Basic Peptide with Insulin-like Activity in Human Serum

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Abstract

A peptide with insulin-like activity was isolated from human plasma. In the purification, insulin-like activity (ILA) was monitored by radioreceptor assay for insulin, using human placental membrane and [125I]-insulin. ILA was extracted from Cohn fraction III with acid-ethanol and chromatographed on Sephadex G-75 in 1% formic acid. When the active fractions were subjected to ion-exchange chromatography with CM-cellulose, the ILA was adsorbed to the column at pH 5.0 and was eluted with a gradient of ammonium acetate. The chromatographic behavior of the ILA was not identical to that of somatomedin A as determined by radioreceptor assay (RRA) for the latter. On isoelectric focusing of the ILA from the CM-cellulose column, insulin-like activity was distributed over a wide pH range. The ILA that was focused at pH 7.5-9.0 was further purified by gel filtration on Sephadex G-50 in 1 M acetic acid. The specific activity of the basic ILA was approximately 200 mU insulin equivalent /mg protein. The apparent molecular weight of the material was estimated to be 7,000. It stimulated [14C]-glucose oxidation in rat epididymal fat cells and had sulfation activity in chick chondrocytes. Furthermore, the basic ILA had a potent mitogenic activity in Balb/c-3T3 cells. Thus, the basic ILA is entitled to be one of the IGFs.

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Insulin-like growth factor (IGF) is the generic designation for serum peptides such as somatomedin A (SM-A) (Hall et al., 1975), somatomedin C (SM-C) (Van Wyk et al., 1974), multiplication stimulating activity (MSA) (Pierson and Temin, 1972) and two forms of non-suppressible insulin-like activity soluble in acid-ethanol (NSILAs I & II or IGF I & II) (Froesch et al., 1975a, b; Rinderknecht and Humbel 1976). These substances share several characteristics, such as insulin-like activity in adipose tissue, sulfation activity in cartilage and mitogenic activity in cultured cells. It has been suggested that IGFs are important in mediating the action of growth hormone (Van Wyk et al., 1974; Daughaday et al., 1975; Froesch et al., 1975; Hall et al., 1975). Purification of these peptides has been cumbersome not only because of low concentration of the materials in serum or plasma, but also because of the time-consuming monitoring by bioassay during purification of the materials. Thus, it is hard to obtain the material even in a partially purified form, and it has been
available only in a limited number of laboratories. These insulin-like peptides are shown to compete with insulin for specific binding sites on human placental membranes (Hintz et al., 1972; Takano et al., 1975), which provided a basis for employment of a radioreceptor assay (RRA) for insulin for monitoring during the purification of peptides with insulin-like activity (ILA). Recently, we have extracted a peptide with insulin-like activity from human plasma protein monitored by RRA for insulin. Posner et al. (1978) purified a slightly acidic peptide with insulin-like activity using RRA for insulin. They called the peptide ILAs. The ILA we have prepared seems to be different in physicochemical characteristics from ILAs. Our peptide appeared to be related rather to IGF-I or SM-C when judged by its molecular weight and basic isoelectric point. In this communication, we describe purification and characterization of this basic peptide with insulin-like activity and cell growth-promoting activity (basic insulin-like activity, basic ILA). A sensitive RRA system for the basic ILA was established using human placental membrane as a receptor and the concentration of the basic ILA in human serum was determined by this RRA. The concentrations of the receptor reactive basic ILA were found to be dependent on growth hormone.

**Materials and Methods**

Cohn fraction III of human plasma was supplied by Chemo-Sero-Therapeutic Research Institute (Kumamoto, Japan). Human growth hormone (hGH, HS-1652C) was a gift from the National Institute of Health (NIAMDD, Bethesda, Md., U.S.A.). Epidermal growth factor (EGF) and nerve growth factor (NGF) were prepared in our laboratory according to the method of Savage and Cohen (1972) and Bocchini and Angeletti (1969), respectively. Fibroblast growth factor (FGF) was purchased from Collaborative Research Inc. (Massachusetts, U.S.A.) and MSA was kindly donated by Dr. M. M. Rechler and Dr. S. P. Nissley (NIH, U.S.A.). Somatomedin A (SM-A; SPE 147-3: 87 U/mg) and somatomedin B (SM-B) were a gift from Dr. K. Takano (Tokyo Women's Medical College). These somatomedins were prepared by Dr. L. Fryklund (AB Kabi, Stockholm). Porcine insulin (25U/mg) and proinsulin were obtained from Eli Lilly Co. (Indianapolis, U.S.A.). \[^{125}I\] - NaI was a product of New England Nuclear (NEN) (Boston, U.S.A.).

RRA for insulin was carried out using the 100,000 g pellet of human placenta as a matrix (Posner, 1974). Radioiodination of insulin was performed by a modified chloramine T method (Greenwood et al., 1963). Two hundred µg of membrane fraction were incubated with 50,000 c.p.m. of labelled insulin (specific activity: 75-100 µCi/µg) and insulin standard or samples at 4°C for 16 hours in a final volume of 0.5 ml of 50 mm Tris-HCl buffer, pH 7.4, containing 0.1% bovine serum albumin (BSA) and 10 mm MgCl₂. After terminating the reaction by adding 2 ml of ice-cold buffer, bound and free \[^{125}I\] - insulin were separated by centrifugation at 1000 g for 30 min at 4°C. The minimum detectable dose for this RRA was 0.2 ng/tube. RRA for SM-A was kindly performed by Dr. K. Takano according to the method described previously (Takano et al., 1976). Radioimmunoassay (RIA) for insulin was carried out with a double antibody method (Morgan and Lazarow, 1963). Protein was determined by the method of Lowry et al. (1951) using BSA as a standard, or by fluorometry.

**Purification of Basic ILA**

Cohn fraction III was extracted as described by Hall for the purification of somatomedins (Hall, 1972). The yield of acid-ethanol (AE) extraction from 10 kg of Cohn fraction III was approximately 35 g. Batches of 1.5 g of AE extract were extracted with 25 ml of 20% (v/v) formic acid for 60 min at room temperature and centrifuged at 10,000 g for 30 min. The precipitate was re-extracted in the same manner and the supernatants combined. The extract (50 ml) was then gel-filtered on a column of Sephadex G-75 (5 cm x 80 cm) equilibrated with 1% (v/v) formic acid. The flow rate was adjusted to 45 ml/hr and 20 ml-fractions were collected. The eluate was determined for insulin-like activity and SM-A concentration by means of the respective RRAAs. Active fractions were pooled, concentrated by ultrafiltration with UM-02 membrane (Amicon) and dialyzed against 0.05 M ammonium acetate buffer, pH 5.0, at 4°C for 24 hours. The fine precipitate was removed by centrifugation at 10,000 g for 30 min at 4°C and the supernatant was subjected to ion-exchange chromatography with a CM-cellulose column (CM 52; Whatman) as described by Humbel et al. (1971) for the purification of non-suppressible insulin-like activity (NSILAs). The elution of insulin-like activity was achieved by a continuous gradient of ammonium
ampholytes, LKB 1809-101, pH 3.5-10.0) in 4 M urea, the LKB 2117 Multiphor, according to application flat-bed isoelectric focusing in a granulated gel with Further purification was carried out by preparative flat-bed isoelectric focusing in a granulated gel with 50 cm) under acidic (1 M acetic acid, pH 2.3) and neutral (50 mm Tris-HCl buffer, pH 7.4) conditions (data not shown) suggests that basic ILA is associated with large molecular weight protein(s) at neutral pH and the complex is dissociated by acid treatment, as reported for other well characterized IGFs (Zapf et al., 1975; Moses et al., 1976; Hintz and Liu, 1977). In order to eliminate the effect of binding protein in serum upon the RRA, basic ILA in individual serum was extracted by the acid-ethanol method described by Mariz et al. (1980). Statistic analyses were carried out by Student’s t-test.

**DNA Synthesis in 3T3 Cell**

Balb/c-3T3 cells (Flow Laboratories Inc., U.S.A.) were cultured in Dulbecco’s modified Eagle medium (DME) containing 10% fetal calf serum (FCS; Gibco) at 37°C in a humidified atmosphere of 95% air and 5% CO₂. Cells grown to subconfluence in 35 mm dishes (Falcon Plastics, U.S.A.) were incubated for 72 hours in DME containing 0.4% FCS. Sixteen hours after the addition of basic ILA, SM-A, EGF or insulin, [³²P-thymidine (43 Ci/mole; Amersham) was added to the dishes (1 µCi/dish). After 4 hours, the medium was removed and the dishes were washed twice with phosphate-buffered saline and once with cold 5% trichloroacetic acid (TCA). The TCA-insoluble materials were solubilized with 2 ml of Protosol (NEN) and 1 ml of the aliquot was counted in 10 ml of Aquasol-2 (NEN) in a beta scintillation counter.

**Characterization of Basic ILA**

Sodium dodecyl sulphate (SDS)-polyacrylamide disc gel electrophoresis of the partially purified basic ILA was carried out at pH 7.2 according to the method described by Weber and Osborne (1969) to determine the molecular weight. Standard proteins used were insulin B chain, aprotinin, cytochrome C and soybean trypsin inhibitor (Boehringer).

**Bioassay for Insulin-like Activity**

Isolated fat cells were prepared from epididymal adipose tissue of male rats (Wistar; 100-150 g) using 0.2% collagenase ( Worthington). Adipocytes (3 x 10⁶ cells) were incubated for 2 hours at 37°C in Krebs-Ringer bicarbonate buffer containing 1 µCi of [¹⁴C]-glucose (NEN, 53 mCi/m mole), 1% BSA, 0.2 mM glucose and several dilutions of insulin standard (porcine insulin, 25 U/mg) or basic ILA prepared by us. ¹⁴CO₂ produced from [¹⁴C]-glucose was determined as described by Rodbell (1964).

**Sulfation Activity**

Sulfation activity of the basic ILA was studied in cultures of chick chondrocytes according to the method of Wasteson et al. (1973), which is based on the specific precipitation of glycosaminoglycans by cetyl pyridinium chloride (CPC). Chick chondrocytes, prepared from sternal rudiments of 12-day-old embryos, were incubated for 16 hours at 37°C in Ham’s F-10 medium containing 20 µCi of carrier free ³⁵S⁰₄ (obtained as sulphate in an aqueous carrier free solution, pH 6-8, from The Radiochemical Center, Amersham) and basic ILA, SM-A or insulin. Incorporation of ³⁵S⁰₄ into glycosaminoglycan in chondrocytes was determined as described by Wasteson et al. (1973).
Results

Purification of Basic ILA

A typical gel filtration profile of insulin-like activity (ILA) on a Sephadex G-75 column is shown in Fig. 1. The ILA was detected in the eluates with $K_{av} = (V_e - V_o)/(V_t - V_o)$, where $V_e$ is the elution volume of the protein, $V_t$ is the total column volume (1600 ml) and $V_o$ is the void volume. $V_t$ and $V_o$ were determined by the elution of Na$^{125}$I and Blue Dextran 2000 (Pharmacia, Sweden), respectively. $[125]$I-insulin was eluted almost in the same position as the receptor reactive insulin-like activity. The serial dilutions of the eluates competed with insulin for placental binding sites in a parallel manner to standard insulin. The ILA was separated from the major protein peak present in the void volume on gel filtration, and the recovery of ILA was approximately 90%. The chromatographic behavior of the receptor reactive ILA in acid in the present study is similar to that described by Posner et al. (1978), but differs in that our ILA is more retarded. As shown in the figure, SM-A activity determined by RRA was not parallel to the insulin-like activity and distribution was wider. The active fractions (1200-1500 ml fractions, shaded area in the figure) were pooled, concentrated and dialyzed against 0.05 M ammonium acetate, pH 5.0. The ILA preparation by Sephadex G-75 column contained less than 0.01% of immunoreactive insulin (IRI) as measured by RIA, and the specific activity of the material ranged from 10 to 15 mU insulin equivalent/mg protein. The ILA was further purified by ion-exchange chromatography with CM-cellulose (Fig. 2). A major part of the protein with little or no insulin-like activity passed through the column, and the insulin-like material was effectively eluted with a gradient of ammonium acetate, which was similar to what was observed by Humbel et al. (1971). We have consistently found two peaks of ILA (peak I and II in the figure). The chromatographic behavior of the ILA was again not parallel to that of SM-A, and the ratio of SM-A/ILA was higher in peak I than in peak II. The more retarded fractions (peak II, shaded area in the figure) were pooled, concentrated and lyophilized. The CM-cellulose purified preparation had a specific activity ranging from 100 to 200 mU/mg, and
Fig. 2. Ion-exchange chromatography on CM-cellulose. The active fractions from a Sephadex G-75 column (shaded area in Fig. 1) were concentrated and dialyzed against 0.05 M ammonium acetate, pH 5.0. The dialysate (50 ml, protein 40 mg) was applied to a CM-cellulose column (2.1 cm×6.0 cm) which had been equilibrated with 0.05 M ammonium acetate buffer, pH 5.0. The column was first washed with 5 volumes of the buffer and was eluted with a continuous gradient of ammonium acetate from 0.05 M, pH 5.0 to 0.3 M, pH 6.8. The flow rate was adjusted to 30 ml/hr and 5 ml-fractions were collected. The eluates were determined for insulin-like activity (indicated as column, SM-A activity (×) and protein (○). The shaded area (peak II) fractions were pooled and further purified.

Fig. 3. Isoelectric focusing of ILA from CM-cellulose column. The insulin-like activity from CM-cellulose column was subjected to preparative flat-bed isoelectric focusing in a granulated gel. 40 mg protein of CM-cellulose fractions (peak II) were dissolved in 95 ml of 5% (v/v) Ampholine solution (pH 3.5-10.0) in 4 M urea, and 5 g of Ultrodex were added to the solution to prepare a 5% (w/v) gel slurry. Focusing was carried out at 8 watts constant power for 36 hours at 4°C. On completion of the electrophoresis, the gel was divided into 30 sections and each was eluted with 2 ml water. The eluate was determined for insulin-like activity (column) by insulin RRA, pH (○) and absorbance at 280 nm (×). The shaded fractions (Basic ILA) were pooled and further purified.

Though the ratio of these insulin-like substances with different pI varied slightly from run to run, the percent of acidic ILA was constantly less than 5%. The ILA focused at pH 7.5-9.5 (basic ILA, shaded area in the figure) was pooled and concentrated by ultrafiltration with UM-02 membrane, and was gel-filtered on a Sephadex G-50 column (1.0 cm×80 cm) equilibrated with 1M acetic acid to remove Ampholine contamination by insulin (IRI) was less than 0.001%. The CM-cellulose fraction was then subjected to isoelectric focusing, as described in Materials and Methods. As shown in Fig. 3, insulin-like activity determined by RRA was found to be distributed over a wide pH range. Approximately 50% of the ILA were focused at basic pH (7.5-9.0), 40% at pH 6.5-7.5 and the remainder at acidic pH (4.5-6.5).
Table 1. Purification of basic ILA

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Recovery (%)</th>
<th>Specific activity (mU*/mg)</th>
<th>Purification (fold)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human plasma</td>
<td>100</td>
<td>0.005</td>
<td>1</td>
</tr>
<tr>
<td>Cohn Fr. III</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Acid ethanol extract</td>
<td>20</td>
<td>0.4</td>
<td>80</td>
</tr>
<tr>
<td>Sephadex G-75</td>
<td>18</td>
<td>12.5</td>
<td>2500</td>
</tr>
<tr>
<td>CM-cellulose (peak II)</td>
<td>3.6</td>
<td>150</td>
<td>30000</td>
</tr>
<tr>
<td>Isoelectric focusing (basic ILA)</td>
<td>0.9</td>
<td>200</td>
<td>40000</td>
</tr>
<tr>
<td>Sephadex G-50</td>
<td>0.8</td>
<td>250</td>
<td>50000</td>
</tr>
</tbody>
</table>

* Insulin equivalent determined by insulin RRA

Table 2. Effect of insulin and basic ILA on the oxidation of [14C]-glucose in rat epididymal adipocytes.

<table>
<thead>
<tr>
<th>Test material</th>
<th>[14C]-glucose oxidized (cpm/10⁵ cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Porcine insulin (μU/ml)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2956 ± 166a</td>
</tr>
<tr>
<td>2.5</td>
<td>6147 ± 452</td>
</tr>
<tr>
<td>6.25</td>
<td>10082 ± 472</td>
</tr>
<tr>
<td>12.5</td>
<td>18242 ± 342</td>
</tr>
<tr>
<td>25.0</td>
<td>18629 ± 377</td>
</tr>
<tr>
<td>62.5</td>
<td>19625 ± 371</td>
</tr>
<tr>
<td>Basic ILA (μU/ml)</td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>5519 ± 34</td>
</tr>
<tr>
<td>6.25</td>
<td>7030 ± 228</td>
</tr>
<tr>
<td>12.5</td>
<td>14673 ± 567</td>
</tr>
</tbody>
</table>

Rat epididymal adipocytes (3 × 10⁵ cells) were incubated with 1 μCi of [14C]-glucose in Krebs-Ringer bicarbonate buffer for 2 hours at 37°C and 14CO₂ production was measured.

a: mean ± half-range of duplicate determinations
b: insulin equivalent in μU/ml, determined by insulin RRA.

Table 3. Incorporation of ³⁵SO₄ into glycosaminoglycan in chick chondrocytes

<table>
<thead>
<tr>
<th>Test material</th>
<th>³⁵SO₄ incorporation (cpm/10⁵ μl proteolyse)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>273 ± 42a</td>
</tr>
<tr>
<td>SM-A (10 ng/ml)</td>
<td>9425 ± 1788</td>
</tr>
<tr>
<td>Insulin (12.5 mU/ml)</td>
<td>1440 ± 908</td>
</tr>
<tr>
<td>Basic ILA (μU/ml)</td>
<td></td>
</tr>
<tr>
<td>125</td>
<td>543 ± 24</td>
</tr>
<tr>
<td>250</td>
<td>7593 ± 626</td>
</tr>
<tr>
<td>500</td>
<td>14441 ± 656</td>
</tr>
</tbody>
</table>

Embryonic chick chondrocytes (5 × 10⁵ cells) were incubated with 20 μCi of carrier free ³⁵SO₄ in Ham’s F-10 medium for 16 hours. After proteolysis, ³⁵SO₄ incorporation into the CPC-insoluble materials was determined.

a: mean ± SEM of triplicate determinations.
b: insulin equivalent in μU/ml.

ampholyte and urea. The final product with specific activity of 100–250 mU/mg was stored at 20°C in 1M acetic acid. The recovery data obtained during purification of basic ILA are shown in Table 1.

Molecular weight of basic ILA

The most purified basic ILA preparation was subjected to SDS-polyacrylamide disc gel electrophoresis (SDS-PAGE). After the separation was complete, one channel of the gel was stained with amido black, and the other gel was divided into 30 thin slices (2.0 mm) and each segment was eluted overnight in 0.5 ml of 0.1 M acetic acid, pH 3.0, containing 0.1% BSA. The eluates were then assayed for insulin-like activity by RRA. The basic ILA was stained as a single but broad band and migrated as a molecule of about 7,000 molecular weight.

Biological activities of the partially purified basic ILA

As expected from its cross-reaction to insulin in RRA, the basic ILA stimulated 14CO₂ production in isolated adipocytes from rat epididymal fat tissue in a dose-dependent manner (Table 2). The ability of the basic ILA to stimulate incorporation of ³⁵SO₄ into glycosaminoglycan, which was specifically precipitated by CPC, was studied in chick chondrocytes (Table 3). This potency of the basic ILA was much greater than insulin, when the activity is compared on the basis of insulin-like activity as determined by RRA for insulin. The basic ILA also stimulated the incorporation of [³H]-thymidine into DNA in stationary Balb/c-3T3 cells in a dose-dependent manner (Table 4). The potency of 750 μU eq./ml of the basic ILA was only 16% of that produced by 10% FCS. Comparison of the potency of the basic ILA with those produced by other growth factors on the basis of protein concentration is difficult, since our basic ILA preparation is not completely...
pure. But the maximum potency of the basic ILA (750 μU eq./ml) was higher than that produced by 25 mU/ml of insulin or 50 ng/ml of SM-A.

**RRA for basic ILA**

The binding of [125I]-basic ILA to the placental particulate fraction was displaced dose-dependently by unlabelled basic ILA between 0.5 μU and 200 μU per tube (Fig. 4, o-o). As shown in the figure, the binding was inhibited in a parallel manner by SM-A or MSA, but only minimally by high concentrations of insulin. Proinsulin, hGH and other growth factors such as SM-B, EGF, FGF and NGF were without effect. Neither SM-C nor NSILAs (IGF-I & II) was available for testing cross-reactivity in the present assay. The RRA for basic ILA was applied to the determination of concentration in human serum. Acid-ethanol extraction yielded a good recovery of added [125I]-basic ILA (approximately 70%), and dilutions of the extract gave a displacement curve parallel to that obtained by the standard basic ILA (figure not shown). Thus, the procedure permitted quantitative measurement of basic ILA in serum. The intra-and inter-assay variations (coefficient of variance) were 6.7% and 20.4%, respectively.

**Serum concentration of basic ILA**

The concentration of receptor reactive basic ILA in various disorders is shown in Fig. 5a. The values in normal males and females were 575±46 μU/ml (mean±SEM, n=20), and 528±27 (n=27), respectively, and no difference according to sex was observed. The basic ILA was significantly higher in 17 acromegalics (3133±245, p< 0.001) and lower in GH-deficient patients (286±47, n=17, p<0.01) than in normals (549±25, n=47). To confirm GH-dependency of serum basic ILA, the effect of GH treatment on serum basic ILA was studied in four patients with GH deficiency (Fig. 5b).
Fig. 5a. Serum basic ILA levels in various disorders. The bars represent the mean values for each group of patients.

Fig. 5b. Twenty four hours after the initial injection of hGH (2mg; i.m.), serum basic ILA was increased slightly, though not significantly, (236±57) compared to pretreatment levels (181±34). And the basic ILA values after three months hGH therapy (2mg hGH, twice a week) were significantly higher (361±50, p<0.05) than pre-treatment levels.

Discussion

A peptide with insulin-like activity (basic ILA) was isolated from human plasma (Cohn fraction III). The effect of the basic ILA was studied in a variety of bioassays. It stimulated $^{14}$CO$_2$ production in isolated adipocytes in a dose-dependent manner. The basic ILA also stimulated sulfate incorporation in chick chondrocytes as well as DNA synthesis in Balb/c-3T3 cells. The material is therefore qualified as an insulin-like growth factor (IGF). In the present study we used a RRA for insulin to follow the purification of an insulin-like peptide from human plasma. Employment of the RRA is reasonable because IGFs can compete with insulin for specific binding sites identified on membrane fractions of human placenta (Hintz et al., 1972; Takano et al., 1975).

Recently Posner et al. (1978) reported partial purification of a peptide with insulin-like activity, sulfation activity and cell growth-promoting activity using insulin RRA. The characteristics of their preparation (ILAs) seem to be different from those of ours in some respects in spite of the similarity in the purification procedure. The molecular weight of ILAs was estimated by chromatography to be approximately
9,500, while our preparation migrated as a molecule with mol. wt. 7,000 on SDS-PAGE. Chromatography on Sephadex G-75 in 1M acetic acid/0.15m NaCl also revealed that the basic ILA was eluted in a position corresponding to mol. wt. 7,000 (data not shown). Furthermore, the results on isoelectric focusing differ from each other. ILAs (CMC-purified ILA) was focused at pH 6.4-6.7, whereas the receptor reactive insulin-like activity of CM-cellulose fraction (peak II) was distributed over a wide pH range. We cannot yet explain this difference but it may possibly be due to a difference in the starting material and/or in the extraction procedure. Posner et al. used Cohn fraction IV-4 and we used fraction III. In gel filtration by Sephadex G-75, they used 1.0 m acetic acid/0.15 m NaCl as the solvent, while we used 1% formic acid. Moreover, the difference in gradient of ammonium acetate on CM-cellulose column was noted (0.05–0.20 m, pH 5.6 vs 0.05 m, pH 5.0–0.3 m, pH 6.8).

Heterogeneity of insulin-like activity in plasma measured by insulin RRA was recently reported by Ginsberg et al. (1979). They developed a new method for purification of IGF using endogenous binding protein, and found two peaks of IGF with apparent molecular weights between 6,000–10,000 in gel filtration on Sephadex G-75. Furthermore the more retarded peak was resolved by isoelectric focusing into two components, one with PI 6.5 and the other with pI 8.3. The basic ILA we have prepared may be similar to the latter, considering their similarities in molecular weight and PI. Their basic IGF (pI 8.3 fraction) showed less potent activity in the MSA bioassay (thymidine incorporation in chick embryo fibroblasts), while our preparation (basic ILA) exhibited potent mitogenic activity in Balb/c-3T3 cells. The difference may possibly be due to the different preparations used and/or the cell species studied. Several peptides with insulin-like activity and cell growth-promoting activity (IGFs) have been purified and characterized. Uthne and his colleagues purified a neutral somatomedin (SM-A) (1973) and Van Wyk et al. have purified a basic peptide (SM-C, pI: 8.6–9.5) (1974). Rinderknecht and Humbel have shown that NSILAs contains two components (IGF-I and II), both with pI 8.2 and similar molecular weight (Rinderknecht and Humbel, 1976), and the characteristics of IGF-I and II have been reported in detail (Zapf et al., 1978a, b). Serum levels of IGF-I are under growth hormone (GH) control, but IGH-II in serum does not show GH dependency. Recently, Van Wyk et al. (1980) and Hintz et al. (1980) observed that IGF-I and SM-C behave identically in their RIAs. Our basic ILA is clearly different from SM-A, since the pI of SM-A is reported to be neutral (7.1–7.5) (Hall et al., 1975). Furthermore the present study revealed that the chromatographic behavior of the ILA determined by insulin RRA was not identical to that of SM-A, by both gel filtration on Sephadex G-75 and ion-exchange chromatography with CM-cellulose. As judged from the molecular weight and pI, the basic ILA we have prepared is very closely related to IGF-I and II or SM-C. Because of the limited amount of the highly purified basic ILA, amino acid analysis of the preparation has not been carried out. Therefore, the structural comparison of our preparation with the well characterized IGF-I and II or SM-C is impossible at present.

As reported for other IGFs (SM-A, SM-C) (Hall et al., 1974; Vay Wyk et al., 1974), the presence of a specific binding site for the basic ILA was demonstrated in the plasma membrane of human placenta. The observation that the binding of [125I]-basic ILA was displaced dose-dependently by unlabelled basic ILA was followed by the development of RRA for the basic ILA. In this system SM-A and MSA cross-reacted considerably, but other growth
factors were without effect. Unfortunately, neither IGF (I and II) nor SM-C was available for us to check the cross-reactivity in this RRA system, and comparison has not been carried out at present.

The serum concentrations of receptor reactive basic ILA in acromegals were six times higher than that in normals. The basic ILA values were approximately half of normal in patients with GH deficiency. Furthermore, hGH treatment in these patients resulted in a significant increase in serum basic ILA levels, indicating that the serum basic ILA is under GH control. Thus, the basic ILA presented in this paper clearly belongs to the somatomedin family. As judged from its physicochemical characteristics, the basic ILA appears to be closely related to IGF-I or SM-C. Further studies will be required to determine their similarity or identity. The isolation procedure for basic ILA as aided by insulin RRA is simple and rapid with a satisfactory yield. The RRA for the basic ILA was rapid and simple, and proved to be useful for the evaluation of patients with GH excess or deficiency.

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