Characterization of Baculovirus-Expressed Human α and β Platelet-Derived Growth Factor Receptors

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ABSTRACT: In an effort to biochemically characterize PDGF receptors and their mechanism of activation, recombinant baculovirus vectors containing the cDNAs of the human α PDGF receptor or β PDGF receptor were engineered. Characterization of recombinant PDGF receptor expression in infected Sf9 insect cells by immunoblot analysis with specific PDGF receptor peptide antisera revealed that the α and β PDGF receptor gene products were translated as 160- and 165-kDa transmembrane proteins, respectively. Ligand binding analysis demonstrated saturable, high-affinity binding of either ^{125}I-labeled PDGF AA or ^{125}I-labeled PDGF BB to Sf9 cells expressing the recombinant α PDGF receptor. In contrast, recombinant β PDGF receptor expressing Sf9 cells showed high-affinity binding only for PDGF BB. Analysis of the kinetics of PDGF receptor expression demonstrated that receptor number increased dramatically from 24- to 48-h postinfection. Early in infection, the PDGF receptors were present in low numbers, lacked tyrosine phosphorylation, and exhibited ligand-dependent tyrosine phosphorylation. However, with increasing time postinfection and increasing receptor number, the PDGF receptors became constitutively tyrosine-phosphorylated in serum-free culture medium. Cross-linking studies revealed that receptor activation involved ligand-independent receptor dimer formation at high receptor number. Thus, these results strongly suggest that PDGF stabilizes and increases the frequency of PDGF receptor interaction, which ultimately results in PDGF receptor activation and intracellular signaling.

Platelet-derived growth factor (PDGF) is a disulfide-linked dimer of two homologous polypeptides chains, designated PDGF A and PDGF B. Three PDGF isoforms (AA, AB, and BB) have been identified in vivo, and shown to possess biologic activity (Johnsson et al., 1982). The PDGF B chain is the homologue of the v-*sis* oncogene (Devare et al., 1983; Doolittle et al., 1983; Waterfield et al., 1983). Although originally isolated from platelets, PDGF has been shown to be produced by a variety of cell types, and represents an important mitogen for mesenchymal cells (Ross et al., 1986).

PDGF exerts its diverse effects (Deuel, 1987) by binding and activating either or both of two distinct high-affinity cell-surface receptors, termed the α and β PDGF receptors (Yarden et al., 1986; Matsui et al., 1989 a,b; Claesson-Welsh et al., 1989). The PDGF receptor subfamily of tyrosine kinase receptors includes the c-*fms* protooncogene product (Coussens et al., 1986), the c-*kit* protooncogene product (Yarden et al., 1987), and the recently identified *fms*-like tyrosine kinase, *flt* (Shibuya et al., 1990). All have extracellular components which exhibit an immunoglobulin-like domain structure with limited sequence homology and a characteristic spacing of cysteine residues. A distinguishing feature of this receptor subfamily is that the tyrosine kinase domains of the receptors are interrupted by a region of varying length termed the kinase insert domain which appears to be important in substrate specificity. PDGF interaction with its cognate receptors leads to activation of the tyrosine kinase and autophosphorylation of the receptor. In addition, there is increasing evidence that PDGF–PDGF receptor interaction is associated with the formation of receptor dimers (Hammacher et al., 1989; Heldin et al., 1989). Whether kinase activation precedes or follows dimer formation as well as the role of the ligands in inducing or stabilizing dimers remains to be elucidated. In the present studies, we have expressed human α and β PDGF receptors independently in a baculovirus expression system to investigate ligand–receptor interactions, as well as mechanisms of receptor activation.

EXPERIMENTAL PROCEDURES

Cell and Virus Culture. The insect cell line *Spodoptera frugiperda* (Sf9) was obtained from the Amercian Type Culture Collection and cultured at 27 °C in supplemented Grace's (Gibco) or EXCELL 400 (JR Scientific) medium. *Autographa californica* nuclear polyhedrosis virus (AcNPV) was a generous gift of Dr. M. Summers, Texas A&M University. Sf9 cells were infected with a multiplicity of infection of ≥10 plaque-forming units/cell for protein expression studies and 0.1–1.0 pfu/cell for virus stock production. NIH 3T3 cells were maintained at 37 °C in Dulbecco's minimal essential medium (DMEM) supplemented with 10% fetal bovine serum.

Antibodies. Rabbit antisera were raised against synthetic peptides corresponding to α PDGF receptor amino acid residues 959–973 or β PDGF receptor residues 967–981. A monoclonal antibody specific for phosphotyrosine, PY20, was purchased from ICN Biochemicals.

Construction of Recombinant Baculovirus Vectors. The baculovirus transfer vector pVL941 was kindly provided by Drs. V. Luckow and M. Summers (Luckow & Summers, 1989). Insertion of the human α and β PDGF receptor cDNAs into pVL941 involved manipulations which removed excess 5′ noncoding sequence. For the α PDGF receptor, oligonucleotides 5′-GTGAATTCGTCGACCCAGAGCTATGGGGACTTC-3′ and 5′-AGCATCTCAGAGAAAAGGAT-3′ were used in the polymerase chain reaction to generate a 124 base pair segment of the α PDGF receptor, which was then digested with *Eco*RI. This fragment was then cloned into pUC18-α PDGF receptor which had been similarly digested

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and purified by isolation on NA45 membranes (Schleicher & Schuell). The modified α PDGF receptor cDNA was isolated as a *Bam*HI fragment and cloned into the unqiue *Bam*HI site of pVL941(pVL-α PDGF receptor).

In the case of the β PDGF receptor, oligonucleotides 5'-CTGAATTCTGTCGACATGCGGCTTCCGGGTGCG-3' and 5'-CTGGCCCCGGGGGTGTGA-3' were used to generate a 140 base pair segment, which was digested with *Eco*RI and *Sma*I. The fragment was directionally cloned into pUC18-β PDGF receptor which had been similarly digested and isolated on NA45 membranes. The modified β PDGF receptor cDNA was removed as a *Sal*I fragment, partially filled in with DNA polymerase I, and cloned into the unique *Bam*HI site of pVL941(pVL-β PDGF receptor).

Recombinant baculovirus were produced by cotransfecting 2×10^6 Sf9 cells with AcNPV DNA (1 mg) and either pVL-α PDGF receptor (2 mg) or pVL-β PDGF receptor (2 mg) by calcium phosphate transfection. The resulting culture supernatants were harvested after 4 days, and screened for homologous recombination by visual inspection of plaques (Summers & Smith, 1987) which were confirmed by dot-blot hybridization using the respective ^{32}P-labeled, nick-translated cDNA probes. Purified recombinant baculovirus was obtained after three rounds of plaque purification, and designated Ac-α PDGF receptor or Ac-β PDGF receptor.

Time Course of Receptor Expression and Phosphorylation. Sf9 cells (9×10^6) infected with Ac-α PDGF receptor or Ac-β PDGF receptor were harvested at the indicated times postinfection by centrifugation, suspended in 2 mL of EXCELL 400 medium with or without PDGF ligand (Amgen), and placed at 37 °C. After 5 min, the cells were collected, washed once with PBS (phosphate-buffered saline)/1 mM Na_3VO_4, and lysed in 500 μL of Staph A buffer (10 mM sodium phosphate, pH 7.5, 100 mM NaCl, 1% Triton X-100, 0.1% SDS, and 0.5% deoxycholate) containing 1 mM Na_3VO_4, 1 mM phenylmethanesulfonyl fluoride, 10 μg/mL aprotinin, 10 μg/mL pepstatin, and 10 μg/mL leupeptin. The lysates were clarified by centrifugation at 16000*g* for 10 min and stored frozen at −70 °C. Total protein concentrations were determined by the method of Bradford (Bio-Rad).

PDGF Receptor Immunoblotting. Whole cell lysates were separated by SDS–PAGE using 5 or 7% gels as indicated, and transferred to Immobilon-P membranes (Millipore) in Towbin's buffer for 3 h at 1 A (Towbin et al., 1979). Nonspecific binding was blocked with 3% dry milk, and the blots were incubated with receptor-specific peptide antisera or PY20 at the indicated concentrations. The filters were washed with Tris-buffered saline/0.05% Tween 20 (TTBS), and bound antibody was detected with ^{125}I-protein A. Densitometric analysis of the autoradiographs was performed with a Bio-Rad Model 620 video densitometer.

PDGF Receptor Binding Assay. Sf9 cells (5×10^4) infected with Ac-α or Ac-β receptor were harvested 30-h postinfection, mixed with 2.95×10^6 uninfected cells, and aliquoted into 1.5-mL Eppendorf tubes. The cells were pelleted (500*g* for 5 min), washed once with ice-cold binding buffer (DMEM/25 mM HEPES/1.0% BSA), and resuspended in binding buffer containing increasing concentrations of ^{125}I-labeled PDGF ligand with or without a 100-fold excess of unlabeled PDGF. After 2 h at 4 °C, the cells were pelleted and washed twice with binding buffer, and the bound radioactivity was counted in a Beckman 5500 γ counter. The data were analyzed by the binding analysis program LIGAND (Munson & Rodbard, 1980).

PDGF Receptor Cross-Linking and Dimerization Studies. At 28- and 48-h postinfection, monolayers of Sf9 cells (9×10^6) infected with Ac-α or Ac-β PDGF receptor were incubated in the presence or absence of 50 ng/mL PDGF at 37 °C for 5 min. Next, the cells were washed once in cross-linking buffer (10 mM HEPES/150 mM NaCl) and placed at room temperature for 15 min in cross-linking buffer with or without 0.1 mM disuccinimidyl suberate (DSS). Then, 0.1 volume of Quench reagent (200 mM glycine/10 mM Tris/4 mM EDTA) was added, and after 2 min, the cells were washed once with Quench reagent diluted 1:10 in cross-linking buffer. Cells were then harvested, lysed in Staph A buffer as described, and analyzed by immunoblotting after separation on 5% SDS–polyacrylamide gels.

RESULTS

Baculovirus Expression of Recombinant PDGF Receptors. Although numerous biological and biochemical parameters are altered by the binding of PDGF to its cognate receptors, the exact mechanism by which this physical interaction brings about these changes is largely unknown. In an effort to better characterize this interaction, and produce sufficient quantities of native and mutant receptors for structural, functional, and biochemical studies, we utilized the baculovirus expression system to produce recombinant human α and β PDGF receptors. The cDNAs for each PDGF receptor were cloned into the baculovirus transfer vector pVL941 and then cotransfected with wild-type AcNPV DNA. Recombinant viruses were identified, purified by visual inspection of plaques, and confirmed by dot-blot hybridization.

To initially characterize baculovirus recombinant PDGF receptor expression, we immunoblotted Ac-α PDGF receptor- or Ac-β PDGF receptor-infected Sf9 whole cell lysates with anti-peptide sera specific for each receptor carboxyl terminus. Whereas receptor-specific bands were not present either in uninfected Sf9 cells or in wild-type virus-infected cells, Ac-α PDGF receptor-infected Sf9 cells expressed a major p160 and a minor p150 species, both of which were identified by α PDGF receptor anti-peptide serum (Figure 1A). Anti-α PDGF receptor serum recognition was specifically blocked by competition with the homologous peptide. Recombinant baculovirus Ac-β PDGF receptor-infected Sf9 cells also produced two translational products, p165 and p150, which were immunoreactive with β PDGF receptor anti-peptide serum and whose recognition was blocked by homologous peptide competition (Figure 1B). In each case, the higher molecular weight form was the predominant species. Moreover, the recombinant PDGF receptors were expressed as a major and a minor species analogous to the mammalian mature and immature PDGF receptors, respectively (Matsui et al., 1989a,b).

To achieve maximal levels of PDGF receptor expression, we analyzed α and β PDGF receptors at various time intervals postinfection. These experiments, performed in parallel for each receptor, demonstrated that infected Sf9 cells under serum-free conditions expressed PDGF receptors at detectable levels by 24-h postinfection. PDGF receptor levels increased rapidly, and peaked between 40 and 48 h. At 48 h, the level of receptor expression was essentially equivalent for either serum-free or serum-supplemented medium (data not shown). The maximal levels achieved were approximately 50–100-fold greater than that detected by immunoblotting an equivalent amount of NIH 3T3 cell lysates.

Characterization of PDGF Binding to Baculovirus-Expressed PDGF Receptors. To determine whether recombinant

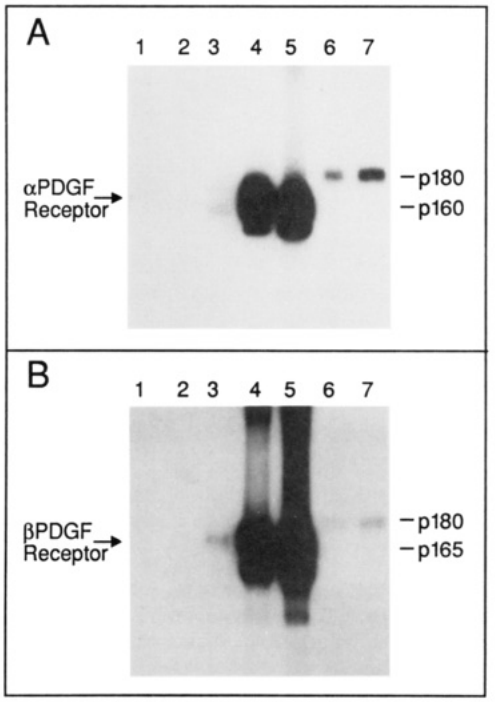

FIGURE 1: Immunochemical identification of human PDGF receptors expressed in baculovirus-infected Sf9 cells. Fifty micrograms of whole cell lysates from uninfected (lane 1), wild-type (lane 2), Ac-α (panel A, lanes 3–5), or Ac-β (panel B, lanes 3–5) infected Sf9 cells was subjected to SDS–PAGE under reducing conditions and immunoblotted with antisera specific for the α (panel A) or β (panel B) PDGF receptors. Ac-α- or Ac-β-infected Sf9 cells were harvested after 24 (lane 3), 36 (lane 4), or 48 (lane 5) h. Relative receptor expression levels were compared directly to those of NIH 3T3 fibroblasts (lanes 6 and 7). In lane 7, 100 μg of NIH 3T3 lysate was loaded. Primary antisera were detected with ^{125}I-protein A. Proteins were visualized after autoradiography.

Table I: Dissociation Constants of Baculovirus-Expressed Human α and β PDGF Receptors Determined by Scatchard Analysis[a]

ligand	α PDGF receptor	β PDGF receptor
PDGF AA	11 nM (13×10^5)	ND[b]
PDGF BB	6.2 nM (9×10^5)	3.3 nM (6×10^5)

[a] Saturable binding was achieved between 250 and 500 ng/mL PDGF for either PDGF receptor. Numbers in parentheses indicate the number of PDGF receptors per Sf9 cell assuming each receptor possesses one PDGF binding site. [b] No specific binding was detected at 500 ng/mL PDGF AA.

PDGF receptors expressed in the baculovirus system were able to functionally interact with PDGF ligand, we performed binding analysis with radiolabeled PDGF AA or BB. These studies also allowed us to assess whether the receptors were located at the cell surface as transmembrane receptors. Analysis of radiolabeled PDGF binding to Ac-α PDGF receptor-infected Sf9 cells demonstrated that both PDGF AA and PDGF BB showed saturable binding consistent with the known binding properties of the α PDGF receptor (data not shown). Scatchard analysis revealed that PDGF AA bound with a dissociation constant of 11 nM while PDGF BB demonstrated a dissociation constant of approximately 6 nM (Table I).

Next, we examined PDGF binding to Ac-β PDGF receptor-infected Sf9 cells. Radiolabeled PDGF binding to the recombinant β PDGF receptor revealed that while PDGF BB exhibited high-affinity saturable binding, PDGF AA failed to detectably bind. Scatchard analysis showed that PDGF BB possessed a dissociation constant of approximately 3 nM (Table I). Although these relative affinities are 3–6-fold lower

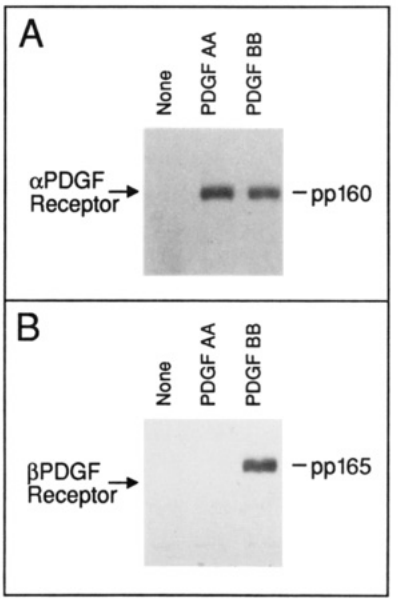

FIGURE 2: Ligand specificity of PDGF receptor tyrosine phosphorylation in baculovirus-infected Sf9 cells. Sf9 cells were infected with Ac-α PDGF receptor (panel A) or Ac-β PDGF receptor (panel B) for 28 h. Cells untreated (lane 1) or treated with 50 ng/mL PDGF AA (lane 2) or PDGF BB (lane 3) were harvested and lysed as described. Fifty micrograms of total protein per lane was loaded on a 7% SDS–polyacrylamide gel. Proteins were separated by SDS–PAGE under reducing conditions, transferred to Immobilon-P, and immunoblotted with anti-phosphotyrosine-specific antibody. Bound primary antibody was detected with ^{125}I-protein A.

than that determined for either mammalian PDGF receptor, each recombinant receptor bound the PDGF homodimers in a highly specific manner, consistent with the known ligand binding properties of PDGF receptors expressed by human or mouse cells (Claesson-Welsh et al., 1989; Heidaran et al., 1990). Furthermore, the baculovirus-expressed PDGF receptors were accessible to ligand binding at the cell surface, indicating that they achieve a cell-surface orientation consistent with their transmembrane nature.

Ligand-Dependent PDGF Receptor Tyrosine Phosphorylation. Analysis of PDGF receptors expressed in human and mouse cells has demonstrated that PDGF AA activates only the α PDGF receptor while PDGF BB activates both the α and β PDGF receptors (Claesson-Welsh et al., 1988, 1989; Matsui et al., 1989a,b). To determine whether human PDGF receptors expressed in baculovirus-infected Sf9 cells possessed similar properties, as well as to analyze the functional integrity of their tyrosine kinase domains, we investigated the responsiveness of these receptors to ligand-stimulated tyrosine phosphorylation. Whole cell lysates of α PDGF receptor-infected Sf9 cells grown in serum-free medium and triggered with PDGF were subjected to SDS–PAGE and immunoblotted with anti-phosphotyrosine monoclonal antibody. While untriggered cells demonstrated little PDGF receptor phosphorylation at 24-h postinfection, PDGF AA or PDGF BB activated the α PDGF receptor as demonstrated by anti-phosphotyrosine monoclonal antibody recognition of the p160 receptor species (Figure 2).

Next, we examined growth factor triggering of β PDGF receptor-infected Sf9 cells. PDGF AA or PDGF BB was added to intact Sf9 cells expressing the β PDGF receptor, and whole cell lysates were prepared. SDS–PAGE followed by immunoblotting with the anti-phosphotyrosine monoclonal antibody showed that PDGF AA failed to activate the β PDGF receptor at concentrations exceeding 250 ng/mL while PDGF BB induced readily detectable tyrosine phosphorylation of

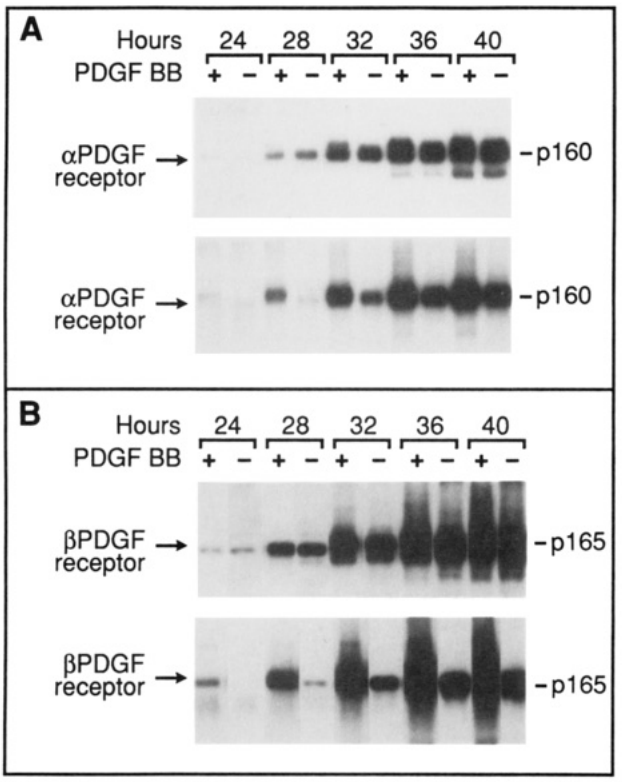

FIGURE 3: Comparison of ligand-dependent and -independent PDGF receptor tyrosine phosphorylation in baculovirus-infected Sf9 cells. Sf9 cells were infected with Ac-α (panel A) or Ac-β (panel B) PDGF receptor and cultured in serum-free medium for the length of time indicated. Infected Sf9 cells were incubated in the presence or absence of PDGF BB. The cells were harvested and lysed, and 50 μg of total protein was loaded per lane on a 7% SDS–polyacrylamide gel. Proteins were separated by SDS–PAGE and, after transfer to Immobilon-P, immunoblotted with anti-α PDGF receptor antisera (panel A, top), anti-β PDGF receptor antisera (panel B, top), or anti-phosphotyrosine monoclonal antibody (panels A and B, bottom). Bound primary antibody was detected as described.

the p165 receptor species. All of these results indicated that the recombinant PDGF receptors expressed in Sf9 cells showed ligand specificities and activation properties indistinguishable from those of receptors expressed in mammalian cells.

Ligand-Independent PDGF Receptor Phosphorylation. While basal levels of PDGF receptor autophosphorylation were barely detectable early in infection, we observed readily detectable tyrosine phosphorylation of the 160-kDa α and 165-kDa β PDGF receptor species in the absence of exogenous PDGF at times later than 24 h. The identity of these proteins as either α or β PDGF reeceptors was further confirmed with peptide antisera specific for either PDGF receptor after anti-phosphotyrosine immunoaffinity purification (data not shown). Detailed analysis revealed tyrosine phosphorylation of the α and β PDGF receptors as early as 28-h post infection in serum-free medium and the absence of PDGF triggering (Figure 3A,B, lower panels). Early in the course of infection (28 h), the relative level of endogenous receptor phosphorylation observed without ligand stimulation was low ($\sim$5%) compared to that observed with PDGF BB-induced receptor phosphorylation. Furthermore, the addition of sodium vanadate did not significantly increase the phosphotyrosine content of either PDGF receptor at 28 h (data not shown). However, both α and β PDGF receptor phosphorylation increased markedly in the absence of ligand (up to $\sim$75% of that triggered with ligand) with increased time and receptor number (Figure 3A,B, lower panels). The PDGF receptor-specific antisera blots demonstrated essentially equivalent receptor protein levels in the presence or absence of ligand (Figure 3A,B, upper panels).

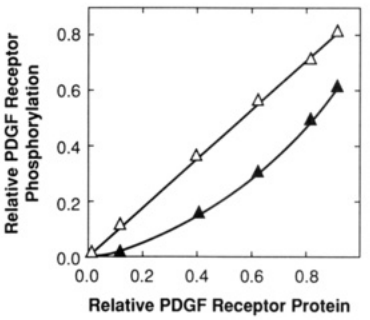

FIGURE 4: Relative PDGF receptor phosphorylation in the presence or absence of PDGF as a function of PDGF receptor protein. Intact Sf9 cells expressing the β PDGF receptor were incubated with (△) or without (▲) 50 ng/mL PDGF BB between 24- and 48-h postinfection. Whole cell lysates at each time interval were prepared, separated by SDS–PAGE, and immunoblotted with either β PDGF receptor-specific antiserum or a monoclonal antibody specific for phosphotyrosine as shown in Figure 4. The autoradiograms were then analyzed densitometrically to determine absolute receptor protein levels as well as phosphorylation levels. In the case of PDGF–treated cells, maximal PDGF receptor phosphorylation was achieved with saturating levels of PDGF BB and normalized to 100% for each time point. Therefore, PDGF receptor phosphorylation as a function of receptor protein has a defined slope of 1 for PDGF-treated cells.

Mechanistic Basis of Ligand-Independent PDGF Receptor Activation. Recent studies have suggested that PDGF-induced receptor dimerization is associated with PDGF receptor activation. Scanning densitometric analysis of α or β PDGF receptor phosphorylation in baculovirus-infected Sf9 cells demonstrated that the signal level for ligand-independent receptor phosphorylation appears to increase exponentially with increasing receptor number while the signal level of ligand-dependent receptor phosphorylation linearly parallels the signal observed for receptor protein (Figure 4). These results are consistent with the concept that PDGF receptor phosphorylation in the absence of ligand follows second-order kinetics.

As an alternative approach to determine whether high concentrations of baculovirus-expressed PDGF receptors interacted in the absence of ligand and whether this might explain their activation, we performed biochemical cross-linking experiments. Recombinant β PDGF receptors from baculovirus-infected Sf9 cells grown in monolayer were untreated or exposed to PDGF BB, and incubated in the presence or absence of DSS cross-linking reagent at 28- and 48-h postinfection. Immunoblot analysis of whole cell lysates revealed that at 28-h postinfection, no PDGF receptor dimers were detected in the absence of PDGF, while dimers were readily identified in PDGF-triggered cells. Thus, detection of cross-linked receptors appeared to be PDGF-dependent at this time (Figure 5). However, late in the course of infection with considerably increased receptor numbers, PDGF receptor dimers were readily identified either in the absence or in the presence of PDGF ligand. These findings support the conclusion that the activation of recombinant PDGF receptors at high levels of receptor expression in the absence of ligand is due to the increased probability of receptor interaction and resulting dimer formation.

DISCUSSION

We have expressed and characterized the human α and β PDGF receptors in baculovirus-infected Sf9 insect cells. These recombinant PDGF receptors were similar to the native α and β PDGF receptors expressed in mammalian cells both by immunochemical analysis and by PDGF isoform binding studies. Like the mammalian PDGF receptors, both α and β PDGF receptors expressed in Sf9 insect cells appear to be

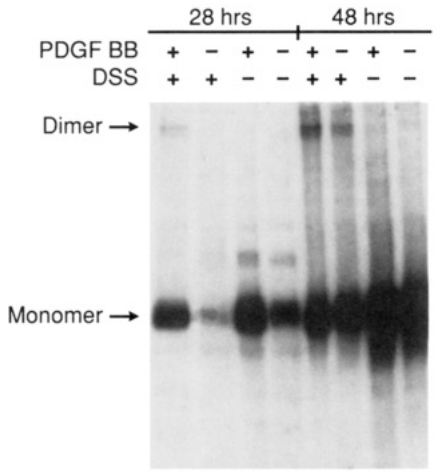

FIGURE 5: Biochemical cross-linking of human PDGF receptor dimers. Intact Sf9 cells infected with the β PDGF receptor were cross-linked with DSS in the presence or absence of PDGF BB triggering at time points early (28 h, 200 μg of total protein per lane) or late (48 h, 25 μg of total protein per lane) in infection. The lysates were then subjected to SDS–PAGE on 5% polyacrylamide gels under reducing conditions, transferred to Immobilon-P, and probed with a monoclonal antibody specific for phosphotyrosine as described. Bound monoclonal antibody was detected as described. In the presence of DSS at 48 h, cross-linked material was also found in the stacking gel which accounted for loss of the receptor monomer relative to the non-DSS-treated receptor.

synthesized and glycosylated as immature receptors which are then processed to mature forms. Furthermore, ligand binding and activation studies demonstrated that both PDGF receptors were oriented properly in the Sf9 cell membrane since their ligand binding domains were readily accessible to ligand stimulation in intact cells. Finally, at expression levels similar to those observed in mammalian cells, PDGF-dependent triggering of the α and β PDGF receptors showed patterns indistinguishable from that observed in mammalian cells.

The baculovirus-expressed PDGF receptors were detected as predominant p160 or p165 species with minor p150 species. These two PDGF receptor species appear to be analogous to the mammalian fully glycosylated mature and core-glycosylated immature PDGF receptors of approximately 180 kDa and 160 or 165 kDa, respectively (Matsui et al., 1989a,b). The observed size differences between recombinant PDGF receptors expressed in Sf9 cells and the native mammalian PDGF receptors appear to be attributable to the known differences in insect versus mammalian patterns of posttranslational modification. This phenomenon has been observed in numerous other recombinant proteins expressed in the baculovirus expression system (Bailey et al., 1989; Quelle et al., 1989; Sissom & Ellis, 1989) and is due to differences in the terminal glycosylation patterns of mammalian and insect cells. Consistent with these observed differences in molecular weight are lectin binding experiments from this laboratory in which the presence of the core oligosaccharide without terminally added complex oligosaccharides was observed on the recombinant PDGF receptors (R. Jensen, unpublished observations). Despite these differences in posttranslational modifications, complex oligosaccharides do not appear essential for PDGF binding, nor did they determine isoform specificity since both PDGF A and B are bound to their cognate receptors with the expected specificity in the absence of these posttranslational modifications. However, the possibility that these differences in glycosylation may account for the slight decrease in binding affinity as compared to the mammalian PDGF receptors cannot be excluded.

One observation which was particularly striking with recombinant PDGF receptors expressed in Sf9 cells was ligand-independent receptor activation at high levels of receptor expression. Ligand-independent activation is perhaps best understood in relation to the postulated models of ligand–receptor interaction (Schlessinger, 1986; Heldin et al., 1989). One potential model suggests that binding of the ligand induces conformational changes within the cognate receptor. Thus, PDGF receptor conformational changes induced by the ligand would be sufficient for receptor activation and signal transduction. An alternative model suggests that the dimeric ligand acts to recruit and stabilize PDGF receptor dimers. This model predicts that ligand-induced receptor dimer or oligomer formation is required for activation of the tyrosine kinase domain by a mechanism that involves interaction between adjacent tyrosine kinase domains. Our present studies have demonstrated that very high receptor expression levels lead to tyrosine phosphorylation in the absence of ligand. In addition, ligand-independent activation of the PDGF receptor involved dimer formation with concomitant phosphorylation by a mechanism which appeared to exhibit second-order kinetics. Thus, while PDGF receptors are able to undergo interactions leading to activation in the absence ligand at high receptor numbers, ligand binding appears crucial for activation of physiological levels of PDGF receptor. This would imply that the process of ligand–receptor binding serves primarily to recruit and/or stabilize receptor dimers.

Other transmembrane tyrosine kinase receptors such as the insulin and EGF receptors have been expressed in Sf9 cells (Greenfield et al., 1988; Paul et al., 1990). In each case, tyrosine phosphorylation in the absence of ligand has not been observed. In fact, tyrosine phosphorylation of these receptors appears to be completely ligand-dependent. In contrast, expression of truncated insulin or EGF receptors lacking their ligand binding domains was associated with ligand-independent phosphorylation (Herrera et al., 1988; Wedegaertner & Gill, 1989). Whether the observed differences between full-length PDGF receptors and other full-length receptor constructs are related to higher levels of expression or less stringent regulation from the PDGF receptor ligand binding domain is not known. In any case, the results presented here demonstrate that human PDGF receptors expressed in the baculovirus system should be suitable for biochemical investigations aimed at identifying regions of the receptor involved in substrate interactions as well as novel substrates involved in PDGF receptor-mediated signal transduction.

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