Platelet-derived growth factor (PDGF), which is stored in the platelet α-granules and released during the platelet release reaction, is a major mitogen for mesenchymal cells in vitro (reviewed in Ref. 1). Its storage site and target cell specificity suggest a role in wound healing. PDGF is a M, 28,000–31,000 dimer of two polypeptide chains, denoted A and B (2), which are 60% similar to each other at the amino acid level (3). The dimer structure seems essential for biological activity since reduction irreversibly inactivates PDGF. The susceptibility of two chains to mild acid cleavage, and two activities. Thus, e.g., A chain homodimers have a lower mitogenic activity on fibroblasts than B chain containing dimers (4).

PDGF was first purified from human platelets (15–18). The purified product contains A and B chains at approximately equal amounts (4), but it has not been known whether it is a heterodimer or a mixture of homodimers. It has been difficult to determine the exact dimeric structure of PDGF due to the extent heterogeneity of the factor, caused by proteolysis during purification. In addition, the two polypeptide chains of PDGF show a high degree of amino acid sequence similarity.

In this communication, we have used antisera specifically recognizing the A and B chains of PDGF, the different susceptibility of the two chains to mild acid cleavage, and two high-resolving chromatographic procedures, and show that the major part of PDGF purified from human platelets is a heterodimer of one A and one B chain.

**MATERIALS AND METHODS**

**Purification of PDGF-like Growth Factors—**PDGF was purified from human platelet lysate, as described (19). To obtain homogeneous PDGF, human platelet pellets were lysed and centrifuged in the presence of protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 1% Trasylol, 3.1 M ε-amino caproic acid, and 20 mM benzamidine), as described (19). A chain homodimers (ODGF and GDGF-I) were purified from the conditioned media of human tumor cell lines and in several cases found to be homodimers of chains similar to the A chain of PDGF (10, 11). In addition, rat smooth muscle cells (12) and mitogen-stimulated human fibroblasts (13) secrete PDGF-like activity of the A homodimer type.

Different PDGF-like factors compete, at least partially, for binding to cell surface receptors. There are, however, indications that different PDGF dimers have dissimilar functional activities. Thus, e.g., A chain homodimers have a lower mitogenic activity on fibroblasts than B chain containing dimers (14).

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The PDGF B chain precursor is almost identical to p28α", the transforming protein of simian sarcoma virus (4–6). p28α" is dimerized after synthesis and then cleaved both in the N and the C termini yielding a biologically active molecule structurally similar to a B chain homodimer (7). PDGF purified from porcine platelets has also been found to be a homodimer of B chains (8). Several normal, as well as malignant, cell types express mRNA for the A and/or the B chain of PDGF (reviewed in Ref. 9). PDGF-like factors have been purified from the conditioned media of human tumor cell lines and in several cases found to be homodimers of chains similar to the A chain of PDGF (10–12). In addition, rat smooth muscle cells (12) and mitogen-stimulated human fibroblasts (13) secrete PDGF-like activity of the A homodimer type.

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1 Platelet-derived growth factor (PDGF) is a disulfide-bonded dimer consisting of two homologous polypeptide chains denoted A and B; it has not been known whether it is a heterodimer or a mixture of homodimers. We present here evidence that a major part of PDGF has a heterodimeric structure. A highly homogeneous, 31-kDa PDGF was purified in the presence of protease inhibitors and shown to contain both chains by means of immunoprecipitations with peptide antisera specific for the A and B chains, respectively. The susceptibility of PDGF to mild acid treatment and its chromatographic behavior in reversed-phase high performance liquid chromatography and immobilized metal ion affinity chromatography, as compared to A and B chain homodimers, is consistent with a heterodimeric structure. Analysis of PDGF purified according to our routine, large scale procedure revealed the major part to have a heterodimeric structure. In addition, B chain homodimers were also found.

With the demonstration that a major part of PDGF purified from human platelets occurs as a heterodimer, all three dimeric forms of PDGF have been identified. The following nomenclature to distinguish the various forms is suggested: PDGF-AA, a homodimer of A chains; PDGF-AB, a heterodimer; PDGF-BB, a homodimer of B chains; PDGF, any dimeric form of A or B chains.

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† The abbreviations used are: PDGF, platelet-derived growth factor; ODGF-I, glioma-derived growth factor-I; HP-LC, high performance liquid chromatography; IMAC, immunobilized metal ion affinity chromatography; ODGF, osteosarcoma-derived growth factor; SDS, sodium dodeyl sulfate.

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1 Hammacher, A., Nistér, M., Westermark, B., and Heldin, C.-H., Eur. J. Biochem. 176, 179–186

2 A. Östman, unpublished data.
Radiolabeling—The factors were labeled with $^{125}$I radioactivity according to the method of Bolton and Hunter (20), which labels both A and B chains, or by the chloramine-T method (21), which labels only A chains.

Synthesis of Peptides—The peptides from the A chain (amino acid residues 157-169; numbering as in Ref. 3) and B chain (amino acid residues 154-170) were chosen according to the method of Hopp and Woods (22). On the B chain peptide, an extra N-terminal tyrosine residue was added to facilitate UV detection. Peptides were synthesized by the solid phase method of Merrifield (23), using a Beckman Model 990 Peptide Synthesizer. The crude peptides were purified by HPLC on a Waters RCM Radial PACK column C18 (8 x 100 mm, 5 \( \mu \)m) in 0.1% trifluoroacetic acid with a gradient of 10-80% acetonitrile.

Immunization Procedure and Characterization of Immune Sera Against the A and B Chains of PDGF—The A and the B chain peptides were coupled to Keyhole Limpet hemocyanin (Calbiochem) with N-ethyl-N'-((3-trimethylaminopropyl)-carbodiimide hydrochloride (Fluka AG) (20 mg/ml) at a weight ratio of 1:1.4 (Keyhole Limpet hemocyanin:peptide) over night at 4 °C in 0.1 M sodium acetate buffer, pH 5. After dialysis against the same buffer, the material was injected with Freund's adjuvant intramuscularly in the hind legs of rabbits. The rabbits were boosted with 0.5 mg of peptide at 2-4-week intervals.

To test the specificity of the immune sera, immunoprecipitations of reduced and alkylated $^{125}$I-labeled human PDGF, porcine PDGF, and ODGF were carried out. The antisera were also tested in the presence of the immunizing peptide or the corresponding peptide from the other PDGF chain (90 \( \mu \)m peptide/ml) for 5 min at 95 °C. The samples were analyzed by SDS-gel electrophoresis and autoradiography.

**RESULTS**

Immunoprecipitation of PDGF with Chain-specific Antisera—Sera obtained from rabbits immunized with synthetic peptides chosen from the PDGF A and B chains were tested for specificity by immunoprecipitation of different radiolabeled dimers of PDGF A and B chains. No material was immunoprecipitated unless the compounds were reduced and alkylated (Fig. 1). When PDGF was $^{125}$I-labeled by the chloramine-T method (21), which labels tyrosine residues present only in the A chain, two bands around 17 kDa were obtained after immunoprecipitation with the A-peptide antiserum. The precipitation of this material was blocked by an excess of A-peptide, whereas the corresponding peptide from the B chain had no effect. No radiolabeled material was precipitated with the B-peptide antiserum (Fig. 1A); this was expected since the B chain is not labeled by the chloramine-T method. When PDGF is $^{125}$I-labeled on amino groups according to the method of Bolton and Hunter (20), both the A and the B chains incorporate radioactivity. From this factor the A-peptide antiserum precipitated two components of similar M substituted in peptide antisera have been established which specifically recognize the B-peptide.

**Human Platelets Contain PDGF-AB**

**Immunoprecipitation with Chain-specific Antisera**

![Characterization of peptide antisera reactive with the A and B chains of PDGF. PDGF-like factors were labeled with $^{125}$I radioactivity by the chloramine-T method (Fig. 1A) or by the Bolton and Hunter method (20) (Fig. 1, B-D). Reduced and alkylated samples were subjected to immunoprecipitation; the precipitated material was analyzed by SDS-gel electrophoresis and autoradiography. Fig. 1, A and B, $^{125}$I-PDGF; Fig. 1C, $^{125}$I-ODGF; Fig. 1D, $^{125}$I-labeled porcine PDGF. Lane A, material before precipitation, unreduced; lane B, material before precipitation, reduced; lane C, normal rabbit serum; lane D, A-peptide antiserum; lane E, A-peptide antiserum + A-peptide; lane F, A-peptide antiserum + B-peptide; lane G, B-peptide antiserum; lane H, B-peptide antiserum + A-peptide; lane I, B-peptide antiserum + B-peptide.**

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Human Platelets Contain PDGF-AB

**FIG. 2.** Immunoprecipitation of highly homogeneous PDGF, 125I-labeled according to the method of Bolton and Hunter (20), with peptide antisera specific for the A and B chains of PDGF. The material was reduced and alkylated before the immunoprecipitation, and precipitates were analyzed by SDS-gel electrophoresis and autoradiography. Lanes A–I are the same as in the legend to Fig. 1.

**FIG. 3.** Schematic representation of PDGF A and B chains assembled into homodimers of A and B chains and a heterodimer of one A and one B chain. Cysteine (C) and histidine (H) residues and the acid-susceptible bond between the aspartic acid and proline residues (DP) are marked as well as the peptides used for immunizations (horizontal bars).

ogonize the A and B chains of PDGF, respectively.

From the experiment described in Fig. 1B, it is clear that purified PDGF contains both A and B chains. The heterogeneity of the material has made it difficult to determine whether these are assembled into a heterodimer structure or as homodimers; to some extent, this is due to proteolysis during the purification. Therefore, an attempt was made to purify more homogeneous material by inclusion of a series of protease inhibitors during the first steps in the purification. This protocol yielded a highly homogeneous 31-kDa component on a silver-stained SDS gel (not shown). The pure factor was radioiodinated according to the method of Bolton and Hunter (20), with peptide antisera specific for the A and B chains of PDGF. The material was reduced and alkylated before the immunoprecipitation, and precipitates were analyzed by SDS-gel electrophoresis and autoradiography. Lanes A–I are the same as in the legend to Fig. 1.

**FIG. 4.** Mild acid cleavage of 125I-labeled ODGF and PDGF. The 125I-labeled compounds were treated with 70% HCOOH for 72 h at 37°C and analyzed by SDS-gel electrophoresis in the absence or presence of reducing agent. The gel was subjected to autoradiography.

**FIG. 5.** Analysis of reversed-phase HPLC. PDGF (---), GDGF-I (---), and B-homodimer (-----), labeled with 125I radioactivity according to the method of Bolton and Hunter (20), were analyzed by reversed-phase HPLC, as described under "Materials and Methods."
were labeled with 125I radioactivity according to the method of Bolton and Hunter (20) and subjected to immunoprecipitation using the chain-specific antisera. Fig. 2 shows that the highly homogeneous 31-kDa PDGF preparation contains both A and B chains; the A-peptide serum brought down a 17-kDa component (lane D), and the B-peptide serum precipitated a component of similar molecular mass (lane G).

**Analysis of PDGF after Mild Acid Hydrolysis**—The experiments described in Figs. 1 and 2 indicate that purified PDGF contains both A and B chains, but do not exclude the possibility that PDGF contains a mixture of A and B homodimers having exactly the same size. In order to further investigate the dimer structure of the PDGF molecule, we took advantage of the fact that the A chain, but not the B chain, contains a peptide bond which should be susceptible to mild acid hydrolysis (Asp-Pro, at positions 25 and 26 from the N terminus) (Fig. 3).

The effect of treatment of radiolabeled, highly homogenous PDGF with 70% HCOOH for 72 h at 37 °C was analyzed by SDS-gel electrophoresis. Analysis under reducing conditions of the A chain homodimer ODGF, revealed that the 17-kDa A chain was cleaved to a species of about 15 kDa at about 70% efficiency after HCOOH treatment (Fig. 4). The unreduced molecule showed a shift in migration from 31 to 28 kDa at about 30% efficiency (Fig. 4). This suggests that in ODGF, the most N-terminal cysteine residue in the A chain of ODGF forms a disulfide bond with the corresponding residue in another chain; cleavage of the Asp-Pro peptide bond between the first and second cysteine residues in both chains then causes a loss of molecular mass of the molecule, which would not happen if the disulfide bonds were arranged otherwise. The cleavage efficiencies, 70% of the monomer, but only 30% of the dimer, are in support of this model.

Analysis of HCOOH-treated PDGF under reducing conditions revealed the conversion of a small amount of the 17-kDa component to 15 kDa. The apparent lower efficiency of the cleavage compared to that of ODGF is expected, since approximately half of the radioactivity in the 17-kDa band of PDGF, after labeling according to the Bolton and Hunter procedure (20), resides in B chains, which should not be susceptible to HCOOH cleavage. Analysis of HCOOH-treated PDGF under nonreducing conditions revealed a homogenous 31-kDa component; no faster migrating species were seen. This indicates that all A chains of PDGF are assembled in heterodimer structures together with B chains.

**Chromatographic Analysis of PDGF**—Attempts were made to identify chromatographic systems which allowed separation of A and B homodimers. As markers were used A and B homodimers purified from conditioned media of a glioma cell line. A, Hammacher, A. Johnsson, and C.-H. Heldin, unpublished observation.

![Fig. 6. Analysis by IMAC of PDGF dimers. All compounds were labeled with 125I radioactivity according to the method of Bolton and Hunter (20). A, undegraded PDGF (---), GDGF-I (- - -), and B-homodimer (- - -) were analyzed by IMAC as described under "Materials and Methods." B, analysis by IMAC of 125I-PDGF, purified according to the routine procedure (19). C, analysis by SDS-gel electrophoresis of undegraded PDGF and of fractions from IMAC (Fig. 6B), before and after immunoprecipitation with chain specific antisera. Lane A, undegraded PDGF, unreduced; lane B, undegraded PDGF, reduced; lane C, PDGF used as starting material in B, unreduced; lane D, pool of flow-through fractions from B, unreduced; lane E, pool of bound fractions from B, unreduced; lane F, PDGF, used as starting material in B, reduced; lane G, pool of flow-through fractions from B, reduced; lane H, pool of bound fractions from B, reduced; lane I, pool of flow-through fractions from B, immunoprecipitated with A-peptide antiserum; lane J, pool of flow-through fractions from B, immunoprecipitated with B-peptide antiserum; lane K, pool of bound fractions from B, immunoprecipitated with A-peptide antiserum; lane L, pool of bound fractions from B, immunoprecipitated with B-peptide antiserum.

![Image: Analysis by IMAC of PDGF dimers]
line (GDGF-I)² and a Chinese hamster ovary cell line transfected with a B chain construct, respectively.

Fig. 5 shows that the two 125I-labeled homodimers could be separated by HPLC reversed-phase chromatography on a Vydac C₄ column eluted with 2 M guanidine-HCl, 1 M acetic acid, and a shallow propanol gradient; the A homodimer eluted before the B homodimer. A similar system has previously been used to separate A and B chain monomers; the A chain monomer was found to elute before the B chain (2), which is consistent with the order of elution of the two homodimers. 125I-PDGF eluted between the two homodimers but overlapped considerably with the B homodimer (Fig. 5).

Chromatography by IMAC takes advantage of the interaction between certain metal ions and surface-exposed histidine residues in proteins. Since the A chain of PDGF contains 3 histidine residues, but the B chain only 1 (Fig. 3), IMAC was tried to separate the various dimeric forms of PDGF. A chelating gel was used to immobilize copper ions. The column was eluted with an increasing gradient of imidazole; an inverse gradient of NaCl was also applied since it was found to increase resolution. In this system the B chain homodimer was found in the flow-through fraction, whereas the A chain homodimer eluted in the imidazole gradient (Fig. 6A), consistent with the differences in histidine contents. PDGF eluted in an intermediate position (Fig. 6A). Taken together, the elution position in reversed-phase HPLC and IMAC and the pattern on SDS gels after acid cleavage indicate that the highly homogenous PDGF has a heterodimeric structure.

When 125I-PDGF, purified according to the routine procedure not involving the use of protease inhibitors (19) and thus more degraded than the PDGF used in Fig. 6A (cf. Fig. 6C), was applied to IMAC, the radioactivity eluted in two peaks (Fig. 6B). Analysis by SDS-gel electrophoresis and autoradiography of these peaks, before or after immunoprecipitation with the chain-specific antisera, revealed that the first peak contained only B chains and the second both A and B chains (Fig. 6C). In addition, N-terminal amino acid sequence analysis of larger amounts of unlabeled PDGF applied to the IMAC column revealed essentially only B chain sequences in the first peak and equimolar amounts of A and B chain sequences in the second peak (data not shown). The amount of B chain homodimers in the preparation was estimated to 30% from the combined analyses by IMAC and N-terminal amino acid sequencing. In conclusion, PDGF purified by our routine procedure contains, in addition to heterodimers, PDGF B chain homodimers.

DISCUSSION

In spite of the fact that PDGF was first purified as early as 1979 (15, 16) and that it shortly thereafter was found to consist of about equal amounts of two homologous but distinct polypeptide chains (2-5), it has until now not been possible to determine whether PDGF is a heterodimer or a mixture of homodimers. The difficulties in determining the exact dimeric structure have been due to the heterogeneity of purified PDGF and to the fact that the two polypeptide chains show a high degree of amino acid sequence similarity; furthermore, the recovery of PDGF is low in many chromatographic systems. In this communication we describe methods to distinguish between the various dimeric forms of PDGF, including immunoprecipitation with chain-specific antisera, susceptibility to mild acid hydrolysis, and two different chromatographic systems, reversed-phase HPLC and IMAC. We used these methods to analyze PDGF purified from human platelets; a highly homogenous preparation, purified in the presence of protease inhibitors, was found to consist of heterodimers, whereas PDGF purified according to our routine, large scale procedure, in addition to heterodimers, also contains about 30% B chain homodimers. It is likely that the dissimilarities between the two preparations are due to slight differences in the purification procedures (19). It should be noted that the activity recovered after purification is only about 10%; the amounts of the different dimeric forms of PDGF in platelet lysate, therefore, remain unknown.

The human genes for the A and B chains are located on chromosomes 7 and 22, respectively (3, 26, 27). Both A and B chains are synthesized as precursor molecules with hydrophobic leader sequences (3, 28, 29) and undergo proteolytic cleavages during maturation. Dimerization seems necessary for the biological activity, but it is not known whether it occurs before or after processing or how the assembly into homodimers or heterodimers is controlled. Interestingly, transforming growth factor-β has also recently been found to consist of two distinct but homologous polypeptide chains, β₁ and β₂; the biologically active molecules are dimers, and both homodimers and the heterodimer have been identified (30).

Two polypeptide chains of PDGF are disulfide-bonded. The fact that the chains have 8 cysteine residues each and that there are no free SH-groups in the molecule suggests that at least 2 of the cysteine residues participate in interchain disulfide bonds. The experiment described in Fig. 4 suggests that one of these bonds is between the most N-terminal cysteines in each chain. The localization of the other bond(s) is not known.

The data presented in this communication, together with previous work (8, 10, 11), indicate that all three possible dimeric combinations of PDGF polypeptide chains occur. To distinguish the various forms, we propose the following nomenclature to be used: PDGF-AA, a homodimer of A chains; PDGF-BB, a homodimer of B chains; PDGF-AB, a heterodimer; PDGF, any dimeric form of A or B chains.

To determine the exact dimeric structure of PDGF-like factors is important, particularly in relation to the recent finding that different dimeric forms have different functional activities (14). The chromatographic procedures described in this communication allow the separation of all three possible dimeric forms of PDGF. As both reversed-phase HPLC and IMAC are applicable also to unlabeled samples, it is anticipated that they will be useful in the characterization of PDGF dimers produced by various cell types.

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