A single-chain insulin-like growth factor I/insulin hybrid binds with high affinity to the insulin receptor

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1. To investigate the structure/function relationship of the interaction between ligand and receptor in the insulin-like growth factor I (IGF-I) and insulin receptor systems we have prepared and characterized a single-chain insulin/IGF-I hybrid. The single-chain hybrid consists of the insulin molecule combined with the C domain of IGF-I. The single-chain hybrid was found to bind with high affinity to both truncated soluble insulin receptors and membrane-bound holoreceptors. The affinity for interacting with the soluble truncated insulin receptors was 55–94 % relative to insulin, and the affinity for membrane-bound insulin receptors was 113 % of that of insulin. Furthermore we found that the affinity of the single-chain hybrid molecule for

IGF-I receptors was 19–28 % relative to IGF-I. 2. The affinity of the single-chain hybrid for chimeric insulin/IGF-I receptors exceeded that of either natural ligand. This indicates that coordinately changing domains of the receptors and the ligands can induce higher affinity of ligand for receptor, supporting the idea that these receptors have a common ligand-binding site [Kjeldsen, Andersen, Wiberg, Rasmussen, Schäffer, Balschmidt, Møller and Møller (1991) Proc. Natl. Acad. Sci. U.S.A. 88, 4404–4408]. 3. In contrast with what was generally assumed about the ligand structure required for binding to the insulin receptor we demonstrate the first single-chain insulin analogue that can bind with high affinity to the insulin receptor

INTRODUCTION

Insulin and insulin-like growth factor I (IGF-I) are highly homologous peptide hormones that are essential for normal regulation of metabolism and growth. Insulin and IGF-I both mediate their biological activity by binding to different, but homologous, receptors on target cells. The insulin and IGF-I receptors are membrane-spanning glycoproteins with intracellular ligand-stimulated tyrosine kinase domains.

The insulin and IGF-I molecules share many structural similarities, 17 of 20 evolutionary invariant residues of the insulin A and B chains are conserved in IGF-I [1], the positions of disulphide bridges are identical and the overall structure is similar in the two hormones [2]. No identity exists, however, in the C domains, and in contrast with proinsulin the C domain of IGF-I is not cleaved from the B and A chains but is retained in the single-chain structure.

The insulin molecule probably changes its conformation upon binding to the insulin receptor. The single-chain insulin precursor miniproinsulin, in which B29Lys (i.e. Lys-29 of the B chain) is coupled directly to A1Gly via a peptide bond, crystallizes into conformations identical to those of insulin [3]. However, the *in vitro* potency of miniproinsulin is less than 0.1 % of that of

insulin [4], probably due to conformational restrictions in this molecule. Other insulin analogues with conformational restrictions have also been shown to exhibit low affinity for the insulin receptor. Insulins that have been chemically cross-linked between B29 and A1 exhibit affinities of 1–7 % relative to insulin [5]. Also proinsulin with the C-peptide connecting B30Thr and A1Gly, binds poorly to the insulin receptor (1–2 %) [6].

Binding of IGF-I to its receptor is less well characterized. Using IGF-I analogues it has been found that replacing the C domain of IGF-I with four glycines resulted in a 30-fold loss of affinity for the IGF-I receptor [7], indicating that the C domain is important for interaction with the IGF-I receptor.

Several studies have addressed how specificity and selectivity is conferred in the homologous insulin and IGF-I ligand/receptor systems. A two-chain hybrid of the insulin and IGF-I hormones that was prepared using semisynthetic methods, has previously been characterized [8]. The two-chain hybrid was essentially insulin with the IGF-I C domain attached to the C-terminal of the B chain. This hybrid bound with high affinity to the insulin receptor (28 %), and to the IGF-I receptor (20 %), relative to the respective cognate ligands [8].

Chimeric receptors have indicated that the insulin and IGF-I receptors have a common binding site [9–11]. The 68 N-terminal

Figure 1 Structure of the single-chain IGF-I/insulin hybrid

The figure illustrates the domain structure and the amino acid sequences of insulin, IGF-I and the single-chain hybrid using the one-letter code. The residues corresponding to that of insulin are shaded in the three peptide sequences.

Abbreviations used: BHK, baby hamster kidney; DMEM, Dulbecco's modified Eagle's medium; IGF-I, insulin-like growth factor I; hIR and hIGFIR, membrane-bound insulin and IGF-I receptors; sIR and sIGFIR, soluble truncated insulin and IGF-I receptors.

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amino acids of the insulin receptor were found to confer high affinity for insulin on this receptor, whereas part of the cysteine-rich region of the IGF-I receptor was found to confer high-affinity binding of IGF-I to the IGF-I receptor [10]. The two-chain IGF-I/insulin hybrid described above was found to bind with higher affinity than either of the natural ligands to chimeric receptors that contained regions determining specificity for both ligands [12].

To investigate further the structure/function relationship of ligand interaction with receptor in the insulin/IGF-I systems we prepared a single-chain insulin/IGF-I hybrid consisting of the A and B chains of insulin connected by the C domain of IGF-I (Figure 1). This single-chain hybrid was found to interact with the insulin receptor with high affinity, demonstrating for the first time that a single-chain ligand conformation is compatible with high-affinity interaction with the insulin receptor.

EXPERIMENTAL

Synthesis of the hybrid cDNA

The IGF-I C-domain cDNA was inserted into insulin cDNA by overlap extension PCR [13,14], with two rounds of PCR. The PCR was performed using *Taq* polymerase (Perkin-Elmer Cetus). Oligonucleotides were prepared on an Applied Biosystems DNA synthesizer. The full-length hybrid cDNA fragment was purified and subcloned into a cPOT plasmid [15] furnished with the *Saccharomyces cerevisiae* α -leader sequence [15]. The hybrid cDNA was sequenced using enzymic chain termination (Sequenase, U.S. Biochemical Corp.). Sequencing was performed on the plasmid recovered from *Escherichia coli*, and later sequencing of plasmid recovered from the expressing yeast cells confirmed the cDNA.

Expression and purification of the single-chain hybrid

The single-chain hybrid peptide was produced in *S. cerevisiae* strain MT663 [15]. Yeast cells were transformed and selected on yeast/peptone/2% glucose plates according to standard methods [16]. Yeast cultures were grown in yeast/peptone medium with 3% (w/v) glucose for 96 h at 30 °C. The cell-free medium was adjusted to pH 3 with HCl and then batch-treated with cation-exchange resin for 16 h at room temperature to adsorb peptides, which were subsequently eluted with 0.5 M ammonia. Fractions containing the single-chain hybrid were pooled and the peptide was purified to homogeneity (>95%) by two rounds of reverse-phase h.p.l.c. using a C_{18} LiChrosorb column (Merck), and a 24.6–34% acetonitrile gradient. The solvent system consisted of A buffer [0.2 M Na_2SO_4 , 0.04 M H_3PO_4 , 10% (w/v) acetonitrile, adjusted to pH 2.3 with ethanolamine] and B buffer [50% (w/v) acetonitrile in water]. The preparation of purified single-chain hybrid was desalted in formic acid/acetonitrile using a C_{18} SEP-PAK column (Millipore), and finally lyophilized.

The molecular mass of the single-chain hybrid peptide was determined by electrospray m.s. (Platform; VG Biotech, with MassLynx version 1.6 software for acquiring and transforming data). The purified peptide was dissolved at 10 pmol/ μl in 0.05% trifluoroacetic acid/50% (w/v) acetonitrile, and injected on to the mass spectrometer at a flow rate of 10 $\mu\text{l}/\text{min}$. Mass spectra were recorded with 3.92 kV capillary voltage in the m/z range 800 to 1800 Da/e with 9.6 s scan duration.

Expression and purification of receptors

The receptor cDNA clones were constructed and stably expressed in baby hamster kidney (BHK) cells, as described previously

[9,10]. The receptors used were insulin and IGF-I receptors and two chimeras of these receptors, sIR.191–290 and sIGFIR.1–68. The chimeric receptor sIR.191–290 is an insulin receptor in which residues 191–290 of the IGF-I receptor replace the corresponding domain of the parent receptor, and sIGFIR.1–68 is an IGF-I receptor in which residues 1–68 of the insulin receptor replace the corresponding domain of the parent receptor [10]. The truncated soluble insulin receptor (sIR) was purified by a previously described procedure [17], modified by keeping the supernatant of the cell culture under nitrogen, which allows purification of receptors with insulin affinity of approx. 0.02 nM (J. Markussen and L. Schäffer, unpublished work). The truncated receptors, sIGFIR, sIGFIR.1–68 and sIR.191–290, secreted from the transfected BHK cells were partially purified by the previously described procedure [10].

Characterizing ligand–receptor interaction

For receptor-binding experiments the single-chain hybrid was labelled with ^{125}I using the lactoperoxidase method [18] and the fraction moniodinated at residue A14Tyr was isolated. The other labelled ligands used (^{125}I -A14Tyr]insulin and ^{125}I -31Tyr]IGF-I [19]), and the unlabelled hormones (insulin and IGF-I), were from Novo Nordisk.

Competition binding assays

Truncated soluble receptors

Assays were performed by incubating the receptors in a total volume of 200 μl with ^{125}I -IGF-I (3–10 pM) or ^{125}I -insulin (3 pM) and increasing concentrations of unlabelled ligand in binding buffer A [100 mM Hepes, pH 8.0, 100 mM NaCl, 10 mM MgCl_2 , 0.5% (w/v) BSA, 0.025% (w/v) Triton X-100] for 48 h at 4 °C. Subsequently bound ligand was precipitated with 0.2% γ -globulin and 500 μl of 25% (w/v) poly(ethylene glycol) 8000, and the radioactivity in the pellet was counted. The concentration of the receptors was adjusted to yield 15–20% binding when no competing ligand was added in the competition assay.

Membrane-bound receptors

Competition binding assays on membrane-bound holoreceptors were performed on BHK cells overexpressing full-length insulin (-exon 11) or IGF-I receptors [11]. Equal number of transfected BHK cells (2000–5000) was seeded in each well of a 24-well plate and grown for 24 h in Dulbecco's modified Eagle's medium (DMEM; GIBCO) containing 10% (v/v) fetal-calf serum (HyClone) before performing the binding assay. Cells were washed once with binding buffer B (DMEM containing 0.5% BSA, 20 mM Hepes, pH 7.8) before adding a total volume of 400 μl with ^{125}I -IGF-I (6.5 pM) or ^{125}I -insulin (6.5 pM) and increasing concentrations of unlabelled ligand in binding buffer B. After 3 h at 16 °C unbound ligand was removed by aspirating the buffer and washing once with 1.2 ml of cold binding buffer B. Cells were solubilized in 500 μl of 1% (w/v) SDS, 100 mM NaCl, 25 mM Hepes (pH 7.8) and counted. The number of cells was adjusted to yield 16–28% binding when no competing ligand was added in the assay.

Tracer binding assay

The tracer binding assay has been described previously [12]. Briefly, the assay was performed by adding the various receptor preparations in sequential dilution to a constant concentration of carrier-free radiolabelled ligand (3 pM in all cases) in 200 μl of

binding buffer A. After 48 h of incubation at 4 °C, bound hormone was separated from free hormone by precipitating with 0.2% (w/v) γ -globulin and 500 μ l of 25% (w/v) poly(ethylene glycol) 8000, and the radioactivity in the pellet was counted. Care was taken to ensure that the binding capacity of the receptor preparation used in each experiment exceeded the amount of ligand–receptor complex obtained by a minimum of 5-fold.

Analysis of data from binding assays

The competition binding data were analysed according to a four-parameter logistic equation to determine IC_{50} values using GraFit software [20]. The tracer binding data were analysed by linear regression using the GraFit software.

RESULTS

Expression and purification of the single-chain hybrid

The single-chain hybrid was purified by cation-exchange chromatography, followed by two rounds of reverse-phase h.p.l.c., yielding single-chain hybrid peptide of more than 95% purity (Figure 2). The purified single-chain hybrid was analysed by SDS/PAGE to ensure that no proteolytic processing of the peptide had occurred (results not shown). The molecular mass of the purified peptide was found to be 7036.26 Da (theoretical molecular mass 7037.96 Da) by electrospray m.s. (Figure 2). The purified peptide was either used directly for receptor displacement assays or labelled with ^{125}I before being used in the tracer binding assay.

Interaction of the single-chain hybrid with receptors

Competition binding assay with soluble truncated receptors

The interaction of the single-chain hybrid with sIR and soluble truncated IGF-I receptor (sIGFIR) was characterized by competition binding experiments. The single-chain hybrid, insulin and IGF-I were tested for their ability to displace ^{125}I -insulin from sIR (Figure 3a). The concentration of unlabelled insulin required for half-maximal inhibition of ^{125}I -insulin binding to sIR was 22 ± 7 pM. The corresponding values for the single-chain hybrid and IGF-I were 23 ± 6 pM and 230 ± 90 pM re-

Figure 2 H.p.l.c. and m.s. analysis of purified single-chain hybrid

Chromatogram showing the elution profile after applying approx. 5 μ g of purified single-chain hybrid peptide in 10 μ l of 0.05 M formic acid on a Lichrosorb h.p.l.c. column. The insert shows a transformed electrospray mass spectrum of purified single-chain hybrid peptide. For details, refer to the Experimental section.

Figure 3 Competition curves for ^{125}I -insulin and ^{125}I -IGF-I binding to soluble receptors

Receptors were incubated with labelled ligand for 16 h at 4 °C together with increasing concentrations of unlabelled insulin ($\circ$), IGF-I ($\bullet$), or single-chain hybrid ($\square$) as described in the Experimental section. The amount of labelled hormone bound, as a percentage of labelled hormone bound in the absence of unlabelled hormone, is plotted against the concentration of unlabelled hormone. (a) ^{125}I -insulin (3 pM) displaced from sIR; (b) ^{125}I -IGF-I (10 pM) displaced from sIGFIR.

spectively (Table 1). The IC_{50} relative to insulin was found to be 94% for the single-chain hybrid and 9% for IGF-I at sIR (Table 1).

The concentration of unlabelled IGF-I for half-maximal inhibition of ^{125}I -IGF-I binding to sIGFIR was 0.37 nM (Figure 3b). The corresponding values for the single-chain hybrid and insulin were 1.9 ± 1.2 and 210 ± 70 nM respectively (Table 1). The effect of introducing the C domain of IGF-I into the insulin molecule is thus a more than 100-fold lower IC_{50} for binding to the sIGFIR.

Competition binding assay with membrane-bound receptors

The interaction of the single-chain hybrid with membrane-bound insulin receptor (hIR) and IGF-I receptor (hIGFIR) was characterized by competition binding experiments using BHK cells overexpressing the receptors. The single-chain hybrid, insulin and IGF-I were tested for their ability to displace ^{125}I -insulin from hIR (Figure 4a). The concentration of unlabelled

Table 1 IC_{50} values for IGF-I and insulin binding to insulin and IGF-I receptors

Data were derived from competition binding studies presented in Figures 3 and 4. Concentrations (nM) of unlabelled ligand required to reduce binding of iodinated ligand to receptors to 50% of maximum ($\pm$ S.D.) are given. Values in parentheses are the affinity relative to native ligand. n denotes the number of experiments.

Receptor	IC_{50} (nM)		
	Insulin	IGF-I	Single-chain hybrid
Soluble			
sIR ($n = 3$)	0.022 ± 0.007 (100)	0.23 ± 0.09 (9)	0.023 ± 0.006 (94)
sIGFIR ($n = 3$)	210 ± 70 (0.2)	0.37 ± 0.16 (100)	1.9 ± 1.2 (19)
Membrane-bound			
hIR ($n = 4$)	1.7 ± 0.5 (100)	133 ± 108 (1)	1.6 ± 0.3 (113)
hIGFIR ($n = 2$)	220 (0.1)	0.22 (100)	0.80 (28)

Figure 4 Competition curves for ^{125}I -insulin and ^{125}I -IGF-I binding to membrane-bound receptors

BHK cells overexpressing receptors were incubated with labelled ligand for 3 h at 16 °C together with increasing concentrations of unlabelled insulin ($\circ$), IGF-I ($\bullet$), or single-chain hybrid ($\square$) as described in the Experimental section. The amount of labelled hormone bound, as a percentage of labelled hormone bound in the absence of unlabelled hormone, is plotted against the concentration of unlabelled hormone. (a) ^{125}I -insulin (6.5 pM) displaced from hIR; (b) ^{125}I -IGF-I (6.5 pM) displaced from hIGFIR.

insulin required for half-maximal inhibition of ^{125}I -insulin binding to cells overexpressing the insulin receptor was 1.7 ± 0.5 nM. The corresponding values for the single-chain hybrid and IGF-

Figure 5 Interactions of radiolabelled ligands with truncated soluble receptors

Sequential dilutions of receptors were incubated with 3 pM of ^{125}I -insulin ($\circ$), ^{125}I -IGF-I ($\bullet$), or ^{125}I -labelled single-chain hybrid ($\square$) as described in the Experimental section. The interactions of radiolabelled ligands with sIR (a) and with the chimeric receptor sIR.191-290 (b) are shown as a function of the sequential dilution of receptor.

I were 1.6 ± 0.3 nM and 133 ± 108 nM respectively (Table 1). The IC_{50} relative to insulin was found to be 113 % for the single-chain hybrid and 1 % for IGF-I at hIR (Table 1).

Table 2 Relative receptor binding affinities of insulin, IGF-I and single-chain hybrid for native and chimeric receptors

Data were derived from tracer binding experiments presented in Figure 5. Affinity for each receptor is related to the labelled ligand*, average of three independent experiments ($\pm$ S.D.).

Receptor	Relative affinity		
	Insulin	IGF-I	Single-chain hybrid
sIR	100%*	9 $\pm$ 2%	55 $\pm$ 11%
sIR.191–290	100%*	28 $\pm$ 4%	174 $\pm$ 29%
sIGFIR.1–68	89 $\pm$ 5%	100%*	141 $\pm$ 16%
sIGFIR	< 1%	100%*	25 $\pm$ 8%

The concentration of unlabelled IGF-I for half-maximal inhibition of 125 I-IGF-I binding to cells overexpressing the IGF-I receptor was 0.22 nM (Figure 4b). The corresponding values for the single-chain hybrid and insulin were 0.80 and 220 nM respectively (Table 1). Introducing the C domain of IGF-I into the insulin molecule, thus results in a more than 250-fold lower IC_{50} for binding to cells overexpressing hIGFIR.

The affinity of IGF-I for sIR was 9% of insulin whereas it was only 1% for hIR. This difference possibly can be ascribed to the high-affinity state of the sIR used here.

Tracer binding assay: binding to chimeric receptors

Plotting the ratio [bound hormone]/[free hormone] versus [receptor] the slope of the line represents the reciprocal of the apparent dissociation constant ($1/K_d$). The concentration of free receptor can be approximated by total receptor concentration when only a small proportion of the receptors are occupied by ligand, and thus [receptor] can be plotted in terms of a simple dilution of receptor [12].

The tracer binding assay was used to analyse binding of insulin, IGF-I and the single-chain hybrid to soluble chimeric insulin/IGF-I receptors and native receptors. The relative apparent affinities calculated from the slopes of the linear plots shown in Figure 5, are presented in Table 2. The single-chain hybrid molecule again was found to bind with high affinity to the insulin receptor. The affinity of radiolabelled single-chain hybrid relative to radiolabelled insulin was 55 $\pm$ 11% for sIR, whereas the affinity of the single-chain hybrid for binding to sIGFIR was 25 $\pm$ 8% relative to IGF-I (Table 2). In contrast the affinities of radiolabelled single-chain hybrid for binding to the two chimeric receptors exceeded that of either radiolabelled natural hormone for these receptors (Figure 5, Table 2).

DISCUSSION

Binding of ligand to receptor in the insulin/IGF-I receptor systems has been studied extensively using naturally occurring and synthetic/recombinant insulin analogues. However, only a few single-chain insulin analogues have been made, probably because they were expected to bind poorly to the insulin receptor and thus have attracted limited attention.

In the present study we have prepared a single-chain hybrid, that consists of the insulin A and B chains connected by the C domain of IGF-I. This new single-chain analogue was found to bind with high affinity to insulin receptors. Relative to insulin the affinity for sIR was 55–94% (Tables 1 and 2), and the affinity for membrane-bound full-length insulin receptors tended to be even higher than the affinity of insulin (Table 1). This high affinity was

surprising, and exceeds that of other single-chain insulin analogues synthesized to date by severalfold. Two features of the single-chain hybrid molecule were expected to interfere with insulin receptor interaction, namely (a) the C domain connection that restricts mobility of the insulin domains and (b) the C domain block of the α -amino group of residue A1Gly.

When interacting with its receptor the insulin molecule is thought to change its conformation, and constraints in the insulin molecule seem to decrease the receptor affinity [22,23]. Proinsulin featuring a 35-amino-acid C-peptide binds with an affinity of 1–2% of insulin [6]. The negative influence of the C-peptide region on binding to the insulin receptor, might be due to restraints introduced to the insulin molecule by the C-domain connection. Several findings of poor receptor binding of insulin analogues with a short chemical or peptide link between residues B29 and A1 [4,22,24], support the notion that structural restraints in the insulin molecule might inhibit insulin-receptor binding. In addition to results showing that short cross-links disrupt binding to the insulin receptor [4,22,24], one study has indicated that binding to the insulin receptor is improved when the length of the cross-link is increased [22]. The affinity of insulin analogues cross-linked between B29 and A1 for the insulin receptor gradually increased from 2% to 11% when the length of the cross-link was extended from two to 12 atoms [22].

Some of these findings may in part be ascribed to blocking of the α -amino group of residue A1Gly, that has been shown to be important for binding to the insulin receptor. Removing A1Gly decreases the affinity for the insulin receptor to less than 1% of that of unmodified insulin [24], and acetylation of A1Gly decreases the affinity 4-fold [24]. Split forms of proinsulin clipped at the B–C junction bound with only 3% affinity relative to insulin, whereas split forms clipped at the C–A junction (i.e. a free A1 residue) exhibited 14% relative affinity [6].

The C domain of the single-chain hybrid is 12 amino acids and thus the length may by itself allow sufficient conformational flexibility for interacting with the insulin receptor. However, in addition to possible intramolecular restraints, the C domain of the single-chain hybrid molecule blocks the α -amino group of A1Gly, and this was thought *per se* to significantly reduce affinity for the insulin receptor (4-fold) [24]. When the single-chain hybrid binds to the insulin receptor the C domain might compensate for the negative effect of blocking the α -amino group of A1Gly, by interacting directly with a specific domain of the insulin receptor. Such an interaction would imply that the insulin receptor has a conserved domain, like the IGF-I receptor, that interacts with the C domain of IGF-I, and thus facilitates binding of the single-chain hybrid to the insulin receptor.

A two-chain insulin/IGF-I hybrid which essentially is insulin with the C domain of IGF-I fused to the C-terminal of the insulin B chain has previously been described [8]. The affinity of this two-chain hybrid relative to insulin has been found to be 28% for placental membrane receptors [8], and 49% for truncated soluble receptors [12]. When interacting with the insulin receptor the affinity of the new single-chain hybrid appears to be similar to that of the two-chain hybrid, despite the fact that the two-chain hybrid also has a free A1Gly residue. The similar affinity of these two analogues might indicate that the insulin receptor-binding site has different domains interacting with either the α -amino group of A1 or the C domain of IGF-I, and the interaction with the IGF-I C domain is favoured by the single-chain conformation.

The affinity of the single-chain hybrid for binding to sIGFIR was 19–25% of IGF-I affinity in the two assays used (Tables 1 and 2), and this was confirmed when using full-length membrane-bound IGF-I receptors (28%, Table 1). This is less surprising,

considering that a total of 39 of the 63 amino acids (62 %) of the single-chain hybrid are identical to IGF-I (Figure 1), and that the single-chain structure also is retained. Furthermore, it has previously been reported that determinants of IGF-I important for binding to the IGF-I receptor are located in the IGF-I C domain [7].

The chimeric receptors have demonstrated that the ligand specificities of the insulin receptor and the IGF-I receptor reside in different domains of a common binding site [9,10]. The N-terminal 68 amino acids of the insulin receptor confer high-affinity binding of insulin, and the cysteine-rich domain (residues 191–290) of the IGF-I receptor confers high-affinity binding of IGF-I [10]. The chimeric receptor sIR.191–290 which is an insulin receptor in which residues 191–290 of the IGF-I receptor replace the corresponding domain of the parent receptor, and sIGFIR.1–68 which is an IGF-I receptor in which residues 1–68 of the insulin receptor replace the corresponding domain of the parent receptor, have previously been shown to bind both insulin and IGF-I with high affinity [10]. The single-chain hybrid binds to these chimeric receptors with an affinity higher than either natural ligand (Table 2), similar to results obtained using the two-chain hybrid [12]. The present findings demonstrate that domains and structures of the two natural ligands can be switched and a single-chain hybrid that binds to both receptors can be obtained. Thus, these findings further support the hypothesis that the insulin and IGF-I receptors have a common binding site that can accommodate both hormones independent of single- or two-chain structure. The difference in affinity of insulin and IGF-I for the receptors results from different residues interacting with various specificity-conferring regions on the two receptors.

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REFERENCES

- 1 Rinderknecht, E. and Humbel, R. E. (1978) *J. Biol. Chem.* **253**, 2769–2776
- 2 Sato, A., Nishimura, S., Ohkubo, T., Kyogoku, Y., Koyama, S., Kobayashi, M., Yasuda, T. and Kobayashi, Y. (1993) *Int. J. Peptide Protein Res.* **41**, 433–440
- 3 Derewenda, U., Derewenda, Z., Dodson, E. J., Dodson, G. G., Bing, X. and Markussen, J. (1991) *J. Mol. Biol.* **220**, 425–433
- 4 Markussen, J., Jørgensen, K. H., Sørensen, A. R. and Thim, L. (1985) *Int. J. Peptide Protein Res.* **26**, 70–77
- 5 Nakagawa, S. H. and Tager, H. S. (1987) *J. Biol. Chem.* **262**, 12054–12058
- 6 Peavy, D. E., Brunner, M. R., Duckworth, W. C., Hooker, C. S. and Frank, B. H. (1985) *J. Biol. Chem.* **260**, 13989–13994
- 7 Bayne, M. L., Applebaum, J., Underwood, D., Chicchi, G. G., Green, B. G., Hayes, N. S. and Cascieri, M. A. (1989) *J. Biol. Chem.* **264**, 11004–11008
- 8 Cara, J. F., Mirmira, R. G., Nakagawa, S. H. and Tager, H. S. (1990) *J. Biol. Chem.* **265**, 17820–17825
- 9 Andersen, A. S., Kjeldsen, T., Wiberg, F. C., Christensen, P. M., Rasmussen, J. S., Norris, K., Møller, K. B. and Møller, N. P. H. (1990) *Biochemistry* **29**, 7363–7366
- 10 Kjeldsen, T., Andersen, A. S., Wiberg, F. C., Rasmussen, J. S., Schäffer, L., Balschmidt, P., Møller, K. B. and Møller, N. P. H. (1991) *Proc. Natl. Acad. Sci. U.S.A.* **88**, 4404–4408
- 11 Andersen, A. S., Kjeldsen, T., Wiberg, F. C., Vissing, H., Schäffer, L., Rasmussen, J. S., De Meyts, P. and Møller, N. P. H. (1992) *J. Biol. Chem.* **267**, 13681–13686
- 12 Schäffer, L., Kjeldsen, T., Andersen, A. S., Wiberg, F. C., Larsen, U. D., Cara, J. F., Mirmira, R. G., Nakagawa, S. H. and Tager, H. S. (1993) *J. Biol. Chem.* **268**, 3044–3047
- 13 Horton, R. M., Hunt, H. D., Ho, S. N., Pullen, J. K. and Pease, L. R. (1989) *Gene* **77**, 61–68
- 14 Ho, S. N., Hunt, H. D., Horton, R. M., Pullen, J. K. and Pease, L. R. (1989) *Gene* **77**, 51–59
- 15 Thim, L., Hansen, M. T., Norris, K., Hoegh, I., Boel, E., Forström, J., Ammerer, G. and Fiil, N. P. (1986) *Proc. Natl. Acad. Sci. U.S.A.* **83**, 6766–6770
- 16 Sherman, F., Fink, G. R. and Lawrence, C. W. (1979) *Methods in Yeast Genetics: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
- 17 Markussen, J., Halstrøm, J., Wiberg, F. C. and Schäffer, L. (1991) *J. Biol. Chem.* **266**, 18814–18818
- 18 Drejer, K., Kruse, V., Larsen, U. D., Hougaard, P., Bjørn, S. and Gammeltoft, S. (1991) *Diabetes* **40**, 1488–1495
- 19 Schäffer, L., Larsen, U. D., Linde, S., Hejnæs, K. R. and Skriver, L. (1993) *Biochim. Biophys. Acta* **1203**, 205–209
- 20 Leatherbarrow, R. J. (1992) *GraFit*, Version 3.01, Erithacus Software Ltd., Staines, U.K.
- 21 Reference deleted
- 22 Nakagawa, S. H. and Tager, H. S. (1989) *J. Biol. Chem.* **264**, 272–279
- 23 Nakagawa, S. H. and Tager, H. S. (1993) *Biochemistry* **32**, 7237–7243
- 24 Gliemann, J. and Gammeltoft, S. (1974) *Diabetologia* **10**, 105–113