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PLATELET-DERIVED GROWTH FACTOR: IDENTIFICATION OF CONSTITUENT POLYPEPTIDE CHAINS

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SUMMARY: Hydrophobic high-performance liquid chromatography of reduced and alkylated ^{125}I-labeled platelet-derived growth factor (PDGF) resulted in the separation of two distinct radioactive components. One of them, designated A, was resolved by polyacrylamide gel electrophoresis in sodium dodecyl sulfate, into four species with the molecular weights 18,000, 15,000, 14,000 and 11,000, respectively. The other component, designated B, showed only one major form with an M_r of about 16,000. Unlabeled PDGF yielded a similar pattern of products. A molecular model is proposed in which each molecule of PDGF consists of one A and one B polypeptide, linked by disulfide bonds. The A chain, but not the B chain, has a variable size, leading to inhomogeneity in the intact PDGF molecule (M_r 28,000 - 33,000). Some molecular weight heterogeneity is compatible with full biological activity.

INTRODUCTION

Platelet-derived growth factor (PDGF)*, a potent mitogen stored in the platelet α-granules (1,2), was recently isolated in near homogeneous form (3-6). Since only minute amounts of PDGF have been available for analysis the structural features of the molecule have not been elucidated in detail. Our previous studies indicated that reduction converted PDGF from a 30,000 dalton form into more low molecular weight components, with M_r ranging from 14,000 to 18,000. On the basis of these findings we suggested that PDGF consists of two different polypeptides, linked by disulfide bridges (5). The present work was undertaken in order to isolate and identify the individual components of this model.

* Abbreviations: PDGF, platelet-derived growth factor; HPLC, high performance liquid chromatography.

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MATERIALS AND METHODS

Partially purified PDGF

PDGF was prepared from human platelets by sequential chromatographies on CM-Sephadex, Blue Sepharose and Bio-Gel P-150, under conditions described previously (5). Occasionally a slight modification of the CM-Sephadex step was applied, in which PDGF was collected from the column by batchwise elution (with 0.5 M NaCl, 0.01 M phosphate buffer, pH 7.4), rather than by gradient elution. Analysis by polyacrylamide gel electrophoresis in sodium dodecyl sulfate of the material resulting after the Bio-Gel P-150 step showed that the modification had no influence on the product. The purity of PDGF was 25-50% at this stage.

High performance liquid chromatography (HPLC)

PDGF purified through Bio-Gel P-150 chromatography (about 600 µg) was lyophilized, dissolved in 200 µl 1 M acetic acid and applied to a 0.4 cm x 25 cm Lichrosorb RP-8 column (particle size 5 µm) (Merck), equilibrated with 2 M HCOOH, 0.8 M pyridine. The column was operated at 42°C at a flow rate of 0.4 ml/min; 1.2 ml fractions were collected. After washing the column with 6 ml 2 M HCOOH, 0.8 M pyridine, it was developed with a non-linear gradient (total volume 56 ml) of propanol (0-26%). The effluent was analyzed for protein (7) and multiplication-stimulating activity (8).

Analytical HPLC was run on a similar column using the same conditions as described above for preparative HPLC. ^{125}I-labeled samples in 1 M acetic acid (100 µl) together with gelatin (1 mg/ml) as a carrier, were applied to the column; the effluent was analyzed for radioactivity in a Packard gamma Spectrometer at 70% efficiency.

Radiolabeling

Pure PDGF (5 µg) was labeled with ^{125}I (0.5 mCi) (NEN, Dreieich, W. Germany) according to Bolton and Hunter (9). A specific activity of about 20,000 cpm/ng was obtained.

Reduction and alkylation

PDGF or ^{125}I-PDGF, in 4 M guanidine, 0.5 M Tris-HCl, pH 8.0, was reduced by a 2 hour treatment with 10 mM dithiothreitol at 37°C and then alkylated with 25 mM iodoacetamide (1 hour at room temperature in the dark).

Gel electrophoresis

Polyacrylamide gel electrophoresis in sodium dodecyl sulfate was carried out according to Blobel and Dobberstein (10) using gradient gels from 13% to 18% acrylamide. Gels were 1 mm thick and 170 mm long and electrophoresis performed at room temperature at a constant current of 15 mA. After the run the gels were fixed in 20% sulphosalicylic acid for 30 min at room temperature, and stained for protein with Coomassie Brilliant Blue R-250. Radioactivity was visualized by autoradiography using intensifying screens (Du Pont Lightning Plus) and preflashed Kodak XOmat AR films. The following molecular weight standards were used (Pharmacia Fine Chemicals, Uppsala): phosphorylase b (M_r 94,000), bovine serum albumin (M_r 67,000), ovalbumin (M_r 43,000), carbonic anhydrase (M_r 30,000) soy bean trypsin inhibitor (M_r 20,100) and α-lactalbumin (M_r 14,400)

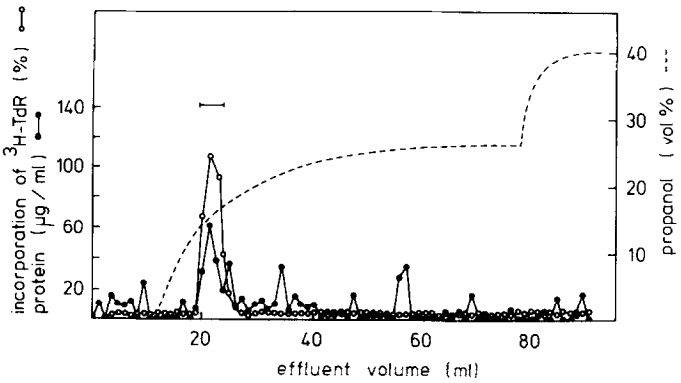

Fig. 1. Preparative HPLC of PDGF. Semipure PDGF (600 µg), prepared as described in Materials and Methods (using the procedure including batch-wise elution from CM-Sephadex) was applied on an RP-8 column. The column was eluted with a gradient of propanol (- - - -) (see Materials and Methods) and the effluent analyzed for protein (●-●) and multiplication stimulating activity (O-O) (8). Active fractions were pooled as indicated.

RESULTS

Purification of PDGF

High performance liquid chromatography of PDGF in 2 M formic acid, 0.8 M pyridine on a hydrophobic matrix (RP-8) led to adsorption of the factor (Fig. 1). The biological activity could be desorbed from the column by elution with a gradient of propanol in the same medium, the factor appearing as a distinct peak of biological activity and protein. Analysis by gel electrophoresis revealed proteinstainable material only in the molecular weight range 28,000 - 33,000, as expected for PDGF (Fig. 2). Extracts from various parts of this region of the gel showed biological activity in approximate proportion to the amount of stainable protein (not shown), indicating that PDGF of different molecular weights had similar specific activities. Gel electrophoresis of PDGF under reducing conditions showed two components of molecular weight 16,000 to 18,000 (Fig. 2), in agreement with earlier observations (5).

Separation of PDGF polypeptide chains

Pure PDGF was labeled with ^{125}I by the Bolton-Hunter procedure and

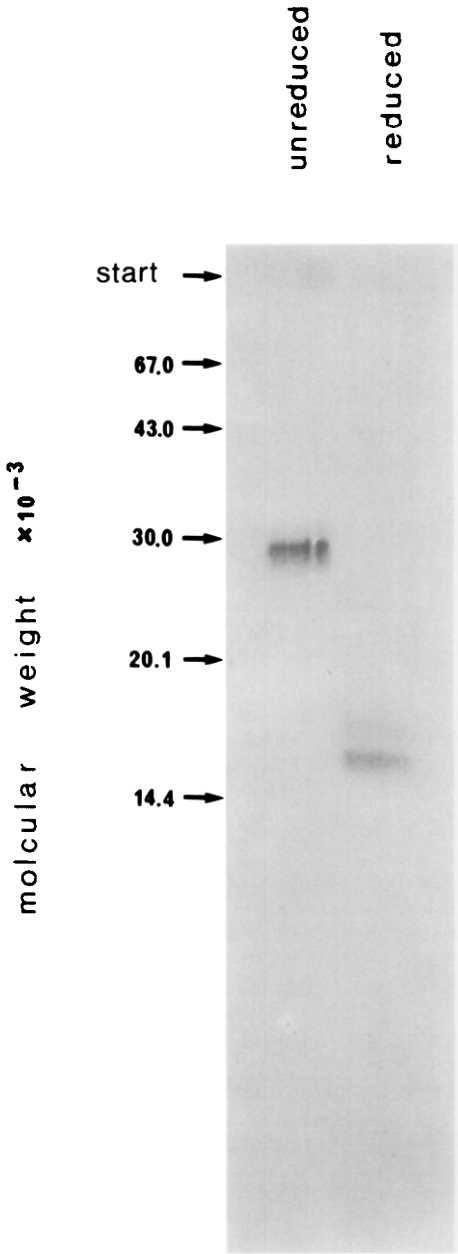

Fig. 2. Gel electrophoresis of PDGF obtained by preparative HPLC, PDGF-containing fractions of the HPLC run were pooled as shown in Fig. 1; samples of 5 μg were analyzed by electrophoresis in sodium dodecyl sulfate on a 13% to 18% gradient gel, before or after reduction and alkylation (see Materials and Methods for further details). The gel was stained for protein with Coomassie Brilliant Blue R-250.

reduced and alkylated. HPLC of this material on a column of RP-8 in 2 M formic acid, 0.8 M pyridine resulted in the chromatogram shown in Fig. 3.

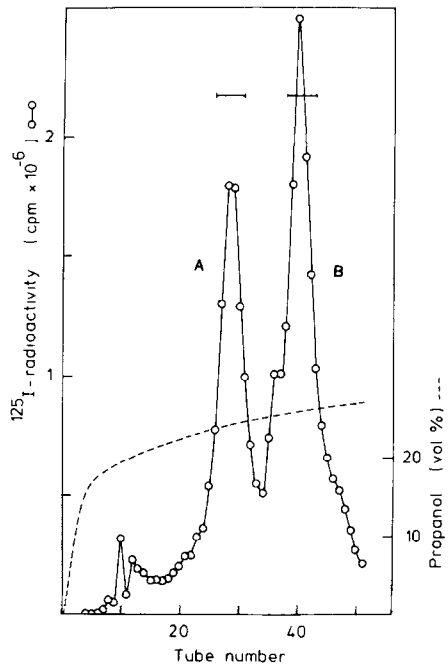

Fig. 3. Fractionation of reduced and alkylated ^{125}I-PDGF on HPLC. ^{125}I-labeled PDGF was reduced and alkylated and applied on an RP-8 column, operated as described in Materials and Methods. The effluent was analyzed for radioactivity and pooled as indicated.

A major part of the radioactivity eluted by the propanol gradient was distributed in two peaks, designated A and B. The yield of ^{125}I-radioactivity in this step was 70-90%. The peak fractions of component A and B, respectively, were pooled and analyzed by gel electrophoresis (Fig. 4). Autoradiography of the gel showed the resolution of component A into one major and three minor bands of radioactivity with mobilities corresponding to molecular weights of 18,000, 15,000, 14,000 and 11,000, respectively, the most high molecular weight species (M_r 18,000) giving the strongest intensity. Component B produced a more homogeneous pattern with only one major component, migrating like a 16,000 dalton polypeptide.

Applying the conditions worked out for ^{125}I-labeled material, reduced and alkylated unlabeled PDGF was similarly fractionated on HPLC. Effluent fractions were analyzed in pairs by gel electrophoresis (Fig. 5). Stainable

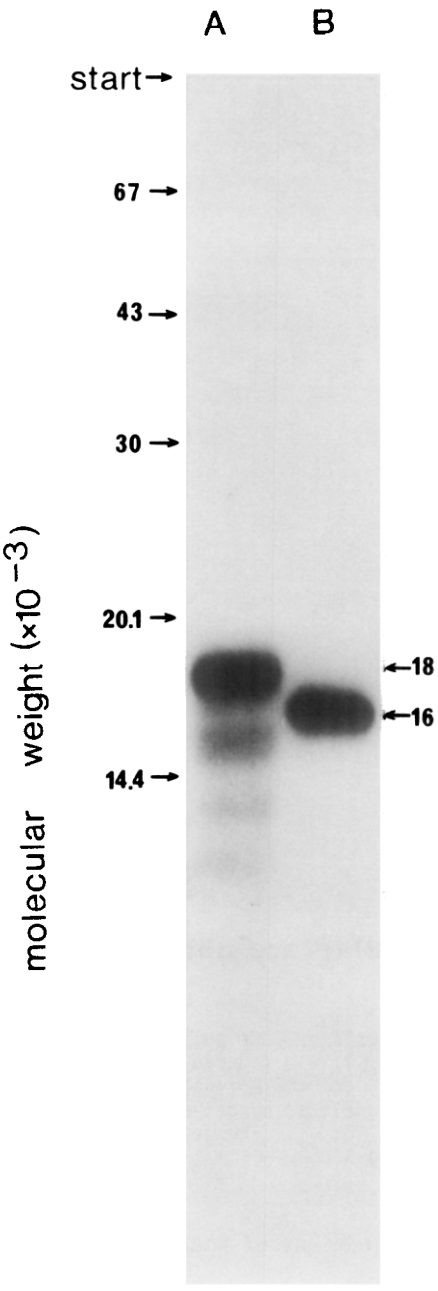

Fig. 4. Gel electrophoresis of ^{125}I-labeled PDGF polypeptide chains. Reduced and alkylated ^{125}I-PDGF was fractionated by HPLC (Fig. 3). Components A and B (Fig. 3) were analyzed by gel electrophoresis in sodium dodecyl sulfate, using a 13% to 18% gradient gel. Radioactivity was visualized by autoradiography.

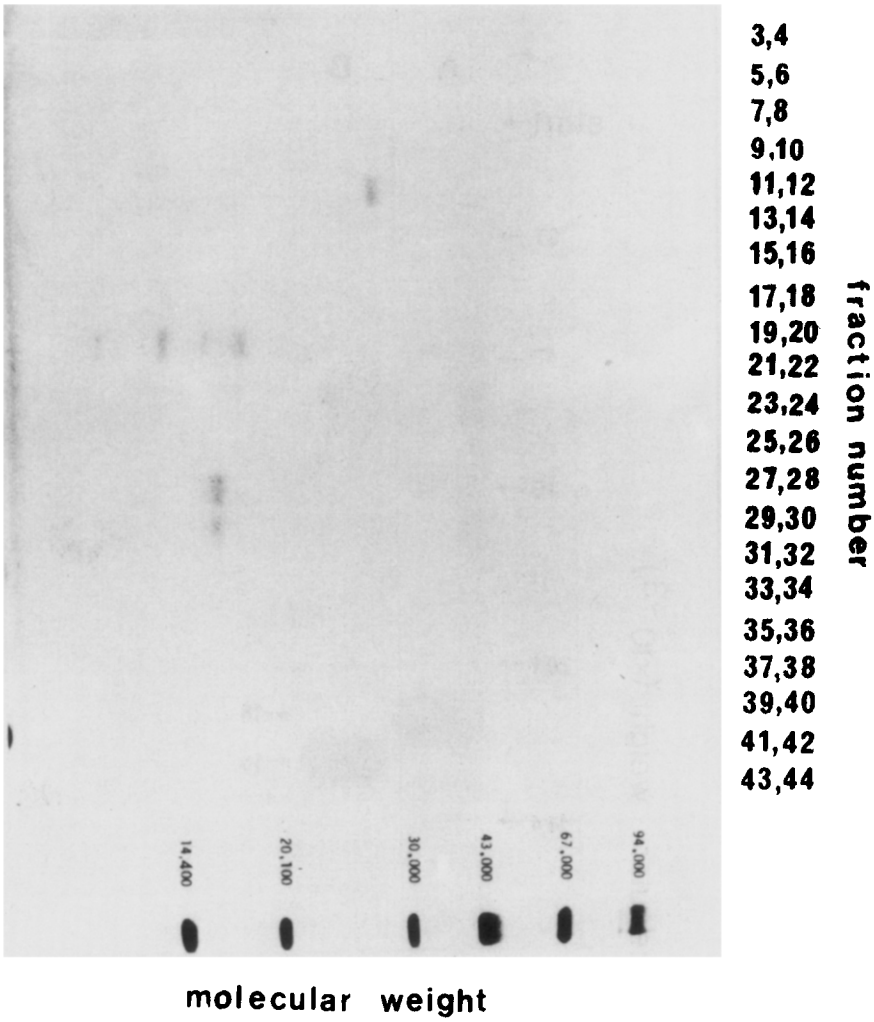

Fig. 5. Gel electrophoresis of PDGF polypeptide chains. Pure PDGF, prepared by HPLC as shown in Fig. 1, was reduced and alkylated and fractionated by a second cycle on the HPLC column. Aliquots of the effluent were pooled in pairs, lyophilized and analyzed by gel electrophoresis in sodium dodecyl sulfate, using a 13% to 18% gel. The gel was stained for protein with Coomassie Brilliant Blue R-250.

material was obtained from regions of the propanol gradient, corresponding to the elution of component A (fractions 19-20) and B (fractions 27-30) of the ^{125}I-labeled material. (The presence of material behaving like native PDGF (fractions 11-12) was not a constant finding but probably due to incomplete reduction in this particular experiment.) Component A gave rise to four stained bands with apparent molecular weights of about 18,000, 15,000,

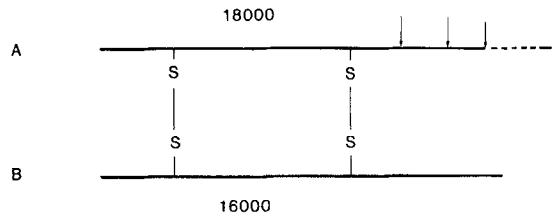

Fig. 6. Tentative model of the PDGF-molecule. The horisontal bars indicate two different polypeptide chains, A and B. The A chain may have a variable length; arrows indicate possible sites of proteolytic attack (cf. Fig. 3). The broken line represents a fragment of the A chain, not indispensable for the biological activity of the intact PDGF molecule. The exact number of disulfide bonds linking the A and the B chains is not known; the total number of half-cystine residues would allow 6 (5) to 9 (6) disulfide bridges, including inter- as well as intrachain bonds.

14,000 and 11,000. Component B showed a molecular weight of about 16,000. It was concluded that HPLC allowed the separation of reduced and alkylated unlabeled PDGF into peptides similar in electrophoretic mobility to those obtained with ^{125}I-labeled material.

DISCUSSION

On the basis of the present findings we suggest the structural model for PDGF shown in Fig. 6. According to this model PDGF is a two-chain protein with a molecular weight of about 30,000; the molecular weight is somewhat variable in the range 28,000 to 33,000 as determined by gel electrophoresis in sodium dodecyl sulfate under non-reducing conditions (4-6). Similar values have been obtained by ultracentrifugation (5) and gel chromatography (4,8). PDGF consists of two different polypeptide chains, designated A and B in the figure. The A chain is heterogeneous in size, showing one major 18,000 dalton form and in addition, distinct fragments of somewhat shorter length (M_r 15,000, 14,000, 11,000, respectively). The B chain is more homogeneous yielding only one major molecular weight class in analytical gel electrophoresis (M_r 16,000). Disulfide bonds link the two polypeptides; the high number of half-cystine residues in PDGF (5,6) would allow for several disulfide bridges in the molecule. These structures have probably a stabi-

lizing effect on the PDGF molecule since native PDGF is remarkably resistant to denaturation by heat (11,12) or dissociative solvents (13), but irreversibly inactivated by reducing agents (3). The variability in the A chain with respect to molecular weight may explain the heterogeneity in intact PDGF observed previously (3-6,8). This feature of the A chain is remarkable, considering the homogeneous appearance of peak A in HPLC. The most likely interpretation is a proteolytic degradation of the A chain, producing fragments of different lengths but with a common hydrophobic determinant. It remains to be clarified whether this process is a specific event, e.g. related to the maturation of the molecule or an unspecific but limited attack by proteases in the course of preparation. It is noteworthy, however, that partial modification, e.g. as indicated by the dashed line in Fig. 6, is compatible with a retained biological activity of the molecule.

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