Binding Domains and Epitopes in Platelet-Derived Growth Factor

S. Vogel and J. Hoppe*

Department of Cytogenetics, GBF—Gesellschaft für Biotechnologische Forschung mbH, Mascheroder Weg 1, D-3300 Braunschweig, FRG

Received April 14, 1988; Revised Manuscript Received November 15, 1988

ABSTRACT: Trypsin treatment of recombinant PDGF-BB from *Escherichia coli* leads to the liberation of a small carboxy-terminal fragment and two internal segments without dissociating the molecule. The remaining core of 21 kDa retained a considerable binding affinity of 8.4 nM. By use of various peptide fragments obtained from monomeric recombinant PDGF-B, a receptor binding domain was assigned to one of these internal trypsin-sensitive segments. This segment is enriched in charged residues, suggesting mainly hydrophilic interactions with the receptor. Circular dichroism measurements of recombinant PDGF-BB showed a high content of random structure and only a small percentage (<10%) of α-helical structures. This structure was very rigid since the addition of 70% trifluoroethanol or 1% SDS did not change the circular dichroism spectrum. On the basis of these results, a tentative structure was generated by computer modeling.

Platelet-derived growth factor (PDGF)[1] is a major mitogen in serum which promotes the proliferation of fibroblasts and smooth muscle cells in vitro (Heldin & Westermark, 1984; Deuel et al., 1985; Ross et al., 1986) and elicits its mitogenic effect at low concentrations (1 ng/mL) by binding with high affinity to specific cell surface receptors. PDGF from human platelets is probably a heterodimer composed of two homologous chains termed A and B (Johnson et al., 1982, 1984; Waterfield et al., 1983; Doolittle et al., 1983; Betsholtz et al., 1986), but it has been shown that homodimers of type A–A and B–B are biologically active (Heldin et al., 1986; Stroobant & Waterfield, 1984; Kelly et al., 1985). The paucity, heterogeneity, and complexity (A–B type) of PDGF from human platelets have hampered so far detailed structure analysis by biochemical and physical methods.

We have recently described the expression of PDGF-B in *Escherichia coli* and its renaturation to full biological activity (Hoppe et al., 1989). This material is available in sufficient amounts for physical and biochemical studies which are greatly facilitated by dealing with a homodimer (type B–B) rather than with a heterodimer (A–B) found in human platelets.

Though it has been generally accepted that charged residues, in particular basic residues, in the sequence of PDGF play an important role in the recognition by the receptor, these residues have not yet been identified. Furthermore, no data are available on the content of the secondary structure and on the flexibility/rigidity of the molecule. These data together with the identification of binding domains would eventually lead to the development of structural models and a better understanding of the mechanisms of recognition by the receptor(s) and the activation of cell division.

Materials and Methods

Binding to Balbc 3T3/A31 Cells. Radioiodination with chloramine T was carried out as described (Heldin et al., 1981). A total of 2×10^4 cells/well were seeded into a 24-well plate (Linbro) and grown for 5 days in DMEM medium containing 10% FCS without changing the medium. After being washed once with Hepes-buffered saline, they were incubated with 0.5 nM ^{125}I-PDGF (30000 cpm/ng) and the respective concentration of the tested substance in 0.5 mL of F-10 medium supplemented with 5 mg/mL BSA and 10 mM Hepes, pH 7.4 (Heldin et al., 1981). Binding was carried for 1 h at room temperature and was terminated by washing (5×) with PBS/0.1% BSA at 4 °C. Cells were solubilized in 500 μL of PBS containing 1% Triton X-100, 10% glycerol, and 0.1% BSA and assayed for ^{125}I-radioactivity. Unspecific binding was determined by addition of a 200-fold excess of unlabeled PDGF. The K_i value was determined according to Dixon and Webb by plotting the reciprocal of the bound ^{125}I-PDGF (y axis) versus the analogue concentration (x axis) and extrapolating the line to the interception with the x axis. The K_i value was calculated by using the following expression: x intercept $= -K_i[1 + ([S]/K_m)]$ (Dixon & Webb, 1953). The obtained data allow a semiquantative comparison of the binding affinities of the test samples. Human PDGF and recombinant PDGF-BB were isolated as described (Hoppe et al., 1989).

Cleavage of rPDGF-B and Isolation of Peptide Fragments. (*A*) *Cleavage with Endoproteinase Lys-C (Boehringer).* To a solution of monomeric (0.3 mg/mL) rPDGF-B was added urea to a final concentrtion of 1 M. The pH was adjusted to 7.0 by addition of 2 M Tris base. Proteinase was then added at a ratio of 1/20 (w/w). Incubation was done at 37 °C overnight.

(*B*) *Iodosobenzoic Acid.* Five hundred micrograms of monomeric rPDGF-B was lyophilized and dissolved in 1.5 mL of 80% acetic acid containing 4 M guanidine hydrochloride and 500 μg of iodosobenzoic acid (Fontana et al., 1983). The mixture was incubated for 20 h at room temperature in the dark.

Peptides generated by Lys-C protease or iodosobenzoic acid cleavage were purified by reversed-phase high-performance liquid chromatography. The whole mixture was applied to a column (0.4 × 25 cm), Vydac 214 TP54, equilibrated in 0.1% aqueous trifluoroacetic acid. The column was washed with the same solvent until the UV absorbance at 220 nm had reached its initial value. Peptides were then eluted with a linear gradient from 15% acetonitrile in 0.1% aqueous trifluoroacetic acid to 42% acetonitrile in 0.1% trifluoroacetic

* Address correspondence to this author at the Institute for Physiological Chemistry, University of Würzburg, Koellikerstrasse 2, 8700 Würzburg, FRG.

[1] Abbreviations: PDGF, platelet-derived growth factor; IBA, iodobenzoic acid; CD, circular dichroism; SDS, sodium dodecyl sulfate; rPDGF-BB, recombinant PDGF-BB.

0006-2960/89/0428-2961$01.50/0

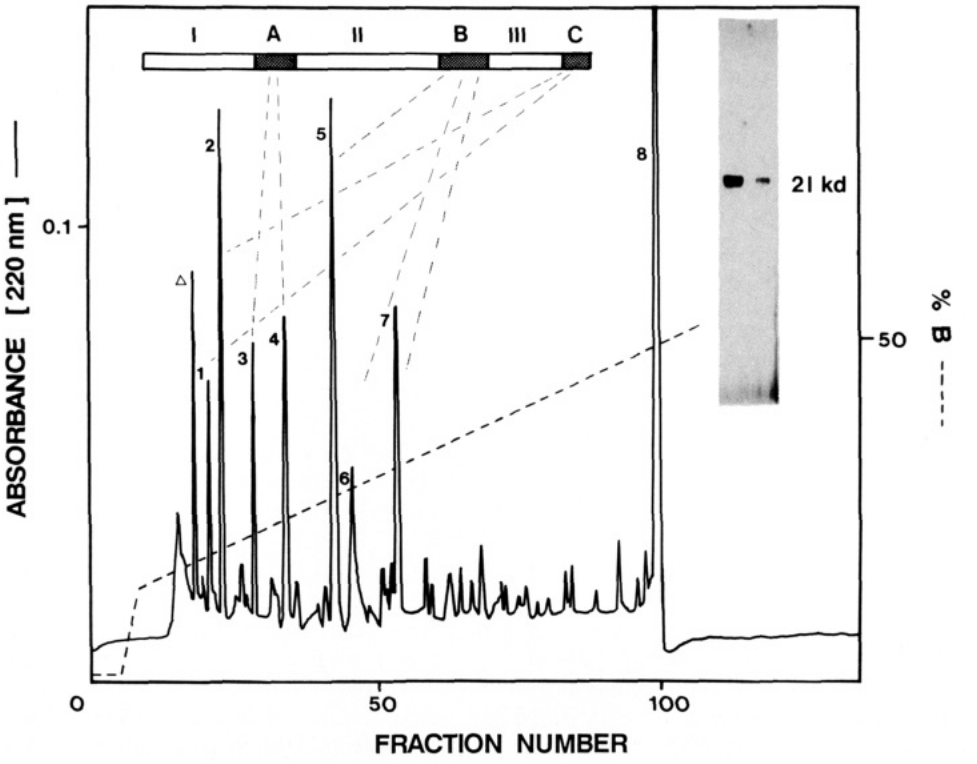

FIGURE 1: Tryptic digest of recombinant PDGF-BB. 200 μg of recombinant (0.4 mg/mL) PDGF-BB in Tris-HCl buffer, pH 8.5, was digested with 20 μg of TPCK-treated trypsin. Peptides were separated by reversed-phase chromatography as described under Materials and Methods. All major peptides were analyzed by gas-phase sequencing. The following sequences were determined: (Δ) no peptidic material; (1) ARPVTR; (2) SPLN; (3) LIDR; (4) TNANF; (5) KKPIFK; (6) KPIF; (7) KIEIVR. The faint lines connect the peptide peaks in the HPLC chromatogram to the tryptic-sensitive domains A, B, and C in the rPDGF-BB. Peak 8 was analyzed by SDS–PAGE (insert). Two different concentrations were applied.

acid during 40 min. The flow rate was 0.7 mL/min. Fractions were collected, each 1 min. The effluent was monitored by the UV absorbance at 220 nm. Fractions were analyzed by SDS–polyacrylamide gel electrophoresis using a 13.5% gel containing 4 M urea. The gels were stained with Coomassie Blue and/or silver nitrate.

(*C*) *Tryptic Digest of rPDGF-BB.* A solution of 200 μg of dimeric rPDGF-BB (0.4 mg/mL) was adjusted with 2 M Tris base at pH 8.5. This mixture was then incubated with 20 μg of TPCK-treated trypsin (Serva) overnight at 37 °C.

(*D*) *Endoproteinase Glu-C Digest.* Cleavage was performed at pH 4.5 in 50 mM ammonium acetate. Monomeric rPDGF-B solution (0.3 mg/mL) was incubated with 1/20 (w/w) proteinase overnight at 37 °C.

For purification of the peptides, the whole mixture of either the tryptic digest or the Glu-C digest was applied to a column (0.4 × 25 cm), Vydac 218 TP54, equilibrated in 0.1% aqueous trifluoroacetic acid. After unadsorbed material was washed out, peptides were eluted by a linear gradient of 7% acetonitrile to 56% acetonitrile in 0.1% aqueous trifluoroacetic acid during 70 min. The flow rate was 0.7 mL/min. Fractions were collected every 30 s, and the effluent was monitored by the UV absorbance at 220 nm.

Fractions were analyzed by thin-layer chromatography using silica gel high-performance plates with concentration areas from Merck. Plates were developed with 1-butanol/pyridine/acetic acid/water, 68/40/14/25 (v/v). Peptides were visualized after fluram staining under UV light. The purity of peptides was further established by gas-phase sequencing or amino acid analysis. The protein content was determined by amino acid analysis or by sequence analysis.

Construction of p13. To shorten the PDGF-B at the C-terminus, plasmid pJLA504 containing the *Sph*I/*Eco*RI fragment (Hoppe et al., 1989; Weich et al., 1986) was completely digested with *Sph*I. A 300 bp fragment was isolated by gel electrophoresis, and protruding 3′ ends were made blunt-ended by T4 polymerase treatment. Plasmid pEx-2 was digested with *Hpa*I/*Sal*I, and protruding 5′ ends were removed by S1 digestion. After ligation, a strain was selected which expressed a fusion protein of M_r 36 000. DNA sequence analysis showed an altered sequence starting from amino acid position 98 including a stop codon at position 102 (Figure 3). The S-sulfonated monomeric form of p13 was prepared essentially as described for rPDGF-B (Hoppe et al., 1989).

Peptide p1 was kindly synthesized by Dr. R. Frank. For computer modeling, the program BRAGI was used which was developed by Dr. D. Schomburg and Dr. J. Reichelt at the Gesellschaft für Biotechnologische Forschung, Braunschweig (Schomburg & Reichelt, 1988). Circular dichroism spectra were recorded on an JASCO J 600 CD spectrometer using a cuvette of 0.1-cm pathway.

RESULTS

Tryptic-Sensitive Domains in rPDGF-BB. The numerous basic residues in the sequence of PDGF-B should give rise to at least 13 tryptic fragments. Indeed, when monomeric S-sulfonated rPDGF-B was treated with trypsin, more than 30 fragments were detected after high-performance liquid chromatography (data not shown). Trypsin treatment of the active dimeric rPDGF-B generated only eight peptides. Peaks 1–7 contained small peptides. Sequence analysis showed that they are released from three distinct regions of the PDGF-B molecule (Figure 1). SDS–PAGE of peak eight showed a homogeneous material with a molecular weight of 21 000.

After reduction of this material by 2-mercaptoethanol, the 21-kDa band completely disappeared. No fragments larger than >4 kDa could be detected by SDS electrophoresis, indicating that trypsin cleavage was complete. These peptides

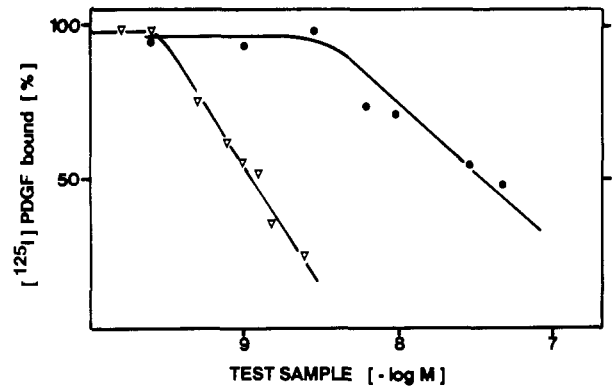

FIGURE 2: Binding properties of the 21-kDa trypsin-resistant core of recombinant PDGF-BB. (▽) Competition of unlabeled PDGF against ^{125}I-PDGF; (●) competition of trypsin-treated rPDGF-BB against ^{125}I-PDGF.

might be isolated by high-performance liquid chromatography and subsequently characterized. On the other hand, it is possible to identify newly generated amino termini in a protein by sequencing the entire material. Furthermore, this method will allow the quantification of the different amino termini. On the basis of the knowledge of the PDGF-B sequence, the following four amino-terminal sequences were readily identified in the core peptide of trypsinized rPDGF-BB (relative amounts are given in parentheses): (a) IAECKTR-, original amino terminus (1); (b) LVWPPCV-, generated by cleavage at Phe-37 (0.6); this terminus is complementary to peptide 4 in segment A; (c) TNANF-, generated by cleavage at Ar-32 (0.5); this terminus is complementary to peptide 3 in segment A; (d) ATVTLEDH-, generated by cleavage at Lys-85 (1); this terminus is complementary to peptides 5–7 in segment B. Trypsin apparently excised peptides from three defined segments of PDGF-B (segments A, B, and C). The other (seven) basic residues which are located in segments I, II, and III are not at all attacked. These residues might be inaccessible to the protease or may adopt a conformation which is resistant to tryptic cleavage. It should be noted that peptide 4 (Figure 1) was generated by a chymotryptic like partial cleavage at Phe-37, indicating that this residue is well accessible to proteases.

The excision of the trypsin-sensitive segments A, B, and C did not lead to a release of the other three fragments which remained linked together as a dimer. This indicates that NH_2-terminal and carboxy-terminal fragments containing one (position 16) and two cysteine (positions 97 and 99) residues, respectively, must be linked in some way to the central cysteine-rich segment.

Table I: Binding Affinities of Various Peptides

peptide	K_i (nM)	peptide	K_i (nM)
PDGF	0.25	IBA	200
rPDGF-BB	1.7	p1	15000
rPDGF-BB-tryp	8.4	Glu-C-77–92	2000
monomeric S-sulfonated rPDGF-B	3.5	Glu-C-93–100	2000
		Glu-C-104–114	10000
p13	3.5	protamine	4000
Lys-C-1	300	whole histone	1000
Lys-C-2	300	BSA	≫2000
Lys-C-3	200	cytochrome *c*	≫6000

Interestingly enough, the 21-kDa core of rPDGF-B retained a considerable binding affinity of 8.4 nM. Compared with rPDGF-BB dimer, this is an only 5-fold increase of the K_i value.

Binding of Peptide Fragments to the PDGF Receptor. Monomeric S-sulfonated rPDGF-B binds with a very high affinity (K_i = 3.5 nM) to the receptor. The masking of cysteine side chains by S-sulfonation effectively suppressed any formation of dimers. Even after prolonged storage, no dimeric form was detected (data not shown). It thus seemed to be feasible to generate peptides from this material for the identification of binding domains. Precautions have to be made with cysteine-containing peptides, since the precise arrangement of those residues in disulfide bridges is unknown. These disulfide bridges might stabilize a certain "active" conformation which will not be adopted by a peptide-containaing cysteine residues or, in our system, S-sulfonated cysteines. Our attempt was therefore to obtain peptides shortened at the NH_2 and carboxy termini or to investigate those segments which are devoid of cysteine residues (Figure 3, Table I).

In p13, the carboxy terminus was modified by a deletion, caused by an *Sph*I cleavage. This reaction generated 1 stop codon at position 102, leading to a deletion of 11 residues, and altered the amino acid sequence from residues 98–101 including Cys-99. This protein bound with an unchanged high affinity of 3.5 nM, indicating that carboxy-terminal residues from position 98 are not part of a binding domain. This assumption is supported by the low binding affinity of peptide Glu-C-104/114 which contains the carboxy terminus of rPDGF-B.

Three large fragments were obtained by cleavage with lysine-specific endoproteinase (Lys-C). Peptides were identified by sequencing the first 10–15 amino-terminal residues and by SDS–PAGE. Peptide Lys-C-1 was generated by cleavage at Lys-17 and -98, peptide Lys-C-2 by cleavage at Arg-32 and Lys-98, and peptide Lys-C-3 by cleavage at Lys-86. All peptides bound with about the same binding affinity of 200–300 nM, which was considerably lower than that of the

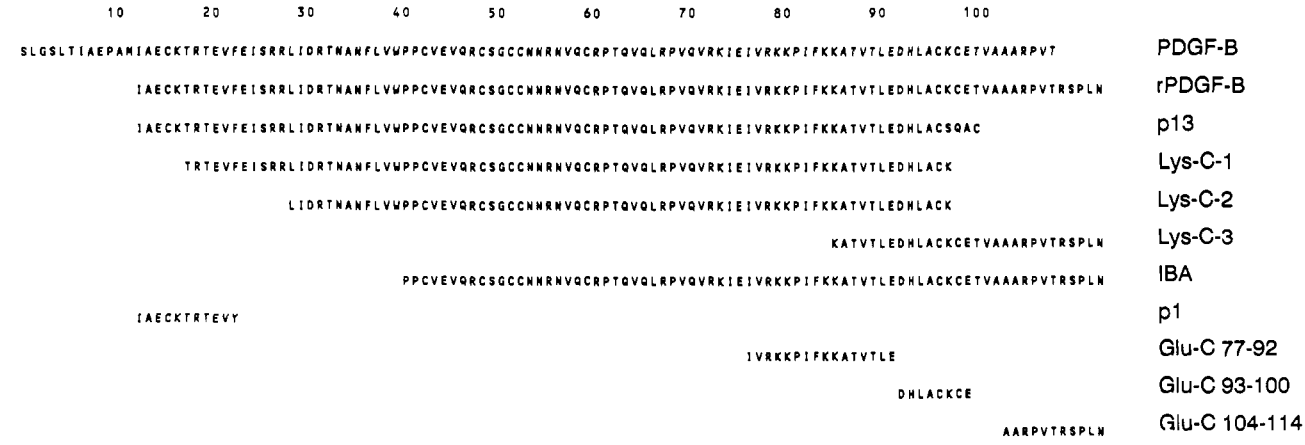

FIGURE 3: Amino acid sequences of the tested proteins and peptides.

Table II: Property of Dimerization of PDGF Variants

peptide	formation of dimers	Cys residues present in sequence							
		16	43	49	52	53	60	97	99
rPDGF-B (m)	+	+	+	+	+	+	+	+	+
p13	-	+	+	+	+	+	+	+	-
IBA	-	-	+	+	+	+	+	+	+
Lys-C-1	-	-	+	+	+	+	+	+	-
Lys-C-2	-	-	+	+	+	+	+	+	-
Lys-C-3	-	-	-	-	-	-	-	+	+

starting monomeric rPDGF-B (K_i = 3.5 nM). To investigate a possible role of the removed five NH_2-terminal residues in peptide Lys-C-1, a corresponding peptide was synthesized (p1). Since this peptide did not show a significant binding affinity, we suggest that this segment is not directly involved in the binding to the receptor but may stabilize an active conformation. A further reduction of the amino-terminal part had no effect on the affinity. Surprisingly, the entire cysteine-rich central segment could be deleted without any loss in affinity. These data were supported by using a peptide generated by iodosobenzoic acid cleavage at Trp-39 which bound the same affinity as peptide Lys-C-3. It thus seems to be reasonable to assume the location of a binding domain in the segment from residue ≈−80 to 97. Indeed, peptides obtained by Glu-C protein cleavage from this segment exhibited a low but significant binding affinity.

The three basic polypeptides protamine, histone, and cytochrome *c* were included in the measurements to investigate the role of positively charged amino acid side chains in the interaction between PDGF and its receptor. As shown in Table I, cytochrome *c* had no effect. Protamine and histone, which like PDGF contain clusters of basic residues, showed moderate binding affinities of 4 and 1 μM, respectively. These binding affinities are significantly lower than those obtained with Lys-C or IBA peptides. Though clearly basic residues play an important role in the recognition of PDGF, the nature of the contact site must be more complex, involving also negatively charged amino acids and hydrophobic residues.

Dimerization of PDGF-B Variants. The large fragments of monomeric S-sulfonated rPDGF-B listed in Figure 3 were assayed for their ability to form dimers using the same conditions which lead to a successful dimerization of monomeric rPDGF-B (Hoppe et al., 1989). None of the investigated fragments were able to undergo a dimerization (Table II). Besides fragment Lys-C-3 which contains only the carboxy terminus including Cys-97 and Cys-99, all fragments bear the cysteine-rich central part from Cys-43 to Cys-60. Apparently, this part alone is not sufficient for dimerization. The property of dimerization seems to be intimately connected with two terminal cysteine residues (Cys-16 and Cys-99) since none of these residues may be deleted without abolishing dimerization.

Circular Dichroism Measurements. As a first attempt to construct a molecular model for PDGF which incorporates the biochemical data regarding binding domains and possible arrangements of disulfide bridges, circular dichroism spectra of PDGF from platelets, rPDGF-B, and monomeric S-sulfonated rPDGF-B were recorded in the range of 185–240 nm in H_2O adjusted to pH 6. A further aspect of these measurements was to establish the renaturation by physical methods and to monitor structural changes during the dimerization (renaturation) of the monomeric rPDGF-B. All spectra showed a strong negative ellipticity at 200–204 nm which is characteristic for a high content of random structure (Figure 4A). The content of the different secondary structures was calculated according to Chen et al. (1974). Since no perfect curve alignment was obtained, the α-helical content

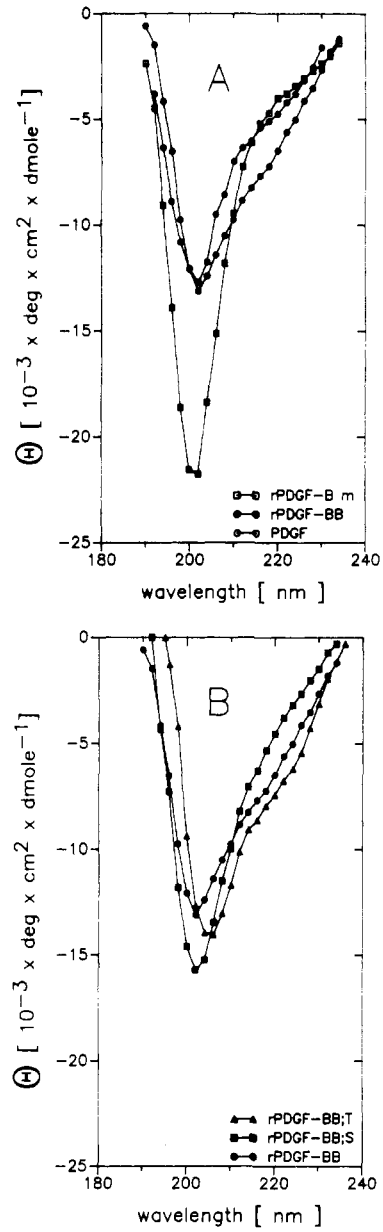

FIGURE 4: Circular dichroism spectra of PDGF and variants. (A) (○) PDGF from platelets; (●) rPDGF-BB; (□) monomeric rPDGF-B. (B) (▲) rPDGF-BB in 70% trifluoroethanol, T; (■) rPDGF-BB in 2% sodium dodecyl sulfate, S.

was estimated by the ellipticity at 223 nm and at 192 nm where θ_β and θ_R are almost zero. In all three species, the α-helical content was particularly low (≤10%). The content of random structure was the highest in monomeric rPDGF-B with about 70–80%. Dimeric structures exhibit a characteristic shoulder at 220 nm which indicates an increased content of β structure. Roughly 60% random structure and 30% β-sheet structure were calculated for PDGF from platelets and rPDGF-BB. It should be noted that the curves for PDGF platelets and rPDGF-BB are very similar, indicating a proper refolding of

the monomeric rPDGF-B into an active dimer. The high content of random structure in PDGF raises the possibility that the conformation of PDGF is highly flexible, thus allowing conformational change during the interaction with its receptor. To investigate the stability/flexibility of the structure, CD spectra were recorded after the addition of solvents which are known to introduce major alterations of the secondary structure in proteins. Trifluoroethanol, a strong α-helix promoter, had little, if any, effect on the conformation of rPDGF-B (Figure 4B). SDS, too, altered the secondary structure composition in rPDGF-B only to a limited extend. These observations indicate a very rigid structure of PDGF which is probably not altered in the complex with its receptor. The existence of a rigid structure furthermore readily explains the known high stability of PDGF toward heat and denaturing agent, e.g., SDS, urea, or acid (Heldin & Westermark, 1984; Deuel et al., 1984).

Secondary Structure Prediction and Computer Modeling. In the next step, we tried to assign the secondary structure elements to defined regions in the polypeptide chain. Therefore, the secondary structure was predicted by using four models (Nagano, 1974; Robson & Suzuki, 1976; Chou & Fasman, 1978; Garnier et al., 1978). Generally, all four applied prediction programs largely overestimated the α-helix content. The highest probability for the occurrence of an α-helix was found from residues 91–104, which comprise about 10–15% of the polypeptide chain. According to the low α-helix content of 10–15% in the PDGF, α-helical structures were assigned only to this segment. All programs suggested four β-sheets interrupted by turns for the central cysteine-rich segment. The predicted content of β-sheet agree fairly well with that calculated from the CD spectra. For the rest of the polypeptide, a random structure was assumed. The computer modeling started with the generation of four antiparallel β-strands (strand 1, residues 37–41; strand 2, residues 43–49; strand 3, residues 61–68; strand 4, residues 70–75) connected by turns. The torsion angles of amino acids in these interrupting turns or loops were adjusted to generate a four-stranded antiparallel β-pleated sheet. To this stable core was joined the α-helix ranging from residue 91 to residue 104 in such a way that a disulfide bridge between Cys-97 (segment III, Figure 1) located on the α-helix and Cys-49 (segment II, Figure 1) located in the cysteine-rich central segment was formed. The core structure (residues 37–75) and the α-helix (residues 91–104) were connected by a large loop which should indicate its trypsin sensitivity (Figure 5a,b; trypsin-sensitive region B). For the amino-terminal residues which comprise the trypsin-sensitive segment A, a second large loop was assumed. Torsion angles of the respective amino acids were adjusted in such a way that the formation of an intramolecular disulfide bridge between Cys-16 (segment I) and Cys-60 (segment II) was possible. This tentative structure was refined by the program AMBER which performs a geometry optimization of a protein structure (Weiner & Kollman, 1981). Due to the high content of random structure, the generation of a more refined structure was impossible. The aim of the work was to present a tentative model which incorporates the results obtained by biochemical methods (e.g., identification of epitopes by proteolysis), physical measurements, and structure predictions. It may serve as a guideline for site-directed mutagenesis to identify residues which directly interact with the receptor's binding site.

Discussion

One attempt to identify essential regions of a protein stems from evolutionary aspects. It is generally assumed that those

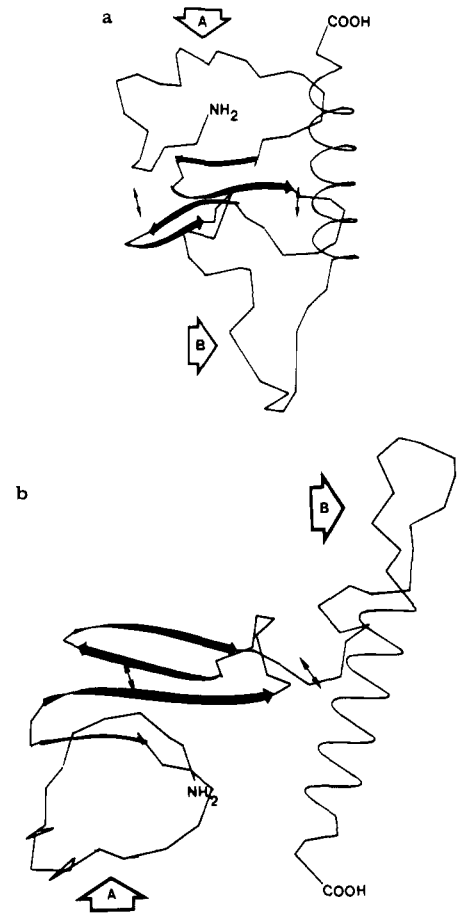

FIGURE 5: Computer-generated model for monomeric rPDGF-B. The arrows indicate β-sheet conformation. The two arrowed lines are the assumed disulfide bridges between Cys-16–60 and Cys-49–97, respectively. A and B are trypsin-sensitive domains.

regions which exert certain functions are conserved during evolution. If sequence data for PDGF are compared (PDGF-A and -B from human and PDGF-B from porcine and feline origins) (Waterfield et al., 1983; Betsholtz et al., 1986; Stroobant & Waterfield, 1984; Van den Ouweland et al., 1987), it is evident that most conserved residues are located in the middle of the sequence starting from the first cysteine-16 until the last cysteine-99. The highest conservation is observed for the central cysteine-rich segment and the basic segment at the carboxy terminus. All eight cysteine residues are strictly conserved. We have shown that a considerable part of the NH_2 terminus may be deleted without affecting the biological activity and the property of the polypeptide chain to form dimers (Hoppe et al., 1989). However, when the terminal cysteine residues (positions 16 and 99) are deleted or mutated, no dimeric structures were reconstituted though a great part of the binding affinity was retained. Recently, Donoghue and co-workers (Hannink et al., 1986; Sauer et al., 1986) have reached similar conclusions by investigating those regions of the PDGF-B homologous transforming protein $p28^{v\text{-}sis}$ of simian sarcoma virus. They found that the first 15 amino acids from the NH_2 terminus and the last 6 amino acids from the carboxy terminus may be deleted without loss of transforming activity and formation of dimers.

Using the same systems, Giese et al. (1987) showed by site-directed mutagenesis of the eight cysteine residues that none of these amino acid residues are dispensable for proper formation of dimers but, interestingly, only mutation of cysteine-16, -49, -60, or -97 led to a loss of transforming activity. In summary, it seems clear that a core comprising residues

16–103 is sufficient for biological activity.

Trypsin released only the C-terminus from this core and excised two segments without dissociation of the dimer. Since the NH_2-terminal segment contains only one cysteine residue (position 16), this residue must be linked to the central part of PDGF. The carboxy-terminal part contains two cysteine residues at positions 97 and 99. The deletion of Cys-99 abolished the formation of dimers. We therefore conclude that this residue is involved in the formation of the dimer. Then Cys-97 must be linked to the central segment. Interestingly, Giese et al. have reached very similar conclusions by mutating the eight cysteine residues. They suggested that cysteines-16, -49, -60, and -97 are involved in intramolecular disulfide bridges.

In a simple monomeric arrangement, the connections 16–97 and 49–60 can be excluded since trypsin would dissect this molecule. Out of the two other possible connections, we have chosen the bridging 16–60 and 49–97 for computer modeling.

Starting from the building of a rigid four-stranded β-pleated sheet comprising the highly conserved cysteine-rich central segment, this connection readily generated two exposed loop structures which were identified by trypsin treatment of the rPDGF-B. The model presented here is in many regards recurrent to those structures proposed by Robson et al. (1985) but seems to be more realistic due to the involvement of the biochemical data and the correction of the α-helical content according to the CD data.

If PDGF adopts such a two-looped structure, it was important to know whether both loops are involved in the binding to the receptor. It should be mentioned here that in the dimer two identical loops probably will be exposed.

The data obtained with peptides indicate that the first NH_2-terminal loop must likely does not interact directly with the receptor. From the carboxy-terminal loop, several peptides could be isolated which showed significant binding affinities. Though clustered basic residues play an important part, as shown by the effect of protamine and histone in these measurements and in data published earlier (Huang et al., 1982), the binding domain extends further, including two acidic residues and probably a histidine (positions 92–94). Interestingly, these residues are not excised by trypsin in rPDGF-BB, which may in part explain the remaining high binding affinity of the trypsin-resistant PDGF-B core.

ACKNOWLEDGMENTS

We thank Drs. Schomburg and Reichelt for introducing us to their modeling system BRAGI, B. Fasel for help during computer modeling, and Dr. R. Frank for the synthesis of peptide p1.

REFERENCES

Betsholtz, C., Johnson, A., Heldin, C.-H., Westermark, B., Lind, P., Urdea, M. S., Eddy, R., Shows, T. B., Philpott, K., Mellor, A. L., Knott, T. J., & Scott, J. (1986) *Nature 320*, 695–699.

Chen, Y. H., Yang, J. T., & Chan, K. H. (1974) *Biochemistry 13*, 3350–3359.

Chou, P. Y., & Fasman, G. D. (1978) *Annu. Rev. Biochem. 47*, 251–276.

Deuel, T. F., Tong, B. D., & Huang, J. S. (1985) *Curr. Top. Cell. Regul. 26*, 51–64.

Dixon, M., & Webb, E. C. (1953) *Biochem. J. 55*, 170–171.

Doolittle, R. F., Hunkapiller, M. W., Hood, L. E., Devare, S. G., Robbins, K. G., Aaronson, A., & Antoniades, H. N. (1983) *Science 221*, 275–277.

Fontana, A., Dalzoppo, D., Grandi, C., & Zombonin, M. (1983) *Methods Enzymol. 91*, 311–318.

Garnier, J., Osguthorpe, D. J., & Robson, B. (1978) *J. Mol. Biol. 120*, 97–120.

Giese, N. A., Robbins, K. C., & Aaronson, S. A. (1987) *Science 236*, 972–974.

Hannink, M., Sauer, M. K., & Donoghue, D. J. (1986) *Mol. Cell. Biol. 6*, 1304–1314.

Heldin, C.-H., & Westermark, B. (1984) *Cell 37*, 9–20.

Heldin, C.-H., Wasteson, A., & Westermark, B. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 3664–3668.

Heldin, C.-H., Johnsson, A., Wennergren, S., Werstedt, C., Betsholtz, C., & Westermark, B. (1986) *Nature 319*, 511–514.

Hoppe, J., Weich, H. A., & Eichner, W. (1989) *Biochemistry* (preceding paper in this issue).

Huang, J. S., Huang, S. S., Kennedy, B., & Deuel, T. F. (1982) *J. Biol. Chem. 257*, 8130–3136.

Johnsson, A., Heldin, C.-H., Westermark, B., & Wasteson, A. (1982) *Biochem. Biophys. Res. Commun. 104*, 66–74.

Johnsson, A., Heldin, C.-H., Wasteson, A., Westermark, B., Deuel, T. F., Huang, J. S., Seeburg, P. H., Gray, A., Ullrich, A., Scrace, G., Stroobant, P., & Waterfield, M. D. (1984) *EMBO J. 3*, 921–928.

Kelly, J. D., Raines, E. W., Ross, R., & Murray, M. J. (1985) *EMBO J. 4*, 3399–3405.

Nagano, K. (1974) *J. Mol. Biol. 84*, 337–372.

Robson, B., & Suzuki, E. (1976) *J. Mol. Biol. 107*, 327–356.

Robson, B., Platt, E., Finn, P. W., Millard, P., Gibrat, J.-F., & Garnier, J. (1985) *Int. J. Pept. Protein Res. 25*, 1–8.

Ross, R., Raines, E. W., & Bowen-Pope, D. F. (1986) *Cell 46*, 155–169.

Sauer, M. K., Hannink, M., & Donoghue, D. J. (1986) *J. Virol. 59*, 292–300.

Schomburg, D., & Reichelt, J. (1988) *J. Mol. Graphics* (in press).

Stroobant, P., & Waterfield, M. D. (1984) *EMBO J. 12*, 2963–2967.

V. d. Ouweland, A. M. W., Van Groningen, J. J. M., Schalken, J. A., Van Neck, H. W., Bloemers, H. P. J., & Van de Ven, W. J. M. (1987) *Nucleic Acids Res. 15*, 959–970.

Waterfield, M. D., Scrace, T., Whittle, N., Stroobant, P., Johnsson, A., Wasteson, A., Westermark, B., Heldin, C.-H., Huang, J. S., & Deuel, T. F. (1983) *Nature 304*, 35–39.

Weich, H. A., Sebald, W., Schairer, H.-U., & Hoppe, J. (1986) *FEBS Lett. 198*, 344–348.

Weiner, P. K., & Kollman, P. A. (1981) *J. Comput. Chem. 2*, 287–303.