Biochem. J. (1990) 266, 513–520 (Printed in Great Britain)



# Effects of a single cleavage in insulin-like growth factors I and II on binding to receptors, carrier proteins and antibodies

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Two somatomedin-like peptides were extracted from Cohn fraction IV of human plasma and brought to homogeneity: one focused at pH 7.8 and the other at pH < 5.6. Each consisted of two peptide chains interlinked by disulphide bonds. The basic peptide was identical to insulin-like growth factor I (IGF-I) and had a single cleavage in the C-domain before $Arg^{37}$ [IGF-I($Arg^{36}$cl)]. The acid peptide showed identity with IGF-II, with a cleavage in the B-domain before $Arg^{30}$ [IGF-II($Ser^{29}$cl)]. The effects of these cleavages on the characteristics of binding to type I and type II receptor sites, to binding proteins and to antibodies was studied. Binding of IGF-I($Arg^{36}$cl) to antibodies directed against the B-domain or against the AD-domain of IGF-I was the same as IGF-I binding. Thus the cleavage does not influence these antigenic sites. In contrast, binding of IGF-I($Arg^{36}$cl) to the type I receptor on human and bovine placental cell membranes was markedly decreased compared with IGF-I binding. Binding to the insulin receptor on human placental cell membranes was slightly diminished, whereas the interaction with specific type II receptors on bovine placental cell membranes was unaffected. There was only a minor influence of the cleavage on the region involved in binding to binding proteins. The cleavage in IGF-II($Ser^{29}$cl) diminished binding to antibodies directed against the C-domain of IGF-II, compared with binding of IGF-II itself. Binding to receptors (type I and type II) was changed less profoundly. With $^{125}$I-labelled IGF-II($Ser^{29}$cl), less insulin was needed in order to obtain 50% displacement of the tracer compared with displacement of $^{125}$I-labelled IGF-II. The cleaved form of IGF-II probably has a greater affinity towards the common receptor population than does native IGF-II. Binding to binding proteins was not affected by the cleavage in IGF-II.

## INTRODUCTION

Somatomedins which can be extracted from human plasma are heterogeneous in charge, as shown by several investigators (Van den Brande & Hoogerbrugge, 1980; Cornell, 1982; Svoboda & Van Wyk, 1985; Blum *et al.*, 1986; Van den Brande *et al.*, 1987). As well as insulin-like growth factors (IGF-I and IGF-II) which focus at pH 8.8 and 6.7 respectively, we have purified to homogeneity two peptides, one focusing at pH 7.8 and the other focusing at pH < 6.0. In contrast to IGF-I and IGF-II, they consist of two peptide chains interlinked by disulphide bonds (Van den Brande *et al.*, 1987). Partial sequence analysis has revealed the basic peptide to be identical to IGF-I, with a single cleavage in the C-domain before $Arg^{37}$ [IGF-I($Arg^{36}$cl)]. The acid peptide showed identity with IGF-II, with a cleavage in the B-domain before $Arg^{30}$ [IGF-II($Ser^{29}$cl)].

Separate binding sites present in the somatomedins are likely to be involved in their specific binding to receptor sites, binding proteins and antibodies (Blundell *et al.*, 1978, 1983; Dafgørd *et al.*, 1985). It has been shown in studies using hybrid molecules containing different portions of insulin and IGF-I that the B-domain of IGF-I is important for the recognition of carrier proteins and certain antibodies (De Vroede *et al.*, 1985; Joshi *et al.*, 1985*a*). It is likely that determinants on the A-domain, and to a certain extent on the B-domain, may be of critical importance in the ability of IGF-I to bind to specific receptor sites and for triggering the mitogenic activity (King *et al.*, 1982; Joshi *et al.*, 1985*b*; De Vroede *et al.*, 1986).

Nothing is known about the effects of cleavage in the IGF molecules on structure–function relationships. Therefore we studied the effects of single cleavages in IGF-I and IGF-II on binding to receptor sites, binding proteins and antibodies.

## MATERIALS AND METHODS

### Peptide preparation

Polypeptides exerting somatomedin activity were derived from Cohn fraction IV of human plasma as described previously (Van Schravendijk *et al.*, 1986; Van den Brande *et al.*, 1987). Briefly, after acidification, ultrafiltration and SP-Sephadex chromatography, active fractions were subjected to isoelectric focusing. Distinct peaks containing somatomedin activity were obtained, as measured by IGF-I radioimmunoassay, radioreceptor assay and somatomedin bioassay. Final purification of the peptides was achieved using h.p.l.c. separation techniques. This resulted in the identification of seven peptides differing in their pI values and/or in their behaviour on h.p.l.c. They were considered to be pure on the basis of the symmetry of the peak on h.p.l.c., the uniqueness of the spot after SDS/PAGE and

Abbreviation used: IGF, insulin-like growth factor.

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autoradiography of the $^{125}$I-labelled peptides, and *N*-terminal amino sequence analysis.

Two peptides were identified as IGF-I (pI 8.8) and IGF-II (pI 6.7); a further two peptides differed in their isoelectric points but otherwise showed barely any differences from IGF-I and IGF-II respectively. One peptide appears to be a new IGF-II variant in which Ser$^{29}$ is replaced by Cys. Details of these substances will be reported elsewhere. The remaining two peptides, one focusing at pH < 5.6 and the other at pH 7.8, appeared to be two-chain forms of IGF-II and IGF-I respectively, as shown by PAGE under reducing conditions (Van den Brande *et al.*, 1987). Partial sequence analysis of the basic peptide revealed two chains coinciding with the amino acid sequences 1–20 and 37–57 of IGF-I, thus indicating a cleavage before Arg$^{37}$. We shall call this substance IGF-I(Arg$^{36}$cl). Sequence analysis of the acid peptide revealed two chains coinciding with amino acids 1–15 and 30–46 of the native IGF-II molecule, suggesting a cleavage before Arg$^{30}$. We shall refer to this substance as IGF-II(Ser$^{29}$cl).

Determination of the molecular masses of both peptides by plasma desorption mass spectrometry gave a difference of 16–18 atomic mass units compared with those found for IGF-I and IGF-II, consistent with a two-chain configuration but with otherwise identical primary structures. The purified peptides were subjected to quantitative amino acid analysis, carried out on a Biotronic LC 7000 analyser (Biotronic AG, Maintal, Germany). These results are used for all indicated amounts of peptide.

Peptides (2.5 $\mu$g portions) were labelled with $^{125}$I using the chloramine-T method (Zapf *et al.*, 1981) to a specific activity of 60–100 $\mu$Ci/$\mu$g. Human insulin, $^{125}$I-labelled according to the lactoperoxidase method of Thorell & Johansson (1971) with some modifications as described by Takano (1975), was a gift from Dr. W. H. L. Hackeng (Bergweg Ziekenhuis, Rotterdam, the Netherlands). Porcine insulin as kindly provided by Dr. C. van Schravendijk (Free University, Brussels, Belgium).

Human serum albumin was from Kabi Vitrum (Stockholm, Sweden) and BSA was from Sigma.

### Radioimmunoassay

The reactivity of the labelled and unlabelled IGF-I-like peptides was studied using two antisera. Firstly, a somatomedin-C/IGF-I antiserum was used. This was obtained from Drs. L. Underwood and J. J. Van Wyk (University of North Carolina, Chapel Hill, NC, U.S.A.), and is distributed for research use by the National Hormone and Pituitary Distribution Programme of NIADDK. The radioimmunoassay method with this antiserum in our laboratory has been described previously (Van Buul-Offers *et al.*, 1988*a*). Secondly, an antiserum was used, which was directed against the AD-fragment of IGF-I [Immunonuclear Corp (INC), Stillwater, MN, U.S.A.]. A slightly modified method of the INC protocol was used: peptides were preincubated in the presence of anti-(IGF-I AD-fragment) antibodies for 2 h in 1% BSA/borate buffer at 4 °C. Then, $^{125}$I-labelled peptides were added (15000 c.p.m./tube) and the incubation was continued for an additional 20 h. Subsequently a second antibody/poly(ethylene glycol) mixture (Immunonuclear Corp.) was added, and after 2 h of incubation the immunocomplexed material was pelleted by centrifugation. The assays were developed or modified by Dr. F. A. Opmeer in our laboratory. The reactivity of IGF-II-like peptides was studied using two different antibodies. The first antibody was a monoclonal (no. 73) against IGF-II, kindly donated by Dr. R. Humbel (Biochemisches Institut der Universität, Zürich, Switzerland). Secondly, a monoclonal antibody from Amano Pharmaceutical Co. Ltd. (Osaka, Japan) was used, which has been proved to have excellent properties for a radioimmunoassay (cross-reactivity with [Thr$^{59}$]hIGF-I was less than 3% (Daughaday *et al.*, 1987). Peptides, $^{125}$I-labelled-IGF-II (10000 c.p.m./tube) and antibody were incubated for 44 h at 4 °C in 300 $\mu$l of assay buffer containing 50 mmol of sodium phosphate/l, 1 mmol of $MgCl_2$/l and 0.2% BSA (pH 7.4). Subsequently, 100 $\mu$l of a second antibody, Sac-cel (Immunodiagnostics), was added. The immunocomplexed material was pelleted by centrifugation (3 min, 2500 ***g***).

### Radioreceptor studies

Binding studies were performed using human placental cell membranes and bovine placental cell membranes. The membrane preparations and incubation conditions were as described previously (van Buul-Offers *et al.*, 1988*b*). Briefly, human placental cell membranes (60–100 $\mu$g/0.5 ml) were incubated with $^{125}$I-labelled (30000 c.p.m.) IGF-I, IGF-II or insulin and unlabelled peptides in a total volume of 500 $\mu$l for 16–20 h at 4 °C. Bovine placental cell membranes at concentrations of 40–60 $\mu$g/0.5 ml or 10–20 $\mu$g/0.5 ml were incubated with $^{125}$I-labelled (25000 c.p.m.) IGF-I or IGF-II respectively. After incubation, an equal volume of cold assay buffer (0.05 mol of Tris/HCl/l and 0.5% BSA, pH 7.4) was added. Tubes were then centrifuged for 10 min at 6000 ***g*** and pellets were washed once with 0.9 ml of cold assay buffer. After recentrifugation, pellets were counted for radioactivity in a Packard gamma counter. Non-specific binding was determined in the presence of a partially purified preparation (40 $\mu$g/ml) containing 3.2 $\mu$g of IGF-I and 20 $\mu$g of IGF-II/mg of protein, and was approx. 5–10% of the total binding. Data are expressed as specific binding (total minus non-specific binding).

### Preparation of IGF-binding proteins

Human serum from normal adult volunteers (8 ml) was chromatographed on a Sephacryl S-200 column (2.5 cm × 100 cm), equilibrated with $NH_4HCO_3$ buffer (50 mmol/l), pH 7.4. Fractions with $K_{av.}$ values of 0.07–0.18 (150 kDa) and 0.31–0.44 (40 kDa) were pooled, lyophilized and used for binding profiles with the different labelled peptides on Sephacryl S-300 columns (see below).

### Binding protein profiles of $^{125}$I-labelled peptides

Gel-filtration chromatography was performed using 1.6 cm × 80 cm columns of Sephacryl S-300 equilibrated with $NH_4HCO_3$ (50 mmol/l), pH 7.4. Whole serum or binding proteins were incubated with $^{125}$I-labelled peptides (100000 c.p.m./400 $\mu$l of plasma) for 24 h at 4 °C before application to the column. Protein content was monitored by absorbance at 280 nm. Fractions of 2–3 ml were collected and the radioactivity was counted in a Packard gamma counter.

**Table 1. Binding of $^{125}$I-labelled IGF-like peptides to anti-IGF antibodies and competition with unlabelled IGFs**

Competition is expressed as the concn. (ng/ml) required to obtain 50% displacement of the tracer. Means ± S.E.M. are given with numbers of experiments in parentheses. n.d., not done.

| $^{125}$I-labelled peptide | Antibody | Specific binding (%) | Competition by unlabelled compounds | | | |
|---|---|---|---|---|---|---|
| | | | IGF-I | IGF-I-(Arg$^{36}$cl) | IGF-II | IGF-II-(Ser$^{29}$cl) |
| IGF-I | IGF-I | 26.2±1.6 (10) | 0.20±0.02 (12) | 0.42±0.12 (6) | 18.36±1.8 (3) | 11.6 (2) |
| | AD-IGF-I | 40 (2) | 4.4 (2) | 11.4 (2) | n.d. | n.d. |
| IGF-II | IGF-II (Amano) | 28.4±1.1 (8) | 4.85±1.07 (3) | 14.9 (2) | 0.19±0.01 (8) | 0.2 (2) |
| | IGF-II (Humbel) | 25.2±5.0 (4) | 295 (2) | 816 (2) | 18.9±6.8 (4) | 167 (2) |

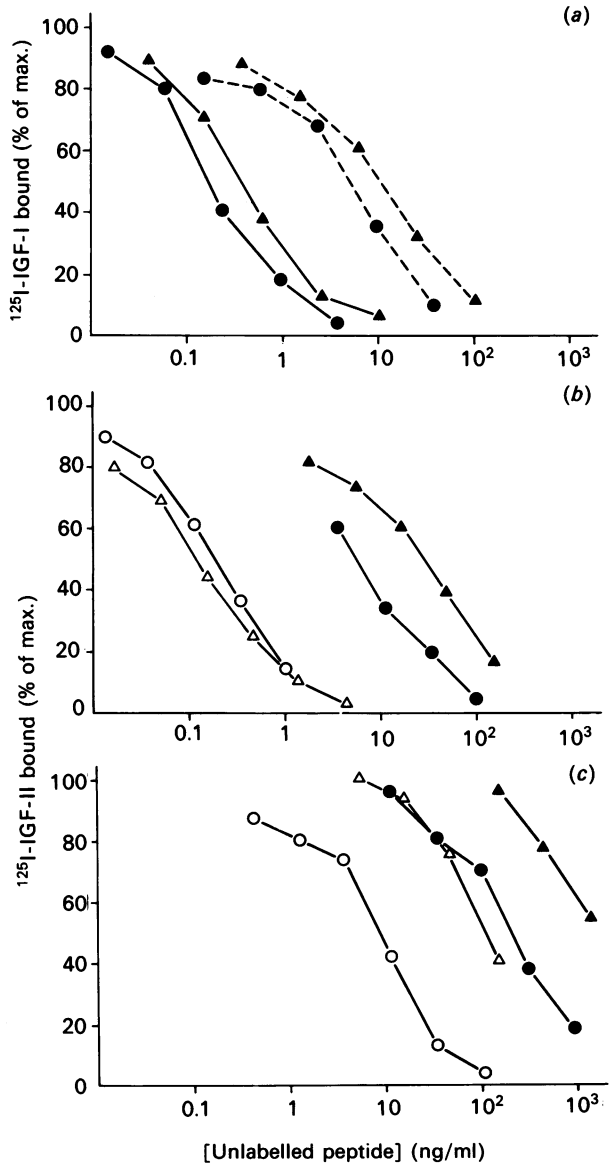


**Fig. 1. Competition between IGFs for binding to anti-IGF antibodies**

The effects of IGF-I (●), IGF-I(Arg$^{36}$cl) (▲), IGF-II (○) or IGF-II(Ser$^{29}$cl) (△) are shown on binding of $^{125}$I-labelled IGF-I (*a*) and IGF-II (*b*,*c*) to antibodies directed against whole IGF-I (*a*, —), the AD-domain of IGF-I (*a*, – –), or anti-IGF-II antibodies from Amano (*b*) and from Humbel (*c*).

## RESULTS

### Interactions with antibodies to IGF-I and IGF-II

The reactivity of IGF-I(Arg$^{36}$cl) with all four antibodies and their appropriate labels was uniformly and only slightly reduced compared with the reactivity of IGF-I (2–3-fold), suggesting a generalized conformational change without disruption of a specific epitope (Table 1, Fig. 1).

In contrast, comparison of IGF-II and IGF-II(Ser$^{29}$cl) shows identical reactivity with both the antibody from Amano and the antibody directed against the B-domain of IGF-I, whereas the reactivity of IGF-II(Ser$^{29}$cl) with the Humbel antibody was reduced. These results suggest that the cleavage has not affected interactions with the B- and the AD-domains but hampers the binding of the C-domain. The antigenic site to antibodies from Amano is unchanged by cleavage, suggesting that these antibodies are directed against another epitope than are the Humbel antibodies.

### Binding to receptor preparations

The binding of the labelled peptides to different receptor preparations was studied using membranes derived from human placental cells containing predominantly type I receptors, and from bovine placental cells, with both type I and type II receptors (van Buul-Offers *et al.*, 1988*b*). All peptides bound to these membrane preparations. Binding of IGF-I(Arg$^{36}$cl) was the least in both systems and IGF-II(Ser$^{29}$cl) bound to a slightly lesser extent to bovine placental cell membranes than did IGF-II (Tables 2 and 3).

With human placental cell membranes (Table 2, Fig. 2), $^{125}$I-labelled IGF-I(Arg$^{36}$cl) was more readily displaced than was $^{125}$I-IGF-I by all substances tested. IGF-I(Arg$^{36}$cl) is less efficient than IGF-I as an unlabelled peptide in all competition studies. This suggests a poor interaction of the cleaved IGF-I peptide with the type I receptor. With bovine placental cell membranes, $^{125}$I-IGF-I(Arg$^{36}$cl) was easily displayed by all peptides, as with human placental cell membranes (Table 3, Fig. 3). The interaction of IGF-I(Arg$^{36}$cl) with the type II receptor of the bovine placental cell membranes was unaffected by the cleavage, as seen from the competition from $^{125}$I-IGF-II.

Binding of $^{125}$I-IGF-II(Ser$^{29}$cl) to human placental membranes is slightly decreased compared with binding of $^{125}$I-IGF-II, but it is not more readily displaced by IGF-II than by itself. On the other hand, IGF-I, IGF-

**Table 2. Binding of IGFs to human placental cell membranes and competition studies**

Competition is expressed as the concn. (ng/ml) required to obtain 50% displacement of the tracer. Means ± S.E.M. are given (numbers of experiments in parentheses).

| $^{125}$I-labelled peptide | Specific binding (%) | Competition by unlabelled peptides (ng/ml) | | | | |
|---|---|---|---|---|---|---|
| | | IGF-I | IGF-I-(Arg$^{36}$cl) | IGF-II | IGF-II(Ser$^{29}$cl) | Insulin |
| IGF-I | 19.6±1.0 (11) | 0.9±0.1 (12) | 43.8 (2) | 6.8±1.1 (6) | 28.1±7.6 (3) | 4190 (2) |
| IGF-I(Arg$^{36}$cl) | 7.8 (2) | 0.2 (1) | 2.2 (1) | 0.9 (1) | 4.0 (1) | 275 (2) |
| IGF-II | 20.3±0.9 (14) | 18.0±3.4 (11) | 165±54 (3) | 3.7±0.4 (17) | 15.4 (2) | $> 2 \times 10^4$ (1) |
| IGF-II(Ser$^{29}$cl) | 12.8±1.2 (7) | 5.7±2.3 (3) | 32.1 (1) | 2.2 (2) | 3.9±1.5 (4) | 72.6±28.6 (3) |
| Insulin | 10.5±0.9 (3) | 611±211 (3) | 1340±461 (3) | 128±39 (3) | 108 (1) | 1.2 (1) |

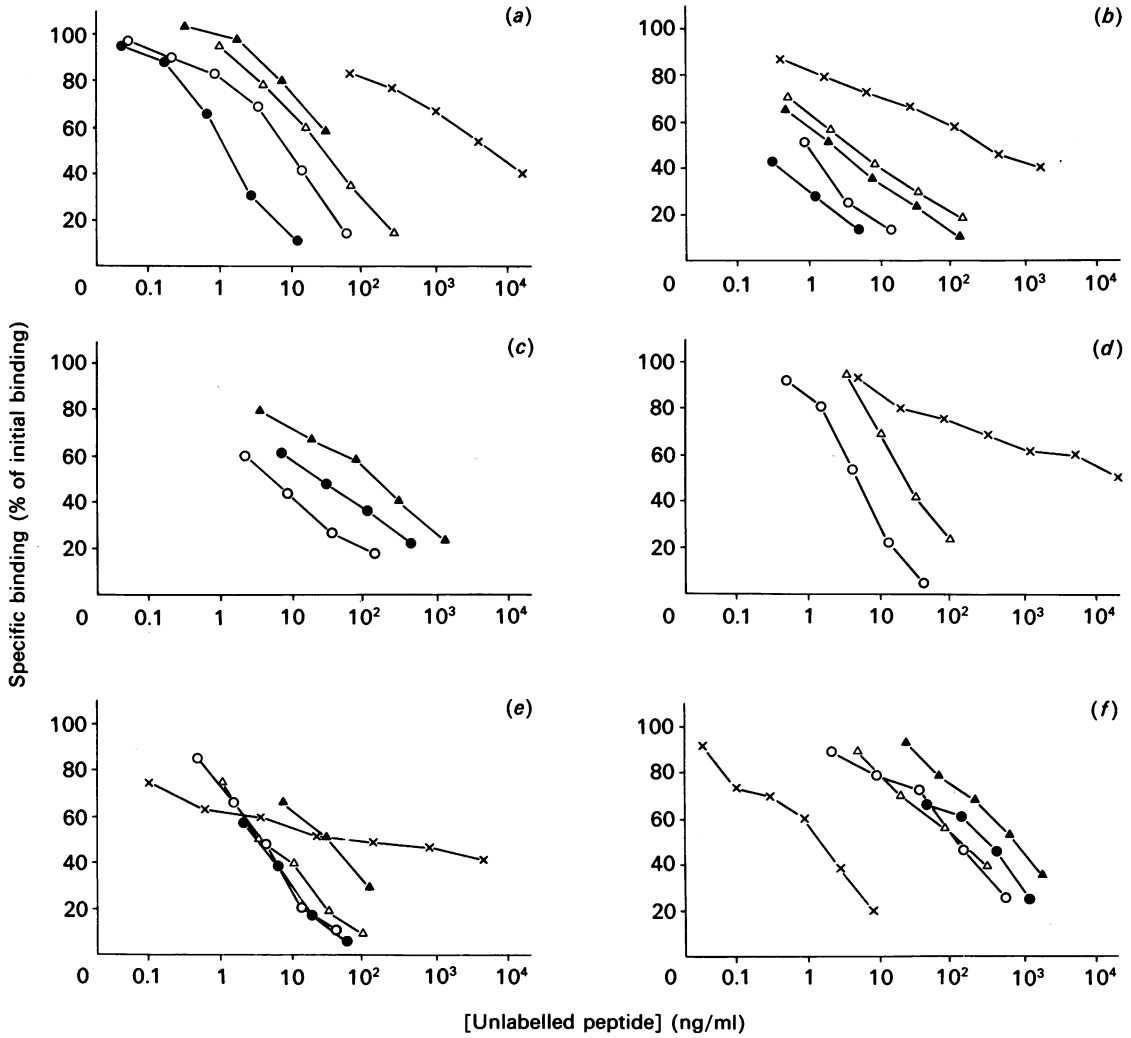


**Fig. 2. Competition between IGFs for binding to human placental cell membranes**

The effects of IGF-I (●), IGF-I(Arg$^{36}$cl) (▲), IGF-II (○), IGF-II(Ser$^{29}$cl) (△) and insulin (×) are shown on the binding $^{125}$I-labelled IGF-I (*a*), IGF-I(Arg$^{36}$cl) (*b*), IGF-II (*c* and *d*), IGF-II(Ser$^{29}$cl) (*e*) and insulin (*f*) to human placental cell membranes. Data are expressed as percentages of the initial binding. Each point is the mean of duplicates. Non-specific binding has been subtracted.

I(Arg$^{36}$cl), IGF-II(Ser$^{29}$cl) itself and insulin remove this tracer molecule more readily than they do $^{125}$I-IGF-II (Table 2, Fig. 2). As an unlabelled peptide IGF-II(Ser$^{29}$cl) tends to be less efficient than IGF-II in the competition for binding with $^{125}$I-labelled IGF-I, IGF-I(Arg$^{36}$cl), IGF-II or IGF-II(Ser$^{29}$cl).

**Table 3. Binding of the IGFs to bovine placental cell membranes and competition studies**

Competition is expressed as the concn. (ng/ml) required to obtaining 50% displacement of the tracer. Means ± S.E.M. are given, with numbers of experiments in parentheses. n.d., not done.

| $^{125}$I-labelled peptide | Specific binding (%) | Competition by unlabelled peptides | | | | |
|---|---|---|---|---|---|---|
| | | IGF-I | IGF-I-(Arg$^{36}$cl) | IGF-II | IGF-II-(Ser$^{29}$cl) | Insulin |
| IGF-I | 18.8±1.0 (4) | 0.6±0.1 (4) | 11.8 (2) | 6.5±0.6 (3) | 22.4 (1) | 3900 (1) |
| IGF-I(Arg$^{36}$cl) | 7.0 (2) | 0.13 (1) | 2.0 (1) | 1.2 (1) | 0.9 (1) | 282.7 (2) |
| IGF-II | 30.6±1.9 (4) | 50.8±2.7 (4) | 90.5 (2) | 1.7±0.2 (5) | 3.3±1.0 (3) | > 2×10$^4$ (1) |
| IGF-II(Ser$^{29}$cl) | 20.5±3.7 (3) | 35.2 (1) | n.d. | 1.1 (2) | 2.3 (2) | > 2×10$^4$ (1) |

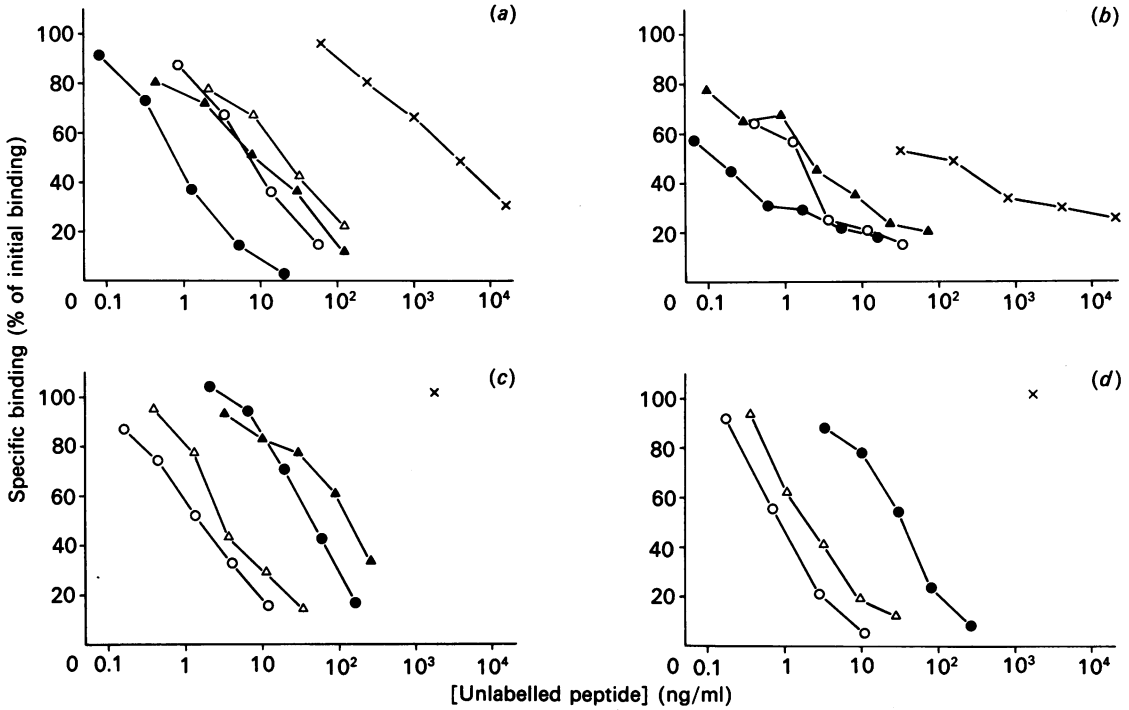


**Fig. 3. Competition between IGFs for binding to bovine placental cell membranes**

The effects of the addition of unlabelled peptides are shown on the binding of $^{125}$I-labelled IGF-I (*a*), IGF-I(Arg$^{36}$cl) (*b*), IGF-II(*c*) and IGF-II(Ser$^{29}$cl) (*d*) to bovine placental cell membranes. For details and symbols, see the legend to Fig. 2 and the Materials and methods section.

Noteworthy is the unexpected potency of insulin in interacting with the binding of $^{125}$I-IGF-II(Ser$^{29}$cl), which is suggestive of an interaction with a receptor population which differs from the type I and type II receptors. No difference was found between the abilities of IGF-II(Ser$^{29}$cl) and IGF-II to displace $^{125}$I-insulin. It should be noted that insulin, when competing with $^{125}$I-labelled IGF-I or IGF-II, exhibits a different slope than that obtained with the IGFs as competing substrates. This is also seen with displacement of $^{125}$I-IGF-II(Ser$^{29}$cl).

Binding of $^{125}$I-IGF-II(Ser$^{29}$cl) to bovine placental cell membranes and the competition by unlabelled peptides is comparable with that of $^{125}$I-IGF-II. Also, unlabelled IGF-II(Ser$^{29}$cl) and IGF-II behave quite similarly in competing with bound labelled peptides (Table 3, Fig. 3).

In contrast with human placental membranes, in bovine placental membranes the amounts of IGF-II(Ser$^{29}$cl) and insulin needed to displace $^{125}$I-IGF-II or $^{125}$I-IGF-II(Ser$^{29}$cl) are similar (Table 3). Binding of $^{125}$I-insulin to these membranes is only 1.4% (160 $\mu$g of protein/ml), compared with 12% binding of $^{125}$I-IGF-I (120 $\mu$g of protein/ml). This suggests the absence of both insulin receptors and so-called 'common' receptors (Hintz *et al.*, 1984) in this tissue. As shown by covalent cross-linking and PAGE under reducing conditions followed by autoradiography, $^{125}$I-IGF-II(Arg$^{36}$cl) and $^{125}$I-IGF-I bound to molecules of identical molecular size in human and bovine placental cell membranes. Using identical methods, $^{125}$I-IGF-II(Ser$^{29}$cl) bound to molecules of the same molecular size as did $^{125}$I-IGF-II on bovine placental membranes. These data are not shown here, since the binding patterns are identical to

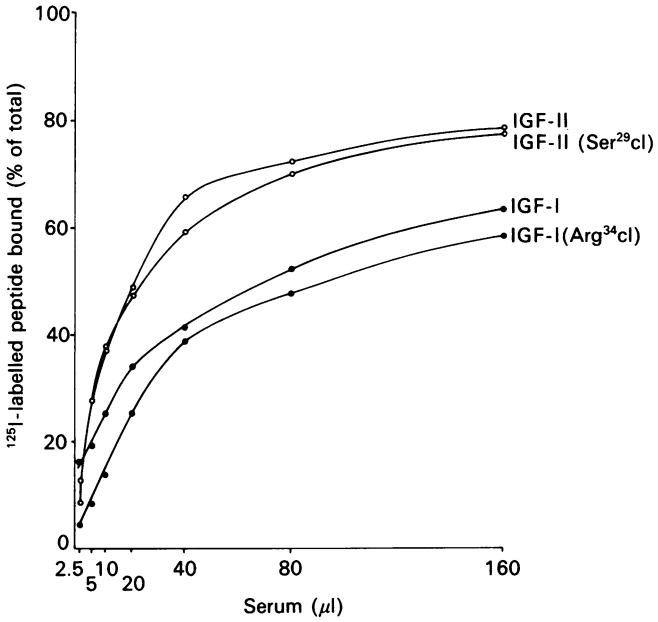


**Fig. 4. Specific binding of the $^{125}$I-labelled peptides to human serum binding proteins**

Binding of IGF-I, IGF-I(Arg$^{36}$cl), IGF-II and IGF-II(Ser$^{29}$cl) is expressed as a percentage of the counts added to human serum binding proteins. Labelled peptides were incubated overnight at 4 °C and processed further as described in the Materials and methods section.

those reported previously (van Buul-Offers *et al.*, 1988*b*).

### Binding to carrier proteins

Total binding of the different labelled peptides to increasing amounts of normal human serum is depicted in Fig. 4. The percentage binding of IGF-II and IGF-II(Ser$^{29}$cl) was higher than that of IGF-I and IGF-I(Arg$^{36}$cl). Cleavage of IGF-I resulted in consistently lower binding at the concentrations tested.

On chromatography (Sephacryl S300) of serum preincubated with the tracers, the distribution patterns of the labelled peptides yield three radioactive peaks: peak I at $K_{av.}$ 0.22 with an apparent molecular mass of 150 kDa, peak II at $K_{av.}$ 0.45 (40–50 kDa) and peak III at $K_{av.}$ 0.7 (7 kDa) (Fig. 5). When preincubation was carried out in the presence of an excess of unlabelled peptide, peaks I and II disappeared (results not shown).

The binding profile of $^{125}$I-IGF-I(Arg$^{36}$cl) is different from that of $^{125}$I-IGF-I: binding to peak I is diminished with a concomitant increase of peak II and free peptide. Binding patterns of $^{125}$I-IGF-II and $^{125}$I-IGF-II(Ser$^{29}$cl) are identical, but quite different from the pattern obtained with $^{125}$I-IGF-I. Binding of IGF-II-like peptides to the 150 kDa binding proteins was much less than to the 40 kDa binding proteins. When all tracers were incubated with serum from a growth-hormone-deficient patient, binding to the 150 kDa protein was, as expected, markedly reduced and reappeared after growth hormone substitution, whereas binding at 40 kDa was unchanged (results not shown). All tracers bind to growth-hormone-dependent binding proteins.

In order to avoid interference between the two classes of binding proteins and the tracers, binding proteins (150 and 40 kDa) of normal serum were incubated separately with the different labelled peptides. Binding of IGF-I and IGF-I(Arg$^{36}$cl) to the 150 kDa binding protein was 2.6 and 2.3% respectively, and to the 40 kDa protein it was 4.2 and 3.6% respectively. For IGF-II and IGF-II(Ser$^{29}$cl), these values were 8.5 and 6.9% for the 150 kDa protein and 14.9 and 16.7% for the 40 kDa protein. Thus the different behaviour of IGF-I(Arg$^{36}$cl) compared with IGF-I in binding to whole serum is absent in the binding profile to the separate binding proteins.

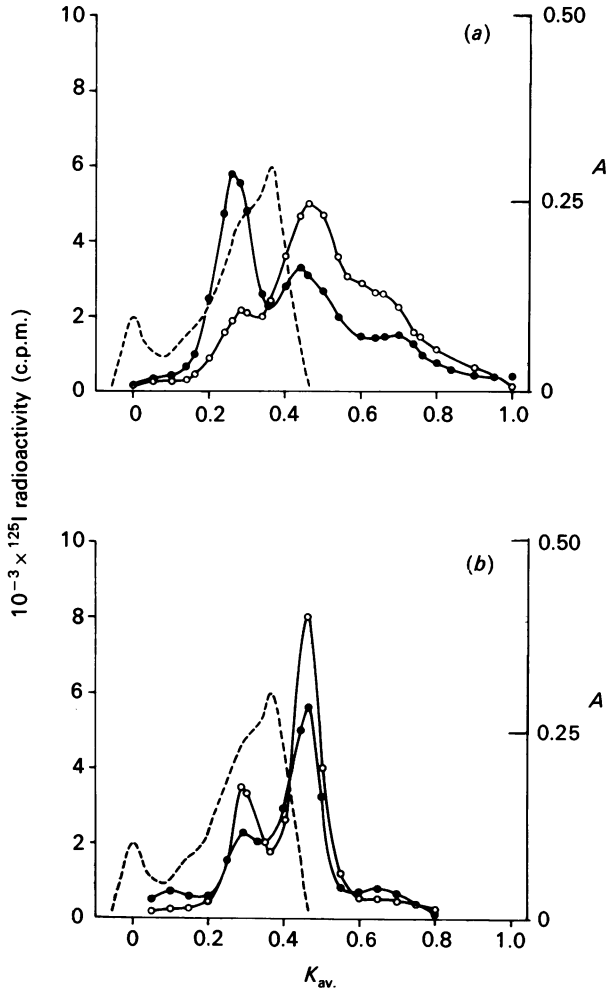


**Fig. 5. Distribution pattern of $^{125}$I-labelled peptides after preincubation with normal human serum and chromatography on a neutral S-300 column**

(*a*) ●, IGF-I; ○, IGF-I(Arg$^{36}$cl); (*b*) ●, IGF-II; ○, IGF-II(Ser$^{29}$cl). The protein concn. is also indicated, measured by absorbance (---).

## DISCUSSION

It is apparent that a single cleavage in either IGF-I or IGF-II results in a different behaviour in their binding to antibodies, receptors and carrier proteins.

With respect to IGF-I with a single cleavage in the C-domain [IGF-I(Arg$^{36}$cl)], binding to antibodies raised either against whole IGF-I [but mainly directed against the B-domain of IGF-I (De Vroede *et al.*, 1986)] and against the AD-domain of IGF-I is somewhat reduced

without loss of interaction with a specific epitope. This contrasts with the binding of IGF-I(Arg$^{36}$cl) to the type I receptor on human and bovine placental cell membranes, which is markedly decreased. Binding to the insulin receptor on human placental cell membranes is slightly diminished, whereas the interaction with specific type II receptors on bovine placental cell membranes seems to be unaffected.

The difference in the binding of $^{125}$I-IGF-I(Arg$^{36}$cl) compared with $^{125}$I-IGF-I to binding proteins of whole serum was absent when the binding to separate 150 and 40 kDa binding proteins was studied. Whereas the differences with whole serum suggest a complex interaction between the binding protein, the cleavage apparently has no influence on the region involved in binding to the individual proteins.

Site-directed mutagenesis of a synthetic gene encoding human IGF-I was used by Bayne *et al.* (1988) to create substitutions in the B-domain. Their data, obtained with four structural analogues of human IGF-I, suggested that the structural determinants of the ligands which are recognized by binding proteins and by type I, type II and insulin receptors are not the same. Our data lead to similar conclusions, since a cleavage in the C-domain of IGF-I causes a loss of affinity for the type I IGF receptor and a moderate change in binding to insulin receptors, whereas binding to the type II receptor was unchanged.

According to the model of Blundell *et al.* (1983), local changes in charge as a consequence of the cleavage may have influenced C7 ion-pairing with A4, causing steric hindrance of the type I receptor site, which is mainly localized in the A-domain and to a certain extent in the B-domain. Such changes may also, but to a lesser degree, influence the binding site for binding proteins, which is localized in the B-domain (De Vroede *et al.*, 1985, 1986; Tseng *et al.*, 1987). Our data are also in accordance with those of Cascieri *et al.* (1988), which showed that substitution of B24 caused a 20–30-fold loss of affinity for type I receptors but no change in that for type II receptors and binding proteins.

Important residues for the binding site for binding proteins are B15 and B16, as are B1–B16 for the type II receptor (Bayne *et al.*, 1988). These are all localized within the area containing disulphide bridges and therefore are perhaps less influenced by conformational changes, in contrast with the residue B24 which is of importance for binding to type I receptors (Cascieri *et al.*, 1988). The C and D regions play a minor role in binding to type I and type II receptor sites and to binding proteins (Zapf *et al.*, 1984; Bayne *et al.*, 1987).

The affinity of IGF-II(Ser$^{29}$cl) for monoclonal antibodies from Humbel, specific for the C-peptide region, is affected by the cleavage (Honegger & Humbel, 1986). This shows that the cleavage in the B-domain has influence on this antigenic site of the C-domain.

The binding properties of IGF-II(Ser$^{29}$cl) to receptors changed less profoundly on cleavage. It had a slightly decreased affinity compared with IGF-II for type I receptors from bovine placental membrane receptors, whereas the interaction with type II receptors was unchanged using these membranes.

In human placental membranes using $^{125}$I-IGF-I as tracer, the potency of IGF-II(Ser$^{29}$cl) for displacement was slightly lower than that of IGF-II. With $^{125}$I-IGF-II(Ser$^{29}$cl), less IGF-I, IGF-I(Arg$^{36}$cl), IGF-II(Ser$^{29}$cl) and especially less insulin was needed in order to obtain 50% displacement of the tracer compared with $^{125}$I-IGF-II. It has been shown by us (van Buul-Offers *et al.*, 1988*b*) that IGF-II binds in these membranes to molecules comparable in molecular size with the type I and insulin receptors. In addition, Casella *et al.* (1986) have described a population of binding sites which bind IGF-I, IGF-II and insulin, the so-called 'common receptor' (Hintz *et al.*, 1984). The presence of such a receptor probably accounts for the discrepancy noticed in the competition between insulin and bound $^{125}$I-IGF-II(Ser$^{29}$cl). The cleaved form of IGF-II probably has much more affinity for this receptor population than does native IGF-II. Insulin binds very poorly to bovine placental membranes and competes with neither $^{125}$I-IGF-II nor $^{125}$I-IGF-II(Ser$^{29}$cl), suggesting the absence of such a population of common receptors as well as the absence of insulin receptors in these membranes. This contrasts sharply with human placental cell membranes.

It can be concluded that the cleavage of IGF-II introduced only minor changes in binding to type I and type II receptors, contrasting with the higher affinity for the so-called 'common receptor' (Hintz *et al.*, 1984). Furthermore, binding to binding proteins was not affected.

Heterogeneity of the somatomedins can originate from post-transcriptional and/or post-translational events, from metabolic degradation in the circulation or from a methodological artefact. The question of whether the cleaved polypeptides are metabolites of physiological importance remains still to be answered. It has been shown that enzymic reduction of the disulphide bonds and proteolytic degradation are important for processing of native IGFs (Enberg & Holmgren, 1985).

In endothelial cells, insulin, IGF-I and IGF-II, after binding to their separate receptors and internalization, are processed by distinct pathways (Bar *et al.*, 1986; Banskota *et al.*, 1986). For insulin and IGF-I, the non-degradative pathway is predominant, whereas for IGF-II the degradative pathway predominates. The role of these pathways in different tissues needs further clarification in order to answer the question of whether partial degradation of IGF molecules plays a role in regulatory mechanisms.

We thank Dr. K. Beyreuther (University of Heidelberg, Heidelberg, Germany) for performing the *N*-terminal amino acid analysis and Dr. P. Roepstorff (Odense Universitet, Department of Molecular Biology, Odense, Denmark) for determination of molecular masses by plasma desorption mass spectrometry. We are most grateful to Mrs. R. Reijnen, Mr. T. de Poorter and Mr. M. Jarmuszewski for their excellent technical assistance and to Dr. M. Jansen and Dr. F. A. Opmeer for critical reading of the manuscript. Thanks are due to Mrs. C. Nierop, Mr. T. Schipper and Mr. H. Kempkes for their help in the preparation of this manuscript. This study was supported by the Dutch Foundation of Medical Research (Medigon) grant no. 900-528-033.

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Received 24 October 1989; accepted 7 November 1989