A key functional role for the insulin-like growth factor 1 *N*-terminal pentapeptide

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In order to elucidate the role of the *N*-terminus of insulin-like growth factor 1 (IGF-1) with respect to its biological properties, we chemically synthesized analogues of IGF-1 truncated by one to five amino acid residues from the *N*-terminus. In a bioassay that measured the stimulation of protein synthesis in rat L6 myoblasts, the concentrations required to produce a half-maximal response were: IGF-1, 13 ng/ml; des-(1)-IGF-1, 10 ng/ml; des-(1-2)-IGF-1, 13 ng/ml; des-(1-3)-IGF-1, 1.5 ng/ml; des-(1-4)-IGF-1, 5.1 ng/ml; des-(1-5)-IGF-1, 1200 ng/ml. When tested for their abilities to compete with ¹²⁵I-IGF-1 binding to L6 myoblasts at 3 °C, the concentrations required for 50% competition were: IGF-1, des-(1)-IGF-1 and des-(1-2)-IGF-1, 20 ng/ml; des-(1-3)-IGF-1, 14 ng/ml; des-(1-4)-IGF-1, 40 ng/ml; des-(1-5)-IGF-1, > 1000 ng/ml. Receptor-binding experiments at 25 °C, however, gave results suggesting that the myoblasts were secreting a binding protein selective for the three longest peptides. This interpretation was confirmed by binding studies with medium conditioned by the L6 myoblasts as well as binding protein purified from MDBK-cell-conditioned medium. In both cases IGF-1, des-(1)-IGF-1 and des-(1-2)-IGF-1 competed for tracer IGF-1 binding at least 60-fold better than did the three shorter peptides. The results obtained account for the increased potency of des-(1-3)-IGF-1 and des-(1-4)-IGF-1, since their activities are not attenuated by the binding protein, and the relatively lower potency of des-(1-4)-IGF-1 is a consequence of this peptide binding less well to the L6-myoblast receptor.

INTRODUCTION

We have purified a variant of IGF-1 from bovine colostrum that lacks the three amino acid residues at the *N*-terminus [1,2]. This des-(1-3)-IGF-1 has a 5–10-fold greater potency than IGF-1 in bioassays with rat L6 myoblasts, but the increased potency cannot be accounted for by a comparable change in the affinity for the IGF-1 receptor of these cells [3]. Rather, the enhanced potency of this peptide apparently results from its lack of binding to a protein secreted by cells in tissue culture [4], a binding that would otherwise decrease the amount of peptide available for interaction with cell receptors.

The dramatic loss of capacity to bind to an IGF-binding protein observed with des-(1-3)-IGF-1 [4] and the increased biological potency of this peptide [2,3] prompted us to investigate *N*-terminal truncations in more detail. The *N*-terminal sequence of IGF-1 is Gly-Pro-Glu-Thr-Leu, with a half-cystine residue at position 6 being presumably essential for maintenance of the correctly folded structure. Hence we have here synthesized IGF-1 as well as analogues lacking one to five amino acid residues and characterized them by bioassay, radioreceptor assay, radioimmunoassay and binding-protein assay in order to define the importance of particular amino acid residues.

MATERIALS AND METHODS

Materials

Recombinant human IGF-1 was generously provided by Dr. H. H. Peters and Dr. K. Scheibli, CIBA-GEIGY, Basel, Switzerland. Carrier-free Na¹²⁵I and [4,5-³H]-leucine (specific radioactivity 40–60 Ci/mmol) were obtained from Amersham International (Amersham, Bucks., U.K.). Tissue-culture reagents and rat L6 myoblasts were obtained as described previously [5]. Binding protein was purified from MDBK cells (Flow Laboratories A.T.C.C. CCL22) as described previously [4]. Conditioned medium was prepared by incubating confluent monolayers of L6 myoblasts in a 24-place multiwell dish with serum-free medium (1 ml/well) for 16 h. Anti-IGF-1 antibodies were produced in rabbits by immunizing them with a bovine IGF-1-ovalbumin conjugate. Goat anti-(rabbit IgG) antibody was obtained by immunization of goats as described in ref. [6]. Reagents for chemical synthesis were obtained from Applied Biosystems (Foster City, CA, U.S.A.) except for *S*-acetamidomethylcysteine, which was obtained from Bachem (Bubendorf, Switzerland). Acetonitrile and trifluoroacetic acid were of h.p.l.c. grade. H.p.l.c. cartridges were obtained from Brownlee Laboratories (Santa Clara, CA, U.S.A.). Bovine ¹²⁵I-IGF-1 with a

Abbreviation used: IGF, insulin-like growth factor.

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specific radioactivity of 100–150 Ci/g was prepared by the chloramine-T method [7].

Growth-factor assays

The assay used to assess the biological activities of IGF peptides was based on the stimulation of protein synthesis in L6 rat myoblasts incubated as monolayers in 24-place multiwell dishes [1]. In this assay the incorporation of [4,5-³H]leucine into trichloroacetic acid-insoluble cell protein was measured over an 18 h period in Dulbecco-modified Eagle's minimal essential medium containing the indicated concentration of growth factor. Incorporation was expressed as a percentage of the maximum stimulation produced by IGF-1, which was usually 2–3-fold higher than in control medium and similar to that produced by 10% (v/v) foetal bovine serum.

Receptor-binding experiments with confluent cultures of L6 myoblasts in 12-place multiwell dishes and [¹²⁵I]-IGF-1 were performed essentially as described by Ballard *et al.* [8]. The labelling periods used were 2 h at 25 °C or 6 h at 3 °C. Before the addition of labelling medium the cell monolayers were washed twice with medium over a 2 h period at the same temperature used for the assay. No correction was made for non-specific binding.

Competition for the binding of [¹²⁵I]-IGF-1 to either binding protein purified from MDBK-cell-conditioned medium [4] or to medium conditioned by L6 myoblasts was carried out by using slight modifications [4] of the assay method described by Martin & Baxter [9].

Radioimmunoassays with rabbit anti-(bovine IGF-1) antibodies and [¹²⁵I]-IGF-1 as radioligand were carried out as previously described [10].

Chemical synthesis of IGF-1 and truncated analogues

Synthetic IGF-1 and *N*-terminally truncated analogues were prepared by using an Applied Biosystems model 430A peptide synthesizer with solid-phase peptide-synthesis chemistry [11] as recommended by the manufacturer except that cysteine side-chain protection was achieved with the *S*-acetamidomethyl protecting group. Material obtained after 63–69 coupling cycles of the human IGF-1 sequence [12] was cleaved from the resin and partially deprotected by trifluoromethanesulphonic acid [13] to give the *S*-acetamidomethyl-protected peptides. These were immediately isolated by reverse-phase h.p.l.c. on a 1 cm × 10 cm butyl-silica cartridge with elution performed at 5 ml/min with a gradient of 25–45% (v/v) acetonitrile over 33 min in the presence of 0.1% (v/v) trifluoroacetic acid. Ninhydrin analysis during the synthesis indicated that on average repetitive coupling yield of 99.4% had been achieved, and direct Edman degradation of the purified *S*-acetamidomethyl-IGF-1 in an Applied Biosystems 470A peptide sequencer indicated a purity of 95%. The purified *S*-acetamidomethyl-peptides were deprotected by using I₂ in 80% (v/v) acetic acid [14]. The crude products were reduced by 50 mM-dithioerythritol in the presence of 6 M-guanidinium chloride, pH 8.0, and purified on the 1 cm × 10 cm butyl-silica cartridge with a gradient of 20–50% (v/v) acetonitrile over 30 min in the presence of 0.1% (v/v) trifluoroacetic acid at a flow rate of 2 ml/min. Oxidation was effected by the glutathione redox buffer system of Ahmed *et al.* [15] in the presence of 2 M-urea at a protein concentration of 0.1 mg/ml. The crude reoxidation products were chromatographed on the

Fig. 1. H.p.l.c. of oxidized IGF-1

Reduced IGF-1 (174 µg) was oxidized as described in the Materials and methods section and chromatographed on a 4.6 mm × 30 mm RP-300 cartridge (Brownlee Laboratories). Solvent A was 0.1% (v/v) trifluoroacetic acid and solvent B was 0.1% (v/v) trifluoroacetic acid in acetonitrile, and elution was performed at 2 ml/min with the indicated gradient of solvent B (----). Absorbance at 215 nm was monitored (—), 0.5 min fractions were collected and 10 µl samples were tested in the L6-myoblast protein-synthesis bioassay (●--●).

butyl-silica cartridge as described for the full IGF-1 peptide (see Fig. 1).

In the reverse-phase chromatogram of oxidized IGF-1, the protein peak that exhibited a retention time equal to that of authentic bovine IGF-1 represented the major peak of protein-synthesis-stimulating activity. A discrete peak of oxidized IGF was observed to be eluted before the main peak of bioactivity. This material, which has been reported by others [16,17], had less than 10% the specific bioactivity of the protein that was co-eluted with authentic IGF-1. Material eluted later exhibited some biological activity and may represent partially reoxidized IGF. The yield of correctly oxidized IGF-1 was less than that observed when reduced recombinant IGF-1 was used as the starting material [16,17], possibly owing to incomplete deprotection of the cysteine residues, even though an extended time (2 h) was used for this reaction. It is also possible that the purified *S*-acetamidomethyl-IGF-1 was contaminated by peptides containing internal deletions or modifications not readily detectable by sequence analysis, since, as indicated by the ninhydrin analysis, any individual contaminant would be probably present at less than 1% of the total amount of peptide.

The oxidation products of peptides lacking up to three amino acid residues gave h.p.l.c. chromatograms similar to that of IGF-1 (see Fig. 1). The oxidation product of des-(1–4)-IGF-1 did not exhibit the early-eluted discrete peak of oxidized IGF, and des-(1–5)-IGF-1 did not oxidize to give a discrete peak of protein corresponding to the bioactivity, a result suggesting that this preparation may contain material lacking the correct disulphide bonds. The des-(1–6)-IGF-1 oxidation product showed no biological activity at 250 ng/ml and accordingly was not tested further.

The most biologically active material from each of the preparations of IGF was re-chromatographed on a 4.6 mm × 30 mm butyl-silica cartridge with a gradient of 20–50% (v/v) acetonitrile over 15 min in the presence of 0.1% (v/v) trifluoroacetic acid at a flow rate of 1 ml/min. In each case the protein content of the major peak (corresponding to at least 80% of the total protein) was calculated from its absorbance at 215 nm ($A_{215}^{1\%} = 150$), and this material was used for the subsequent studies. The biologically active fractions from the chromatogram of the reoxidized des-(1–5)-IGF-1 were rechromatographed in order to measure the protein amount, but this did not result in any further purification.

For each synthetic IGF peptide, at least two separate oxidations and purifications were carried out (denoted A and B). Except for des-(1–5)-IGF-1, all the synthetic peptides had a similar purity to the IGF-1, as indicated by analytical reverse-phase h.p.l.c. Synthetic IGF-1 preparations were found to be full agonists of natural (bovine) IGF-1 in the protein-synthesis bioassay and the radioreceptor assay performed at 3 °C, but exhibited only 80% the potency.

RESULTS

The cross-reactivities of the synthetic IGFs were compared by using the polyclonal anti-IGF-1 serum (Fig. 2). This experiment showed that all IGFs competed for tracer binding. The des-(1–3)-IGF-1 and des-(1–4)-IGF-1 peptides competed slightly less effectively than did IGF-1, especially at high concentrations of competing ligand, perhaps suggesting that there may be a small subpopulation of antibodies to which these IGFs do not

Fig. 2. IGF-1 radioimmunoassay of various IGF peptides

The ability of IGF-1 and analogues from reference preparation B to compete for the binding of ^{125}I -IGF-1 to antiserum was measured as described in the Materials and methods section. The competing peptides were IGF-1 (●), des-(1)-IGF-1 (○), des-(1–2)-IGF-1 (△), des-(1–3)-IGF-1 (■), des-(1–4)-IGF-1 (▲) and des-(1–5)-IGF-1 (□). Total binding was 35% after a blank determined in the absence of antibody equal to 1.9% of total added radioactivity had been subtracted. Data plotted represent the means of triplicate determinations and are expressed as percentages of the binding evident in the absence of competing ligand. As an indication of assay variability, standard errors of the mean are indicated for the des-(1–5)-IGF-1 data when they are sufficiently large in relation to the symbols.

bind. The des-(1–5)-IGF-1 was approx. 4-fold less potent than IGF-1 in the assay.

In order to determine which analogue was the most biologically active, we tested the synthetic growth factors in the L6-myoblast protein-synthesis bioassay. Peptides from each batch were assessed in at least two separate experiments, with representative experiments being shown in Fig. 3. Although there were differences in the potencies of the two batches of growth-factor preparations evaluated, as assessed by the concentrations required to produce half-maximal effects, within each assay the relative potencies of the various peptides were similar. The potencies, expressed as the geometric mean of the two experiments in which the concentrations required for half-maximal effects were determined, were: des-(1–3)-IGF-1, 1.5 ng/ml; des-(1–4)-IGF-1, 5.1 ng/ml; des-(1)-IGF-1, 10 ng/ml; IGF-1 and des-(1–2)-IGF-1, 13 ng/ml. Des-(1–5)-IGF-1 was approx. 100-fold less potent than IGF-1.

Previous reports [18,19], in which a foetal-brain radioreceptor assay was used, have suggested that the enhanced potency of the des-(1–3)-IGF-1 compared with

Fig. 3. Protein-synthesis bioassay of various IGF peptides

This was performed as described in the Materials and methods section. (a) Bioassay of peptides of preparation A. (b) Bioassay of peptides of preparation B. The peptides tested were IGF-1 (●), des-(1)-IGF-1 (○), des-(1–2)-IGF-1 (△), des-(1–3)-IGF-1 (■), des-(1–4)-IGF-1 (▲) and des-(1–5)-IGF-1 (□). Data represent the means of triplicate determinations and are expressed as percentages of the maximum stimulation caused by IGF-1. As an indication of assay variability, standard errors of the mean are indicated for the des-(1–3)-IGF-1 data.

IGF-1 was due to its 5–10-fold greater affinity for binding to the type 1 receptor. On the other hand, we have noted that des-(1–3)-IGF-1 was equipotent to [3] or only slightly more potent than [20] IGF-1 in the L6-myoblast receptor assay. The truncated peptides were therefore tested for their abilities to compete with ^{125}I -IGF-1 for binding to L6 cells (see Fig. 4). At 3 °C all the peptides competed effectively, with the concentration required for half-maximal displacement being approx. 20 ng/ml for IGF-1, des-(1)-IGF-1 and des-(1–2)-IGF-1. Des-(1–3)-IGF-1 was 1.5-fold more potent and des-(1–4)-IGF-1 2-fold less potent than IGF-1. Des-(1–5)-IGF-1 was approx. 100-fold less potent than IGF-1. These results confirm that the enhanced biological potency of des-(1–3)-IGF-1 in myoblasts is only minimally due to an increased affinity for the receptor.

When the radioreceptor assay was performed at 25 °C, IGF-1, des-(1)-IGF-1 and des-(1–2)-IGF-1 produced anomalous displacement curves in which low concentrations of IGF (4–20 ng/ml) stimulated radioligand binding up to 150% of the control. Des-(1–3)-IGF-1 and des-(1–4)-IGF-1 did not elicit this effect, but exhibited displacement curves more similar to those obtained at 3 °C. Des-(1–5)-IGF-1 caused no displacement of labelled IGF-1 at concentrations up to 1 $\mu\text{g/ml}$. The binding measurements carried out at 25 °C suggested that there

Fig. 4. IGF-1 radioreceptor assay with L6 cells

The abilities of IGF-1 and analogues from preparation B to inhibit binding of ^{125}I -IGF-1 to L6-cell monolayers were determined as described in the Materials and methods section. The competing peptides were IGF-1 (●), des-(1)-IGF-1 (○), des-(1–2)-IGF-1 (△), des-(1–3)-IGF-1 (■), des-(1–4)-IGF-1 (▲) and des-(1–5)-IGF-1 (□). (a) Receptor assay incubation performed at 3 °C for 6 h; 13.6% of added radioactivity was bound in the control wells. (b) Receptor assay incubation performed at 25 °C for 2 h; 6.9% of added radioactivity was bound in the control wells. Non-specific binding has not been subtracted. Data plotted represent the means of triplicate determinations and are expressed as percentages of the binding evident in the absence of competing ligand. As an indication of assay variability, standard errors of the mean are indicated for the des-(1–3)-IGF-1 data when they are sufficiently large in relation to the symbols.

may have been an IGF-binding protein present that modified the binding of IGF-1, des-(1)-IGF-1 and des-(1–2)-IGF-1 but not that of des-(1–3)-IGF-1 or des-(1–4)-IGF-1 to the cell receptor.

Specific IGF-binding proteins, secreted by cells in tissue culture, have been shown to alter the binding of IGF-1 to the cell surface [21,22]. Accordingly, we tested the abilities of IGF-1 and its analogues to compete with ^{125}I -IGF-1 for binding to a purified preparation of binding protein from MDBK-cell-conditioned medium, since this protein could be conveniently purified to homogeneity [4]. As shown in Fig. 5a, IGF-1, des-(1)-IGF-1 and des-(1–2)-IGF-1 exhibited similar potencies in this assay, whereas des-(1–3)-IGF-1, des-(1–4)-IGF-1 and des-(1–5)-IGF-1 were at least 100-fold less potent for binding to the MDBK-cell protein. When the assay was performed with medium conditioned by L6 cells as a source of binding proteins, IGF-1, des-(1)-IGF-1 and des-(1–2)-IGF-1 again exhibited similar potencies (see Fig. 5b). On the other hand, des-(1–3)-IGF-1 was 60-fold less potent and des-(1–4)-IGF-1 was 280-fold less potent than IGF-1.

Fig. 5. IGF-1 competitive binding assay with MDBK-cell binding protein and L6-myoblast-conditioned medium

Inhibition of ^{125}I -IGF-1 binding to MDBK-cell binding protein or L6-myoblast-conditioned medium by IGF-1 and analogues from preparation B (except where noted) was measured as described in the Materials and methods section. The competing peptides were IGF-1 (●), des-(1)-IGF-1 (○), des-(1–2)-IGF-1 (△), des-(1–3)-IGF-1 (■), des-(1–4)-IGF-1 (▲) and des-(1–5)-IGF-1 (□). (a) Binding assay with MDBK-cell binding protein; total binding was 23% after a blank determined in the absence of binding protein equal to 19% had been subtracted. (b) Binding assay with L6-myoblast-conditioned medium; in this assay recombinant IGF-1 was substituted for synthetic IGF-1 and a different preparation of des-(1–3)-IGF-1 was used; total binding was 26% after a blank of 7.8% had been subtracted. Data plotted represent the means of duplicate determinations and are expressed as percentages of the binding evident in the absence of competing ligand. As an indication of assay variability, ranges are indicated for the des-(1–4)-IGF-1 data.

DISCUSSION

The truncated IGF-1 peptides compared in this study fit into three groups. The first group, comprising IGF-1 together with des-(1)-IGF-1 and des-(1-2)-IGF-1, bind equally to the L6-myoblast receptor, have equal potencies in the myoblast protein-synthesis assay and bind equally both to a binding protein purified from MDBK-cell-conditioned medium and to a crude preparation of binding protein secreted by L6 myoblasts. Moreover, all three peptides exhibit an anomalous binding pattern when the myoblast receptor assay is performed at 25 °C (Fig. 4b). The second group of truncated peptides consists of des-(1-3)-IGF-1 and des-(1-4)-IGF-1. These are more potent than the intact molecule in the protein-synthesis bioassay, do not significantly compete for ^{125}I -IGF-1 binding to either preparation of binding protein and do not exhibit the anomalous receptor binding at 25 °C. Of these peptides, des-(1-3)-IGF-1 is the more potent biologically because it exhibits a higher affinity for the cell receptor. The third group of peptides tested comprises molecules with five or six amino acid residues omitted from the *N*-terminus. Neither peptide shows significant biological activity, no doubt because they bind so poorly to the cell receptors. However, it is unlikely that our preparation of des-(1-5)-IGF-1 possesses the correct disulphide bonds, since a discrete peak of folded peptide was not found upon chromatography of the material after reoxidation. Generation of des-(1-5)-IGF-1 by an alternative means such as proteolysis of des-(1-4)-IGF-1 may lead to a preparation exhibiting a greater receptor-binding activity. Des-(1-6)-IGF-1 lacks the cysteine residue at position 6 and therefore was also unable to form the correct disulphide bonds.

Only a small part of the increased potency of des-(1-3)-IGF-1 can be explained by enhanced binding of the growth factor to receptors on L6 myoblasts, in accord with previous studies with both natural and synthetic preparations of this peptide [3,20]. On the other hand, Sara and co-workers, who are probably investigating the same peptide on the basis of their published *N*-terminal sequences [18,19], find that it competes for ^{125}I -IGF-1 binding to the human brain receptor some 5–10-fold more effectively than IGF-1. This increased receptor potency matches the higher biological potency [19]. Perhaps the differences in binding potencies in the two series of investigations can be explained by true molecular differences between receptors, because the type 1 receptor in brain has an altered carbohydrate composition [23].

The modest decrease in receptor binding with des-(1-4)-IGF-1 suggests that there is an involvement of the *N*-terminal region when IGF-1 binds to the cell receptor. Studies with insulin, which binds to a homologous receptor [24], have suggested the major receptor-binding region of this molecule consists of the C-terminal pentapeptide of the B-chain together with various residues of the A-chain [25,26]. The removal of up to four residues from the *N*-terminus of the insulin B-chain caused only a slight decrease in biological activity, whereas the removal of His-B5 to give des-(B1-B5)-insulin gave a protein that exhibited only 5% the activity of insulin in an isolated fat-cell assay [27]. The histidine residue, B5, which is highly conserved between species [28], is analogous to Thr-4 of IGF-1 and may represent a portion of the insulin molecule that is important for distinguishing it functionally from IGF-1. The threonine

residue at position 4 of IGF-1 appears to play only a minor role in receptor binding, since des-(1-4)-IGF-1 binds to the receptor only 3-fold less well than des-(1-3)-IGF-1. Moreover, this conclusion is in agreement with studies in which [Gln³,Ala⁴]IGF-1 was found to be equipotent to IGF-1 for binding to the type 1 IGF receptor [29] whereas on the other hand the replacement of residue Tyr-24 by a non-aromatic residue caused a large decrease in affinity for the type 1 receptor [30]. Other workers have implicated the C-terminal (A) domain [31] and the tyrosine residues at positions 24, 31 and 60 in the binding of IGF-1 to cell receptors [32].

Although affinity is the major determinant of receptor-binding activity, we found that L6 myoblasts produced a substance that caused cell-associated ^{125}I -IGF-1 to be increased by the addition of low concentrations of unlabelled IGF-1, des-(1)-IGF-1 or des-(1-2)-IGF-1 in a radioreceptor assay performed at 25 °C. Similarly, De Vroede *et al.* [21] showed that low concentrations of IGF-1 or an insulin-IGF hybrid caused an increase in the binding of radioligand to the cell surface of fibroblasts. They proposed that the extra binding resulted from displacement of tracer from a secreted binding protein, thus allowing more to bind at the cell surface. They simultaneously measured the amount of radioligand bound to the cell surface and to medium proteins in the presence of increasing concentrations of unlabelled ligand and found that maximal binding of the radioligand and the cell surface occurred at concentrations of unlabelled ligand that caused 30–80% displacement of radioligand from medium proteins. De Vroede *et al.* [21] further demonstrated that the medium conditioned by the fibroblasts had an adequate binding capacity for such a process to occur. This interpretation is supported by our results because only those peptides that were able to bind significantly to proteins present in L6-myoblast-conditioned medium could cause an increased association of radioligand with the cell surface.

The binding differences between the various truncated IGF-1 peptides observed with either the MDBK-cell binding protein or the myoblast-conditioned medium are dramatic. Thus virtually a total loss of binding occurs when Glu-3 is absent. The possibility that the effect of removal of Glu-3 is due to the perturbation of the IGF tertiary structure seems unlikely, since, firstly, the reduced protein was able to be oxidized correctly to give a protein of the expected chromatographic properties, and, secondly, this protein bound well in the radioreceptor assay performed at 3 °C. Our results support the proposal by Blundell *et al.* [33] that the surface analogous to the hexamer formation region of insulin might be involved in binding to carrier proteins. This region, centred around residues Ala-13, Phe-16, Val-17 and Leu-54 also includes residues Glu-3, Leu-5, Glu-9, Gln-15, Glu-58, Arg-55 and Asp-53 of IGF-1. Moreover, studies with hybrid insulin-IGF-1 molecules have identified the *N*-terminal (B) domain of IGF-1 as being important for peptide association with binding proteins [34]. In this respect, the MDBK-cell and L6-myoblast binding proteins seem to differ from the acid-stable binding proteins of human serum, which bind [Gln³,Ala⁴]IGF-1 only 4-fold less well than IGF-1 [29].

Our findings point to the lack of an association between an IGF-1 peptide and a medium binding protein, i.e. lack of sequestration into an inactive form, as leading to enhanced biological potency. Thus both des-(1-3)-IGF-

1 and des-(1-4)-IGF-1, which are more potent than IGF-1 in the bioassay, do not bind significantly to the binding protein present in L6-myoblast-conditioned medium. Moreover, the enhanced biological response is particularly evident at low concentrations of these truncated peptides (Fig. 3), conditions where binding protein secreted into the medium would be expected to lower more effectively the concentration of free IGF-1, des-(1)-IGF-1 or des-(1-2)-IGF-1. This postulate, that it is the concentration of free IGF-1 peptide that determines the biological potency, seems at variance with a report by Elgin *et al.* [35] in which the binding protein from human amniotic fluid potentiated IGF-1 stimulation of DNA synthesis in several fibroblast cell lines and in aortic smooth-muscle cells. However, it is noteworthy that the cells used by Elgin *et al.* [35] were particularly insensitive to IGF-1 in the absence of binding protein. Also, the IGF-1 binding to the cell monolayer was predominantly to a surface protein other than the type 1 receptor, since it had a lower molecular mass than expected and neither anti-(type 1 receptor) antibodies nor insulin inhibited IGF-1 binding [22]. In another study, Ritvos *et al.* have demonstrated that a 34 kDa binding protein from human decidua inhibits both the binding of IGF-1 to a choriocarcinoma cell line and also IGF-1-stimulated [^3H]-aminoisobutyrate uptake [36].

We have identified des-(1-3)-IGF-1 as being the most potent *N*-terminally truncated form of IGF-1 in L6 myoblasts and propose that the increased potency is due to its inability to bind to IGF-binding proteins produced by the cells. As yet, no physiological role for des-(1-3)-IGF-1 has been established. This peptide has only been detected in human brain [18,19], foetal circulation [37] and bovine colostrum [1,2]. It is not clear whether des-(1-3)-IGF-1 is produced by action of a specific proteinase in the liver, by a tissue-derived proteinase near the sites of IGF-1 action, or as an artifact during the purification procedures. In this respect, it will be of interest to determine the specificities of other binding proteins such as the serum carrier proteins and to test other biological fluids for the presence of des-(1-3)-IGF-1.

This work was supported by the National Health and Medical Research Council of Australia and the National Biotechnology Program.

REFERENCES

- Francis, G. L., Read, L. C., Ballard, F. J., Bagley, C. J., Upton, F. M., Gravestock, P. M. & Wallace, J. C. (1986) *Biochem. J.* **233**, 207-213.
- Francis, G. L., Upton, F. M., Ballard, F. J., McNeil, K. A. & Wallace, J. C. (1988) *Biochem. J.* **251**, 95-103.
- Ballard, F. J., Francis, G. L., Ross, M., Bagley, C. J., May, B. L. & Wallace, J. C. (1987) *Biochem. Biophys. Res. Commun.* **149**, 398-404.
- Szabo, L., Mottershead, D. G., Ballard, F. J. & Wallace, J. C. (1988) *Biochem. Biophys. Res. Commun.* **151**, 207-214.
- Ballard, F. J. (1982) *Biochem. J.* **208**, 275-287.
- Krantz, M. J., Laferte, S. & Ariel, N. (1982) *Methods Enzymol.* **84**, 32-48.
- Van Obberghen-Schilling, E. & Pouyssegur, J. (1983) *Exp. Cell Res.* **147**, 369-378.
- Ballard, F. J., Read, L. C., Francis, G. L., Bagley, C. J. & Wallace, J. C. (1986) *Biochem. J.* **233**, 223-230.
- Martin, J. L. & Baxter, R. C. (1986) *J. Biol. Chem.* **261**, 8754-8760.
- Read, L. C., Ballard, F. J., Francis, G. L., Baxter, R. C., Bagley, C. J. & Wallace, J. C. (1986) *Biochem. J.* **233**, 215-221.
- Merrifield, R. B. (1963) *J. Am. Chem. Soc.* **85**, 2149-2154.
- Rinderknecht, E. & Humbel, R. E. (1978) *J. Biol. Chem.* **253**, 2769-2776.
- Bergot, B. J., Noble, R. L. & Geiser, T. (1986) *Applied Biosystems Peptide Synthesizer User Bulletin*, vol. 16, pp. 1-19, Applied Biosystems, Foster City.
- Kamber, B., Hartmann, A., Eisler, K., Riniker, B., Rink, H., Sieber, P. & Rittel, W. (1980) *Helv. Chim. Acta* **63**, 899-915.
- Ahmed, A. K., Schaffer, S. W. & Wetlaufer, D. E. (1975) *J. Biol. Chem.* **250**, 8477-8482.
- Peters, M. A., Lau, E. P., Snitman, D. L., Van Wyk, J. J., Underwood, L. E., Russell, W. E. & Svoboda, M. E. (1985) *Gene* **35**, 83-89.
- Saito, Y., Yamada, H., Niwa, M. & Ueda, I. (1987) *J. Biol. Chem.* **101**, 123-134.
- Carlsson-Skwirut, C., Jörnvall, H., Holmgren, A., Andersson, C., Bergman, T., Lundquist, G., Sjögren, B. & Sara, V. R. (1986) *FEBS Lett.* **201**, 46-50.
- Sara, V. R., Carlsson-Skwirut, C., Andersson, C., Hall, E., Sjögren, B., Holmgren, A. & Jörnvall, H. (1986) *Proc. Natl. Acad. Sci. U.S.A.* **83**, 4904-4907.
- Ballard, F. J., Ross, M., Upton, F. M. & Francis, G. L. (1988) *Biochem. J.* **249**, 721-726.
- De Vroede, M. A., Tseng, L. Y.-H., Katsoyannis, P. G., Nissley, S. P. & Rechler, M. M. (1986) *J. Clin. Invest.* **77**, 602-613.
- Clemmons, D. R., Elgin, R. G., Han, V. K. M., Casella, S. J., D'Ercole, J. A. & Van Wyk, J. J. (1986) *J. Clin. Invest.* **77**, 1548-1556.
- Burgess, S. K., Jacobs, S., Cuatrecasas, P. & Sahyoun, N. (1987) *J. Biol. Chem.* **262**, 1618-1622.
- Ullrich, A., Gray, A., Tam, A. W., Yang-Feng, T., Tsubokawa, M., Collins, C., Henzel, W., Le Bon, T., Kathuria, S., Chen, E., Jacobs, S., Francke, U., Ramachandran, J. & Fujita-Yamaguchi, Y. (1986) *EMBO J.* **5**, 2503-2512.
- Pullen, R. A., Lindsay, D. G., Wood, S. P., Tickle, I. J., Blundell, T. L., Wollmer, A., Krail, G., Brandenburg, D., Zahn, H., Gliemann, J. & Gammeltoft, S. (1976) *Nature (London)* **259**, 369-373.
- Nakagawa, S. H. & Tager, H. S. (1987) *J. Biol. Chem.* **262**, 12054-12058.
- Schwartz, G. & Katsoyannis, P. G. (1978) *Biochemistry* **17**, 4550-4556.
- Blundell, T. L. & Wood, S. P. (1975) *Nature (London)* **257**, 197-203.
- Bayne, M. L., Applebaum, J., Chicchi, G. G., Hayes, N. S., Green, B. G. & Cascieri, M. A. (1988) *J. Biol. Chem.* **263**, 6233-6239.
- Cascieri, M. A., Chicchi, G. G., Applebaum, J., Hayes, N. S., Green, B. G. & Bayne, M. L. (1988) *Biochemistry* **27**, 3229-3233.
- Tseng, L. Y.-H., Schwartz, G. P., Sheik, M., Chen, Z. Z., Joshi, S., Wang, S.-F., Nissley, S. P., Katsoyannis, P. G. & Rechler, M. M. (1987) *Biochem. Biophys. Res. Commun.* **149**, 672-679.
- Maly, P. & Lüthi, C. (1988) *J. Biol. Chem.* **263**, 7068-7072.
- Blundell, T. L., Bedarkar, S. & Humbel, R. E. (1983) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **42**, 2592-2597.

34. De Vroede, M. A., Rechler, M. M., Nissley, S. P., Joshi, S., Burke, G. T. & Katsoyannis, P. G. (1985) *Proc. Natl. Acad. Sci. U.S.A.* **82**, 3010–3014
35. Elgin, R. G., Busby, W. H. & Clemmons, D. R. (1987) *Proc. Natl. Acad. Sci. U.S.A.* **84**, 3254–3258
36. Ritvos, O., Ranta, T., Jalkanen, J., Suikkari, A.-M., Voutilainen, R., Bohn, H. & Rutanen, E.-M. (1988) *Endocrinology (Baltimore)* **122**, 2150–2157
37. Carlsson-Skwirut, C., Andersson, C. & Sara, V. R. (1987) *Acta Endocrinol. (Copenhagen)* **114**, 37–40

Received 19 August 1988/14 November 1988; accepted 23 November 1988