of the Src family of cytoplasmic tyrosine kinases, phospholipase C-\(\gamma\), the regulatory \(\beta\)5 subunit of phosphoinositide 3-kinase, the adapter Grb2, and the Src homology-containing phosphatase 2 (Shp-2) (Ref. 11 and reviewed in Refs. 12 and 13). Binding of SH2 domain proteins in turn leads to initiation of signaling cascades involving mitogen-activated protein kinase (MAPK) and protein kinase B (PKB/Akt) that provide survival signals for the cell.

The PDGF-A chain appears as two variants, a longer form (PDGF-A\(_1\)) and a shorter form (PDGF-A\(_3\)), that are generated through alternative splicing of exons 6 and 7 of the PDGF-A gene. The PDGF-B chain, on the other hand, is proteolytically processed into a shorter form and a longer form. The PDGF-A\(_3\) and the short form of PDGF-B are effectively secreted into the medium, whereas PDGF-A\(_1\) and the long form of PDGF-B are retained at the cell surface (14, 15). The retention of the long PDGF polypeptides is due at least in part to binding to glycosaminoglycans, particularly those of heparan sulfate proteoglycans (16, 17). Heparan sulfate proteoglycans are expressed on most cell types but are also secreted and deposited in the extracellular matrix. The binding of proteins to HS and other glycosaminoglycans is largely electrostatic in nature and involves the negatively charged carboxyl and sulfate groups in the HS chains and basic amino acid residues in the protein. Sulfation of HS may occur at the N-, 3-O and 6-O positions of the glucosamine units and at the 2-O position of the hexuronic acid residues (18). Notably, the sulfation patterns of HS are tissue-specific, developmentally regulated, and apparently designed to accommodate selective interactions with a spectrum of proteins.
of proteins (19). In the present study, we have investigated effects of heparin on PDGF-BB-stimulated PDGF receptor activation and determined the influence of oligosaccharide chain length and O-sulfation on receptor activation and downstream signaling events.

MATERIALS AND METHODS

Cell Culture—Wild-type Chinese hamster ovary (CHO) KI cells and mutated, HS-deficient CHO 677 cells (20) were cultured in Ham’s F-12 medium (Invitrogen) supplemented with 10% fetal bovine serum (In- vatrogen) at 37°C and 5% CO₂.

Scatchard Analysis—Confluent CHO 677 cells were washed with PBS-B/BSA (PBS plus 0.077 mM CaCl₂ and 0.083 mM MgSO₄ supplemented with 1% BSA) and incubated for 1 h on ice with increasing amounts of unlabeled PDGF-BB (24,300 Da; Peprotech) in the presence or absence of 100 ng/ml heparin. The cells were then incubated for 1 h with 1 ng/ml PDGF-BB (20,000 cpm/ng; Amersham Biosciences). The cells were washed three times with PBS-B/BSA and then lysed for 15 min on ice in 20 mM Tris-HCl, pH 7.5, 1% Triton X-100, and 10% glycerol. Cell-associated 125I was estimated using a gamma counter.

Flow Cytometry—For detection of cell surface HS and chondroitin sulfate (CS), CHO KI and CHO 677 cells were suspended at a concentration of 1 × 10⁶ cells/ml in RPMI 1640 medium and 10% fetal calf serum and treated with 50 μg/ml PDGF-BB in 20 mM Tris-HCl, pH 7.5, 1% BSA and incubated with fluorescein isothiocyanate-labeled goat anti-mouse IgG (Dako) for 30 min on ice. Cells were washed with RPMI 1640/10% fetal calf serum, incubated with fluorescein isothiocyanate-labeled goat anti-mouse IgM (Dako) for 30 min on ice, and then washed with PBS/2% BSA. The cells were analyzed by fluorescence-activated cell sorting.

Glycosaminoglycan (GAG) Preparations—Purification of heparin from pig intestinal mucosa (22) and selective chemical O-desulfation followed by re-N-sulfation of bovine lung heparin (23) were performed as described. In the 2-O-desulfated heparin, 1% of the iduronic acid residues were 2-O-sulfated, whereas >80% of the glucosamine residues were 6-O-sulfated. In the 6-O-desulfated preparations, the degree of glucosamine 6-O sulfation was <10%, but the treatment also resulted in the removal of ~30% of the 2-O sulfate groups. The preparations were subjected to high-resolution gel filtration and sterile-filtered to remove possible contamination. There was no sign of toxicity of these preparations in the tissue culture. Chemical depolymerization of bovine lung heparin was performed by limited deamination with nitrous acid and glucuronic acid as a standard (25). A factor of 3 was arbitrarily employed to convert values to saccharide mass.

Filter Binding Assay—Radiolabeled heparin, 2-O-desulfated heparin, and preferentially 6-O-desulfated heparin (0.75 μM) of >20-mer size were incubated at room temperature for 60 min with PDGF (0–10 μM) in a final volume of 40 μl of Tris-HCl, pH 7.4, 150 mM NaCl, and 0.1% BSA. Protein, along with protein-bound oligosaccharides, was trapped on nitrocellulose filters (2.5 cm, diameter) (Schleicher & Schuell) through vacuum suction, whereas nonbound oligosaccharides were washed off with phosphate-buffered saline (26). The protein-bound oligosaccharides were dissociated from the filter in 2 ml of 2 M NaCl and quantitated by scintillation counting.

PDGF-BB Treatment and Immunoprecipitation—Cell cultures at 106 cells/ml in RPMI 1640 medium and 10% fetal calf serum were starved in Ham’s F-12 medium containing 0.25% BSA (starvation medium) for 16 h. Cells were detached using a nonenzymatic cell detachment solution (2 mg/ml Trypsin, 0.2% EDTA, 0.02% pancreatin, and 0.001% NaB₃H₄) and then separated with regard to size by gel filtration on a column (1 × 146 cm) of BioGel P-10 in 0.1 M NH₄HCO₃. All nonlabeled GAGs were quantified by colorimetric determination of hexuronic acid using the meta-hydroxydiphenyl method with glucuronic acid as a standard (25). A factor of 3 was arbitrarily employed to convert values to saccharide mass.

RESULTS

Tyrosine Phosphorylation of PDGF α- and β-Receptors in PDGF-BB-treated HS-deficient CHO Cells—We analyzed HS-deficient CHO 677 cells for their ability to respond to PDGF-BB stimulation with increased tyrosine phosphorylation of PDGF α- and β-receptors. Both receptor types were expressed on the cells, and ligand stimulation led to increased tyrosine phosphorylation of the receptors, indicative of activation of their intrinsic tyrosine kinase activities (Fig. 1A). To verify the phenotype of the cells with regard to cell surface proteoglycans, fluorescence-activated cell-sorting analysis was performed after incubation of the cells with antibodies against HS or, as a control, against CS. As seen in Fig. 1B, the CHO 677 cells lacked expression of HS but showed CS expression comparable to that of the wild-type CHO KI cells. Based on indications in the literature that HS-related polysaccharides may affect PDGF function, we decided to examine in more detail the potential effects of heparin on PDGF receptor activation. PDGF-BB was chosen as the ligand because it binds to both PDGF receptors.

Effect of Heparin on PDGF-BB-induced PDGF α- and β-Receptor Tyrosine Phosphorylation in HS-deficient CHO Cells—We first characterized the heparin binding ability of the commercial 24,300-Da PDGF-BB in nitrocellulose filter trapping assays. PDGF-BB clearly bound to ¹⁴C-labeled heparin oligosaccharides in a dose-dependent fashion. Thus, incubation of 0.75 μM heparin (≥20-mer fragments) with PDGF-BB at physiological ionic strength resulted in saturation of the saccharide at a ~5-fold molar excess of the protein and an estimated dissociation constant in the micromolar range. Similar results were obtained with 2-O- or 6-O-desulfated heparin oligosaccharides, although these experiments could not be pursued to define the relative affinities of the various saccharides for the growth factor. Nevertheless, the results obtained suggest that significant proportions of the PDGF-BB added to cells in subsequent experiments were complexed to heparin or to its partially O-desulfated derivatives. Furthermore, stable complex formation between PDGF-BB and heparin was obtained in...
co-immunoprecipitation experiments. Immunoprecipitation was performed using antibodies against PDGF-BB or a mixture of antibodies (10E4 and HepSS-1) against HS proteoglycans, followed by immunoblotting for PDGF-BB. The 10E4 antibody probably recognizes an L-iduronic acid epitope. The HepSS-1 antibody epitope is likely an N-O-sulfated glucoronic acid-rich sequence that recognizes N-sulfates, but not free amino groups, L-iduronic acid, N-acetyl groups, or O-sulfates. There was appreciable co-precipitation of PDGF-BB with heparin, using the anti-HS monoclonal antibodies, whereas the isotype-matched control mouse serum essentially failed to precipitate PDGF-BB (Fig. 2). Washing the immobilized precipitate with 0.5 M NaCl eliminated co-precipitation with the HS antibodies.

Next, HS-deficient CHO 677 cells were treated with PDGF-BB and heparin at increasing concentrations to analyze the effects on PDGF-α and β-receptor tyrosine phosphorylation. Cells were lysed, divided equally, and immunoprecipitated with antibodies specific for PDGF-β (Fig. 3A) or α-receptors (Fig. 3B), and samples were subjected to immunoblotting with anti-phosphotyrosine antibody. The results showed that PDGF α-receptor tyrosine phosphorylation was amplified by heparin in a dose-dependent manner, with a maximal 4-fold effect at 100 ng/ml heparin. At higher concentrations of heparin, the level of PDGF α-receptor tyrosine phosphorylation returned to the basal level. In contrast, PDGF-BB stimulated PDGF β-receptor tyrosine phosphorylation efficiently in the absence of heparin, and the addition of the polysaccharide did not augment the reaction, set in relation to the loading control. Similar results were obtained in at least three repeated experiments; the augmenting effect of heparin on PDGF β-receptor activation was small or nonexistent, whereas the effect on the PDGF α-receptor was stable and significant. Furthermore, there was a small effect or no effect of heparin on PDGF-AA-stimulated PDGF α-receptor tyrosine phosphorylation (Fig. 3C). PDGF-AA does not bind appreciably to the PDGF β-receptor, and this combination was therefore not tested. Heparin alone, in the absence of PDGF, did not induce phosphorylation of either PDGF receptor (Fig. 3C).

For comparison, wild-type CHO KI cells were treated similarly with heparin in the presence or absence of PDGF-BB, followed by immunoprecipitation of PDGF receptors and immunoblotting (Fig. 4). In these HS-expressing cells, heparin had no effect on PDGF-BB-induced PDGF α- or β-receptor tyrosine phosphorylation.

The possibility that heparin may increase the affinity of PDGF-BB binding for the PDGF α-receptor was tested in a Scatchard analysis. CHO 677 cells were incubated in the presence of 1 ng/ml 125I-PDGF-BB and increasing concentrations of unlabeled ligand. As shown in Fig. 5, the affinity of PDGF-BB binding to PDGF receptors expressed on CHO 677 cells was similar in the presence and absence of heparin.

**Effect of Heparin Desulfation on PDGF-BB-induced Receptor Tyrosine Phosphorylation**—We further tested the effects of selectively desulfated heparin preparations on PDGF-α- and β-receptor tyrosine phosphorylation. CHO 677 cells were treated with PDGF-BB in the absence or presence of 2-O-desulfated heparin (Fig. 6, A and B) or 6-O-desulfated heparin (Fig. 6, C and D) at different concentrations. The cells were lysed, and lysates were immunoprecipitated with PDGF-β- (Fig. 6, A and C) or α-receptor (Fig. 6, B and D) antibodies. There was no appreciable effect of 2-O- or 6-O-desulfated heparin on the PDGF β-receptor, in agreement with the lack of effect of native heparin on this receptor (cf. Fig. 3A). On the other hand, 2-O- as well as 6-O-desulfated heparin amplified PDGF-BB-induced PDGF α-receptor phosphorylation, albeit to a lower extent than native heparin. However, contrary to the pattern seen with native heparin, there was no dose-dependent decrease in PDGF α-receptor activation at higher concentrations of the desulfated heparin preparations.

**Heparin Fragments of Four Monosaccharide Units Amplify PDGF-BB-induced PDGF α-Receptor Phosphorylation**—We examined the effect of heparin oligosaccharide size on PDGF-BB-induced PDGF receptor tyrosine phosphorylation. As expected, heparin fragments from four monosaccharide units to full-length heparin showed little or no effect on PDGF β-receptor tyrosine phosphorylation in the CHO 677 cells (Fig. 7A). In
Fig. 3. Heparin enhances PDGF-BB-induced PDGF α-receptor phosphorylation in HS-deficient CHO 677 cells in a dose-dependent manner. CHO 677 cells were stimulated with 50 ng/ml PDGF in the absence (−) or presence (+) of heparin at defined concentrations or treated with heparin alone. Immunoprecipitated PDGF-BB-stimulated PDGF β-receptors (A) and PDGF α-receptors (B) (A and B were from the same lysate) and PDGF-AA-stimulated PDGF α-receptors (C) were subjected to SDS-PAGE, followed by immunoblotting with anti-phosphotyrosine antibody PY99, and, after stripping, with anti-PDGF β- and anti-PDGF α-receptor antibodies, as indicated. Fold induction of PDGF receptor phosphorylation was quantified (right panels).

Fig. 4. Heparin does not affect PDGF-BB-induced PDGF α- and β-receptor tyrosine phosphorylation in CHO K1 cells. CHO K1 cells were stimulated with 50 ng/ml PDGF-BB in the absence (−) or presence (+) of heparin at defined concentrations. Immunoprecipitation was performed with anti-PDGF α or anti-PDGF β-receptor antibodies as indicated, followed by SDS-PAGE and immunoblotting with the anti-phosphotyrosine antibody PY99. As a control for equal loading, blotting with the anti-PDGF β-receptor antibody was performed.

Fig. 5. Heparin does not affect the affinity of PDGF-BB binding to CHO 677 cells. CHO 677 cells were incubated with 125I-PDGF-BB in the presence of increasing concentrations of unlabeled PDGF-BB (0–500 ng/ml) on ice. After washing away unbound PDGF, the amount of bound and free PDGF-BB fractions was estimated and used for Scatchard analysis.

contrast, PDGF α-receptor tyrosine phosphorylation in the same cells was induced 4-fold by PDGF-BB in the presence of the 4-mer at 100 ng/ml (Fig. 7B). Treatment with the decasaccharide fragment led to 6-fold amplification of PDGF α-receptor tyrosine phosphorylation by PDGF-BB, whereas longer saccharide fragments were slightly less efficient.

Heparin Augments PDGF-BB-induced Signal Transduction and Biological Responsiveness of the CHO 677 Cells—To test potential effects of heparin on signal transduction, CHO 677 cells were treated with heparin at different doses in combination with a low dose of PDGF-BB. Heparin augmented MAPK
and PKB/Akt phosphorylation in a dose-dependent manner (Fig. 8), indicative of HS-modulated activation of these signaling components in the intact, PDGF-BB-stimulated cell.

To determine whether co-treatment with heparin would increase cellular responsiveness to PDGF-BB, we analyzed directed migration of CHO 677 cells, compared with CHO KI cells, under different conditions as shown in Fig. 9. The HS-expressing CHO KI cells displayed a relatively high basal migration and only a slight increase in migration toward PDGF-BB; there was no appreciable additional stimulation when cells also received heparin. The CHO 677 cells also migrated poorly toward PDGF-BB, but the addition of heparin significantly augmented the chemotactic migration of the cells.

DISCUSSION

In this study, we show that the addition of heparin augmented PDGF-BB-induced activation of the PDGF α-receptor, but not PDGF β-receptor, in HS-deficient CHO 677 cells expressing endogenous PDGF receptors. Moreover, heparin promoted PDGF-BB-induced intracellular signaling and increased chemotaxis of such cells. Heparin alone had no effect on PDGF receptor tyrosine phosphorylation. The lack of effect of heparin on PDGF β-receptor tyrosine phosphorylation is in agreement with previous data (27). Moreover, heparin had no effect on PDGF-AA-stimulated PDGF α-receptor tyrosine phosphorylation.

The effect of heparin on the PDGF-BB-stimulated PDGF α-receptor did not appear to be due to increased binding affinity. However, the CHO 677 cells express both PDGF α- and β-receptors, and it is possible that PDGF-BB binding to the PDGF β-receptor obscured any heparin-related changes in affinity for the PDGF α-receptor. The dose-dependent effect of heparin was maximal at around 100 ng/ml heparin, whereas higher concentrations of 1–5 μg/ml lacked effect. Short oligosaccharide fragments (4-mers and longer) were active in this model. Heparin lacking either the 2-O-desulfation on iduronic acid units or the 6-O-desulfation groups on glucosamine units retained the ability to augment PDGF-BB-induced PDGF α-receptor activation, although the effect was reduced, and the dose-response pattern was changed. Notably, there was no absolute requirement for heparin in PDGF α-receptor activation by PDGF-BB. This is in agreement with previous data on chlorate-treated fibroblasts deficient in cell surface HS, which still respond to PDGF-BB with increased mitogenic activity (28).

Previous reports in the literature indicate that PDGF-BB does indeed bind heparin and that biological responses to PDGF may depend on interaction with HS. Thus, heparin-binding fragments from fibronectin (29) or apolipoprotein E (30) negatively modulate proliferative responses to PDGF-BB. Furthermore, PDGF-BB may be deposited in the matrix.

**Fig. 6.** Effect of 2-O- and 6-O-desulfation on the ability of heparin to augment PDGF-BB-induced PDGF α-receptor tyrosine phosphorylation. CHO 677 cells were exposed to 50 ng/ml PDGF-BB in the presence (+) or absence (−) of 2-O-desulfated (A and B) or 6-O-desulfated (C and D) heparin at the concentrations indicated. PDGF β-receptors (A and C) and PDGF α-receptors (B and D) were immunoprecipitated. Samples were subjected to SDS-PAGE and immunoblotting with anti-phosphotyrosine monoclonal antibody PY99, followed by stripping and immunoblotting with anti-PDGFP β-receptor antibodies or anti-PDGFA α-receptor antibodies.
through binding to heparan sulfate proteoglycans because treatment with heparitinase I allows release of biologically active growth factor (16). Binding of heparin to the long PDGF-AA isoform is dependent on N-, 2-O-, and 6-O-sulfation (31); the minimal size binding to PDGF-AA L is an octasaccharide. The long PDGF-A isoform contains an 18-amino acid residue polybasic stretch encoded by the alternatively spliced exon 6 in the PDGF-A chain gene. The PDGF-B isoform contains a similar but not identical polybasic stretch encoded by exon 6 in the PDGF-B chain gene. This stretch is proteolytically removed to generate the mature processed PDGF-BB. The removal of the polybasic stretch does not preclude heparin binding because the short PDGF-AA isoform, which lacks this stretch, still binds heparin, although with reduced affinity (32). Furthermore, three basic residues in the loop III receptor-binding domain present in the short and long form of PDGF-BB have been identified as important for heparin binding (33). The commercially available short form of PDGF-BB (Mr 24,300) used in this study presumably lacked the exon 6-encoded polybasic heparin-binding sequence but nevertheless retained binding capacity for heparin and modified heparin fragments as shown in a filter binding assay.

The mode of action of heparin/HS in relation to PDGF-BB and its PDGF/α-receptor remains unclear. The effect appears highly specific for this combination of ligand and receptor be-
cause heparin did not modulate PDGF-AA-stimulated PDGF α-receptor tyrosine phosphorylation. This is compatible with the observation that PDGF-AA and -BB bind with different affinities and induce different conformational changes in the PDGF α-receptor extracellular domain (34). The possibility that heparin/HS may physically interact not only with growth factors but also with their receptors has been argued for fibroblast growth factors (FGFs) and the corresponding receptors (FGFRs) (35–38). Indeed, x-ray crystallography studies of ternary complexes show heparin oligosaccharides in contact with both FGF and FGFR proteins (35, 39). We do not know whether PDGF receptor ectodomains bind heparin/HS. The interaction between heparin and FGFR-1 appears to involve a basic stretch, denoted K18K, in the FGFR-1 extracellular domain (residues Lys160 to Lys177) (40). Although there is no obvious polybasic region in the PDGF α-receptor extracellular domain, there is some sequence similarity between the FGFR-1 K18K sequence and regions in the PDGF α-receptor (data not shown); such a similarity is not recorded for the PDGF β-receptor in homology searches. Additional studies are needed to show whether the PDGF α-receptor binds heparin with any measurable affinity.

Our observation that PDGF α-receptor activation by PDG-BB is augmented not only by full-sized heparin but also by relatively short oligosaccharides would seem to argue against a bridging function for the saccharide in a ternary complex with growth factor and receptor. However, we note that similar (and unexplained) effects of small saccharides have been observed also in connection with FGF action (41, 42). Several additional possibilities may be considered. Saccharide binding may change the conformation of PDG-BB in such a way that its interaction with the PDGF α-receptor, but not with the PDGF β-receptor, is promoted. Alternatively, receptor binding of the saccharide may selectively make the PDGF α-receptor more receptive to the growth factor. On the other hand, the involvement of a more extended “bridging” domain could explain why the receptor-stimulatory effect decreases at higher heparin concentrations; under these conditions, the probability of binding PDG-BB and its receptor to the same polysaccharide chain will decrease. Conversely, the partially O-desulfated heparin derivatives may present fewer binding sites along the chain, thus explaining the persistent stimulation at higher saccharide concentration. Finally, we cannot exclude the possibility that the selective effect of heparin on PDG-α-receptor phosphorylation is mediated by an as yet unidentified protein ligand(s).

Notably, FGF-2 stimulation of FGF receptor-1 in the absence of heparin/HS elicits FGF receptor activation and signal transduction, but the spectrum of autophosphorylation sites employed and the range of signal transduction pathways that become initiated are limited compared with stimulation in the presence of heparin. These data indicate that signal transduction by receptor tyrosine kinases can be directed by heparin-mediated changes in receptor conformation or by effects on other properties of the kinase. Our data indicate that signal transduction and cellular responses to PDG-BB are augmented by heparin. Whether this effect is purely quantitative or also qualitative is an interesting issue for future studies.
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