Primary Structure of Rat Insulin-like Growth Factor-I and Its Biological Activities*

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Rat insulin-like growth factor-I (IGF-I), a serum polypeptide with growth promoting activity, was isolated from rat serum by a combination of acid/ethanol extraction, affinity chromatography, and a series of reversed phase high performance liquid chromatography, cation exchange, and reversed phase. All peptide fragments produced by chymotrypsin digestion of reduced and carboxymethylated rat IGF-I were amino acid sequenced and compared with the sequence of human IGF-I. Three out of 70 of the rat amino acid residues differed from those of human IGF-I as follows: Asp⁰ → Pro, Ser³⁵ → Ile and Ala⁶⁷ → Thr. Purified rat IGF-I cross-reacted with polyclonal anti-human IGF-I antibody 75% as compared to human IGF-I, but it cross-reacted only 3% with monoclonal anti-human IGF-I antibody. Thus, it is possible to monitor the metabolic fate of human IGF-I, when injected into rats, without interference by endogenous rat IGF-I. Rat IGF-I showed 65% activity in the radioreceptor, 28.6% activity in the lipogenesis and 22.5% activity in the free fatty acid release inhibition assays as compared to human IGF-I on a protein quantity basis.

Insulin-like growth factors (IGFs) or somatomedins are a family of serum polypeptides with insulin-like and growth promoting activity and play major roles in mammalian development and growth (1).

Although IGFs were originally recognized in rat serum (2), two forms of human counterparts, IGF-I (or somatomedin C) and IGF-II, have been purified from human plasma and their primary structures reported (3, 4). Human IGF-I (h-IGF-I) is a polypeptide with 70 amino acid residues, whereas human IGF-II (h-IGF-II) is a polypeptide with 67 amino acid residues. Marquardt et al. (5) isolated multiplication stimulating activity from rat serum and demonstrated its close homology with h-IGF-I (62 amino acid residues out of 67). The amino acid sequence of rat IGF-I has been reported by Rubin et al. (6) to be identical to h-IGF-I up to 29 residues from the N-terminal end. Since then, no report on the primary structure of rat IGF-I has been reported from peptide studies except the amino acid composition data by Canalis et al. (7).

Recently, bovine IGF-I was purified and its primary structure was reported to be the same as that of h-IGF-I (8). The structures of rat IGF-I and mouse IGF-I remained undetermined because of the scarcity of rat and mouse serum until the primary structure of mouse IGF-I was determined recently from its cDNA (9). Its structure differed from that of h-IGF-I by 4 residues; Asp⁰ → Pro, Ser³⁵ → Ile, Ala⁶⁷ → Thr, and Ser⁶⁷ → Ala. During our research on rat IGF-I its primary structure was reported from two laboratories; one by Roberts et al. (10) from a study with a liver cDNA library, and the other by Shimatsu and Rotwein (11) from a study with a genomic gene library. Roberts et al. (10) reported that rat IGF-I differed from human IGF-I in 6 residues, whereas Shimatsu and Rotwein (11) reported a difference only in 3. The amino acid composition of rat IGF-I reported by Canalis et al. (7) did not coincide with the composition calculated from either of the primary sequences of Roberts et al. (10) or Shimatsu and Rotwein (11). As these contradictory findings have left a question on the exact primary structure of rat IGF-I to the protein chemical work, we describe here its complete purification from rat serum, its complete primary structure and its biological and immunological activities in comparison with h-IGF-I.

MATERIALS AND METHODS

Human IGF-I was produced by recombinant DNA technique in our laboratory as reported by Niwa et al. (12, 13). Polyclonal (pAb) and monoclonal antibody (mAb) used in this paper were obtained in our laboratory, and the epitopes recognized by these antibodies have already been reported (14). Briefly, pAb was produced in rabbit by multiple immunization of ovalbumin-h-IGF-I conjugate and purified by DEAE column chromatography. mAb (mAb-121) was produced by hybridoma cells obtained by fusion of mouse myeloma cells with the spleen cells of mice immunized several times with Val⁶⁷-h-IGF-I, and was purified by DEAE column chromatography. Bovine serum albumin (BSA) was purchased from Armour (Kankakee, IL), polyethylene glycol 6000, chymotrypsin (three times crystallized and lyophilized), bacitracin, and porcine insulin from Sigma, collagenses from Cooper Biomedical, and CNBr-activated Sepharose from Pharmacia (Uppsala, Sweden). Na¹⁹¹ and [methyl-³H]Thymidine were obtained from Du Pont-New England Nuclear, TSK-CM-2SW column (4.5 × 300 mm) from Toyo Soda Co., Ltd (Tokyo, Japan), and C-18 reversed phase column YMC AP-302 ODS (4.6 × 150 mm) from Yamamura Chemicals Inc. (Kyoto, Japan). Fetal calf serum (FCS) was purchased from HyClone, goat anti-rabbit γ-globulin from Daiich Radioisotope Institute (Tokyo, Japan), and goat anti-mouse γ-globulin from ICN Immunobiologics. NEFA-Kit U used for the determination of free fatty acid concentration was purchased from Nihon Shoji Co., Ltd. (Osaka, Japan). All other solvents and reagents were either analytical grade or the best available grade unless otherwise indicated.
Preparation of Affinity Column

DEAE-purified polyclonal anti-h-IGF-I antibody (rabbit) was coupled to CNBr-activated Sepharose according to the method suggested by the manufacturer. Antibody bound to the resin was 1.22 mg/ml.

Purification of Rat IGF-I

Rat serum was prepared from Sprague-Dawley rat blood. Serum (385 ml) was treated with acid/ethanol solution (2 N HCl, 192.5 ml, and ethanol, 1347.5 ml) for 30 min at room temperature (15). The precipitates were removed by centrifugation at 9000 × g for 1 h, the supernatant was concentrated to 930 ml under reduced pressure, and the concentrate was dialyzed with 930 ml of 0.01 M PBS (pH 7.0) and dialyzed against changes of 20 liters of 0.01 M PBS (pH 7.0). The precipitates were removed by centrifugation at 105,000 × g for 1 h to give a supernatant (2385 ml) which was applied to an anti-h-IGF-I pAb affinity column (2.5 × 54 cm, 46 ml) at a flow rate of 1.5 ml/min. The column was washed with 300 ml of 0.01 M PBS (pH 7.0), and the adsorbed rat IGF-I was eluted with 0.2 M glycine-HCl buffer containing 0.5 M NaCl (pH 2.0) at a flow rate of 1.5 ml/min. Fractions from the column were monitored by UV%λnm and pAb RIA (Fig. 1A). The active fractions detected by pAb RIA were collected and subjected to C-18 reversed phase HPLC. The active fractions were purified by C-18 reversed phase HPLC. The adsorbed rat IGF-I was eluted with a 40-min linear gradient of acetonitrile from 25 to 35% containing 0.05% trifluoroacetic acid (Fig. 1B). Active fractions by RIA were collected and subjected to ion-exchange HPLC (TSK-CM-2SW) directly. The active fractions were purified by C-18 reversed phase HPLC. The adsorbed rat IGF-I was eluted with a linear gradient of NaCl (from 0 to 0.2 M) in 0.05 M PBS (pH 6.5) at a flow rate of 2 ml/min. The elution was monitored by UV%λnm and pAb RIA (Fig. 1C). The main peak gave 60 μg of rat IGF-I and was further purified by C-18 reversed phase HPLC under the same conditions as above, to yield 33.8 μg of pure rat IGF-I (Fig. 2B).

Structure Determination

Reduction and Carboxymethylation—Purified recombinant human (12, 13) or purified rat IGF-I, 32 and 30 μg, respectively, was dissolved in 200 μl of 8 M urea, 0.3 M Tris-HCl (pH 8.2) buffer containing 1% 2-mercaptoethanol and left at room temperature for 90 min. Monoiodoacetic acid sodium salt, 5.3 mg, was added to the reaction mixture, which was stirred for 25 min at room temperature to give reduced-carboxymethylated h-IGF-I or reduced-carboxymethylated rat IGF-I. Reduced-carboxymethylated IGF-I (human or rat) was purified by C-18 reversed phase HPLC with a 40-min linear gradient of acetonitrile from 0 to 60% containing 0.05% trifluoroacetic acid as a mobile phase and subjected to chymotrypsin digestion.

Chymotrypsin Digestion—Reduced-carboxymethylated h-IGF-I or reduced-carboxymethylated rat IGF-I, 25 μg for both species, was digested with 0.2 μg of chymotrypsin in 0.2 ml of 2% (NH4)HCO3 for 90 min at 37 °C. The peptides were separated by C-18 reversed phase HPLC with a 40-min linear gradient of acetonitrile from 0 to 60% containing 0.05% trifluoroacetic acid at a flow rate of 2 ml/min (Fig. 3, A and B). The separation was monitored by UV%λnm and all the peaks were collected manually and subjected to gas phase amino acid sequencing (gas phase amino acid sequencer model 470A, Applied Biosystems).

Radioimmunoassay (RIA) and Immunoreactivity

RIA by pAb or mAb and immunoreactivity of h-IGF-I and pure rat IGF-I were performed according to the method reported (16) (Fig. 5, A and B). Briefly, binding of serially diluted h-IGF-I or rat IGF-I to pAb or mAb was measured in the presence of [3H]-h-IGF-I (0.02 μCi/μl, 106 cpm), and B/B0 was calculated in the usual manner.

Receptor Assay

One hundred microliters of a BALB/c 3T3 fibroblast cell suspension (5 × 106 cells) in 1% BSA/PBS was incubated with 100 μl of different dilutions of h-IGF-I or pure rat IGF-I (0-105 μg/ml) and 100 μl of [3H]-h-IGF-I (16) (106 cpm) at 4 °C overnight. The cells were washed twice with 0.1% BSA/PBS and centrifuged at 3000 × g for 10 min. The 3H bound to the cells was counted by a γ-counter (Fig. 6).

[3H]Thymidine Incorporation into BALB/c 3T3 Cells

After 24 h incubation at 37 °C under 5% CO2 in 10% FCS-Dulbecco’s modified Eagle’s medium (FCS-DMEM), BALB/c 3T3 fibroblasts were washed twice with DMEM. The medium was changed to 0.5% FCS-DMEM and the plate was incubated at 37 °C for 24 h under 5% CO2. The cells were washed 3 times with DMEM. After the addition of 100 μl of pure IGF-I (0-105 ng/ml), the cells were incubated at 37 °C for 8 h under 5% CO2 and pulsed for 16 h with [methyl-3H]thymidine (0.1 μCi/100 μl of DMEM well). The cells were then detached from the plate by the addition of 0.025% trypsin containing 5 mM EDTA in PBS, and harvested on a glass fiber with a semiautomatic multiple cell harvester (Labo Mash®). Labo Science Co., Ltd., Tokyo, Japan) and [methyl-3H]thymidine incorporation was counted by scintillation counter (Fig. 7).

Ligogenesis Assay

Fat cells were isolated from epididymal fat pads of normal male Zbh Cara rats, weighing 100–120 g, according to the method reported earlier (17). The assay was performed in the presence or absence of h-IGF-I or pure rat IGF-I as described (17), except that the cells were incubated for 45 min in 0.5 ml of Krebs-Ringer-Hepes buffer containing 0.2% human serum albumin. [U-14C]Glucose incorporation into total lipids was counted. In this assay, natural h-IGF-I (lot 1/4, kindly provided by Dr. R. Humbel, Biochemisches Institut der Universität Zürich) was used instead of recombinant h-IGF-I (Fig. 8).

Free Fatty Acid (FFA) Release Inhibition Assay

FFA release inhibition assay was performed according to the method reported by Rodbell and Jones (18) with some modifications. Briefly, fat cells were isolated as above from overnight fasted rats and suspended in 1% BSA-Krebs-Ringer-Hepes buffer (pH 7.4) at 2.7 × 105 cells/ml. The cell suspension (0.9 ml) was incubated with bacitracin (10 μl, 50 mg/ml in saline) in the presence or absence of recombinant h-IGF-I, rat IGF-I or porcine insulin (each 50 μl) were mixed in an LSA vial (Packard Japan Inc., Tokyo, Japan) at 37 °C for 15 min. DL-1-isoproterenol (50 μl, 50 mg/ml in saline) was added to the cell suspension which was further incubated at 37 °C for 1 h. FFA released into the medium from the fat cells was measured by NEFA-Kit U which measured FFA based on ACS-ACO enzymatic method (19).

Calculation of Specific Activity Ratio between Peptides

Specific activity ratio between two peptides was calculated according to the following equation.

\[
\text{Specific activity ratio} = \frac{\text{50% competitive dose of rat IGF-I}}{\text{50% competitive dose of h-IGF-I in the assay}} \times 100 (\%)
\]

IGF-I Protein Quantity

Rat IGF-I quantities in the purification and in the biological activities were expressed in the weight of recombinant h-IGF-I standard, using C-18 reversed phase HPLC and supposing that the molar absorption coefficient of rat IGF-I at UV%λnm was the same as that of h-IGF-I. The quantity of standard h-IGF-I had been firmly determined by the dry weight method; weighing the standard sample following the subtraction of impurities (percentage) over C-18 reversed phase and TSK-CM-2SW ion-exchange HPLC, moisture, organic solvents, and ignited ashes.

RESULTS

Purification—The results of the different purification steps are summarized in Table I. Acid/ethanol treatment of the serum was introduced to separate rat IGF-I from its binding protein and to precipitate other serum proteins (15).

Affinity chromatography of rat serum using anti-h-IGF-I-pAb-Sepharose was shown to be very effective for the purification of rat IGF-I (20), and our analysis of rat IGF-I in serum by pAb and mAb RIA (Table I) suggested that mAb could not be used for the purification of rat IGF-I owing to differences in the reactivity. Still, when the affinity purified rat IGF-I was subjected to C-18 reversed phase HPLC (Fig. 1B) a lot of impurities were found. Rat IGF-I fractions were collected and applied to TSK-CM-2SW HPLC to give a single peak of rat IGF-I (retention time = 23 min). This step also
TABLE I

Purification of rat IGF-I

The data presented in this table are representative of 7 runs.

| Volume       | pAb RIA | mAb RIA | HPLC |
|--------------|---------|---------|------|
| Serum        | 385     | ND*     | ND   |
| Acid/ethanol treatment | 2395    | 145.9 (100)* | 7.3  |
| pAb-Sepharose affinity column | 500     | 71.5 (49.0) | 2.9  |
| Reversed phase HPLC | 0.33    | ND      | 101.1|
| TSK-CM-2SW HPLC | ND      | ND      | 60.0 (29.0) |
| Reversed phase HPLC | 0.20    | ND      | ND   |

* ND, not determined.
* Number in parentheses is the recovery yield of rat IGF-I from the acid/ethanol treatment step.

FIG. 1. Purification of rat IGF-I. A, affinity column chromatography purification of rat serum. Sprague-Dawley rat serum was applied to pAb-coupled Sepharose column after treating the serum with an acid/ethanol. The adsorbed rat IGF-I was eluted with 0.2 M glycine·HCl buffer containing 0.5 M NaCl (pH 2.0). The elution pattern was monitored by UV at 210 nm (—) and by pAb RIA (■). B, first C-18 reversed phase HPLC. Rat IGF-I containing fraction from the affinity column was applied to C-18 reversed phase HPLC, and the IGF-I was eluted by the linear gradient of acetonitrile. Elution was monitored by UV at 214 nm. The IGF-I containing fraction was detected by pAb RIA (the arrow in the figure). C, TSK-CM-2SW ion-exchange HPLC. Rat IGF-I containing fraction from the first reversed phase HPLC was applied to TSK-CM-2SW HPLC. Elution was monitored by UV at 214 nm. The IGF-I containing fraction was detected by pAb RIA. As stated in the text, sometimes two other peaks, denoted as Peaks 1 and 2 in C, were seen. Main Peak 3 was subjected to further purification by reversed phase HPLC, and the structure and the biological characters were determined.

FIG. 2. HPLC pattern of purified h-IGF-I (A) and rat IGF-I (B). IGF-I was analyzed by C-18 reversed phase HPLC (column: YMC AP202 ODS, 4.6 × 150 mm) with a 40-min linear gradient of acetonitrile from 25 to 35% containing 0.05% trifluoroacetic acid at a flow rate of 1 ml/min.

sometimes gave two minor peaks at a shorter retention time, and these were designated as Peaks 1 and 2 in Fig. 1C. Rat IGF-I (peak 3, retention time = 23 min) was collected and purified further by C-18 reversed phase HPLC. IGF-I fractions were collected and concentrated by Speed-Vac concentrator to give 33.8 µg of purified rat IGF-I, which showed a single peak both on reversed phase and TSK-CM-2SW HPLC. The final protein quantity was obtained from the HPLC peak area using recombinant h-IGF-I as a standard. Purity of purified rat IGF-I was over 99%, to which Met⁵⁹-sulfoxide derivative appearing before rat IGF-I was counted (Fig. 2B).

In the chymotryptic peptide maps of native form of IGFs under reducing conditions, very similar maps were obtained (data not shown). However, chymotryptic digestion of reduced carboxymethylated IGF-I gave different chromatograms between rat and h-IGF-I (Fig. 3). The peptide designated as C-5 from reduced-carboxymethylated rat-IGF-I was clearly shifted to a longer retention time, suggesting hydrophobic substitution. In addition to C-5, three minor peaks (C-3, C-4, and C-7) were newly formed. All these peaks from C-1 to C-9 were isolated and sequenced by gas phase sequencer. The results are summarized in Fig. 4. The amino acid sequence
Rat IGF-I

Hmn I

Rat C-8  c-2  IC c-5 I c-9 I

Retention Time

FIG. 3. Chymotryptic peptide maps on C-18 reversed phase HPLC of reduced-carboxymethylated h-IGF-I (upper panel) and reduced-carboxymethylated rat IGF-I (lower panel). HPLC conditions were described under “Materials and Methods.”

GPETLCAELVDALQFVGPRGYPFKPTGYGSSI

C-9 C-8 C-7 C-6 C-5 C-4 C-3 C-2

RRAPQTGIVDECCFRSCDLRLEMYCAPKPKSA

C-8 C-7

FIG. 4. Amino acid sequence of rat IGF-I and arrangement of chymotryptic digestion fragments of C-1 to C-9 which correspond to the fragment peaks in Fig. 3. The arrows indicate the residues sequenced. The primary structure of h-IGF-I is shown in parentheses where the amino acid residues are different from rat IGF-I.

and the chymotryptic peptide arrangement are shown together with those of h-IGF-I. The differences in sequence were Asp30 → Pro, Ser35 → Ile, and Ala67 → Thr. Although Ser35 in h-IGF-I was replaced by Ile in rat IGF-I, and Ala67 by Thr, the peptides from rat and h-IGF-I containing the respective residues (C-1 and C-8) eluted at the same retention time in C-18 reversed phase HPLC.

Immunocross-reactivity—Using pAb and mAb against h-IGF-I, immunological cross-reactivity of pure rat IGF-I was compared to h-IGF-I (Fig. 5, A and B). Purified rat IGF-I showed very similar immunoreactivities as h-IGF-I to pAb (Fig. 5A). Cross-reactivity was calculated to be 75%. Our mAb against h-IGF-I did show very weak reactivity (about 3%) toward rat IGF-I, despite only three amino acid substitutions (Fig. 5B).

Radioceptor Assay—The results of the radioreceptor assay are shown in Fig. 6. Binding affinity of rat IGF-I was calculated to be 65% of that of h-IGF-I.

[3H]Thymidine Incorporation into BALB/c 3T3

FIG. 5. Immunocross-reactivity of h-IGF-I (●) and rat IGF-I (○) to pAb (A); immunocross-reactivity of h-IGF-I (●) and rat IGF-I (○) to mAb-121 (B). A, all dilutions were performed with PBS containing 0.025 M EDTA-Na2 and 0.5% BSA (SD buffer). Serial dilutions of purified h-IGF-I or purified rat IGF-I (100 µl) were incubated with 100 µl of rabbit polyclonal anti-h-IGF-I antiserum (1:104 dilution) and 400 µl of 0.5% polyethylene glycol 6000, the mixture was incubated at 4 °C for 3 h and centrifuged at 3000 × g for 30 min. The 125I count in the precipitates was determined by γ-counter. The between-assay CV (percentage) ranged from 3.3 to 11.7% with h-IGF-I concentrations of 3.96 to 17.1 ng/ml, and the within-assay CV (percentage) was from 3.1 to 5.1% with h-IGF-I concentrations of 3.70 to 18.7 ng/ml. The data represent one of three experiments, all of which gave similar results. B, RIA of purified h-IGF-I and purified rat IGF-I to mAb-121 were performed in the same way as the RIA by pAb described above except mAb-121 was used at 1:5 × 106 dilution of the ascites fluid. The data represent one of three experiments, all of which gave similar results.

FIG. 6. Radioreceptor assay of h-IGF-I (●) and rat IGF-I (○) to BALB/c 3T3 fibroblast cells in the presence of [125I]-h-IGF-I. Serial dilutions of purified h-IGF-I or purified rat IGF-I were prepared in PBS containing 1% BSA and tested in radioreceptor assay as described under “Materials and Methods.” Each point is the result of single replicate in one of four experiments, all of which gave similar results. CV (percentage) of this assay is within 5%.
Cells—The results of [methyl-3H]thymidine incorporation assay are shown in Fig. 7. The activity of rat IGF-I could not be distinguished from h-IGF-I.

Lipogenesis Assay—The results of the lipogenesis assay are shown in Fig. 8. The EC50 of h-IGF-I in the fat cell assay was 130 ng/ml and that of rat IGF-I >430 ng/ml. Thus, the reactivity of rat IGF-I was only 28.6% as potent as h-IGF-I.

FFA Release Inhibition Assay—The results are shown in Fig. 9. The IC50 of h-IGF-I was 2.7 nm, and that of rat IGF-I was 13 nm. The activity of rat IGF-I was only 22.5% as potent as h-IGF-I in the FFA release inhibition assay and is comparable to the result in the lipogenesis assay.

DISCUSSION

The discrepancy between reports by Roberts et al. (10) and Shimatsu and Rotwein (11) on the primary structure of rat IGF-I has left the question as to the correct sequence open. We therefore purified rat IGF-I from serum by a combination of acid/ethanol treatment, affinity, ion-exchange, and reversed phase HPLC (Table I). Ion-exchange chromatography (TSK-CM-2SW) occasionally gave three peaks (Peaks 1, 2, and 3 in Fig. 1C). The main component (Peak 3) was finally purified and sequenced although the other two peaks have not yet been identified. We suppose they are deamidated forms (6) of rat IGF-I at Gln16 and/or Gln21. The amino acid sequence of the main peak was identical to the sequence determined from cDNA by Shimatsu and Rotwein (11). What was interesting in the peptide maps was the formation of C-3 and C-4 peptides which were not formed in the chymotryptic digestion of h-IGF-I (Fig. 3, A and B). Although the amino acid sequences around the chymotrypsin cleavage site in rat and h-IGF-I were the same, new cleavage was observed at Phe23 in rat IGF-I to form C-3 and C-4 peptides. Substitution of Asp20 to Pro is thought to cause a slight conformational change and render rat IGF-I susceptible to chymotrypsin cleavage at Phe23. Although longer digestion time (90 min) caused a loss of the C-8 peptide with a concomitant increase of C-6 and C-7, contrary to Klapper et al. (21), C-8 (Gly3'-Tyr20) was still the main fragment in our chymotryptic maps of both rat and h-IGF-I.

As is shown in Fig. 5B, rat IGF-I showed little crossreactivity (about 3%) with anti-h-IGF-I mAb even though the se-
quence difference was only 3 residues (Fig. 4). This suggests that the metabolic fate of h-IGF-I in rat can be monitored without interference by endogenous rat IGF-I.

In the RRA (Fig. 6) rat IGF-I was 65% as active as h-IGF-I. This weak activity of rat IGF-I, however, was not reflected in [methyl-3H]thymidine uptake stimulation activity (Fig. 7), probably, because of the less sensitivity of the [methyl-3H] thymidine incorporation assay. In the lipogenesis assay and in the FFA release inhibition assay, the activity of rat IGF-I was only 28.6% (Fig. 8) and 22.5% that of h-IGF-I (Fig. 9), respectively. In our in vitro assays, all purified proteins were added on the protein quantity basis measured by reversed phase HPLC. The assumption in our assay was that the same quantity of rat IGF-I would have the same molar absorption coefficient at UV280 nm. Based on this assumption, rat IGF-I had weaker specific biological activities than h-IGF-I in four assays tested except [nethyl-3H]thymidine uptake incorporation assay, and this is the first report which shows the definitive biological activities of rat IGF-I.

In the radioreceptor and [methyl-3H]thymidine assays with BALB/c 3T3 fibroblast cells, IGF-I interacts with the type I IGF receptor, whereas in the lipogenesis and the FFA release inhibition assays it interacts with the insulin receptor. Two explanations to explain the difference between the relative specific activities of rat IGF-I to h-IGF-I (65% or comparable activity through the type I IGF receptor, while 28.6 and 22.5% through the insulin receptor) may be possible. One is that, although unlikely, conformation of insulin receptor binding site of rat IGF-I was specifically less stable than human IGF-I under the purification conditions. The other possible idea to explain this difference is that it may reflect the different sensitivities of the respective receptor-ligand interaction to the conformational change, possibly caused by the single amino acid residue substitution at Asp29 to Pro.

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