Human platelet lysates contained potent mitogenic activities for MCF-7 human breast-cancer cells in serum-free-defined media. Because these activities were not replaced by known platelet mitogens, such as platelet-derived growth factor or transforming growth factor β, we sought to identify the breast cancer cell mitogens by purification and N\textsuperscript{α} amino acid sequencing. Acetic acid extracts of outdated human platelets were concentrated by ammonium sulfate precipitation and fractionated on Sephadex G-50 and Bio-Gel P-10 columns in 0.5 mol/L acetic acid. Two major activities were resolved by molecular sieve methods and fractionated further by reverse-phase high-performance liquid chromatography (HPLC). Purifications (70,000 to 870,000-fold) were accomplished yielding mol wt 7,400 products that were homogeneous as determined by iodination, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and autoradiography. The factors were identified as insulin-like growth factor I (IGF-I) and II (IGF-II) and truncated IGF-I by N\textsuperscript{α} amino acid sequencing. In dose-response experiments, platelet-derived IGF-I and IGF-II promoted multiple divisions of the MCF-7 cells with ED\textsubscript{50} values of 12 and 100 pg/mL, respectively. The specific activities and other bioassay characteristics of platelet-derived IGF-I and IGF-II were similar to those of recombinant-produced human growth factors. This is the first report of the purification of insulin-like growth factors from human platelet lysates.

Previously this laboratory demonstrated that lysates of outdated human platelets contained growth-factor activities for rat and human breast-cancer cells in culture. Microgram/milliliter concentrations of neutral pH extracts promoted continuous division of MTW9/PL rat and MCF-7 human cells under serum-free conditions. Lysates were shown also to be mitogenic for a wide variety of established epithelial and mesenchymal origin cell lines as well as containing growth factors for short-term cultures of human diploid fibroblasts. Because of the diversity of cell types stimulated, we proposed that platelets contained many growth factor activities.

Earlier reports had shown that platelets contained a potent mitogen that was later purified to homogeneity by several methods and that was established to be an A:B heterodimer with B-chain amino acid sequence homology to the expected protein product of the v-sis oncogene. This factor, known as platelet-derived growth factor (PDGF), has since been identified in a homodimeric form containing A chains. Further heterogeneity of PDGF types was possible within the A chain structure. Although mol wt 26,000 to 31,000 PDGF usually was considered a mitogen for mesenchymal cells, our previous study showed platelets contained a mammary epithelial cell activity of approximately mol wt 38,000, which might have been one of the forms of PDGF.

Platelets are also an important source of transforming growth factor β (TGFβ). This dimeric mol wt 25,000 factor has been identified in both TGFβ1 and TGFβ2 forms and has been shown to influence mesenchymal cell growth positively and epithelial cells negatively. Other studies confirmed that epidermal growth factor (EGF)/urogastrone was present in the platelet structure, as was a mol wt 45,000 endothelial-cell growth factor.

To determine which mitogens in platelets promoted growth of human breast-cancer cells, we used reverse phase high performance liquid chromatography (RP-HPLC) and a newly developed serum-free cell-culture bioassay method to purify the activities. The bioassay with MCF-7 and T47D human breast-cancer cells allowed a direct comparison of the potencies of five major functional families of nonlymphoid growth factors. The heterodimeric form of PDGF, TGFβ1, and TGFβ2 were not mitogenic for these cell lines, whereas insulin-like growth factors I (IGF-I) and II (IGF-II), EGF, transforming growth factor α (TGFα), and basic fibroblast growth factor (bFGF) promoted continuous cell divisions at picomolar to nanomolar concentrations. Therefore platelet-derived breast-cancer cell growth factor(s) might have been related functionally or structurally to a homodimeric form of PDGF or to any of the other mitogen groups that were active in this assay. Another possibility was a novel mitogen not previously characterized. To establish identity conclusively, we undertook the complete purification and identification by N\textsuperscript{α} amino-acid sequencing. A preliminary report of these results has been presented.

MATERIALS AND METHODS

Cell culture. Stock cultures of MCF-7 cells (American Type Culture Collection, Rockville, MD) were maintained in a 1:1 (vol/vol) mixture of Ham’s F12 and Dulbecco’s Modified Eagle’s media (DMEM, high-glucose formulation) supplemented with 2.2 g/L sodium bicarbonate (F12/DMEM), 15 mmol/L HEPES, pH 7.2, and 10% (vol/vol) fetal bovine serum (FBS) in a humidified atmosphere of 95% (vol/vol) air and 5% (vol/vol) CO\textsubscript{2} at 37°C. Cultures were passed every four to six days and seeded at 1.75 × 10\textsuperscript{4}/10-cm diameter plastic culture dish in 20 mL of medium. Stock cultures were assayed bimonthly for mycoplasma contamination using the MycoTect system purchased from GIBCO (Grand Island, NY); the tests were negative throughout this investigation.

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Supported by National Cancer Institute Grant CA-38024, American Cancer Society Grant BC-255, and Grant No. 2225 from The Council for Tobacco Research, USA, Inc., New York.

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0006-4971/89/7403-0024$3.00/0


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Labeled thymidine incorporation assay. The methyl-\(^3\)H thymidine (\(^{3}\)HTdR) incorporation assay described previously\(^{21}\) was used to monitor column chromatography fractions for biological activity. Briefly, cells were harvested from stock culture plates, washed three times with F12/DMEM supplemented with 2.2 g/L sodium bicarbonate, 15 mmol/L HEPES, pH 7.2, 10 \(\mu\)g/mL human transferrin (Tf; Collaborative Research, Bedford, MA), and 200 \(\mu\)g/mL bovine serum albumin fraction V (BSA; Sigma, St Louis; hereafter referred to as Tf/BSA). Cells were seeded at 1.0 \(\times\) 10\(^6\)/0.5 mL of Tf/BSA medium in 12 \(\times\) 75-mm sterile polystyrene culture tubes containing the sample (\(\pm 50 \mu\)L) to be assayed. After one-hour in a 37\(^\circ\)C cell-culture incubator, 0.5 \(\mu\)Ci/ml \(^{3}\)HTdR (60 to 90 Ci/mmol, ICN Biomedicals, Costa Mesa, CA) was added to each tube in 10 \(\mu\)L of 2.5 mg/mL gentamicin sulfate (Sigma). After a 48-hour incubation at 37\(^\circ\)C, the cells were collected by centrifugation, precipitated, and washed twice with 10% (wt/vol) trichloroacetic acid. The radioactivity in the pellet was determined by liquid scintillation counting.

Aliquots of column fractions from conventional chromatography steps were assayed directly for their ability to cause \(^{3}\)HTdR incorporation into DNA after dilutions of \(\leq\)1000-fold. Samples of RP-HPLC fractions were dried in a Speed Vac Concentrator (Savant Industries, Hicksville, NY) in the presence of BSA and dissolved in glass-distilled water. This drying procedure stabilized activities, minimized sample losses, and eliminated toxic solvents. The additional BSA introduced into the \(^{3}\)HTdR and cell number assays was \(\pm 25 \mu\)g, which did not affect results\(^{23}\). Chromatography fractions were collected into either siliconized borosilicate glass or polypropylene tubes to maximize recoveries.

Cell number assay. Mitogenic activity was quantified by monitoring MCF-7 growth by an increase in cell number over eight days. These methods have been described in detail elsewhere\(^{21,23}\). The cell population doublings (cpd) were pooled and stored at 2.5 mg/mL gentamicin sulfate (Sigma). After a 48-hour incubation at 37\(^\circ\)C, the cells were collected by centrifugation, precipitated, and washed twice with 10% (wt/vol) trichloroacetic acid. The radioactivity in the pellet was determined by liquid scintillation counting.

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Labelling of purified growth factors and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Aliquots of each purified growth factor (\(\geq 10 \mu\)g of total protein) were placed separately in 1.5 mL microcentrifuge tubes and dried in a Speed Vac Concentrator. Each sample was dissolved in 20 \(\mu\)L of 0.5 mol/L sodium phosphate, pH 7.5, and iodinated by addition of 1.0 mCi of carrier-free sodium \(^{125}\)I in 5 \(\mu\)L of 0.5 mol/L sodium phosphate, pH 7.5, and 5.0 \(\mu\)g chloramine T in 10 \(\mu\)L of 10 mmol/L sodium phosphate, pH 7.0. This combination was mixed for 30 seconds at room temperature and the reaction terminated by the addition of 10 \(\mu\)L of 5.0 mol/L sodium metabisulfite in 10 mmol/L sodium phosphate (pH 7.0) and 45 \(\mu\)L of phosphate-buffered saline (PBS) containing 1.0% (wt/vol) potassium iodide and 16% (wt/vol) sucrose. The reaction products were separated on a 0.5 \(\times\) 15-cm Sephadex G-25 column (siliconized glass) that was equilibrated and eluted with 0.5 mol/L acetic acid. Fractions containing \(\approx 125\)I-labeled growth factors (void volume, \(V_a\)) were pooled and stored at \(-20^\circ\)C. Aliquots of these labeled proteins (approximately 12,000 cpm/sample) were dried in a Speed Vac Concentrator, dissolved in sample buffer, and analyzed by SDS-PAGE according to the method of Laemmli\(^{22}\) using a 5% (wt/vol) stacking and 15% (wt/vol) acrylamide resolving gel. After the gel was dried and exposed to x-ray film (X-Omat XR-5, Eastman Kodak, Rochester, NY) for 26 hours at \(-70^\circ\)C.

Human recombinant 71 amino acid N\(^{\text{2}}\)-Met-IGF-I\(^{27}\) was a gift from Dr B. Daniel Burleigh, IMC Pitman-Moore (Terra Haute, IN) or was supplied courtesy of Charles E. Seeley, IMCERA Bio.
Pool B, from the Bio-Gel column, was applied to a 0.78 steps.

at

with fractions stored

Pool A activity.

activity. (Table 1). No attempt was made to further characterize the Pool A activity.

Purification of Bio-Gel pool B by RP-HPLC. All RP-HPLC chromatography was performed at room temperature with fractions stored at 4°C or –20°C between purification steps. Pool B, from the Bio-Gel column, was applied to a 0.78 × 30-cm μBONDAPAK C18 column (Waters Division of Millipore, Milford, MA). A multilinear gradient of acetonitrile in trifluoroacetic acid (TFA) was used to elute the column (Fig 2A). The broad peak of biological activity that eluted was well separated from the major UV-absorbing components. The active fractions were pooled (Fig 2A, shaded area), concentrated by lyophilization in a siliconized flask, dissolved in 0.1% (vol/vol) TFA (4.0 mL), and applied again to the same column. Elution was accomplished with a multilinear gradient of 2-propanol. The peak of biological activity eluted immediately before a series of UV absorbance peaks. The pooled fractions (Fig 2B, shaded area, 30 mL) were concentrated by lyophilization in a siliconized flask and dissolved in 0.1% (vol/vol) TFA (3.4 mL). This sample was divided into three equal portions and each applied to a Vydac C4 RP-HPLC column (Separations Group, Hesperia, CA) equilibrated with 0.1% (vol/vol) TFA. Biological activity was determined using a final sample dilution of 1:2,000. Shaded fractions were pooled and concentrated. (B) Elution of the active pool from Fig 2A by solvent B, which was 90% (vol/vol) 2-propanol and 0.1% (vol/vol) TFA. Selected fractions were assayed at a final dilution of 1:2,000 (vol/vol). Shaded fractions were pooled and concentrated.

Fig 2. Purification of Bio-Gel pool B on a C4 RP-HPLC column. The eluates (flow rate 2.5 mL/min) were monitored at 280 nm. (A) Elution of Bio-Gel pool B by solvent B, which was 90% (vol/vol) acetonitrile and 0.1% (vol/vol) TFA. Biological activity was determined using a final sample dilution of 1:2,000. Shaded fractions were pooled and concentrated. (B) Elution of the active pool from Fig 2A by solvent B, which was 90% (vol/vol) 2-propanol and 0.1% (vol/vol) TFA. Selected fractions were assayed at a final dilution of 1:2,000 (vol/vol). Shaded fractions were pooled and concentrated.

Peak 1 was diluted 1:1 (vol/vol) with 0.1% (vol/vol) TFA and applied again to the same Vydac C4 column eluted with a gradient of 2-propanol. The activity was associated with a single protein peak and was pooled as indicated (Fig 4A, shaded area). This material (peak B1) was subjected to Nα amino acid sequence analysis.

Peak 2 material was subjected to the same Vydac C4 RP-HPLC fractionation as peak 1. Peak B2 activity, which was associated with a very small UV-absorbing peak (Fig 4B, shaded area), was subjected to Nα amino acid microsequencing.

Purification of Bio-Gel pool C by RP-HPLC. Pool C from the Bio-Gel P-10 column was applied to a μBONDAPAK C8 RP-HPLC column equilibrated with 0.1% (vol/vol) TFA and eluted with a multilinear gradient of acetonitrile (Fig 5). The major peak of activity eluted between 160
column, and eluted with a gradient of 2-propanol. A single area of activity corresponded to a well-separated absorbance peak (Fig 6B, shaded area). The active fractions (peak C) were pooled and subjected to N-terminal amino-acid sequencing.

Identification of purified mitogens by amino-acid sequence analysis. The results of the amino-acid sequence analysis of the purified fractions are shown in Fig 7. Peak B1 was the final Vydac C2 RP-HPLC fractionation of Bio-Gel Pool B activity (from Fig 4A). It contained an N* truncated form of IGF-I that lacked the first three to six amino acids of intact human IGF-I.36 Also, the sample contained a signifi-
The N\textsuperscript{a} amino-acid sequence of the Bio-Gel P-10 peak C material (from Fig 6B) was identical to IGF-II throughout the 20 residues identified beginning with the N\textsuperscript{a} terminus established by Rinderknecht and Humbel\textsuperscript{11} (Fig 7).

**Mol wt determinations and evaluation of homogeneity.** Samples of each sequenced growth factor were radiodinated separately and analyzed by SDS-PAGE and autoradiography. The relative mobilities of bands on the autoradiogram were compared with those of \textsuperscript{125}I-labeled recombinant IGF-I and prestained proteins of known mol wt (Fig 8). All of the sequenced IGF-I preparations (lanes 2 through 4) demonstrated a major single component of mol wt equivalent to recombinant IGF-I (lane 1). Truncated IGF-I (lane 4) had a mobility similar to \textsuperscript{125}I-labeled recombinant IGF-I. Apparently, the SDS-PAGE method used\textsuperscript{26} was unable to resolve mol wt differences of ±600.

The band pattern in lane 4 demonstrated the purity of the truncated IGF-I product following additional purification of peak B1 material by Vydac C\textsubscript{18} RP-HPLC to remove the HSA peptide as described above. In contrast, lane 5 showed the presence of a mol wt 9,000 component in the sample of truncated IGF-I before additional RP-HPLC purification. Presumably this material was the HSA peptide. The bands present at the bottom of lanes 3 and 4 were due to free \textsuperscript{125}I migrating with the dye front. Their appearance increased with prolonged storage of the labeled materials and/or incomplete separation of reaction products on the Sephadex G-25 column following iodination.

**Determination of specific activity during purification by the cell-number assay.** Growth-factor specific activity was monitored initially at each step of the isolation by the MCF-7 \textsuperscript{3}HTdR incorporation assay. Samples of each pooled fraction were stored frozen until the isolation was completed and assayed together by the cell number method for determination of final fold purification and specific activity. A representative set of assays is shown in Fig 9. The protein concentrations required to achieve ED\textsubscript{50} progressively decreased from crude extracts to homogeneous preparations. At every step, growth was confirmed to equal or approach that promoted by a saturating concentration of insulin (100 ng/mL). Addition of the platelet-derived mitogens at sufficiently high concentrations caused 2.5 to 3.0 cpd above the Tf/BSA controls (C\textsubscript{0}). In all cases concentrations of mitogens five times those of ED\textsubscript{50} promoted the same level of growth as 100 ng/mL insulin (data not shown).

The crude acetic acid extract had an ED\textsubscript{50} of 7.2 µg/mL, whereas platelet-derived truncated IGF-I (peak B1), IGF-I (peak B2), and IGF-II (peak C) had a values of 8, 12, and 100 pg/mL, respectively. These values were within the same range as the ED\textsubscript{50} concentrations we reported before with recombinant human IGF-I and IGF-II.\textsuperscript{25} Only truncated IGF-I from platelets appeared somewhat less active than expected. Our other recent studies\textsuperscript{29} of new forms of insulin-like factors from uterus showed DES I ÷ 3 (6) IGF-I had an ED\textsubscript{50} of 0.8 to 3.3 pg/mL with MCF-7 cells in the Tf/BSA cell number assay. The differences in ED\textsubscript{50} values between platelet and uterine-derived truncated IGF-I were most probably related to the methods used to estimate the amounts of protein isolated.

**Fig 7.** Comparison of N\textsuperscript{a} amino acid sequences of platelet purification products IGF-I and IGF-II. Peak B1 refers to the product derived from Bio-Gel pool B carried through the Vydac C\textsubscript{8} RP-HPLC step (Fig 4A). Peak B2 refers to the product derived from Bio-Gel pool B and carried through the Vydac C\textsubscript{8} RP-HPLC (Fig 4B). Peak C refers to the product of Bio-Gel pool C carried through the Vydac C\textsubscript{8} RP-HPLC step (Fig 4B). X, not determined.

**Fig 8.** Mol wt analysis of RP-HPLC purified radiiodinated insulin-like growth factors and human recombinant IGF-I by SDS-PAGE and autoradiography. Lane 1, \textsuperscript{125}I-human recombinant IGF-I; lane 2, peak B2 \textsuperscript{125}I-IGF-I; lane 3, peak C \textsuperscript{125}I-IGF-II; lane 4, \textsuperscript{125}I-truncated IGF-I (peak B1) after additional Vydac C\textsubscript{8} RP-HPLC purification; and lane 5, \textsuperscript{125}I-truncated IGF-I (peak B1) before removal of the HSA contaminant. The Coomassie blue-prestained mol wt markers were ovalbumin (43,000), α-chymotrypsinogen (25,000), β-lactoglobulin (18,000), lysozyme (15,000), bovine trypsin inhibitor (6,000), and insulin (A and B chains, 3,000).
Table 1. Summary of the Purification of IGF-I and IGF-II From Human Platelets

<table>
<thead>
<tr>
<th>Step</th>
<th>Total Protein (mg)</th>
<th>ED&lt;sub&gt;50&lt;/sub&gt; (ng/mL)</th>
<th>Total Units</th>
<th>Yield (%)</th>
<th>Fold Purification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid extract</td>
<td>17,450</td>
<td>7,160</td>
<td>2,400,000</td>
<td>100.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Ammonium sulfate precipitation</td>
<td>5,249</td>
<td>6,493</td>
<td>808,410</td>
<td>33.7</td>
<td>1.1</td>
</tr>
<tr>
<td>Sephadex G-50</td>
<td>554</td>
<td>729</td>
<td>759,770</td>
<td>31.7</td>
<td>9.8</td>
</tr>
<tr>
<td>Bio-Gel P-10 (pool B)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(acetonitrile)</td>
<td>131</td>
<td>197</td>
<td>665,990</td>
<td>27.7</td>
<td>36.3</td>
</tr>
<tr>
<td>(2-propanol)</td>
<td>30.0</td>
<td>48.1</td>
<td>625,050</td>
<td>26.0</td>
<td>149</td>
</tr>
<tr>
<td>(peaks 1, 2)</td>
<td>2.44</td>
<td>5.94</td>
<td>411,110</td>
<td>17.1</td>
<td>1,200</td>
</tr>
<tr>
<td>Bio-Gel P-10 (pool C)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(acetonitrile)</td>
<td>0.0575</td>
<td>ND</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>(2-propanol)</td>
<td>0.0324</td>
<td>ND</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>(peaks 1, 2)</td>
<td>0.000013*</td>
<td>0.008</td>
<td>16,030</td>
<td>0.7</td>
<td>873,170</td>
</tr>
<tr>
<td>RP-HPLC C&lt;sub&gt;18&lt;/sub&gt; (2-propanol, peak B1)</td>
<td>0.000017*</td>
<td>0.011</td>
<td>14,000</td>
<td>0.6</td>
<td>716,000</td>
</tr>
<tr>
<td>RP-HPLC C&lt;sub&gt;18&lt;/sub&gt; (2-propanol, peak B2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RP-HPLC C&lt;sub&gt;18&lt;/sub&gt; (2-propanol, peak C)</td>
<td>29.5</td>
<td>90.8</td>
<td>324,520</td>
<td>10.4</td>
<td>78.8</td>
</tr>
<tr>
<td>RP-HPLC C&lt;sub&gt;18&lt;/sub&gt; (acetonitrile)</td>
<td>0.122</td>
<td>ND</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>RP-HPLC C&lt;sub&gt;18&lt;/sub&gt; (acetonitrile)</td>
<td>0.090</td>
<td>ND</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>RP-HPLC C&lt;sub&gt;18&lt;/sub&gt; (2-propanol, peak C)</td>
<td>0.010</td>
<td>0.101</td>
<td>99,010</td>
<td>4.1</td>
<td>70,890</td>
</tr>
</tbody>
</table>

Abbreviation: ND, not determined.

*These values were corrected for the content of HSA fragment determined from the quantification of amino-acid residues identified during N<sup>1</sup> sequence analysis.
recombinant growth factors as the criteria for positive identification. Whereas our first report of the properties of platelet-derived mammary growth factor had indicated a mol wt of 38,000 to 45,000, the study described here using acidic dissociating conditions during isolation yielded mitogens of mol wt 7,400 consistent with IGF-I and IGF-II. Further confirmation of IGF-I-like properties was obtained by radioimmunoassay (RIA) and radioreceptor assay as described in an accompanying report.

The data presented in Table 1 were used to estimate the amounts of insulinlike growth factors present in outdated platelets. The calculated amounts of IGF-I and IGF-II per 200 units of platelets were $\approx 7 \mu g$ and $\approx 30 \mu g$, respectively, based on the total units of activity in the appropriate pools of Bio-Gel P-10 fractions. These values were underestimations because significant amounts of the mitogens might have been released from the platelets during storage, and approximately 60% of the total activity was lost in isolation steps before Bio-Gel P-10. Indeed, RIA quantification presented in the accompanying report indicated that as much as 90% of the total IGF-I was lost from outdated platelets before beginning the purification. Nevertheless, the amounts of IGF-I and IGF-II were less than the 0.5 to 0.75 mg of TGF$\beta$ purified from 250 U of outdated platelets. The quantities of insulinlike factors more closely approximated the 100 $\mu g$ of PDGF isolated from 500 blood bank units.

The quantities of IGF-I and IGF-II isolated raised the question of the origin of these factors. Normal human plasma contains $\approx 170$ ng/mL IGF-I, and $\approx 800$ ng/mL IGF-II. Although these substantial concentrations suggested that the mitogens purified might have been nonspecifically associated with the platelets, other studies with thoroughly washed cells (ie, three times with 100-fold volumes of buffer) showed the activity was still present at approximately the same specific activity as crude extracts of larger scale preparations initiated without washing (data not shown). These observations support the conclusion that residual plasma was not the source of the IGF-I in platelet extracts. Furthermore, in large-scale isolations, the volume of the initial packed platelet pellet from 200 U was $\approx 100$ mL. Since 7 $\mu g$ of IGF-I were isolated from this number of units and IGF-I in human plasma is $\approx 170$ ng/mL, nearly 50% of the pellet volume necessarily would have been plasma. This was highly improbable.

Nevertheless, our data could not exclude that IGF-I associated with platelets nonspecifically in amounts necessary to explain 7 $\mu g$/200 U. For this reason we conducted the study described in the accompanying report. These experiments provided the strongest evidence that IGF-I was an integral component of the platelet structure because the factor was localized to $\alpha$-granule-containing fractions of sucrose density sedimentation experiments and was released during degranulation in response to thrombin treatment.

As noted above, several growth factors were candidates for the human breast-cancer growth activity in platelets. Our acidic extract and TFA RP-HPLC methods only identified insulinlike factors. It was possible that the minor activities not characterized further were the material in peak A of the Bio-Gel P-10 column (Fig 1B) were acid stable growth factors such as EGF or TGF$\alpha$. Because these factors were mitogenic for MCF-7 cells in serum-free TF/BSA, we expected to identify them if present in amounts comparable to IGF-I, IGF-II, or PDGF. Although EGF was found in platelets by immunologic and biological activity methods, the form identified was mol wt 31,000 to 45,000, which may not be active with human breast-cancer cells. High mol wt EGF may require activation for maximum biologic potency. For example, latent, high mol wt TGF$\beta$ has been purified from platelets and shown to require extreme conditions for activation.

In another study we attempted to identify aFGF and bFGF-like activities in pH 7.2 buffer lysates using the purification methods of Gospodarowicz. No FGF-like activity was found by Heparin-Sepharose chromatography in experiments done in parallel with the isolations of bovine brain aFGF and bovine and porcine pituitary bFGF. Furthermore, we have shown that pH 7.2 and 0.5 mol/L acetic acid extracts of platelets had the same total numbers of units of growth-factor activity/blood bank unit of platelets (data not shown). These observations supported the conclusion that acid labile bFGF was not a major contributor to the activity-promoting MCF-7 cell growth.

Finally, IGF-I and IGF-II isolated from platelets appeared by amino-acid sequencing and mol wt estimation to be the 70 and 67 residue forms, respectively, characterized by others. However, one of the components of peak B from the Bio-Gel P-10 fractionation was tentatively identified as a truncated form of IGF-I (Fig 7, peak B1). It lacked from three to six residues at the $N^\alpha$ terminus. Although more characterization of this sample will be required before a definite assignment of truncation can be made, it is interesting to note that DES $\rightarrow 3$ IGF-I has been shown by several groups to be considerably more biologically active than the usual 70 amino acid form of IGF-I. Our data confirmed that DES $\rightarrow 3$ to DES $\rightarrow 6$ IGF-I isolated from porcine uterus was 35 to 100 times more active than recombinant 71 amino acid human IGF-I in serum-free bioassays with human MCF-7 cells and Balb/c 3T3 mouse embryo fibroblasts.

Although increased receptor-binding affinity was first thought to explain the increased activity of DES $\rightarrow 3$ IGF-I, more recent data suggested that the truncated factor was more potent because it did not associate with cell-secreted high mol wt carrier proteins, which cause inactivation. The terminal tripeptide appeared to be required for binding to carrier protein. In any case, the presence of a form of IGF-I in platelets that is not inactivated by binding to carrier proteins might have special significance with regard to repair of tissue damage. Release of truncated IGF-I at wound sites might cause a greater stimulation of cell proliferation than release of the intact 70 amino-acid molecule.

ACKNOWLEDGMENT

The authors thank Judy M. Roscoe and Beto Zuniga for technical assistance and the preparation of illustrations. We thank Dr Terry L. Riss for advice regarding RP-HPLC procedures and Dr Betty H. Stewart for aiding in the preparation of this manuscript.
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KP Karey, H Marquardt and DA Sirbasku