Purification, amino acid sequence and characterisation of kangaroo IGF-I

C A Yandell1,2, G L Francis1, J F Wheldrake2 and Z Upton1

1Cooperative Research Centre for Tissue Growth and Repair, PO Box 10065, Gouger St, Adelaide, South Australia 5000, Australia and 2Flinders University of South Australia, School of Biological Sciences, Sturt Rd, Bedford Park, South Australia 5042, Australia

(Requests for offprints should be addressed to C A Yandell)

Abstract

Insulin-like growth factor-I (IGF-I) and IGF-II have been purified to homogeneity from kangaroo (Macropus fuliginosus) serum, thus this represents the first report of the purification, sequencing and characterisation of marsupial IGFs. N-Terminal protein sequencing reveals that there are six amino acid differences between kangaroo and human IGF-I. Kangaroo IGF-II has been partially sequenced and no differences were found between human and kangaroo IGF-II in the 53 residues identified. Thus the IGFs appear to be remarkably structurally conserved during mammalian radiation. In addition, in vitro characterisation of kangaroo IGF-I demonstrated that the functional properties of human, kangaroo and chicken IGF-I are very similar. In an assay measuring the ability of the proteins to stimulate protein synthesis in rat L6 myoblasts, all IGF-I proteins were found to be equally potent. The ability of all three proteins to compete for binding with radiolabelled human IGF-I to type-1 IGF receptors in L6 myoblasts and in Sminthopsis crassicaudata transformed lung fibroblasts, a marsupial cell line, was comparable. Furthermore, kangaroo and human IGF-I react equally in a human IGF-I RIA using a human reference standard, radiolabelled human IGF-I and a polyclonal antibody raised against recombinant human IGF-I. This study indicates that not only is the primary structure of eutherian and metatherian IGF-I conserved, but also the proteins appear to be functionally similar.

Journal of Endocrinology (1998) 156, 195–204

Introduction

Insulin-like growth factor-I (IGF-I) and IGF-II are structurally related single-chain proteins which share considerable identity with proinsulin (Froesch et al. 1985). IGFs regulate the proliferation and differentiation of a multitude of cell types (Lowe 1991) and are thought to exert their effects in both an endocrine and autocrine/paracrine manner (Daughaday & Rotwein 1989). In mammals, IGF-I has been shown to mediate many of the growth-promoting actions of growth hormone (Humbel 1984, Froesch et al. 1985). IGF-II function on the other hand is less clearly understood but studies in rats suggest that it plays a role in prenatal and neonatal development (Brown et al. 1986, Stylianopoulou et al. 1988, Filson et al. 1993). The physiological actions of both IGFs are mediated by interactions with cell surface receptors. In mammals there are two receptors that specifically bind IGFs. The type-1 IGF receptor, structurally related to the insulin receptor, binds both IGF-I and IGF-II with high affinity. The type-2 IGF receptor, which has been found to be identical with the cation-independent mannos-6-phosphate receptor (CI-MPR), binds IGF-II with much greater affinity than IGF-I. While the CI-MPR is present in chickens and frogs, it does not contain the high-affinity IGF-II-binding site (Canfield & Kornfeld 1989, Clairmont & Czech 1989, Duclos & Goddard 1990). Interestingly, studies of the CI-MPR in the American opossum (Dahms et al. 1993) reveal that the receptor appears to contain an IGF-II-binding site. However, the opossum receptor appears to have a much lower affinity for bovine IGF-II than the bovine receptor.

While IGFs have been extensively studied in mammalian species, IGF structure and function in non-mammals is less well understood. IGF proteins or cDNA transcripts have been described in the chicken (Dawe et al. 1988, Ballard et al. 1990, Kallincos et al. 1990), several species of fish (Cao et al. 1989, Duguay et al. 1995, 1996, Moriyama et al. 1995, Liang et al. 1996, Kinhult 1996) and the frog, Xenopus laevis (Kajimoto & Rotwein 1990). N-Terminal protein sequencing and the cDNA-deduced amino acid sequences indicate that there is a high degree of conservation of IGF structure throughout vertebrate evolution. However, the role of IGFs in these species has not been extensively characterised and it remains to be determined if IGFs have been functionally as well as structurally conserved throughout vertebrate evolution. While in vitro characterisation of salmon (Moriyama et al. 1993), trout (Moriyama et al. 1995) and chicken (Dawe et al. 1988, Armstrong et al. 1990, Kallincos et al. 1990, Upton et al.
1992) IGF-I has been described, very few in vivo studies using homologous proteins have been performed.

Marsupials (metatherians) diverged most recently from placental mammals (eutherians) and are thought to have potential as developmental models (Tyndale-Biscoe & Janssens 1988). As most of marsupial development occurs in the pouch, marsupials are a more accessible model for the study of early development of the mammalian foetus than the eutherian models currently in use. However, they have not been exploited as developmental models because of the lack of data on marsupial endocrinology and physiology. The present study was designed to isolate and subsequently characterise IGFs from serum of the Western grey kangaroo (Macropus fuliginosus). We now report the purification of IGF-I and IGF-II to homogeneity from kangaroo serum. The complete amino acid sequence, in vitro binding interactions and biological activities of kangaroo (k)IGF-I are also described.

**Materials and Methods**

**Materials**

Kangaroo blood was collected from wild Western grey kangaroos (Macropus fuliginosus) at Burra, South Australia, Australia. Bulk C18 silica resin and Cellufine resin were purchased from Amicon, Danvers, MA, USA. SP Sephadex C-25 and Sephadex G-50 were from Pharmacia, North Ryde, New South Wales, Australia. The Prep-pak C4 column and the Nova-pak C18 column were from Waters, Lane Cove, New South Wales, Australia while the C4 Brownlee Aquapore column was purchased from Applied Biosystems, Santa Clara, CA, USA. Endoproteinase Glu-C was purchased from Boehringer-Mannheim, North Ryde, New South Wales, Australia. Recombinant human (h) and chicken (c) IGF-I and antiserum to human IGF-I were supplied by GroPep Pty Ltd, Adelaide, South Australia, Australia. Sheep anti-rabbit IgG was purchased from Silenus, Hawthorn, Victoria, Australia and rabbit IgG was from Sigma/Aldrich, Castle Hill, New South Wales, Australia. Radioiodinated recombinant hIGF-I and hIGF-II were prepared to specific activities of between 30 and 60 μCi/μg with chloramine-T, and separated from reaction components by chromatography on a Sephadex G-50 column as described previously (Francis et al. 1989a). Tissue culture components have been previously described by Francis et al. (1986). Smilthopsis crassiodonta transformed lung fibroblasts were purchased from Commonwealth Serum Laboratory (CSL), Parkville, Victoria, Australia.

**Purification**

IGFs were purified from two batches of serum (2·5 and 4·4 litres) collected from wild western grey kangaroos using procedures similar to those previously described for the purification of IGFs from bovine colostrum (Francis et al. 1986). The serum was mixed with 0·33 volumes of 1,1,2-trichloro-1,2,2-trifluoroethane, centrifuged (3500 g, 15 min) and the supernatant adjusted to pH 2·7 with glacial acetic acid and 11·6 M HCl. An equal volume of acetic acid (1 M) containing NaCl (0·25 M) was added and the mixture was stirred for 2 h at 4 °C. The acidified serum was passed through a 1 μm glass fibre filter before the addition of 100 g SP Sephadex C-25 gel in acetic acid (1 M) at pH 2·7. This mixture was stirred for a further 18 h at 4 °C before the gel was allowed to settle and the supernatant decanted. The gel slurry was poured into a column (50 mm × 300 mm), allowed to settle and the supernatant applied at a flow rate of 20 ml/min. The column was washed with acetic acid (1 M), followed by ammonium acetate (50 mM) at pH 5·5 before elution of the IGFs with NaCl (1 M) containing NH4OH (0·25 M) at pH 11·3 at a flow rate of 10 ml/min.

Acetonitrile, trifluoroacetic acid (TFA) and concentrated HCl were added to the SP Sephadex eluate to achieve a final concentration of 10% (v/v), 0·1% (v/v) and a pH of 2·1 respectively. The mixture was stirred for 18 h and passed through a 1 μm glass fibre filter. This was applied to a C18 silica column (26 mm × 200 mm) equilibrated with 10% acetonitrile/0·1% TFA (v/v) at a flow rate of 20 ml/min. The IGFs were eluted with 50% acetonitrile/0·1% TFA (v/v) and dried under vacuum before being redissolved in acetic acid (1 M).

The reconstituted material from the C18 column was applied at 2 ml/min to a gel-filtration Cellufine column (50 mm × 1000 mm) which was equilibrated with acetic acid (1 M). The IGFs were eluted with acetic acid (1 M) at 2 ml/min, and 15 min fractions were collected. Activity in these fractions and fractions from subsequent purification steps was monitored by protein synthesis assay in rat L6 myoblasts, IGF-I RIA and type-2 IGF radioreceptor assay (RRA). Fractions from the Cellufine column containing IGF bioactivity were pooled and adjusted to 10% acetonitrile/0·1% TFA before being applied to the next column.

A C4 Prep-pak column (25 mm × 100 mm) was equilibrated with 0·1% TFA and the pooled fractions from the Cellufine column applied at 5 ml/min. The IGFs were eluted at 5 ml/min with a two-step linear gradient of 0–24% acetonitrile/0·1% TFA in 20 min and 20–50% acetonitrile/0·1% TFA in 300 min, and 2 min fractions were collected. Analysis of these fractions in the protein synthesis assay revealed the presence of two bioactive peaks. The first of these peaks (pool A) contained IGF-I immunoreactivity while the second (pool B) competed for binding of tracer in the type-2 IGF RRA. The fractions from the two peaks were pooled separately and diluted 1:1 in 0·13% (v/v) heptfluorobutyric acid (HFBA). These two pools were processed separately in the subsequent steps.

The material from pool A was applied at 5 ml/min on the same C4 column as above or applied at 1 ml/min on a
C18 Nova-pak column (10 mm × 100 mm) during the purification from 4-4 or 2-5 litres serum respectively. The columns were equilibrated with 10% n-propanol/0-13% HFBA (v/v) and the IGFs eluted at the flow rates described above with a two-step linear gradient of 10–20% n-propanol/0-13% HFBA in 5 min and 20–50% n-propanol/0-13% HFBA in 300 min, and 2 min fractions were collected. The activity was eluted as two peaks as measured by protein synthesis assay and IGF-I RIA. The two peaks were pooled separately and further processed (pool A1 and A2). The material from pool A2 was further purified by separation on the C4 Brownlee Aquapore column as described above for the IGF-I purification. The material from pool A2 was processed only in the purification from 4-4 litres serum.

After dilution 1:2 with 0-1% TFA, the pools were injected separately on to a C18 Nova-pak column, equilibrated with 0-1% TFA. The IGFs were eluted at a flow rate of 1 ml/min with a two-step linear gradient of 0–24% acetonitrile/0-1% TFA in 24 min and 24–35% acetonitrile/0-1% TFA in 330 min with 1 min fractions collected. The IGF-I activity from the major pool (pool A1) was eluted as one peak, and the pooled fractions were dried under vacuum and stored at −20 °C. The minor IGF-I pool (pool A2) was shown to contain several bioactive peaks containing both protein synthesis activity and IGF-I immunoreactivity. The fractions from the major peak only were pooled (pool A2-1) and further purified.

The material from pool A2-1 was diluted 1:1 with 0-1% TFA and injected on to a C4 Brownlee Aquapore column (Applied Biosystems; 2-1 mm × 100 mm) equilibrated with 0-1% TFA at 0-5 ml/min. The IGFs were eluted with a linear gradient of 25–35% acetonitrile/0-1% TFA in 600 min at a flow rate of 0-5 ml/min, and 1 min fractions were collected. The fractions from the one peak containing protein synthesis activity and IGF-I immunoreactivity were pooled, dried under vacuum and stored at −20 °C.

IGF-II (pool B) was purified as described for IGF-I (pool A1); however, an additional step was required as the IGF-II peak from the final C18 Nova-pak column was not homogeneous. Thus the protein pool containing IGF-II was further purified by separation on the C4 Brownlee Aquapore column as described above for the IGF-I material in pool A2-1.

Quantification and sequencing

kIGF-I and kIGF-II were assessed for purity by N-terminal sequencing. Quantification of kIGF-I and kIGF-II was performed as described by Upton et al. (1995) using the calculated absorption coefficient 33-38 mol⁻¹ cm⁻¹ for kIGF-I. As the complete sequence of kIGF-II was not determined the absorption coefficient calculated for hIGF-II (31-701 mol⁻¹ cm⁻¹) was used. Proteins were sequenced from the N-terminus using a gas-phase sequencer by the method of Hunkapiller et al. (1983). Additional portions of each growth factor were reduced, S-carboxymethylated, cleaved with endoproteinase Glu-C and the resultant peptides isolated for sequencing.

S-Carboxymethylation and endoproteinase Glu-C digestion

S-Carboxymethylation and endoproteinase Glu-C digestion were carried out as previously described in Francis et al. (1988).

IGF assays

IGF activity was followed in each purification step by testing the fractions in a rat L6 myoblast protein synthesis assay, IGF-I RIA and type-2 IGF RRA. Kangaroo, human and chicken IGF-I were also characterised in a number of in vitro assays. Before analysis the samples were dried under vacuum, dissolved in HCl (10 mM) and resuspended in the appropriate assay buffer.

The IGF-I RIA was carried out as described in Read et al. (1986). However, the radioligand used was recombinant hIGF-I, and Tween 20 (0-05%, v/v) replaced BSA in the binding buffer. The polyclonal antibody was supplied by GroPep and used as described by the supplier. The amount of labelled hIGF-I bound was measured and the data were expressed as a percentage of the control samples with no added growth factors.

Protein synthesis was determined using confluent cultures of rat L6 myoblasts in serum-free medium as described in Ballard et al. (1986). Protein synthesis was measured as the incorporation of [3H]leucine into total cell protein over a 16 h incubation period. Responses to the IGFs are expressed as the percentage stimulation above the response measured in cells with medium alone.

Binding of IGFs to receptors (RRA) in rat L6 myoblasts and S. crassicaudata transformed lung fibroblasts was measured as described by Ross et al. (1989). Briefly, radioligand and unlabelled growth factors were added to cell monolayers and incubated for 16 h at 4 °C. The monolayers were washed and the bound radioactivity measured. The radioligands used for determining competition for binding to the type-1 or type-2 IGF receptors were 125I-hIGF-I and 125I-hIGF-II respectively.

All assays for the characterisation of IGF-I were performed in triplicate, with each experiment repeated at least three times as indicated in the figure legends. Statistical analysis was performed using a repeated-measures ANOVA. A P<0-05 was considered an indication of a significant difference between means.

Results

Purification

IGF-I and IGF-II were isolated from kangaroo serum on two occasions, from starting quantities of 2-5 and 4-4 litres serum. IGF-I and IGF-II were co-purified until the first reverse-phase HPLC step (Fig. 1a). Analysis of the fractions eluted from the C4 Prep-pak column by protein synthesis assay and type-2 IGF RRA suggested that IGF-I...
Figure 1 (a) Elution of IGF-I and IGF-II from a reverse-phase C₄ Prep-pak column with an acetonitrile gradient. The dashed line represents the percentage of acetonitrile (CH₃CN) and the solid line the absorbance at 214 nm ($A_{214}$). Activity in the fractions (2 ml) was detected by L6 myoblast protein synthesis assay (solid bars) and type-2 IGF RRA (●). Pooled fractions are indicated by the solid lines above the peaks. Pool A was further processed to purify IGF-I and pool B was processed to purify IGF-II. (b) Elution of IGF-I (pool A1) from a reverse-phase C₁₈ Nova-pak column using an acetonitrile gradient. Activity in the fractions (1 ml) was detected by L6 myoblast protein synthesis assay (solid bars).
and IGF-II had been separated by this chromatography step. The two protein pools A and B containing IGF-I and IGF-II respectively were then processed separately.

Subsequent processing of pool A revealed two biologically active peaks, both containing IGF-I immunoreactivity. The major peak, pool A1, was further processed to homogeneity (Fig. 1b) and determined to be IGF-I. Yields of 26 and 158 µg IGF-I were obtained from 2·5 and 4·4 litres of serum respectively. The minor pool, pool A2, contained several bioactive peaks when further separated by reverse-phase HPLC (not shown), of which one peak (pool A2.1) was also purified to homogeneity. A yield of 60 µg of the latter minor form was obtained from 4·4 litres serum.

The putative IGF-II activity (pool B) was processed to yield 40 µg homogeneous IGF-II from 4·4 litres serum. Processing of the 2·5 litres serum, on the other hand, yielded only 21 µg IGF-II which was 75% pure as determined by N-terminal protein sequencing. This was not processed further because of the small amount of protein obtained.

**Sequencing**

N-Terminal sequencing of kIGF-I was performed and the sequence identified up to residue 40 (Fig. 2a). Endoproteinase Glu-C cleavage of the reduced and S-carboxymethylated protein resulted in four peptides, which were isolated and then sequenced. These peptides provided the identity of residues 21–46, 47–58, 59–70 and confirmed the identities of residues 1–9 and 10–20. The sequence revealed six differences between kIGF-I and hIGF-I (Fig. 2b). These differences are the substitution of Glu for Asp at position 20, Ser for Asn at position 26, Leu for Ala, His for Pro and His for Glu at positions 38, 39 and 40 respectively and Ile for Leu at position 64 when compared with the hIGF-I sequence (Rinderknecht & Humbel 1978a). A partial sequence was also obtained for...
the minor IGF-I protein (pool A2·1) with the first 40 amino acids identified. There were no differences between this and the major kIGF-I species characterised. In addition, a partial amino acid sequence for kIGF-II was determined with residues 1–46 and 57–67 identified (Fig. 2c). There were no differences between kIGF-II and hIGF-II (Rinderknecht & Humbel 1978b) in the 53 residues identified by N-terminal sequencing.

Characterisation

In order to assess the biological and receptor-binding characteristics of kIGF-I, it was compared with recombinant hIGF-I and cIGF-I in a variety of in vitro assays. kIGF-II was not functionally characterised because of the small amount remaining after the structural studies had been performed. In an assay measuring the ability of the IGFs to stimulate protein synthesis in rat L6 myoblasts, all IGF-I proteins were found to have a similar potency (P>0·05) (Fig. 3). Half-maximal effects were observed with 9·5 ± 1·6, 9·3 ± 1·5 and 10·9 ± 2·3 ng/ml for kIGF-I, hIGF-I and cIGF-I respectively.

Affinity of binding to the type-1 IGF receptors in cultured rat L6 myoblast and S. crassicaudata fibroblast monolayers was assessed by measuring competition for binding of 125I-labelled hIGF-I to receptors on these cells (Fig. 4). All IGF-IIs bound similarly to L6 cells (P>0·05) with half-maximal competition for binding observed at 0·81 ± 0·08, 1·5 ± 0·18 and 2·1 ± 0·15 ng/0·5 ml for kIGF-I, cIGF-I and hIGF-I respectively (Fig. 4a). Like-wise, all IGF-I proteins competed similarly in S. crassicaudata transformed lung fibroblasts at the concentrations tested (Fig. 4b). Owing to the large amount of protein required, half-maximal competition for binding to IGF receptors in this cell line was not determined.
The cross-reactivity of kIGF-I in an RIA using antiserum raised against hIGF-I was also examined (Fig. 5). hIGF-I and kIGF-I reacted similarly ($P > 0.05$) with half-maximal binding observed at $439.9 \pm 27.6$ and $330 \pm 19.7$ pg/0.35 ml respectively.

**Discussion**

We have described the purification, complete amino acid sequence and *in vitro* characterisation of kIGF-I. We have also purified and partially sequenced kIGF-II. This represents the first report of the sequencing and characterisation of marsupial IGFs. N-Terminal sequencing of the IGFs yielded a single sequence for both IGFs, indicating that they were of high purity.

kIGF-I and kIGF-II were separated by reverse-phase HPLC using a C$_4$ column and an acetonitrile gradient. In previously reported purifications of IGFs from other sera, IGF-I and IGF-II were not separated by a similar reverse-phase HPLC step (Francis *et al.* 1986, 1989a,b, Dawe *et al.* 1988, Ballard *et al.* 1990, Moore *et al.* 1993). Instead, an additional cation-exchange chromatography step has been required to separate the two IGFs. Although the overall charge of kIGF-I remains the same as hIGF-I, the residue changes that have occurred may have altered the hydrophobicity of the molecule, making it sufficiently different from kIGF-II for them to be separated on this column. Differences in the kIGF-I sequence could explain why the two IGFs in kangaroo serum can be separated at this early stage in the purification. However, determination of the complete IGF-II sequence may also help to explain the differences in chromatographic properties between kIGFs and those of other species. Fortuitously, this separation procedure also minimises cross-contamination of IGF-I preparations with IGF-II, which has been reported to be a major problem with other methods for purifying IGFs from biological sources (Blum *et al.* 1986, Francis *et al.* 1988, Bayne *et al.* 1991). Yields of 10.4 and 36.0 µg/l IGF-I and 8.4 and 9.0 µg/l IGF-II were obtained from 2.5 and 4.4 litres serum respectively. Both preparations of IGF-I were found to be homogeneous; however, IGF-II was purified to homogeneity on one occasion only. These yields compare favourably with those reported for many other purifications from serum (Francis *et al.* 1986, 1989a,b, Dawe *et al.* 1988, Ballard *et al.* 1990, Moore *et al.* 1993).

The sequence of kIGF-I differs from that of hIGF-I by six amino acids, with five of these changes at positions 26, 38, 39, 40 and 64 being identical with the differences observed between cIGF-I and hIGF-I (Ballard *et al.* 1990). The difference of Glu at position 20 also occurs in IGF-I from several species of fish including salmon (Cao *et al.* 1989), barramundi (Kinhult 1996) and sea-bream (Duguay *et al.* 1996). This change is not seen in IGF-I from all other species of fish characterised, with IGF-I from carp (Liang *et al.* 1996) and shark (Duguay *et al.* 1995) containing Asp, a similarly charged amino acid, at position 20. Interestingly, this substitution has not been observed in IGF-I from any avian species sequenced thus far (Ballard *et al.* 1990, Kida *et al.* 1994). The change from Glu to Asp results from a single nucleotide difference at the third position of the codon and represents a conservative charge change. kIGF-I also exhibits similar structural changes to the non-mammalian species at position 38–40. These changes must have occurred before the divergence of marsupials from eutherians approximately 100 million years ago. However, kIGF-I does not have the characteristic amino acid changes observed at residues 41 and 50 in non-mammalian species, hence in this respect at least it is more similar to eutherian IGF-I.

We have previously reported the isolation of a potent, N-terminally truncated form of IGF-I, des-(1–3)-IGF-I, from bovine colostrum (Francis *et al.* 1988), while others have also isolated this protein from human brain (Sara *et al.* 1986, Carlsson-Skwirut *et al.* 1987). Hence, we were interested to know whether such a species exists in kangaroo serum. N-Terminal protein sequencing of the putative IGF-I variant (pool A2-1) isolated from kangaroo serum indicates that it is not the N-terminally truncated form. Thus far, protein sequencing has shown no differences between this protein and the major IGF-I protein isolated. There are also a number of other possible variants.
IGF-I proteins in kangaroo serum which were detected in the third reverse-phase HPLC step (pool A2). However, these were not purified any further because of the small quantity of protein present in this pool. A number of variant forms of IGFs have been reported in other studies, including larger forms of IGF-II identified in human plasma (Zumstein et al. 1985, Gowen et al. 1987). Minor forms of IGF-I which may be due to alternative splicing of mRNA and have different isoelectric points from the major form have also been shown to exist in human sera (Blum et al. 1986, 1987). The site of post-translational modification and the possible physiological role for these variants is unclear at this stage.

kIGF-I was compared with recombinant hIGF-I and cIGF-I in a variety of in vitro assays. The functional significance of the residues that differ between these proteins is not clear as the properties of kIGF-I are very similar to those of the human and chicken IGFs in the assays undertaken in this study. The ability of all three IGF-IIs to stimulate protein synthesis in rat L6 myoblasts and to bind to the type-I IGF receptor in both the L6 myoblasts and S. crassicaudata transformed lung fibroblasts was similar. However, it was apparent from these studies that much greater concentrations of IGF-IIs were required to displace the hIGF-I radiolabel from the receptors on the S. crassicaudata cells, suggesting that there may be differences in the eutherian and metatherian type-I IGF receptors. Nevertheless, further investigations are clearly necessary to identify the functional significance of the amino acid changes found in kIGF-I. The fact that similar changes are also found in these regions of all non-mammalian IGF-I sequences suggests that these residues are functionally important.

The RIA for human IGF-I detected hIGF-I and kIGF-I equally efficiently despite sequence differences, inferring that the antigenic site/s recognised by the human polyclonal antibody are to the conserved regions of IGF-I. Furthermore, these results suggest that measurements of IGF-I activity in marsupials using a heterologous reference standard and the antibody employed in these studies may give accurate estimates of circulating IGF-I levels in marsupials. Indeed, IGF-I levels have been measured in the milk of the tammar wallaby using a polyclonal antibody raised against bovine IGF-I and recombinant hIGF-I as the reference standard (Ballard et al. 1995). The results here validate the significance of those measurements. It was shown that IGF-I levels in the milk of the tammar wallaby rise during lactation and reach a peak at the time that the joey (young) starts to leave the pouch. IGF-I levels then decrease again until weaning. This pattern of secretion of IGF-I into the milk differs from that reported for eutherian mammals where the IGF-I levels are high in colostrum and then drop for the remainder of the lactation period (Baxter et al. 1984). It was suggested that IGF-I in tammar milk may be important for mammary gland maturation.

The partial sequence of kIGF-II reveals no differences between hIGF-II and kIGF-II in the 53 residues identified. However, the most variable region of IGF-II, positions 31–40 (C domain), is yet to be sequenced in the kangaroo protein. The IGF-II/CI-MPR in the American opossum has been shown to have 75 times lower affinity for bovine IGF-II than the bovine receptor (Dahms et al. 1993). One possible explanation for the lower affinity is the use of a heterologous assay and possible amino acid differences between the opossum and bovine IGF-II. Although kIGF-II is yet to be functionally characterised, its partial sequence indicates that the reduced binding of bovine IGF-II to opossum receptors is probably due to evolutionary changes occurring in the amino acids that constitute the ligand-binding site of the receptor, rather than the ligand itself. The residues in IGF-II that have been proposed to be important for the binding of IGF-II to the IGF-II/CI-MPR, residues 48–50 and 54–55 (Burgisser et al. 1991, Sakano et al. 1991), have been conserved in kIGF-II, and it is possible that opossum IGF-II has also been similarly conserved. Studies are currently underway to purify the IGF-II/CI-MPR from kangaroo liver and to examine, in a homologous assay, the binding affinities of IGF-II for the IGF-II/CI-MPR in the kangaroo. Development of a method for the isolation of kIGF-II from serum now makes it possible to conduct studies comparing eutherian and metatherian IGF-II/CI-MPRs. Ascertainment of the differences and similarities between the IGF-II/CI-MPR from these two groups of mammals may help us to elucidate the role of this receptor in the IGF system.

Acknowledgements

We would like to thank T Skelton and M Richardson for giving up their time to help us collect kangaroo blood and D Turner for the amino acid sequence analysis.

References


Canfield WM & Kornfeld S 1989 The chicken liver cation-independent mannose 6-phosphate receptor lacks the high affinity binding site for insulin-like growth factor II. Journal of Biological Chemistry 264 7100–7103.


Clairmont KB & Czech MP 1989 Chicken and Xenopus mannose 6-phosphate receptors fail to bind insulin-like growth factor II. Journal of Biological Chemistry 264 1173–1183.

Dahms NM, Bryzcki-Wessell MA, Ramajumal KS & Seetharam B 1993 Characterization of mannose 6-phosphate receptors (MPRs) from opossum liver: opossum cation-independent MPRs binds insulin-like growth factor II. Endocrinology 133 440–446.


Li YH, Cheng CH & Chan KM 1996 Insulin-like growth factor Ia2 is the predominantly expressed form of IGF in common carp (Cyprinus carpio) Molecular Marine Biology and Biotechnology 5 145–152.


Received 19 March 1997
Revised manuscript received 28 July 1997
Accepted 26 August 1997