Altered affinity of insulin-like growth factor II (IGF-II) for receptors and IGFB-binding proteins, resulting from limited modifications of the IGF-II molecule

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The binding affinities of seven analogues of recombinant human insulin-like growth factor II (hIGF-II) were characterized for the IGF type-I and type-II receptors and insulin receptors, as well as for IGF-binding protein (IGFBP)-1, IGFBP-2, IGFBP-3 and human serum IGFBPs. A switch of two of the three cysteine bridges in hIGF-II, 9–47 and 46–51 to 9–46 and 47–51, severely impaired the binding of this analogue to all receptors and to the IGFBPs. The affinities for the IGF type-I receptor and the IGFBPs were decreased over 100-fold, while the binding to the insulin receptor and the IGF type-II receptor was less affected, with a 6–10-fold decrease in affinity. Slight modifications of the N-terminus had only minor effects upon the binding of IGF-II to the IGFBPs or to the receptors. Deletion of both the N-terminal amino acid and the two C-terminal amino acids resulted in moderate decreases in affinity, with a 60 % decrease in affinity for IGFBP-1 and the IGF type-I receptor. Acetylation of the N-terminus of Ala4 and the ε-nitrogen of Lys16 decreased the affinity, by 60–90 %, of hIGF-II for all of the IGFBPs and receptors. The experiments involving acetylation of IGF-II or switching of its cysteine bridges indicate that these modifications (no substitution, deletion or addition of any of the 67 amino acids of hIGF-II) may lead to a severe impairment of the binding affinity of IGF-II for both the IGFBPs and the receptors. Acetylation of the ε-nitrogen of Lys4, which causes a charge change, or alteration of the three-dimensional structure, as shown by the cysteine bridge switch, lead to a severe impairment of the binding affinity for the binding proteins and for the receptors. In general, care should be taken with the synthesis of analogues and the interpretation of resulting binding data, since affinity alterations ascribed to amino acid changes may instead be caused by alterations of the charge or the three-dimensional structure of the protein.

INTRODUCTION

The insulin-like growth factors, IGF-I and IGF-II, are peptide mitogens which share structural identity with insulin and may play an important role in early postnatal growth and development (Van Wyk, 1984). In humans, IGF-I appears to be primarily involved in regulating cell replication and the growth of the organism (Rosenfeld & Hintz, 1986; Rechler et al., 1987), in contrast with insulin, which primarily regulates rapid anabolic responses, such as glucose transport, and glycogen and fat biosynthesis (Kahn, 1985). The specific physiological role of IGF-II is not yet clear, but it may play a role in fetal growth (Froesch et al., 1985).

Each IGF interacts with a distinct high-affinity receptor. The IGF type-I receptor is a member of the tyrosine kinase family of growth factor receptors (Ebina et al., 1985; Froesch et al., 1985; Ulrich et al., 1986) and is structurally similar to the insulin receptor. The IGF type-II receptor, on the other hand, consists of a monomeric polypeptide which has no intrinsic tyrosine specific kinase activity (Czech, 1982; MacDonald et al., 1984, 1985; Kahn, 1985; Rechler & Nissley, 1985; Corvera et al., 1986). IGF-I binds to the type-I receptor with high affinity and to the insulin receptor and type-II receptor with significantly lower affinities. IGF-II binds to both type-I and type-II receptors with similar high affinities and to the insulin receptor with lower affinity (Roth et al., 1988). The IGFs also have high affinity for a family of IGFB-binding proteins (IGFBPs), several of which have been cloned and sequenced (Brewer et al., 1988; Lee et al., 1988; Binkert et al., 1989; Brown et al., 1989; Wood et al., 1989; Shimasaki et al., 1991). These specific binding proteins are found in many body fluids and in the conditioned media of a wide variety of cell types, and they modulate IGF peptide activity in a complex manner (Elgin et al., 1987; Busby et al., 1989; Cascieri et al., 1988, 1989a; Ross et al., 1989; Rosenfeld et al., 1990; Lamson et al., 1991).

The primary sequence of human IGF-I and the subsequent modelling of its secondary and tertiary structure based on its identity with insulin have made it possible to predict which structural features of IGF-I are involved in its binding to the IGF type-I and type-II receptors and to IGFBPs (Blundell et al., 1978, 1983). Furthermore, a series of IGF-I analogues made by site-directed mutagenesis have defined binding affinities and binding sites for IGF receptors and IGFBPs at different IGF-I domains (Cascieri et al., 1988, 1989a, b; Cascieri & Bayne, 1989; Ross et al., 1989). Recently, Bagley et al. (1989) have shown that des-(1-3)-IGF-I had a markedly lower affinity for the IGFBPs compared with the parent molecule. These data indicate that modification of amino acids in the N-terminus and the C-terminus of IGF-I results in altered affinity for the IGFBPs, and for the insulin and IGF receptors. Because of the considerable structural similarities between IGF-I and IGF-II, it is of interest to examine the relative affinity of IGF-II analogues which are modified in similar regions.

In the present investigation, we have examined the ability of

Abbreviations used: CM, conditioned medium; DPP-I, dipeptidyl peptidase I; f.a.b./m.s., fast atom bombardment/mass spectrometry; hIGF-I, human insulin-like growth factor I; hIGF-II, human insulin-like growth factor II; IGFBP, insulin-like growth factor binding protein; rIGFBP, rat IGFBP; IC50, conc. causing half-maximal inhibition.

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seven different IGF-II analogues, prepared by disulphide interchange reactions, acetylation or limited enzymic digestion of either recombinant hIGF-II or Met-His-Trp-IGF-II, to bind to insulin and IGF receptors and IGFBPs (IGFBP-1, IGFBP-2, IGFBP-3 and human serum IGFBPs). These studies should assist in identifying the BP- and receptor-binding domains of IGF-II.

MATERIALS AND METHODS

Materials

Recombinant [Th240]IGF-I was purchased from Amgen Biologicals (Thousand Oaks, CA, U.S.A.). Recombinant hIGF-II, IGF-II analogue and insulin were provided by Dr. Michele Smith (Eli Lilly, Indianapolis, IN, U.S.A.). The IGF-II analogues (Fig. 1) were prepared by a disulphide-interchange reaction during the generation of recombinant hIGF-II, by acetylation or by limited enzymic digestion of either recombinant hIGF-II or recombinant Met-His-Trp-IGF-II, which contains the N-terminal addition Met-His-Trp.

Analogue 1 contains a cysteine bridge switch (9-46, 47-51) in two of the three native cysteine bridges (originally 9-47, 21-60 and 46-51), and was prepared as described (Smith et al., 1989). Analogue 2, which contains the N-terminal addition Met-His-Trp, was overexpressed in Escherichia coli and purified as described (Cantrell et al., 1991).

Analogue 3-6 were prepared by digestion of either IGF-II or Met-His-Trp-IGF-II with dipetidyl peptidase I (DPP-I) (McDonald & Barrett, 1986). The digests were carried out in 50 mm-sodium acetate/5 mm-NaCl/5 mm-β-mercaptoethanol, pH 3.8, with 3 mg of substrate/ml. Analogues 3 and 4 were prepared from Met-His-Trp-IGF-II digested with DPP-I at 4 °C, with an enzyme/substrate molar ratio of 1:500, for 137 min. Analogue 5 was prepared from IGF-II digested at 37 °C, with an enzyme/substrate molar ratio of 1:1000, for 7 min. Analogue 6 was prepared by digesting Met-His-Trp-IGF-II with an enzyme/substrate molar ratio of 1:500 at 37 °C for 6.6 h, followed by 16.6 h at room temperature. All reactions were quenched by the addition of 1 M-HCl so that the final pH was 2.0. Analogues 3-6 were purified over a Zorbax C-8 reversed-phase h.p.l.c. column in 0.2 M-(NH4)2SO4/0.1 M-H2SO4 containing isocratic acetonitrile concentrations between 25% and 27%, depending on the analogue.

These analogues were characterized by amino acid analysis and N-terminal sequencing. Analogue 6 was further characterized by N-terminal sequencing of the C-terminal peptide from a pepsin digest as previously described (Smith et al., 1989).

Analogue 7 was prepared as described (Means & Feeney, 1971). The acetylated product was purified over the reversed-phase h.p.l.c. system described above, with an acetonitrile concentration of 27.4%. The product was characterized by peptide mapping and those peptides which differed from the peptide map of IGF-II were analysed by fast atom bombardment (f.a.b.)/m.s. as described (Smith et al., 1989).

IGF-II, IGF-I and insulin were iodinated by a modification of the chloramine-t method (Hunter & Greenwood, 1962) to a specific radioactivity of 150-300 μCi/μg.

Human serum from healthy male adult volunteers was collected and pooled. This pooled serum was chromatographed over a G50 Sephadex column in 1% formic acid to separate IGF peptides from binding proteins, as previously described (Liu et al., 1990).

Cell culture

All cell cultures were maintained at 37 °C in a 5% CO2 environment. HEPG2 human hepatoma cells were cultured as previously described and conditioned medium (CM) was collected as a source of hIGFBP-1 (Lamson et al., 1989). BRL-3A rat hepatoma-derived cells were cultured and CM was collected as a source of rat IGFBP-2 (DONOVAN et al., 1989). Monolayer cultures of purified human trophoblast cells were prepared and CM was collected as a source of hIGFBP-3 (Deal et al., 1991). Briefly, at confluency, the cells were washed and incubated with serum-free medium for 72 h. At the end of the incubation period, CM was centrifuged at 2000 g at 4 °C for 30 min and frozen at −20 °C until the time of assay.

Membrane preparation

Membranes were stripped from fresh human and rat placenta, and blood and blood vessels were removed. The placentas were cut and washed in 0.25 M-sucrose and homogenized with a Polytron homogenizer (Casella et al., 1986). Crude microsomal preparations were prepared by differential centrifugation as described by Marshall et al. (1974).

Binding assay for IGFBPs

The conditioned media (1 μl of HEPG2 CM, 1 μl of human serum, 50 μl of BRL-3A CM, 8 μl of human trophoblast CM) were incubated with [125I]-IGF-II in the presence of unlabelled IGF-II or IGF-II analogues at concentrations indicated in the text, at 4 °C for 18 h in a total volume of 500 μl of 50 mm-Tris buffer/0.5% BSA, pH 7.4. Following incubation, 1 ml of 0.5% activated charcoal (Sigma, St. Louis, MO, U.S.A.) in phosphate-buffered saline (pH 7.4) containing 1% BSA with protamine sulphate (Sigma; 0.2 mg/ml) was added for 15 min at 4 °C. Protamine sulphate was not added to HEPG2 CM because of its inhibitory effect on binding to IGFBP-1 (Conover et al., 1989). Samples were centrifuged at 2000 g for 30 min at 4 °C and the supernatants were counted for radioactivity in a γ-radiation counter. Experiments have been performed three times in duplicate, unless otherwise indicated.

Receptor binding assay

Microsomal membrane protein (50 μg) was incubated with 2000 c.p.m. of radioligand ([125I]-insulin, IGF-I or IGF-II) with or without unlabelled insulin, IGF-I, IGF-II or IGF-II analogues, at concentrations indicated in the text, in a total volume of 500 μl of assay buffer (50 mm-Hepes/0.5% BSA, pH 7.4, 0.2 M-NaCl, 0.1 M-mercaptoethanol, 0.4% BSA, 0.1% Triton X-100, 0.1% CHAPS, 0.1% deoxycholic acid, 1 mm-EDTA, 5 mm-thiamine, 5 mm-poly(3-glutamic acid), 1 mm-chloramphenicol, 1 mm-phenylmethylsulphonyl fluoride, 5 μl of 100 μM-palmitic acid, 0.05 μCi of [125I]-insulin, IGF-I or IGF-II) for 18 h at 4 °C.
Altered affinity of analogues of insulin-like growth factor II

pH 7.4) for 18 h at 4 °C. At the end of the incubation period, 1 ml of cold assay buffer was added and tubes were centrifuged at 2000 g for 30 min at 4 °C. Membrane pellets were counted in a Micromedic γ-radiation counter. All experiments were performed in duplicate and were repeated three times, unless otherwise indicated.

Statistical analysis

Means and s.d.s for the relative potency of hIGF-II and analogues were calculated and significant differences between hIGF-II and analogues were determined using Student’s t test.

RESULTS

The amino acid sequences of IGF-II and the characteristics of seven analogues are shown in Fig. 1, and the presumed three-dimensional structure is depicted in Fig. 2. These IGF analogues (analogues 2–6) have been prepared by limited DPP-I digestion of either recombinant hIGF-II or recombinant Met-His-Trp-IGF-II, which contains the N-terminal addition Met-His-Trp. Analogue 1 has been prepared by switching two (9–46, 47–51) of the three putative native cysteine bridges (originally 9–47, 21–60, 46–51). Analogue 7 has been prepared by acetylation of the N-terminus and ε-nitrogen of Lys46.

All seven analogues have been examined for binding to the insulin and IGF receptors and IGF-binding proteins. Determinations of the affinities of these analogues for the IGF-binding proteins were performed utilizing the CM of HEPG2 cells, BRL-3A cells, human trophoblast cells and acid-chromatographed human serum for IGFBP-1, IGFBP-2, IGFBP-3 and total human serum IGFBPs respectively. Binding affinities for the receptors were determined in binding assays using particulate membranes. Human placental membranes were employed as a source of insulin receptors and IGF type-I receptors, whereas rat placental membranes were used for the IGF type-II receptor.

Fig. 3 shows representative competitive binding curves of 125I-IGF-II binding to IGFBPs and the IGF type-II receptor in the presence of various concentrations of the IGF-II analogues. Also shown are the displacement curves for the IGF type-I receptor and the insulin receptor obtained by determination of the displacement of 125I-IGF-I and 125I-insulin respectively by these analogues.

In Table 1 the relative potency of each analogue in competition studies is presented as the mean IC50 (concen. causing half-maximal inhibition) of IGF-II divided by the mean IC50 of the IGF-II analogue; the actual mean IC50 of IGF-II for each IGFBP and receptor preparation is also presented. The IC50 values of IGF-II for hIGFBP-3, hIGFBP-2 and hIGFBP-1 were 0.3, 1.0 and 5.4 ng/ml respectively. As anticipated, IGF-II bound with high affinity to the IGF type-II receptor in rat placental membranes, with an IC50 of 2.0 ng/ml. IGF-II bound with significantly lower affinity to the human placental and insulin receptors, with an IC50 of 34.0 ng/ml.

Analogue 1, in which the cysteine bridges have been switched from 9–47 and 46–51 to 9–46 and 47–51, had a severely impaired affinity for all the IGFBPs tested. Relative affinities for IGFBP-2 and IGFBP-3 were significantly lower than those of IGF-II (P < 0.005, P < 0.05 respectively). In general, the affinity was decreased by 2–3 orders of magnitude for these binding proteins. The affinity of this analogue for the IGF type-I receptor was also decreased over 100-fold. The affinities for the IGF type-II and the insulin receptors were somewhat better preserved, with relative affinities of 10% and 16% respectively compared with IGF-II.

Modifications of the B domain, represented by analogues 2–5, demonstrated affinities comparable with those of hIGF-II for both the IGFBPs and the receptors. Specifically N-terminal additions or deletions had little effect on binding to IGFBPs or receptors.

Analogue 6, in which Ala1, Ser44 and Glu47 were deleted, showed moderate decreases in affinity, especially in the IGFBP-1 and type-I receptor binding assay (60% decrease) (Table 1). Acetylation of Ala1 and Lys46, represented by analogue 7, decreased the affinity for all the binding proteins and receptors. The affinity of this analogue was decreased by 60–90% compared

Fig. 2. Three-dimensional structure of human IGF-II

The modifications, as present in the analogues, are as described in the legend to Fig. 1. □, Affected part of IGF-II; , natural cysteine bridges of IGF-II. (Modified from Blundell et al., 1983.)
with hIGF-II. In particular, the relative affinities for IGFBP-2 and IGFBP-3 were significantly decreased (P < 0.05). The affinity of this analogue for the hsIGFBPs was, however, comparable with the affinity of hIGF-II.

DISCUSSION

Seven hIGF-II analogues have been investigated for their affinities for the IGF type-I and type-II insulin receptors, as well as for IGFBP-1, IGFBP-2, IGFBP-3 and human serum IGFBPs. Consistent with former studies (Roth et al., 1988), hIGF-II itself has high affinity for the IGF type-II receptor in rat placental membranes and an equivalent affinity for the IGF type-I receptor present in human placental membranes.

There are three putative native disulphide bonds, Cys9-Cys47, Cys51-Cys60 and Cys48-Cys51, in human IGF-II, as illustrated in Fig. 1. Analogue 1, containing the cysteine bridge switch (9-46, 47-51), showed a markedly lowered affinity for all the receptors and IGFBPs, indicating that the native three-dimensional structure of IGF-II is essential for its interaction with receptors and IGFBPs (Fig. 2). These results extend the findings of Smith et al. (1989), who reported a 160-fold difference in affinity between analogue 1 and hIGF-II for binding to bovine plasma binding proteins. The present study shows that the switch of two of the
Table 1. Relative potency of hIGF-II and analogues for IGFBPs, IGF receptors and insulin receptor

Relative potency is the ratio of IC50 of IGF-II/IC50 of the analogue. Data are presented as the means ± s.d. Experiments with IGFBP-1, IGFBP-2, IGF type-II receptor and insulin receptor have been performed three times in duplicate; all other experiments have been performed twice in duplicate. **P < 0.005; *P < 0.05 versus IGF-II.

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Human serum BPs</th>
<th>BP-1</th>
<th>BP-2</th>
<th>BP-3</th>
<th>Type-II</th>
<th>Type-I</th>
<th>Insulin</th>
</tr>
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<tbody>
<tr>
<td>Analogue 1</td>
<td>0.01 ± 0.01</td>
<td>&lt; 0.02</td>
<td>&lt; 0.01**</td>
<td>&lt; 0.03*</td>
<td>0.1 ± 0.04</td>
<td>&lt; 0.01</td>
<td>0.2 ± 0.1</td>
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<tr>
<td>Analogue 2</td>
<td>0.8 ± 0.1</td>
<td>0.9 ± 0.3</td>
<td>0.8 ± 0.2</td>
<td>0.9 ± 0.5</td>
<td>1.1 ± 0.6</td>
<td>1.6 ± 0.2</td>
<td>0.7 ± 0.3</td>
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<tr>
<td>Analogue 3</td>
<td>1.3 ± 0.03</td>
<td>0.9 ± 0.3</td>
<td>1.1 ± 0.9</td>
<td>0.8 ± 0.2</td>
<td>0.9 ± 0.5</td>
<td>1.4 ± 0.4</td>
<td>1.0 ± 1.0</td>
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<tr>
<td>Analogue 4</td>
<td>1.7 ± 0.3</td>
<td>0.6 ± 0.3</td>
<td>0.7 ± 0.5</td>
<td>0.8 ± 0.4</td>
<td>1.4 ± 0.3</td>
<td>0.8 ± 0.4</td>
<td>0.8 ± 0.6</td>
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<tr>
<td>Analogue 5</td>
<td>1.7 ± 0.3</td>
<td>0.6 ± 0.1</td>
<td>0.7 ± 0.3</td>
<td>0.8 ± 0.01</td>
<td>1.1 ± 0.5</td>
<td>0.8 ± 0.1</td>
<td>0.6 ± 0.4</td>
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<tr>
<td>Analogue 6</td>
<td>1.6 ± 0.1</td>
<td>0.4 ± 0.2</td>
<td>0.8 ± 0.4</td>
<td>0.8 ± 0.2</td>
<td>1.2 ± 0.9</td>
<td>0.4 ± 0.2</td>
<td>1.0 ± 0.04</td>
</tr>
<tr>
<td>Analogue 7</td>
<td>0.8 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>0.4 ± 0.3*</td>
<td>0.1 ± 0.02*</td>
<td>0.4 ± 0.4</td>
<td>0.2 ± 0.1</td>
<td>0.4 ± 0.01</td>
</tr>
<tr>
<td>IGF-II (IC50 in ng/ml)</td>
<td>(0.7 ± 0.6)</td>
<td>(5.4 ± 4.7)</td>
<td>(1.0 ± 0.3)</td>
<td>(0.3 ± 0.1)</td>
<td>(2.0 ± 1.9)</td>
<td>(2.1 ± 1.2)</td>
<td>(34.0 ± 22.6)</td>
</tr>
<tr>
<td>Insulin</td>
<td>6.9 ± 4.5</td>
<td></td>
<td></td>
<td></td>
<td>0.2 ± 0.05</td>
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<td>15.2 ± 2.0</td>
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| Relative potency |
|------------------|---------------|
| IGFBPs           | Receptors     |
|                  |               |
| Type-II          | Type-I        |
| Insulin          |               |

three disulphide bonds greatly impairs the binding of IGF-II to IGFBP-1, IGFBP-2, IGFBP-3 and the hIGFBP.

The modifications, both deletions and additions, of the N-terminal region of IGF-II, as represented by analogues 2–5, did not cause major alterations in the affinities of these analogues for either the receptors or the binding proteins tested. In contrast, experiments with des-(1–3)-IGF-I have shown that all or some of these three N-terminal amino acids are necessary for high-affinity binding to the IGFBPs, as demonstrated by the severe decrease in affinity upon removal of these amino acids (Szabo et al., 1988; Ross et al., 1989). In addition, Cascieri et al., (1989a) have shown that substitution of the amino acids Glu4 and Thr4 in IGF-I with the equivalent amino acids of insulin results in a marked decrease in the affinity of IGF-I for hIGFBPs and for the rat liver IGF type-II receptor.

The current data show that the two N-terminal amino acids of IGF-II are not involved in binding to the IGFBPs; analogues 2–5 retained their binding affinity for all of the IGFBPs tested. Comparison of the structures of IGF-I and IGF-II shows that amino acids Glu4 and Thr4 of IGF-I are the two amino acids which are preserved in the N-terminus of IGF-II (Glu4 and Thr4), suggesting that a substitution of these two amino acids in IGF-II with the equivalent amino acids of insulin might lead to the synthesis of an IGF-II analogue which has decreased affinity for the IGFBPs, similar to that seen with des-(1–3)-IGF-I (Bagley et al., 1989).

Analogue 6, in which the first amino acid of the N-terminus and the last two amino acids of the C-terminus were deleted, showed quantitatively similar decreases in affinity for binding to IGFBP-1 and to the IGF type-I receptor. The fact that the affinity for IGFBP-I and the type-I receptor are decreased by more than is the affinity for the other binding proteins and receptors suggests that the binding sites for IGFBP-I and for the type-I receptor may be located in the same part of the IGF-II molecule. Analogue 4, which is only missing the N-terminal alanine, did not show as large a decrease in affinities, suggesting that the C-terminus might be particularly important for native binding. Furthermore, analogue 7, in which Ala1 and LysK6 are acetylated, had an even lower affinity than analogue 6 for the various binding proteins and receptors. The largest decreases in affinities among the analogues in which the sequence was altered or the side-chains modified were seen with analogues 6 and 7. The negatively charged Glu61 residue was removed in analogue 6, and the positive charge on LysK6 was eliminated through acetylation in analogue 7. These results suggest that the local charge at the C-terminus of IGF-II may be involved in binding to receptors and binding proteins, perhaps through the formation of salt bridges.

Comparison of the binding profiles of the individual IGFBPs with the human serum IGFBPs shows that none of the individual binding protein profiles are identical to the profile found in human serum. Additional binding proteins present in human serum, such as IGFBP-4 (Shimasaki et al., 1991) and, perhaps, the recently demonstrated IGFBP in human cerebrospinal fluid (Roghani et al., 1989), might explain these findings. Alternatively, the human serum binding proteins may differ from the individual ones that we have obtained from CM, due to altered post-translational modifications such as differential glycosylation.

IGF-II for example shows a 2–3-fold higher affinity for the IGFBP-3 found in the CM of human trophoblasts than for the IGFBP-3 isolated from human serum (Deal et al., 1991). A third possibility is that the binding proteins affect each other’s binding profiles in a co-operative manner.

The experiments with analogues 1 and 7 show that these modifications (no substitution, deletion or addition of any of the 67 amino acids of IGF-II) may lead to a severe impairment of the binding affinity of IGF-II for both the IGFBPs and the receptors.

These data, particularly for analogue 1, indicate that a decrease in affinity for binding proteins or receptors may be caused by an alteration of the three-dimensional structure, without any actual change in the primary structure. In this regard, acetylation also causes a severe decrease in binding affinity. Acetylation of the ε-nitrogen of LysK6 causes a charge change, abolishing the positive charge, and extends the side chain by two carbon atoms.

In general, care should be taken with the synthesis of analogues and with the interpretation of resulting data, since the affinity alterations ascribed to amino acid changes may instead have been caused by alterations of the three-dimensional structure or by charge changes of the protein. Nevertheless, careful production of IGF-II analogues with altered affinities for individual IGFBPs or receptors may prove to be important for our understanding of the complex physiology of the IGF system.
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