Mutants of Human Insulin-like Growth Factor I with Reduced Affinity for the Type 1 Insulin-like Growth Factor Receptor

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ABSTRACT: Four mutants of human insulin-like growth factor I (hIGF I) have been purified from the conditioned media of yeast transformed with an expression vector containing a synthetic gene for hIGF I altered by site-directed mutagenesis. hIGF I has the sequence Phe-23–Tyr-24–Phe-25 which is homologous to a region in the B-chain of insulin. [Phe^{23},Phe^{24},Tyr^{25}]IGF I, in which the sequence is altered to exactly correspond to the homolgous sequence in insulin, is equipotent to hIGF I at the types 1 and 2 IGF and insulin receptors. [Leu^{24}]IGF I and [Ser^{24}]IGF I have 32- and 16-fold less affinity than hIGF I at the human placental type 1 IGF receptor, respectively. These peptides are 10- and 2-fold less potent at the placental insulin receptor, respectively. [Leu^{24}]IGF I and [Ser^{24}]IGF I have similarly reduced affinities for the type 1 IGF receptor of rat A10 and mouse L cells. Thus, the importance of the interaction of residue 24 with the receptor is conserved in several species. In three cell-based assays, [Leu^{24}]IGF I and [Ser^{24}]IGF I are full agonists with reduced efficacy compared to hIGF I. Desoctapeptide [Leu^{24}]IGF I, in which the loss of aromaticity at position 24 is combined with the deletion of the carboxyl-terminal D region of hIGF I, has 3-fold lower affinity than [Leu^{24}]IGF I for the type 1 receptor and 2-fold higher affinity for the insulin receptor. In contrast to their lack of affinity for the type 1 receptor, [Leu^{24}]IGF I, [Ser^{24}]IGF I, and desoctapeptide [Leu^{24}]IGF I have normal affinities for the rat liver type 2 IGF receptor and human serum binding proteins. Thus, our data clearly show that the structural determinants of hIGF I required to maintain binding to the type 1 IGF receptor and to the type 2 IGF receptor and serum binding proteins are different.

Human insulin-like growth factor I (hIGF I) is a 70 amino acid peptide with a high degree of sequence homology with insulin (Rinderknecht et al., 1978). This peptide has been shown to be a growth factor for cells of mesenchymal origin and is identical with the growth hormone dependent serum factor somatomedin C (Klapper et al., 1983). The peptide interacts with two distinct cell surface receptors (Massague & Czech, 1982). Insulin cross-reacts weakly with the type 1 IGF receptor but has no measurable affinity for the type 2 IGF receptor (Massague & Czech, 1982). Affinity labeling of the type 1 IGF receptor suggests that this protein shares a high degree of structural homology with the insulin receptor (Massague & Czech, 1982; Chernausek et al., 1981). In contrast, affinity labeling of the type 2 IGF receptor suggests that this protein is unrelated to the type 1 IGF receptor and the insulin receptor (Massague & Czech, 1982). The recent elucidation of the primary structures of the type 1 IGF receptor (Ullrich et al., 1986) and the type 2 IGF receptor (Morgan et al., 1987) has confirmed this.

In addition to binding to its cell surface receptors, hIGF I binds with high affinity to at least two types of soluble binding proteins. Human serum contains a 150-kDa growth hormone dependent binding protein which binds hIGF I and other homologues but does not bind insulin (Martin & Baxter, 1986). A 35-kDa protein has been detected in serum which is not growth hormone dependent but which also binds hIGF I but not insulin (Wilkins & D'Ercole, 1985).

Blundell and his colleagues have proposed a model for the tertiary structure of hIGF I on the basis of the homology of hIGF I and insulin (Blundell et al., 1978, 1983). This model predicts that certain insulin receptor binding domains are conserved in hIGF I and may account for the observed binding of hIGF I with the insulin receptor. In particular, alteration of the aromatic residues at positions 24, 25, and 26 in the B-chain of insulin has been shown by several groups to drastically alter affinity for the insulin receptor (Tager et al., 1980, Kobayashi et al., 1982). This region is conserved as residues Phe-23, Tyr-24, and Phe-25 of hIGF I.

In the present study, we have used site-directed mutagenesis of a synthetic gene encoding hIGF I (Bayne et al., 1987) to test if this region is also important for maintaining high-affinity binding of hIGF I with its receptors and binding proteins. We have previously shown that fully active hIGF I can be purified from the conditioned media of yeast transfected with an α 1 mating factor expression vector containing this gene (Bayne et al., 1988). We have also shown that this recombinantly derived hIGF I has the correct amino-terminal sequence, confirming that processing of the prepro α 1 mating factor sequence has occurred. In addition, we have shown that desoctapeptide hIGF I, produced by mutation of the synthetic gene, is a fully active ligand of the type 1 IGF receptor but has 4-fold higher affinity for the insulin receptor. We now show that mutants of hIGF I altered at residue 24 have reduced affinity for the type 1 IGF receptor but have normal affinity for the type 2 IGF receptor and serum binding proteins.

Materials and Methods

Materials. Restriction endonucleases and T4 DNA ligase were from New England Biolabs and used according to their recommendations. Crystalline bovine serum albumin (BSA) came from Sigma. Bio-Rex 70 and Bio-Gel P-10 were from

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Bio-Rad. [*methyl*-^{3}H]Thymidine (20 Ci/mmol) and [^{14}C]-glucose (10 mCi/mmol) were from New England Nuclear Corp. ^{125}I was from Amersham Corp. Purified multiplication stimulating activity (MSA, rat IGF II) was a gift from Dr. James Florini, Syracuse University. [Thr^{59}]IGF I was from Amgen. The rat clonal aortic smooth muscle cell line A10 and the mouse muscle cell line BC3H1 were obtained from the American Type Culture Collection and were maintained as previously described (Cascieri et al., 1986a,b). Mouse L cells were maintained as described previously (Bayne et al., 1987).

DNA Manipulations. Standard DNA manipulations were carried out as described elsewhere (Maniatis et al., 1983). Oligodeoxynucleotides were synthesized by phosphoramidite chemistry on an Applied Biosystems Model 380A synthesizer and gel purified as previously described (Bayne et al., 1987). DNA sequences were determined on M13 templates by the dideoxynucleotide chain termination method (Sanger et al., 1977). Plasmid strains were propagated in *Escherichia coli* strain DH 5 following standard transformation procedures (Hanahan, 1983).

Construction of Expression Vector. By use of a standard site-directed mutagenesis technique (Morinaga et al., 1984), a synthetic oligonucleotide, 5′-TATCCAA-AGGGATCCCTTCTTC-3′, was used to change codons 80 and 81 of the yeast α factor prepro sequence from GTATCT to ATCCCT, creating a *Bam*HI restriction endonuclease site six amino acids upstream from the KR endoprotease processing site (Kurjan & Herskowitz, 1982). A 213 base pair *Pst*I/*Bam*HI fragment from the mutated gene was cloned into partial *Pst*I/*Bam*HI-digested pJC197 (Schultz et al., 1987) to create expression vector pα2.

The plasmid phIGF containing the synthetic gene for IGF I (Bayne et al., 1987) was digested with *Bam*HI and *Acc*I; the ends were blunted then ligated to give the new vector pJY1. The 5′ end of a synthetic human IGF I gene was modified for expression in pα2 by replacement of the *Nde*I/*Sac*I fragment of pJY1 with a synthetic fragment formed by the annealing of oligonucleotides 5′-TATGCCGGATCCTTTCCTTG-GATAAAAGAGGTCCGCAAGCTTTGTGTGGTGCT-GAGCT-3′ and 5′-CAGCACCACACAAAGTTTCCG-GACCTCTTTTATCCAAGGAAAGGATCCGGCA-3′ as previously described (Bayne et al., 1987). After two rounds of transformation into *E. coli* DH5, a clone designated pJY2 was identified by *Hpa*II digestion and DNA sequencing. Further modification of pJY2 to prepare genes coding for the mutant peptides is described in detail below. The *Bam*HI cassette from pJY2 was ligated into *Bam*HI-digested pα2 by standard procedures. Proper orientation for pα2IGF was determined by *Pst*I mapping.

Construction of the Mutant hIGF I Genes. pJYX vectors were prepared by replacing the *Bst*EII/*Xba*I fragment of pJY2 with the synthetic DNA fragments containing small base-pairing mismatches described in Figure 2. After two rounds of transformation into *E. coli* DH5, clones were identified by *Nla*IV digestion and DNA sequencing.

Expression and Purification of Mutant hIGF I Peptides. *Saccharomyces cerevisiae* strain BJ1995 (MATα, leu2, trp1, ura3, prb1–1122, pep4–3, cir^0) was a gift from Dr. Elizabeth Jones, Carnegie Mellon University. Yeast were transformed as described previously (Hinnen et al., 1978), and transformants were selected on leucine-minus plates. Cells were grown to saturation in 1 L of 5× leu$^-$ media, pH 4.8, containing 0.85% yeast nitrogen base without amino acids and ammonium sulfate supplemented with 4% glucose, 1% ammonium sulfate, 0.6% sodium hydroxide, 0.03% L-isoleucine, 0.03% L-phenylalanine, 0.025% L-tyrosine, 0.02% L-lysine, 0.02% L-tryptophan, 0.02% uracil, 0.02% adenine, 0.01% L-arginine, 0.005% methionine, 0.005% L-histidine, 29 μM ferric chloride, 25 μM zinc sulfate, and 1% succinic acid. Cells were removed by centrifugation at 3000*g*. The cleared supernatant was mixed with 10 g of Bio-Rex 70 equilibrated in 1% succinic acid, pH 4.8. After being stirred for 3 h at 4 °C, the resin was poured into a 2.5-cm column and washed with 1 L of 1% succinic acid, pH 4.8. Peptide was eluted with 1 M ammonium acetate, pH 8. Receptor-active material was pooled, concentrated to 4 mL, and then applied to a 2.5 × 90 cm Bio-Gel P-10 (200–400 mesh) column equilibrated in 1 N acetic acid. Gel filtration was carried out at 30 mL/h. Twelve-milliliter fractions were collected and assayed for IGF-like activity by the radioreceptor assay. Active fractions were pooled and lyophilized. The activity was reconstituted in 0.2 mL of 0.05% trifluoroacetic acid–15% acetonitrile and loaded onto a C18 μBondapak (0.46 × 25 cm, 10 μm, Waters) reverse-phase HPLC column. The peptides were eluted from the column with a 15–50% acetonitrile gradient in 0.05% trifluoroacetic acid. The flow rate was 1 mL/min, and 1-min fractions were collected and assayed by receptor assay. Active fractions were pooled and lyophilized. The purified peptide was quantitated by amino acid analysis and stored at −20 °C in 0.1 N acetic acid at a concentration of 0.1 mM.

Characterization of the Biological Activities of Mutant hIGF I Peptides. (A) Type 1 IGF Receptor. [Thr^{59}]IGF I was used for iodination throughout these studies. ^{125}I-IGF I (50–80 Ci/g, 50 fmol) and human placental membranes (0.2 mg) were incubated in the presence or absence of competing peptides in 0.2 mL of 0.1 M Hepes, pH 8, containing 120 mM NaCl, 5 mM KCl, 0.12 mM $MgSO_4$, and 0.1% BSA for 1 h at 20 °C. Samples were filtered over Whatman GF/F filters presoaked in 0.1% poly(ethylenimine) to separate bound from free ligand. The incubation tubes and filters were washed 4 times with 2.5 mL of cold assay buffer (minus BSA). Less than 5% of the ligand bound to filters in the absence of membranes. Placental membranes bound 38% of the ligand in the absence of competing peptides. Nonspecific binding of the ligand to placental membranes was measured by adding excess amounts of unlabeled [Thr^{59}]IGF I (0.3 μM) to the incubation, and this routinely represented 5% of the total binding of ligand to membranes.

Type 1 IGF receptor binding to A10 and L cells was measured in confluent monolayer cultures in 24-well plates. Cells were incubated as above for 16 h at 4 °C and then washed 4 times with 1 mL of ice cold assay buffer. Cells were lysed with 1% SDS and counted. Insulin (5 μM) inhibited greater than 80% of the binding of ^{125}I-IGF I to cells.

(B) Insulin Receptor. ^{125}I-Insulin (receptor grade, NEN, 30 nCi) and placental membranes (0.025 mg) were incubated in 0.05 mL of the assay buffer described above for 1 h at 20 °C. Samples were filtered over EHWP filters, and the incubation tubes and filters were washed 4 times with 2.5 mL of cold assay buffer. Less than 5% of the ligand bound to the filters in the absence of competing peptides. Nonspecific binding of the ligand to placental membranes was measured by adding excess amounts of insulin (1 μM) to the incubation and was less than 1% of the total binding of ligand to membranes.

(C) Type 2 IGF Receptor. ^{125}I-MSA (30–50 Ci/g, 50 fmol) and rat liver membranes (0.2 mg) were incubated in 0.15 mL of the assay buffer described above and insulin (20 μM) for 90 min at 20 °C. Samples were filtered over Whatman GF/F

filters presoaked in 0.1% poly(ethylenimine), and then the incubation tubes and filters were washed 4 times with 2.5 mL of cold assay buffer. Less than 15% of the ligand bound to filters in the absence of membranes. Rat liver membranes bound 22–25% of the ligand in the absence of competing peptides. Nonspecific binding of the ligand to liver membranes was measured by adding excess amounts of MSA (0.1 μM) to the incubation and was less than 1% of the total binding of ligand to membranes.

(*D*) *Serum Binding Protein.* ^{125}I-IGF I (50 fmol) was incubated with 0.1 mg of protein of acid-treated human serum (Bayne et al., 1987) for 24 h at 4 °C. Free ^{125}I-IGF I was removed from the media by the addition of charcoal, and after centrifugation, an aliquot of the supernatant was removed and counted. The charcoal treatment removed greater than 95% of the free ligand in the absence of crude binding protein. In contrast, 30% of the ligand remained in the supernatant in the presence of crude binding protein and in the absence of competing peptides. An excess of [Thr^{59}]IGF I (0.1 μM) inhibited greater than 99% of the binding of ligand to the crude binding protein.

Cell-Based Assays. The measurement of [*methyl*-^{3}H]thymidine incorporation into DNA in A10 and L cells has been described in detail elsewhere (Cascieri et al., 1986a). Glycogen synthesis in BC3H1 cells was measured in monolayer cultures in 24-well plates at 5–7 days postconfluence. Cells were incubated in 0.2 mL of 20 mM Hepes, pH 7.4, containing 0.12 M NaCl, 0.1 mM $CaCl_2$, 0.5 mM $MgCl_2$, 1.5 mM KH_2PO_4, 2.6 mM KCl, 8.1 mM Na_2HPO_4, 0.5 mM glucose, and 0.1% BSA at 37 °C for 1 h. Peptides were added for 10 min at 37 °C, and then [^{14}C]glucose (NEC-042H from NEN, 0.4 μCi) was added for an additional 30 min at 37 °C. Monolayers were washed 2 times with 1 mL of ice-cold assay buffer, and then cells were digested with 1 mL of 5 N KOH. The digest was transferred to a glass tube, 0.1 mg of glycogen was added, and samples were boiled for 15 min. After the addition of 0.4 mL of 2% Na_2SO_4, the glycogen pellets were recovered by centrifugation, and the pellets were washed with 66% ethanol and then dissolved in water and counted.

Results

The synthetic gene for hIGF I was altered to prepare peptides in which (a) residues 23, 24, and 25 were changed to give the sequence corresponding to that of insulin in this region ([Phe^{23},Phe^{24},Tyr^{25}]IGF I), (b) the tyrosine at residue 24 was replaced with leucine ([Leu^{24}]IGF I) or serine ([Ser^{24}]IGF I), and (c) the eight amino acids at the carboxyl terminal of [Leu^{24}]IGF I were deleted (desoctapeptide [Leu^{24}]IGF I). A schematic showing the construction of the expression vector used to direct synthesis and secretion of the mutant peptides is shown in Figure 1. The mutant genes were prepared by replacing the *Bst*EII/*Xba*I fragment of pJY2 with the synthetic oligonucleotide fragments shown in Figure 2 to form pJYX. The *Bam*HI cassette of pJYX was then inserted into the *Bam*HI-digested pα2 vector to give the expression vector pα2IGFX.

The expression vectors were introduced into a protease-deficient strain of yeast (BJ1995) which was grown to saturation in a 1-L culture. The conditioned media contains receptor-active IGF I like activity. The peptides were purified by batch treatment with Bio-Rex 70 followed by Bio-Gel P-10 chromatography. The peptides were purified to homogeneity by reverse-phase HPLC (Figure 3). The purity of each peptide preparation was confirmed by SDS–polyacrylamide gel electrophoresis followed by colloidal gold protein staining (data not shown). The final yields of peptide from 1-L of

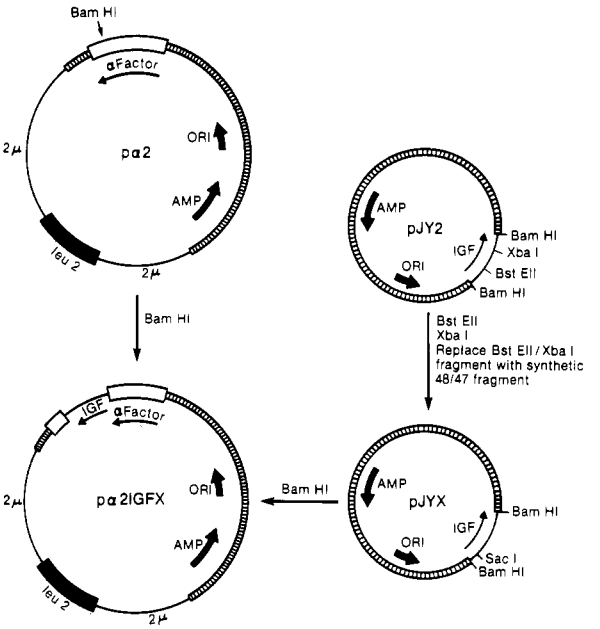

FIGURE 1: Construction of expression vectors pα2IGFX. Plasmid pα2 contains the ampicillin-resistance gene (AMP) and the DNA origin of replication (ORI) derived from pBR322 (hatched lines), yeast *Leu* 2 gene (black bars), yeast 2μ sequences, and the *MF*α1 promoter and prepro polypeptide. Plasmid pJY2, a pBR322 derivative (hatched lines) contains the chemically synthesized human IGF I gene inserted between the *Bam*HI sites. Mutations were introduced into the synthetic gene as described under Materials and Methods to form the vectors pJYX. The *Bam*HI cassette was inserted into the unique *Bam*HI site of pα2 to form pα2IGFX.

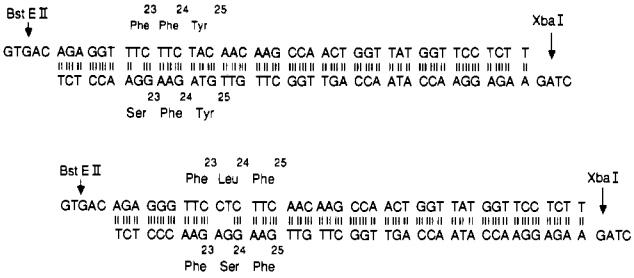

FIGURE 2: Sequences of the synthetic oligonucleotide fragments inserted into the *Bst*EII/*Xba*I-digested pJY2.

conditioned media are 244 μg of [Phe^{23},Phe^{24},Tyr^{25}]IGF I, 171 μg of [Leu^{24}]IGF I, 170 μg of [Ser^{24}]IGF I, and 375 μg of desoctapeptide [Leu^{24}]IGF I.

We have shown that the hIGF I produced in this expression system is a fully active ligand of the type 1 IGF receptor and of human serum binding proteins (Bayne et al., 1988). The aromatic region of hIGF I has the sequence Phe-Tyr-Phe at residues 23, 24, and 25. This region was changed to give the sequence corresponding to that of insulin in this region. [Phe^{23},Phe^{24},Tyr^{25}]IGF I is equipotent to hIGF I at both the type 1 IGF receptor and the insulin receptor (Figure 4).

Changing residue 24 to Leu or Ser results in peptides which have 32-fold and 17-fold lower affinity for the type 1 IGF receptor, respectively (Figure 4A). [Leu^{24}]IGF I and [Ser^{24}]IGF I have 10-fold and 2-fold reduced affinity for the insulin receptor, respectively (Figure 4B). Desoctapeptide [Leu^{24}]IGF I, which combines a loss in aromaticity in residue 24 with deletion of the carboxy-terminal D-region, is 3-fold less active than [Leu^{24}]IGF I at the type 1 IGF receptor and 2-fold more active at the insulin receptor (Figure 4).

In contrast to the effects of loss of aromaticity at residue 24 on binding to type 1 IGF receptors and insulin receptors, there is little difference in the affinity of these peptides and

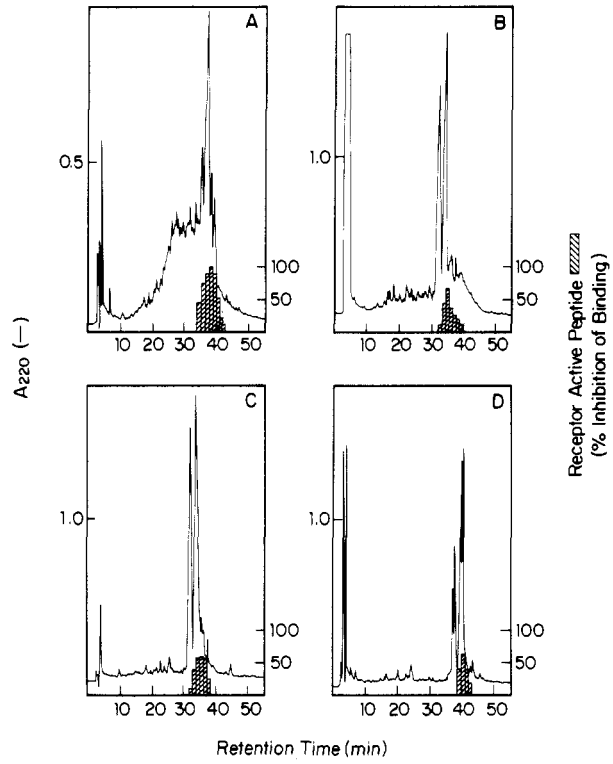

FIGURE 3: Reverse-phase HPLC purification of [Phe^{23},Phe^{24},Tyr^{25}]IGF I (A), [Leu^{24}]IGF I (B), [Ser^{24}]IGF I (C), and desoctapeptide [Leu^{24}]IGF I (D). Active fractions from Bio-Gel P-10 chromatography were applied to a C18 μBondapak column and eluted with a 15–50% acetonitrile gradient in 0.05% trifluoroacetic acid. Aliquots of each fraction were assayed for their ability to inhibit the binding of ^{125}I-IGF I to human placental membranes. The data are shown as percent inhibition of binding.

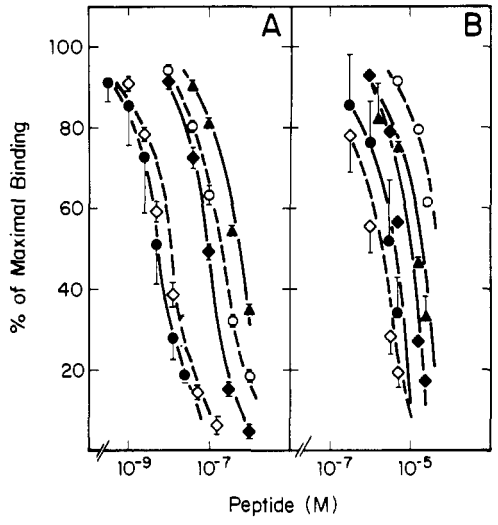

FIGURE 4: Inhibition of ligand binding to human placental type 1 IGF receptors (A) and insulin receptors (B) by hIGF I (●), [Phe^{23},Phe^{24},Tyr^{25}]IGF I (◇), [Leu^{24}]IGF I (○), [Ser^{24}]IGF I (◆), and desoctapeptide [Leu^{24}]IGF I (▲). Data are expressed as the percent of maximal specific binding of ligand determined in the absence of added peptide. Each point represents the mean ± SD for two determinations.

hIGF I for the type 2 IGF receptor and human serum binding protein. The affinities of hIGF I and the four mutant peptides for the receptors and binding proteins are summarized in Table I.

In contrast to the selective loss in type 1 IGF and insulin receptor affinity by mutation of the aromatic residue at position 24, mutation of the aromatic residue at position 23 results in a loss in affinity for all of the receptors and the binding

Table I: Effects of Modification of the Aromatic Region (Residues 23–25) of IGF I on Binding to the Human Placental Type 1 IGF Receptor, the Rat Liver Type 2 IGF Receptor, the Human Placental Insulin Receptor (IR), and Human Serum Binding Proteins (hBP)

peptide	IC_{50}[a] type 1 (nM)	type 2 (μM)	IR (μM)	hBP (nM)
hIGF I	5.6 ± 0.8	0.3 ± 0.2	2.8 ± 1	0.48 ± 0.02
[Phe^{23},Phe^{24},Tyr^{25}]IGF I	7.7 ± 0.8	0.1 ± 0.04	1.5 ± 0.3	1.2 ± 0.4
[Leu^{24}]IGF I	180 ± 10	0.9 ± 0.6	29 ± 4	0.45 ± 0.02
[Ser^{24}]IGF I	95 ± 6	0.6 ± 0.2	6.2 ± 0.7	0.45 ± 0.02
desoctapeptide [Leu^{24}]IGF I	490 ± 5	0.3 ± 0.03	13 ± 1	0.27 ± 0.01

[a] Mean ± SD; n = 2.

protein. [Ser^{23},Phe^{24},Tyr^{25}]IGF I has 14-, >30-, >10-, and 12-fold lower affinity than hIGF I for the type 1 IGF receptor, the type 2 IGF receptor, the insulin receptor, and human serum binding proteins, respectively. These data are most consistent with the hypothesis that the loss of aromaticity at residue 23 destabilizes the tertiary structure of hIGF I. Structural alterations of insulins mutated in this position have been demonstrated previously (Wollmer et al., 1981).

We have previously shown that IGF I stimulates DNA synthesis in the rat clonal aortic smooth muscle cell line, A10, through the type 1 IGF receptor (Cascieri et al., 1986a). hIGF I stimulates DNA synthesis in rat A10 cells with ED_{50} = 2 nM. [Leu^{24}]IGF I and [Ser^{24}]IGF I are 5 times less potent than hIGF I with ED_{50} = 10 nM (data not shown). Thus, the potencies of these peptides in this system do not quantitatively reflect their potencies at the human type 1 IGF receptor. The ED_{50} of desoctapeptide [Leu^{24}]IGF I is 100 nM, which does reflect its lack of potency at the human type 1 IGF receptor.

In order to see if the unexpected potency of [Leu^{24}]IGF I and [Ser^{24}]IGF I in rat A10 cells was the result of species differences in the selectivity of the type 1 IGF receptor, we measured the affinity of these peptides for the type 1 IGF receptor on rat A10 cells and mouse L cells (data not shown). [Leu^{24}]IGF I has 26- and 45-fold less affinity than IGF I for the type 1 receptor of A10 cells and L cells, respectively. [Ser^{24}]IGF I has 15- and 18-fold less affinity than IGF I for the type 1 receptor of A10 cells and L cells, respectively. Thus, the selectivity of the rat and mouse receptors in this region is very similar to that of the human receptor.

hIGF I stimulates DNA synthesis in L cells with ED_{50} = 1.6 nM (Figure 5A). [Leu^{24}]IGF I and [Ser^{24}]IGF I are 29-fold less potent with ED_{50} = 46 nM (Figure 5A). hIGF I stimulates glycogen synthesis in BC3H1 cells through the type 1 IGF receptor (Cascieri et al., 1986b) with ED_{50} = 4.7 nM (Figure 5B). [Ser^{24}]IGF I and [Leu^{24}]IGF I are 13- and 100-fold less potent with ED_{50} = 59 nM and 500 nM, respectively. Thus, in these two assays, the potency of the mutants correlates more closely with their respective potencies at the type 1 IGF receptor.

Discussion

The alteration of the hIGF I sequence from the wild-type Phe-23, Tyr-24,Phe-25-containing IGF I to give the mutant [Phe^{23},Phe^{24},Tyr^{25}]IGF I does not result in major changes in affinity for either the type 1 or the type 2 IGF receptors or human serum binding protein. Thus, the tyrosine hydroxyl group at residue 24 does not have a direct effect on binding affinity. However, mutation at residue 24 to the nonaromatic residues leucine or serine results in drastic loss in type 1 IGF receptor affinity. The combination of this mutation with the deletion of the eight amino acid carboxyl-terminal D-region

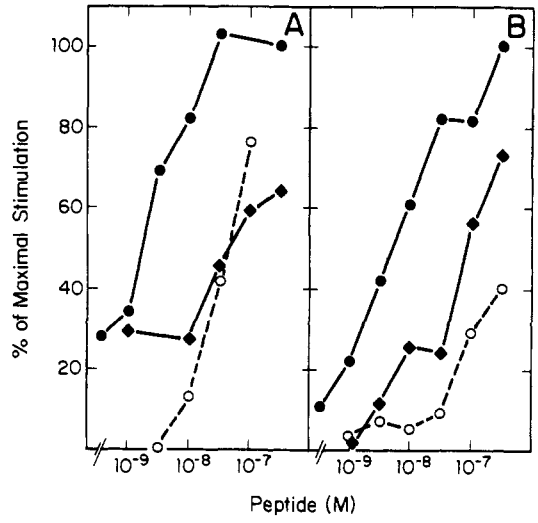

FIGURE 5: Stimulation of DNA synthesis in mouse L cells (A) and glycogen synthesis in BC3H1 cells (B) by hIGF I (●), [Leu^{24}]IGF I (○), and [Ser^{24}]IGF I (◆). hIGF I stimulated [^{3}H]thymidine incorporation into DNA 3–5-fold and [^{14}C]glucose incorporation into glycogen 3–5-fold in these systems. Data are expressed as the percent of the maximal response seen with 300 nM hIGF I. Each point is the average of three determinations.

of hIGF I results in a 3-fold further loss in type 1 receptor affinity.

These particular amino acid substitutions were chosen because a naturally occurring mutant of insulin occurs in this position containing leucine (Kobayashi et al., 1982). Substitution of the phenylalanine at residue 25 of the B-chain of insulin, which is analogous to residue 24 of hIGF I, with serine or leucine results in analogues with 50–100-fold reduced potency at the insulin receptor (Kobayashi et al., 1982; Haneda et al., 1985; Wollmer et al., 1981). Thus, our studies show that the homologous region of hIGF I is important for maintaining affinity at the type 1 IGF receptor.

The mutation of hIGF I at residue 24 results in a loss of affinity for the insulin receptor which is not as marked as the loss in type 1 IGF receptor affinity. In addition, the loss in insulin receptor affinity is not as great as is the loss in affinity of [Leu^{B25}]insulin and [Ser^{B25}]insulin. This may be explained by the work of Nakagawa and Tager (1986), who have shown that the role of phenylalanine-B25 in maintaining a high-affinity interaction with the insulin receptor is modulated by the structures of nearby domains including the carboxyl-terminal domain of the B-chain. Thus, the loss of aromaticity at this residue in the framework of the tertiary structure of hIGF I may not have as dramatic an effect on insulin receptor affinity as predicted.

While [Leu^{24}]IGF I and [Ser^{24}]IGF I have reduced affinity for the type 1 IGF receptor, they have normal affinity for both the type 2 receptor and serum binding proteins. Therefore, this residue is not involved in the binding of hIGF I to these proteins. Our data clearly show that the structural determinants for maintaining high-affinity binding to the types 1 and 2 IGF receptors and human serum binding proteins are different.

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Registry No. hIGF I, 68562-41-4; IGF I, 67763-96-6; [Phe^{23},Phe^{24},Tyr^{25}]hIGF I, 113507-17-8; [Leu^{24}]hIGF I, 113507-16-7; [Ser^{24}]hIGF I, 113507-15-6; desoctapeptide [Leu^{24}]hIGF I, 113490-27-0.

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