The Crystal Structure of Human Placenta Growth Factor-1 (PlGF-1), an Angiogenic Protein, at 2.0 Å Resolution*

Received for publication, September 4, 2000, and in revised form, November 6, 2000
Published, JBC Papers in Press, November 7, 2000, DOI 10.1074/jbc.M008055200

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The angiogenic molecule placenta growth factor (PlGF) is a member of the cysteine-knot family of growth factors. In this study, a mature isoform of the human PI GF protein, PI GF-1, was crystallized as a homodimer in the crystallographic asymmetric unit, and its crystal structure was elucidated at 2.0 Å resolution. The overall structure of PI GF-1 is similar to that of vascular endothelial growth factor (VEGF) with which it shares 42% amino acid sequence identity. Based on structural and biochemical data, we have mapped several important residues on the PI GF-1 molecule that are involved in recognition of the fms-like tyrosine kinase receptor (Flt-1, also known as VEGFR-1). We propose a model for the association of PI GF-1 and Flt-1 domain 2 with precise shape complementarity, consider the relevance of this assembly for PI GF-1 signal transduction, and provide a structural basis for altered specificity of this molecule.

Angiogenesis, the process of new blood vessel formation, is essential for development, reproduction, wound healing, tissue regeneration, and remodeling (1). It also plays a major role in tumor progression, diabetic retinopathy, psoriasis, and rheumatoid arthritis (2). Angiogenesis involves proliferation of endothelial cells (ECs)1 in an organized fashion and is most likely regulated by polypeptide growth factors (3, 4) such as acidic and basic fibroblast growth factors (aFGF and bFGF, Ref. 5), vascular endothelial growth factor (VEGF, Refs. 6–10), and placenta growth factor (PI GF, Refs. 11–14). PI GF, VEGF (VEGF-A), VEGF-B (15), VEGF-C (16), VEGF-D (17), VEGF-E (18), and Fos-induced growth factor (PI GF, Ref. 19) are members of a family of structurally related growth factors. Intra- and interchain disulfide bonds among eight characteristically spaced cysteine residues are involved in the formation of these active dimeric proteins and hence termed as cysteine-knot proteins. They also share a number of biochemical and functional features (for a review, see Ref. 20) such that PI GF and VEGF can form heterodimeric molecules in cells in which both genes are expressed (21, 22).

Alternative splicing of the PI GF primary transcript leads to three forms of the mature human PI GF protein (22–24). The two predominant forms, PI GF-1 and PI GF-2 (also known as PI GF-131 and PI GF-152, respectively), differ only by the insertion of a highly basic 21-amino acid stretch at the carboxyl end of the protein. This additional basic region confers upon PI GF-2 the ability to bind to heparin (13, 23).

The exact role of PI GF in vascular development is yet to be established. However, purification of PI GF-1 from overexpressing eukaryotic cells and measurement of angiogenic activity of the purified PI GF-1 in vivo in the rabbit cornea and chick chorioallantoic membrane (CAM) assays showed induction of a strong neovascularization process that was blocked by affinity-purified anti-PI GF-1 antibody. In the avascular cornea, PI GF-1 induced angiogenesis in a dose-dependent manner and seemed to be at least as effective (if not more effective) as VEGF and bFGF under the same conditions and at the same concentration. PI GF-1 was shown to induce cell growth and migration of endothelial cells from bovine coronary postcapillary venules and from human umbilical veins (HUVECs). In these two in vitro assays, PI GF-1 seemed to have a comparable effect on the cultured microvascular endothelium (e.g. capillary venule endothelial cells, CVECs) to that of VEGF and bFGF. These results clearly demonstrate that PI GF-1 can induce angiogenesis in vivo and stimulate the migration and proliferation of endothelial cells in vitro (25). In the case of PI GF-2 it has been established that the recombinant, purified protein is able to stimulate bovine aortic endothelial cells (BAEC, Ref. 13) and HUVECs but not the ECs from hepatic sinusoids (26).

The VEGF homodimer binds to and induces autophosphorylation of two distinct kinase receptors: the fms-like tyrosine kinase, Flt-1 (also known as VEGFR-1) and the kinase insert domain-containing receptor/fetal liver kinase, KDR/Flk-1 (also known as VEGFR-2). Conversely, the PI GF-1 and -2 homodimer bind only to the Flt-1 receptor (22, 26–28). Likewise, VEGF-B also binds selectively to Flt-1 and hence appears to be...
a closer homolog of PIGF in its receptor-binding profile (29). Purified heterodimeric VEGF/PIGF has been shown also to bind KDR/Flk-1 (22). The extracellular portion of both receptors consists of seven immunoglobulin (IgG)-like domains, and the receptors share 44% amino acid sequence homology. The IgG-like domain 2 of the Flt-1 receptor is responsible for the binding specificity of PIGF-1 and -2 (30–32). Furthermore, it has been reported that only PIGF-2 can recognize neuropilins-1 and -2, receptor molecules found at the endothelial surface, in a heparin-dependent fashion (33, 34).

Since PIGF has been shown to bind and induce autophosphorylation of Flt-1 but not KDR/Flk-1, it appears that PIGF should exert its mitogenic and chemotactic effects on ECs through the activation of the Flt-1 intracellular signaling pathway. PIGF induces DNA synthesis but not migration of porcine aortic ECs (PAE) overexpressing Flt-1 (28). However, recent findings that PIGF is mitogenic and chemotactic for CVECs and HUVECs in vitro (25) (discussed above), raise the question of whether PIGF induces Flt-1 directly to transduce mitogenic and chemotactic signals inside the cell or whether PIGF acts indirectly through a mechanism of decoy, as previously proposed by Park et al. (14).

The recent observation that Flt-1 is able to mediate signaling in HUVECs in response to both PIGF and VEGF, leading to distinct biological responses, suggests that Flt-1 does not act as...

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**FIG. 1.** Structural comparison of PIGF-1 and other members of the cysteine-knot super family. A, representation of the PIGF-1 homodimer structure. Disulfide bonds are shown in a ball-and-stick representation. The inset presents the organization of three intra- (in yellow) and one interdisulfide bridge (in green) in the cysteine-knot motif. Each monomer in the homodimer is colored differently to enhance clarity. Orange, monomer A; cyan, monomer B. B, representatives of known structures from the cysteine-knot protein family of dimeric molecules. a, VEGF (PDB code 2VPF, Ref. 39); b, PDGF-BB (PDB code 1PDG, Ref. 51); c, TGF-β2 (PDB code 1TFG, Ref. 52); and d, NGF (PDB code 1BTG, Ref. 53). With the exception of NGF, the homodimer 2-fold axis is perpendicular to the plane of the β-sheet. The cysteine knots are highlighted.

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**TABLE I**

Crystallographic statistics

| Dataset | PIGF-1 |
|---------|--------|
| **Data collection statistics** | |
| Unit cell dimensions (P43, 1 homodimer/a.u.) | a = b = 62.6 Å, c = 84.1 Å |
| Resolution (Å) | 40–2.0 |
| Reflections measured | 161,044 |
| Unique reflections | 21,067 |
| Rsym (%) | 6.3 |
| I/σ(I) (outermost shell) | 19.6 (3.6) |
| Completeness (outermost shell) (%) | 99.4 (98.8) |
| **Refinement statistics** | |
| Rcryst (%) | 21.6 |
| Rfree (%) | 24.7 |
| Number of protein atoms (homodimer) | 1,546 |
| Number of solvent molecules (homodimer) | 132 |
| R.m.s. deviation in bond lengths (Å) | 0.010 |
| R.m.s. deviation in bond angles (°) | 1.5 |
| Average B-factor for protein atoms (Å²) | 32.6 |
| Average B-factor for side-chain atoms (Å²) | 32.7 |
| Average B-factor for solvent molecules (Å²) | 44.8 |
| B-factor (from Wilson plot) (Å²) | 33.9 |

* Rsym = Σ(|Ij| - 〈I〉)/Σ(〈I〉) where Ij is the observed intensity of reflection j, and 〈I〉 is the average intensity of multiple observations.

* Rcryst = Σ(|Fo| - |Fc|)/Σ(|Fo|), where Fo and Fc are the observed and calculated structure factor amplitudes, respectively.

* Rfree is equal to Rcryst for a randomly selected 4% subset of reflections not used in the refinement.
Inhibition of PlGF translation by antisense mRNA in the human dermal microvascular endothelial cells in culture results in the inhibition of cell proliferation under hypoxic conditions (36). These new findings assign a role to PlGF in the direct control of endothelial cell proliferation, probably competing with VEGF for binding to Flt-1 and thereby forcing the binding of VEGF to the KDR/Flk-1 and activating cell proliferation. In addition, both PlGF and VEGF are able to induce migration of 39% and 51% of monocytes, respectively, through activation of Flt-1 (35, 39). This suggests that PlGF may induce EC migration and proliferation through activation of Flt-1, although the existence of a yet unknown PlGF receptor cannot be ruled out.

A considerable amount of structural information is now available. A structure-based sequence alignment of PlGF-1 with VEGF (38, 39). Amino acid residues that form part of the secondary structural elements (β-strands and helices) as determined by DSSP (60) are shown in blue and red, respectively. The cysteine residues are shaded pink. VEGF residues involved in Flt-1 (VEGFR-1) binding (40), and the equivalent residues in PlGF-1 (based on a modeling study) are boxed and shaded in yellow. The conserved glycine residue in both structures is underlined. This figure was created with the program ALSCRIPT (61). D, stereo view displaying the Cα traces of PlGF-1 (orange) and VEGF (cyan) (39) homodimers after alignment of the two structures with the program “O” (49). A, B, and D were created with the program MOLSCRIPT (59).
available for VEGF (VEGF-A). Muller et al. (38, 39) reported the crystal structures of the receptor binding domain of VEGF in different crystal forms and have identified the KDR binding site using mutational analysis. Also, Wiesmann et al. (40) have reported the crystal structure of VEGF in complex with domain 2 of Flt-1 (Flt-1D2). To understand the specific molecular details of the receptor binding site and critical components of the homodimer, which will consequently help in understanding the differences in specificity and cross-reactivity among the VEGF homologs, we have embarked on a three-dimensional structural study of PlGF. Here we report the crystal structure of PlGF-1 at 2.0 Å resolution. As anticipated, the structure is
similar to that of VEGF. However, it shows subtle differences in molecular interactions at the receptor recognition site that appear to be relevant to signaling.

**EXPERIMENTAL PROCEDURES**

**Protein Expression and Purification**—By polymerase chain reaction, the region of the human PlGF-1 gene coding for the mature protein was cloned into a prokaryotic expression vector as described previously (11). The recombinant vector was used to transform a DE3 Escherichia coli strain, and the synthesis of PlGF-1 was induced by 1 mM isopropyl-2-thiogalactopyranoside. After preparation and refolding of the inclusion bodies, the PlGF-1 protein was purified first by anion exchange chromatography followed by reverse phase chromatography. Final recovery of the active protein was about 140 mg per liter of initial bacterial culture. The identity of the protein was checked by various assays such as immunoblotting, SDS-polyacrylamide gel electrophoresis under reducing and nonreducing conditions, two-dimensional electrophoresis, reverse phase chromatography, and amino-terminal sequencing. The angiogenic activity was tested using a CAM assay (41); the purified protein was found to induce a strong and dose-dependent angiogenic response (42).

**Crystallization**—Crystals of recombinant PlGF-1 were grown using the hanging drop vapor diffusion method from drops containing 8 mg/ml protein at pH 6.0 in 0.05 M MES buffer, 10 mM CaCl₂ and 7.5% (v/v) 2-methyl-2,4-pentanediol (MPD) equilibrated against reservoirs containing 0.1 M MES buffer (pH 6.0), 20 mM CaCl₂ and 15% (v/v) MPD. Single crystals appeared after 5–6 days at 16 °C. These crystals could be flash-frozen at 100 K using a cryoprotectant solution containing 0.1 M MES buffer (pH 6.0), 20 mM CaCl₂ and 15% (v/v) MPD, with unit cell dimensions a = b = 62.6 Å, and c = 84.1 Å. There was one PlGF-1 homodimer per crystallographic asymmetric unit and ~50% of the crystal volume was occupied by solvent.

**Data Processing and Reduction**—X-ray diffraction data to 2.0 Å were collected at 100 K from a single crystal using the Synchrotron Radiation Source (station FX 9.5) at Daresbury (United Kingdom). Seventy images were collected (λ = 1.0 Å, oscillation range of 1.5°, 45 s exposure time) using a MAR-CCD detector system. Data processing was performed with the HKL package (43). Data reduction was carried out using the program TRUNCATE of the CCP4 suite (44). Details of data processing statistics are presented in Table I.

**Phasing**—The structure of PlGF-1 was determined by molecular replacement with the program AMoRe (45) using a polyalanine (homodimer) model based on the structure of VEGF at 1.93 Å resolution (PDB code 2VPF, Ref. 39). Data in the range 15.0–3.0 Å were used for both the rotation and the translation function searches. No solution was found in space group P4₁. In space group P4₃, the best solution after fitting had a correlation coefficient of 56% and an R-factor of 51%. Rigid-body refinement with CNS version 0.9 (46) of this model corresponding to the highest peak using data in the range 40.0–2.0 Å, resulted in an R cryst of 44.6 and 40.6%, respectively.

**Refinement**—All crystallographic refinement was carried out using the program CNS version 0.9 (46). Procedures carried out with CNS included simulated annealing using a maximum likelihood target function, restrained individual B-factor refinement, conjugate gradient minimization, and bulk solvent correction. The behavior of the R cryst value (811 reflections) was monitored throughout refinement. Several rounds of refinement (using all reflections) and model building were performed until the R cryst for the model could not be improved any further. During the final stages of refinement, water molecules were inserted into the model at positions where peaks in the Fobs − |Fcalc| electron density maps had heights greater than 3σ and were at hydrogen bond forming distances from appropriate atoms. 2 |Fobs|−|Fcalc| maps were also used to verify the consistency in peaks. Water molecules with a temperature factor greater than 65 Å² were excluded from the model and subsequent refinement. One bound MPD molecule per monomer from the crystallization medium was identified (interacting with the main-chain carbonyl oxygen atom of Thr-104 at one end and a water molecule at the other end) and was included in the final stages of the refinement. The details of refinement are presented in Table I. Map calculations were performed with CNS with the SigmaA protocol (47), using all the reflections in the resolution range 40.0–2.0 Å. The program PROCHECK (48) was used to assess the quality of the final structure. Analysis of the Ramachandran (φ-ψ) plot showed that all residues lie in the allowed regions. The program O (49) was used for map visualization and model building.

**Accession Number**—Final atomic coordinates of human PlGF-1 have been deposited with the RCSB Protein Data Bank under the accession code 1FZV.
RESULTS AND DISCUSSION

Quality of the Structure—The crystal structure of PlGF-1 was determined at 2.0 Å resolution. Details of the data collection and refinement statistics are shown in Table I. The protein crystallizes as a homodimer in the asymmetric unit. As in the VEGF structure (38), the first 17 amino-terminal residues of both monomers are not visible in the electron density map and were excluded from crystallographic refinement. Both monomers A and B contain residues 18–117. Also, residues Ser115, Glu49, Glu73, Asn74, and Ser94 in both molecules and residues Glu84 and Arg57 in molecule A have been modeled as alamines because of lack of sufficient density beyond β atoms. The arrangement of the homodimer and the nomenclature used throughout the text are shown in Fig. 1A. The final model (homodimer) includes 1,546 non-hydrogen protein atoms, 132 water molecules, and two MPD molecules with a crystallographic R-factor (Rcryst) of 21.6% in the resolution range 40.0–2.0 Å. The Rfree value is 24.7% with 4% of the reflections excluded from the refinement (50). The mean coordinate error calculated from a plot of ln σa versus (sin θ/λ)2 is 0.3 Å. The root mean square (r.m.s.) deviation in Ca atoms between each monomer of the pair is 0.43 Å (for 100 Ca atoms). Regions that deviate most include residues 18–19 from the amino-terminal tail, part of the loop connecting strands β3 and β4 (residues 72–73), and the carboxyl-terminal residue 117. Excluding these residues improves the r.m.s. deviation to 0.17 Å (for 94 Ca atoms). Examination of the Ramachandran plot shows 91.5% of the residues in most favorable regions and no residues in disallowed regions.

Overall Structure—The crystal structure of PIGF-1 consists of a homodimer, organized in an antiparallel arrangement with the 2-fold axis perpendicular to the plane of the β-sheet (Fig. 1A). The homodimer is covalently linked by two interchain disulfide bonds between Cys60 and Cys89. The most prominent feature of the structure is the presence of a cysteine-knot motif, positioned symmetrically opposite at one end of each monomer. This motif is found in other closely related growth factors such as VEGF (38, 39), platelet-derived growth factor-BB (PDGF-BB, Ref. 51), transforming growth factor-β2 (TGF-β2, Ref. 52) and nerve growth factor (NGF, Ref. 53) (Fig. 1B). The knot consists of an eight-residue ring formed by one interchain (Cys60–Cys89) and three intrachain (Cys35–Cys77, Cys66–Cys111, Cys70–Cys118) disulfide bonds (Fig. 1A). The ring structure is formed between two adjacent β-strands, β3 and β7, with the third intrachain disulfide bond penetrating the covalent linkage and connecting strands β1 and β4. The cysteine ring contains a conserved glycine residue at position 68, which seems to be important in optimizing the conformation of the sidechains in the knot. As in the VEGF structure (38, 39), this residue adopts positive dihedral φ angles of 141 and 149° in monomers A and B, respectively. Thus the cysteine-knot motif appears to be important for the stabilization of the dimer as there are only a few contacts between the β-strands (β1 and β4) at the dimer center. One peptide bond in the PIGF-1 structure adopts a cis conformation: that connecting Ser57 and Pro86 in both monomers.

The structural core of the PIGF-1 monomer consists of a four-stranded, highly irregular, solvent-accessible β-sheet (Fig. 1A). The total buried surface area at the interface between the two monomers is 2,627 Å2. A considerable proportion of this (1,830 Å2 or 69%) is accounted for by the extensive intermolecular hydrophobic core interactions at the interface on the opposite end of the cysteine-knot and provides additional stability to the central portion of the structure. The hydrophobic core is formed by residues from both monomers and is known to be part of the receptor binding region of PIGF-1 (see under “Receptor Recognition”). Fourteen potential hydrogen bond interactions were observed between the two monomers (Table II). Two water-mediated hydrogen bonds between Glu49 from each monomer form a bridge between two strands (β1 and β4) across the center of the dimer interface.

Comparison with VEGF Structure—Overall, the structure of PIGF-1 exhibits remarkable topological identity with that of VEGF (38, 39) (with which it has 42% amino acid sequence identity) despite significant functional diversity (Fig. 1, B–D, r.m.s. deviation of 1.47 Å using 95 Ca atoms). The mode of dimerization for PIGF-1 is similar to that of VEGF. Conformational differences between PIGF-1 and VEGF are observed at the amino-terminal residues (18–25), some residues from loop regions (loops connecting β3–β4, β5–β6, and α2–β2) and the carboxyl-terminal residues (116–117). Interestingly, these loop regions appear to be part of the receptor-binding face in both molecules (see below). Approximately 70 water molecules are conserved in PIGF-1 and VEGF and appear to be important for the structural integrity of the homodimer in both molecules.

Receptor Recognition—The extracellular domain of both KDR and Flt-1 receptors consist of seven immunoglobulin domains. Mutational analysis of VEGF has revealed that symmetrical binding sites for KDR are located at each pole of the VEGF homodimer (38). Each site appears to contain two functional regions composed of binding determinants presented across the intermolecular interface. This experimental evidence suggested that only a small number of VEGF residues are important for binding to KDR, and the binding epitope for KDR contains two hot-spots, each of which extends across the dimer interface (39, 54–56). Furthermore, analysis of the conformational variability of VEGF (based on the high resolution structure of VEGF, Ref. 39) showed that the loop connecting strands β5 to β6 undergoes a concerted movement. This loop is important for binding to both Flt-1 and KDR, suggesting that these receptor molecules have overlapping binding sites on the target molecule. It has also been established that minimally domains 2 and 3 of Flt-1 are necessary and sufficient for binding VEGF with near native affinity, and domain 2 alone binds to VEGF (60-fold less tightly than wild-type, Ref. 38). Similar results have been found for deletions in the KDR (56).

Recently, the crystal structure of VEGF in complex with Flt-1z (1.7 Å) has revealed that domain 2 is predominantly involved in hydrophobic interactions with the poles of the VEGF dimer (40). Based on this structure and previous mutagenesis data, Wiesmann et al. (40) have proposed a model of VEGF bound to the first four domains of Flt-1. In the case of PIGF, it has been shown that binding of PIGF to human ECs revealed a high affinity site and a low affinity site (35, 37). The high affinity site is for Flt-1 and PIGF can displace VEGF from

| Molecule A | Molecule B | Distance (Å) |
|------------|------------|--------------|
| Val64–N   | Thr66–O    | 2.89         |
| Val74–O   | Glu84–N    | 2.89         |
| Phe26–N   | Glu86–Oc1  | 3.06         |
| Arg52–Nc2 | Glu92–Oc1  | 2.79         |
| Arg52–Nc2 | Glu92–Oc2  | 3.22         |
| Ser7–Oc3  | Cys76–O    | 2.78         |
| Arg56–Nc2 | Glu86–Oc1  | 2.45         |
| Glu99–Oc1 | Arg52–Nc2  | 3.02         |
| Glu99–Oc1 | Arg52–Nc1  | 2.77         |
| Glu99–Oc2 | Arg52–Nc2  | 3.26         |
| Cys86–Oc4 | Ser91–Oc5  | 2.78         |
| Thr92–Oc6 | Val64–N    | 2.89         |
| Gln98–Nc6 | Val64–Oc1  | 2.88         |
| Gln98–Oc6 | Phe86–N    | 2.86         |
both truncated and full-length Flt-1 receptors. However, at the present time it is yet to be established whether both PlGF-1 and VEGF bind identically to Flt-1.

**Flt-1 (VEGFR-1) Receptor Interactions**—The structure reported here for PlGF-1 is an unliganded structure and hence it is not possible to establish the precise nature of the interaction of PlGF-1 with Flt-1. However, using the structural data from the VEGF-Flt-1D2 complex, we have been able to construct a model to visualize the binding mode between PlGF-1 and Flt-1. The PlGF-1-Flt-1D2 complex was modeled by superimposing the PlGF-1 Flt-1 D2 complex (Ref. 40, PDB 12159) pre- dict that binding between PlGF-1 and Flt-1 D2 might also be mediated through hydrophobic interactions involving planar surfaces from both the ligand and the receptor (Table III). Such structures from both the ligand and the receptor (Table III). Such

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### TABLE III

Putative intermolecular contacts

| PIGF-1 | Flt-1D2 | Contacts | VEGF | Flt-1D2 | Contacts |
|--------|---------|----------|------|---------|----------|
| **van der Waals contacts** | **van der Waals contacts** |
| **Mol A** | **Mol A** |
| Phe17 | Ile142, Pro143(2), Leu221 | 4 | Phe17 | Ile142, Pro143(3) | 3 |
| Asp19 | Leu221 | 9 | Leu174 | Ile142 | 2 |
| Tyr21 | Gly203, Leu204(6) | 2 | Pro106 | Tyr199 | 7 |
| Glu22 | Phe172 | 3 | Pro106 | Tyr199 | 7 |
| Asp24 | Arg224 | 2 | Pro106 | Tyr199 | 7 |
| Leu25 | Pro143(4), Leu221(2) | 6 | Pro143(2), Leu221(2) | 6 |
| Gly27 | Glu141(3), Ile142, Pro143(9), Leu204, Asn219(3) | 17 | Glu141(3), Ile142, Pro143(9), Leu204, Asn219(3) | 17 |
| Trp30 | Leu204 | 1 | Leu204 | Pro143(4), Leu221(2) | 12 |
| Gly30 | Phe172 | 2 | Phe172 | Pro143(4), Leu221(2) | 12 |
| Tyr34 | Pro106 | 3 | Pro106 | Tyr199 | 7 |
| Tyr100 | Leu221 | 3 | Leu221 | Arg224 | 2 |
| Gly121 | Ile142, Pro143(2) | 12 | Gly121 | Ile142, Pro143(2) | 12 |
| Pro115 | Tyr199 | 9 | Pro115 | Tyr199 | 9 |

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*van der Waals distances are the maximum allowed values of C–C, 4.1 Å; C–N, 3.8 Å; C–O, 3.7 Å; O–O, 3.3 Å; O–N, 3.4 Å; and N–N, 3.4 Å.

* Numbers in parentheses represent the number of contacts made with the indicated Flt-1 residue. Hydrogen bond parameters and van der Waals distances are the maximum allowed values of C–C, 4.1 Å; C–N, 3.8 Å; C–O, 3.7 Å; O–O, 3.3 Å; O–N, 3.4 Å; and N–N, 3.4 Å.

* Glu73 and Asn74 are modeled as alanines due to insufficient electron density for the sidechain atoms in the free PlGF-1 structure.

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**atomic coordinates of the VEGF-Waals contacts** were calculated with the program CONTACT (44). PlGF-1 to visualize the binding mode between PlGF-1 and Flt-1. The resultant model showed a reasonable fit between PlGF-1 and Flt-1 without any obvious stereochemical impediments between the two proteins (Fig. 2A). The interface of the putative PlGF-1-Flt-1D2 complex appears to include some 22 amino acids from the PlGF-1 molecule: residues from the a1 helix, β3-β4 loop, and β7 strand of one monomer, and residues from strands β5, β6, and the β5-β6 loop of the second monomer. In the modeled complex, nineteen residues from the Flt-1D2 segments 141–147, 171–175, 199–204, and 219–226 form part of this contact surface. Modeling studies based on the VEGF-Flt-1D2 complex structure (40) predict that binding between PlGF-1 and Flt-1D2 might also be mediated through hydrophobic interactions involving planar surfaces from both the ligand and the receptor (Table III). Such
shape complementarity is energetically favorable for maximizing the contribution of van der Waals contacts.

Based on the PI GF-1-Flt-1D2 model, we speculate that both PI GF-1 and Flt-1D2 form extensive contacts through sidechain interactions (Table III, Fig. 2B). The contact residues from the two individual components of the modeled complex are shown in Fig. 2C and D. Asp72 in PI GF-1 appears to be the only residue to make direct H-bond interactions with Arg224 of Flt-1D2. (In the VEGF-Flt-1D2 complex, the conserved VEGF residue Asp65 makes similar interactions with Flt-1, Ref. 40, Table III.) PI GF-1 residues Glu27, Tyr34, Ala74, Tyr100, and Glu112 in one molecule (either A or B) are predicted to make both polar and van der Waals interactions with Flt-1, whereas residues Phe26, Trp30, Glu31, Gly73, Leu75, Glu84, Leu90, Ile92, and Pro115 seem to participate in van der Waals interactions with Flt-1 residues (Table III). Additional PI GF-1 residues Pro25, Cys70, Gly71, Pro96, Cys111, Cys113, and Arg114 also appear to be part of the interface.

A general mechanism of Flt-1 recognition by PI GF-1 can be postulated based on the proposed dimeric model of the receptor binding domain of VEGF in complex with domains 1–4 of Flt-1 (40). A similar picture may be visualized for PI GF-1-Flt-1 recognition with domain-1 pointing away from PI GF-1, the domain 2–3 linker region occupying the groove (6.8 Å wide) between the two monomers, and domain 3 making contact with its bottom face, which would bring domain 4 into direct inter-receptor contacts and hence involved in dimer formation. In the PI GF-1 structure, the walls of the groove are formed by residues Asp72, Glu73, Val52, Met55, Val45, Asp43, and Ser90. The corresponding residues Pro25, Cys70, Gly71, Pro96, Cys111, Cys113, and Arg114 could be useful for the design of small mimics of PI GF-1. Such agonists could be useful for the design of PI GF antagonists, which prevent the interaction with the receptor, and may serve to be important for the treatment of pathological disorders involved in neovascularization during tumor growth.

Acknowledgments—We thank the staff at the Synchrotron radiation source, Daresbury (UK) for their help with X-ray data collection and members of the Acharya laboratory for constructive criticism of the manuscript.

REFERENCES

1. Folkman, J., and Shing, Y. (1992) J. Biol. Chem. 267, 10931–10934
2. Folkman, J. (1985) Nat. Med. 1, 27–31
3. Folkman, J., and Klagesmier, M. (1987) Science 235, 442–447
4. Risau, W. (1990) Prog. Growth Factor Res. 2, 71–79
5. Klagsbrun, M. (1989) Prog. Growth Factor Res. 1, 207–235
6. Ferrara, N., and Henzel, W. J. (1989) Biochem. Biophys. Res. Commun. 161, 851–858
7. Keck, P. J., Hauser, S. D., Krivi, G., Sanzo, K., Warren, T., Feder, J., and Connolly, D. T. (1989) Science 246, 1309–1312
8. Godspadoriavich, D., Abraham, J. A., and Schilling, J. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 7311–7315
9. Levy, A. P., Tamargo, R., Brem, H., and Nathans, D. (1989) Growth Factors 2, 9–19
10. Connolly, D. T. (1989) Acc. Chem. Res. 22, 186–192
11. Maglione, D., Guerriero, V., Viglietto, G., Risau, W., Delli-Bovi, P., and Persico, M. G. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 9267–9271
12. Maglione, D., Guerriero, V., Viglietto, G., Risau, W., Delli-Bovi, P., and Persico, M. G. (1992) in Growth factors of the vascular and nervous system (Lendzian, C., Paoletti, R., and Albertini, A., eds), pp. 28–33, Karger, Basel
13. Hauser, S., and Weich, H. A. (1993) Growth Factors 9, 259–268
14. Park, J. E., Chen, H. H., Winer, J., Houck, K. A., and Ferrara, N. (1994) J. Biol. Chem. 269, 25646–25654
15. Olofsson, B., Pajusola, K., Kaipainen, A., van Euler, G., Joukov, V., Sakela, O., Orpana, A., Petersson, R. F., Altaito, K., and Eriksson, U. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 2523–2528
16. Joukov, V., Pajusola, J., Kaipainen, A., Chilov, D., Lahtinen, I., Kukk, E., and Sakela, O. (1996) EMBO J. 15, 299–308
17. Achen, M. G., Jeltsch, M., Muihn, M., Vitali, A., Wilks, A. F., Altaito, K., and Stacke, S. A. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 548–553
18. Merler, M., Claus, L., Leppl-Wienhues, A., Wallenberger, J., Augustin, H. G., Zinche, M., Lanz, C., Buttner, M., Rizha, H-J., and Dehio, C. (1999) EMBO J. 18, 363–374
19. Orlandini, M., Marconcini, L., Ferruzzi, R., and Oliviero, S. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 11675–11680
20. Bussolino, F., Mantovani, A., and Persico, M. G. (1997) Science 271, 255–256
21. Di Salvo, J., Bayne, M. L., Conn, G., Kwock, P. W., Trivedi, P. G., Soderman, D. D., Palisi, T. M., Sullivan, K. A., and Thomas K. A. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 2628–2632
22. Cao, Y. H., Chen, H., Zhou, L., Chiang, M. K., Anand-Apte, B., Weatherbee, J. A., Wang, Y. D., Fang, P. Y., Flanagan, J. G., and Tsang, M. L. S. (1996) J. Biol. Chem. 271, 7717–7723
23. Cao, Y., Chen, H., Zhou, L., Chiang, M. K., Anand-Apte, B., Weatherbee, J. A., Wang, Y. D., Fang, P. Y., Flanagan, J. G., and Tsang, M. L. S. (1996) J. Biol. Chem. 271, 3154–3162
24. Maglione, D., Guerriero, V., Viglietto, G., Ferraro, M. G., Aprelikova, O., Altaito, K., Del Vecchio, S., Lei, K. J., Chou, J. Y., and Persico, M. G. (1993) Oncogene 8, 925–931
25. Cao, Y., Ji, W. R., Qi, P., Rosin, A., and Cao, Y. (1997) Biochem. Biophys. Res. Commun. 235, 493–498
26. Lichtenstein, M., Maglione, D., Rihatti, D., Morbidelli, L., Lago, C. T., Battistini, M., Paoletti, I., Barra, A., Tucci, M., Parise, G., Vincenti, V., Granger, H. J., Viglietto, G., and Persico, M. G. (1997) Lab. Invest. 76, 517–531
27. Sawa, A., Takahashi, T., Yamaguchi, S., Asumiya, M., and Shibuya, M. (1996) Cell Growth and Diff. 7, 213–221
28. Terman, B. I., Khadive, L., Dougher-Vermazan, M., Maglione, D., Lassam, N. J., Godspadoriavich, D., Persico, M. G., Bohlen, P., and Eisengr, M. (1994) Growth Factors 11, 187–195
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28. Landgren, E., Schiller, P., Cao, Y., and Claesson-Welsh, L. (1998) Oncogene 16, 359–367
29. Oldfors, B., Korpelainen, E., Pepper, M. S., Mandriota, S. J., Aase, K., Kumar, V., Gunji, Y., Jeltsch, M. M., Shibuya, M., Alitalo, K., and Eriksson, U. (1996) Proc. Natl. Acad. Sci. U. S. A. 95, 11709–11714
30. Davis-Smyth, T., Chen, H., Park, J., Presta, L. G., and Ferrara, N. (1996) EMBO J. 15, 4919–4927
31. Olofsson, B., Korpelainen, E., Pepper, M. S., Mandriota, S. J., Aase, K., Kumar, V., Gunji, Y., Jeltsch, M. M., Shibuya, M., Alitalo, K., and Eriksson, U. (1996) Proc. Natl. Acad. Sci. U. S. A. 95, 11709–11714
32. Barleon, B., Totzke, F., Herzog, C., Blanke, S., Kremmer, E., Siemeister, G., Marme, D., and Matiny-Baron, G. (1997) J. Biol. Chem. 272, 10382–10388
33. Midgal, M., Huppertz, B., Tessler, S., Comforti, A., Shibuya, M., Reich, R., Baumann, H., and Neufeld, G. (1998) J. Biol. Chem. 273, 22272–22278
34. Clau, M.D., Weich, H., Breier, G., Knes, U., Roel, W., Waltenberger, J., and Riau, W. (1996) J. Biol. Chem. 271, 17629–17634
35. Cunningham, S. A., Stephan, C. C., Arrate, M. P., Ayer, K. G., and Brock, T. A. (1997) Biochem. Biophys. Res. Commun. 231, 596–599
36. Yonekura, H., Sakurai, S., Liu, X., Migita, H., Wang, H., Yamagishi, S., Nomura, M., Abedin, M. J., Unoki, H., Yamamoto, Y., and Yamamoto, H. (1999) J. Biol. Chem. 274, 35172–35178
37. Barleon, B., Sozzani, S., Zhou, D., Weich, H. A., Montovani, A., and Marme, D. (1996) Blood 87, 3336–3343
38. Muller, Y. A., Li, B., Christinger, H. W., Wells, J. A., Cunningham, B. C., and de Vos, A. M. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 7192–7197
39. Muller, Y. A., Christinger, H. W., Keyt, B. A., and de Vos, A. M. (1997) Structure 5, 1325–1338
40. Wissmann, C., Fuh, G., Christinger, H. W., Eigenh, C., Wells, J. A., and de Vos, A. M. (1997) Cell 91, 695–704
41. Ribatti, D., Vasca, A., Roncali, L., and Dammacco, F. (1996) Int. J. Dev. Biol. 40, 1189–1197
42. Maglione, D., Battisti, M., and Tucci, M. (2000) Il Farmaco 55, 165–167
43. Otwinowski, Z., and Minor, W. (1997) Methods Enzymol. 276, 307–326
44. Collaborative Computational Project, Number 4 (1994) Acta Crystallogr. Sect. D 50, 760–763
45. Navaza, J. (1994) Acta Crystallogr. Sect. A 50, 157–163
46. Brung, A. T., Adams, P. D., Clore, G. M., DeLano, W. L., Gros, P., Grosse-Kunstleve, R. W., Jiang, J. S., Kuszewski, J., Nilges, M., Pannu, N. S., Read, R. J., Ree, L. M., Simonson, T., and Warren, G. L. (1998) Acta Crystallogr. Sect. D 54, 905–921
47. Read, R. J. (1996) Acta Crystallogr. Sect. A 52, 140–149
48. Laskoski, R. A., MacArthur, M. W., Mass, D. S., and Thornton, J. M. (1993) J. Appl. Crystallogr. 26, 283–291
49. Jones, T. A., Zou, J. Y., Cowan, S. W., and Kjeldgaard, M. (1991) Acta Crystallogr. Sect. A 47, 110–119
50. Brung, A. T. (1992a) Nature 355, 472–475
51. Oefner, C., D'Arcy, A. D., Winkler, F. K., Eggimann, B., and Hosang, M. (1992) EMBO J. 11, 3921–3926
52. Schluenegg, M. P., and Grutter, M. G. (1992) Nature 358, 430–434
53. Holland, D. R., Coons, L. S., Meng, W., and Matthews, B. W. (1994) J. Mol. Biol. 239, 385–400
54. Keyt, B. A., Berleau, L. T., Nguyen, H. V., Chen, H., Heinsohn, H., Vanden, R., and Ferrara, N. (1996) J. Biol. Chem. 271, 7788–7795
55. Keyt, B. A., Nguyen, H. V., Berleau, L. T., Duarte, C. M., Park, J., Chen, H., and Ferrara, N. (1996) J. Biol. Chem. 271, 5638–5646
56. Fuh, G, Li, B., Crowley, C, Cunningham, B., and Wells, J. A. (1998) J. Biol. Chem. 273, 11197–11204
57. Brung, A. T. (1992b) X-FLOR Version 3.1 Manual: A System for X-ray Crystallography & NMR. Yale University Press, New Haven
58. Stacker, S. A., Vitali, A., Caeser, C., Domagala, T., Groenen, L. C., Nice, E., Achen, M. G., and Wilkes, A. F. (1999) J. Biol. Chem. 274, 34884–34892
59. Kraulis, P. J. (1991) J. Appl. Crystallogr. 24, 946–950
60. Kabsch, W., and Sanders, C. (1983) Biopolymers 22, 2577–2587
61. Barton, G. J. (1993) Protein Eng. 6, 37–40