Vol. 157, No. 1, 1988
November 30, 1988

BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
Pages 196-202

CLASSIFICATION OF THE INSULIN-LIKE GROWTH FACTOR BINDING PROTEINS INTO THREE DISTINCT CATEGORIES ACCORDING TO THEIR BINDING SPECIFICITIES

Briony Forbes, Laszlo Szabo, Robert C. Baxter*, F. John Ballard**
and John C. Wallace

Department of Biochemistry, University of Adelaide, Adelaide, SA 5000, Australia
*Department of Endocrinology, Royal Prince Alfred Hospital, Camperdown, NSW 2050, Australia
** CSIRO Division of Human Nutrition, Adelaide, SA 5000, Australia

Received September 8, 1988

Summary: Competitive binding experiments with insulin-like growth factor (IGF)-1, IGF-2 and des-(1-3)-IGF-1 have confirmed the interpretation based on limited amino-terminal sequence analysis that at least three types of IGF binding protein occur. In addition to the acid stable subunit of the large serum binding protein which exhibits des-(1-3)-IGF-1 binding only slightly less than IGF-1, the small IGF binding proteins can be separated into two classes based on differences in des-(1-3)-IGF-1 and IGF-2 binding potencies.

Insulin-like growth factors (IGFs) are found in mammalian blood in association with various binding proteins. Two categories of binding proteins can be defined by molecular weight. The first is a growth hormone-dependent form of 150-200 kDa which dissociates under acid conditions to yield a 53 kDa subunit (BP53) that is active in binding IGF (1). A second group of growth hormone-independent binding proteins of 25-40 kDa has been isolated. The binding proteins can also be classified on the basis of their amino terminal amino acid sequences. BP53 isolated from rat and human serum are identical in 11 of the first 15 amino terminal residues (2). However, neither shows amino terminal sequence similarity to the small binding proteins isolated from several different sources. These include a form from human amniotic fluid (3-5) or medium conditioned by human HEP G2 cells (6,7) as well as others from conditioned medium of bovine kidney (MDBK) cells (8) and rat BRL-3A cells (9). Furthermore, the human amniotic fluid BP (BP28) and the MDBK BP have only limited similarity in their amino terminal amino acid sequences, while the MDBK and the

Abbreviations: IGF-1, insulin-like growth factor-1; hIGF-1, human IGF-1; rhIGF-1, recombinant human IGF-1: IGF-2, insulin-like growth factor-2; hIGF-2, human IGF-2; bIGF-2, bovine IGF-2; des-(1-3)-IGF-1, human/bovine IGF-1 with the amino terminal tripeptide omitted; BP, binding protein; BP53, acid stable binding subunit of the human serum large BP; BP28, human amniotic fluid BP; MDBK BP, BP from medium conditioned by MDBK cells; HPLC, high performance liquid chromatography.

0006-291X/88 $1.50

BRL-3A binding proteins are homologous (8,9). Our aim in the present investigation is to provide further information on the binding proteins by a comparison of their binding properties with different IGF peptides.

MATERIALS AND METHODS

Materials: Human BP53 (1), BP28 (4), MDBK BP (8), bIGF-2 (10,11) and hIGF-1 (12) were isolated as previously described. Recombinant human IGF-1 was a gift from Drs. H. H. Peter and K. Scheibli, Ciba-Geigy, Basle, Switzerland. Synthetic des-(1-3)-IGF-1 was prepared as previously described (13). The concentration of each IGF was quantified against a weighed amount of the rhIGF-1 by HPLC analysis, assuming that the absorbance at 215nm of similar concentrations of IGF-1, IGF-2 and des-(1-3)-IGF-1 were equivalent. The purity of each IGF preparation was confirmed by amino terminal sequence analysis. Bovine IGF-2, hIGF-2, hIGF-1 and rhIGF-1 were iodinated with ^{125}I using the Chloramine T method to a specific activity of 100-150 Ci/g (14). Antibody directed against BP28 (antibody A2) was prepared as previously described (4).

Competitive Binding Studies: Each binding protein was tested in a competitive binding study using several unlabelled ligands and either ^{125}I-labelled IGF-1 or IGF-2 as tracers. Initially, each binding protein was titrated against IGF-1 and IGF-2 tracers to determine the amount of binding protein which bound at least 30% of the tracer. Competition for a standard amount of tracer (~4000 dpm/tube) by the chosen amount of binding protein was subsequently tested with increasing amounts of unlabelled IGF. Separation of bound and unbound tracer was performed in one of two ways: (a) charcoal binding (8) for BP53 and MDBK BP, and (b) antibody A2 precipitation for BP28 (4).

RESULTS

The three binding proteins (BP28, BP53, MDBK BP) were initially tested for their IGF binding characteristics using rhIGF-1 tracer. We found that bIGF-2 competed at slightly lower concentrations than rhIGF-1 for ^{125}I-rhIGF-1 binding to the small bovine binding protein (MDBK BP) (Fig. 1A), the small human binding protein (BP28) (Fig. 1B), and the large human BP53 (Fig. 1C). In each case the competition curves with human IGF-2 were identical to those found with bovine IGF-2.

Competitive binding studies were also performed using bovine IGF-2 tracer. Again, bIGF-2 competed better than rhIGF-1 for binding to MDBK BP, but in this case the difference was approximately 40 fold, much greater than observed with rhIGF-1 tracer (Fig. 2A). Competition for BP28 and BP53 by rhIGF-1 and bIGF-2 was similar to that seen using IGF-1 tracer (Fig. 2B, 2C). As noted for the IGF-1 tracer, hIGF-2 and bIGF-2 behaved identically with bIGF-2 tracer.

An additional series of competitive binding experiments was performed using human IGF-1 and IGF-2 tracers and the MDBK BP, to see whether the binding of bIGF-2 tracer and hIGF-2 tracer to this binding protein differed (Fig. 3). When hIGF-2 tracer was used instead of bIGF-2 tracer, the pattern of competition for binding of the various IGFs to the MDBK BP was very similar to that seen previously (Fig. 3B compared to Fig. 2A). Purified hIGF-1 tracer also gave similar curves to that obtained with rhIGF-1 tracer (Fig. 3A compared to Fig. 1A).

Very different binding patterns were found when des-(1-3)-IGF-1 was tested with each binding protein (Figs. 1 and 2). Des-(1-3)-IGF-1 did not compete significantly for the

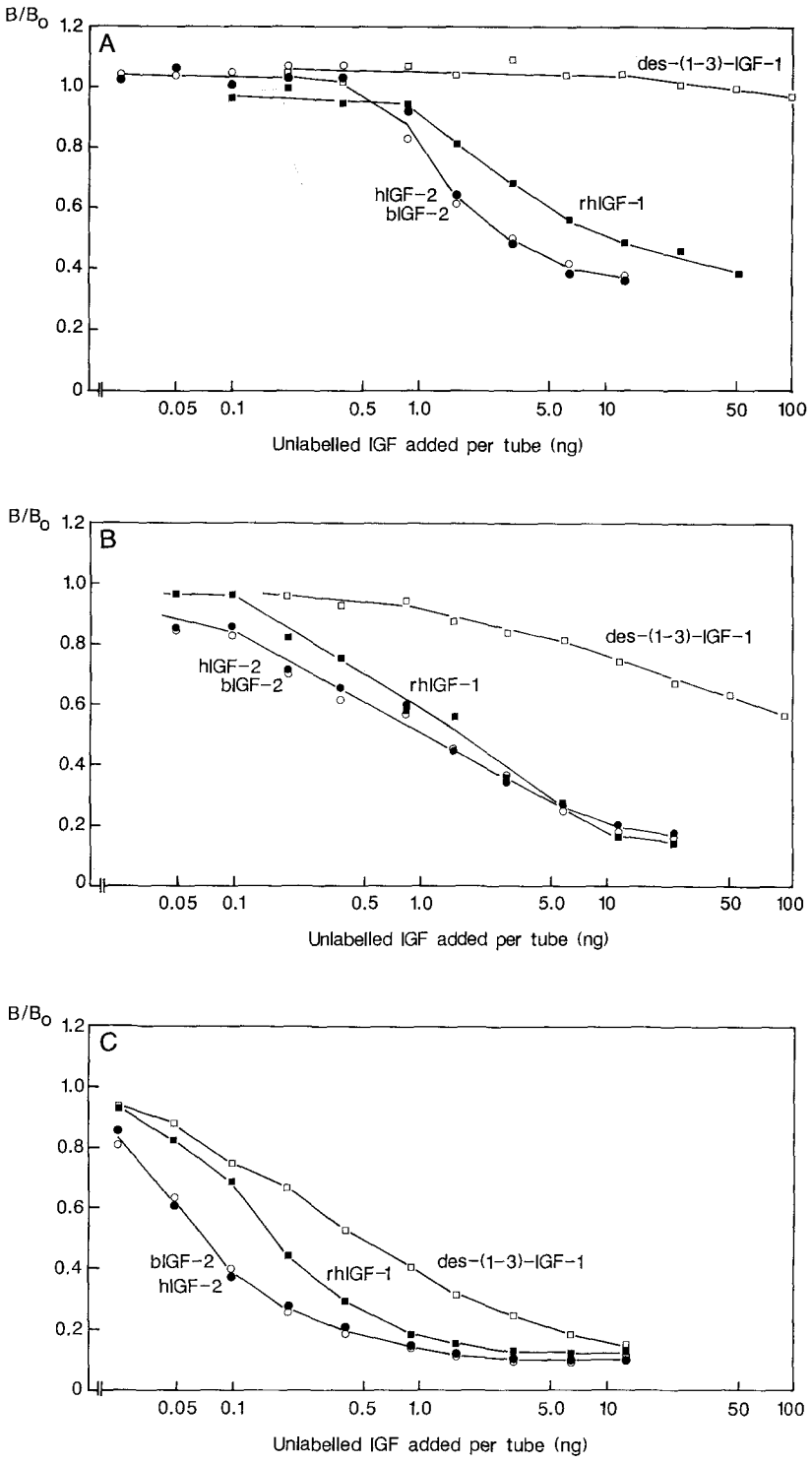

Figure 1. Competitive binding of recombinant human ^{125}I-IGF-1 tracer and rhIGF-1 (■), bIGF-2 (○), hIGF-2 (●) and des-(1-3)-IGF-1 (□) to three binding proteins. Competition with (**A**) 10ng MDBK BP per tube (Bo=31% total counts), (**B**) 2.5ng BP28 per tube (Bo =35% total counts), and (**C**) 1.0ng BP53 per tube (Bo =58% total counts).

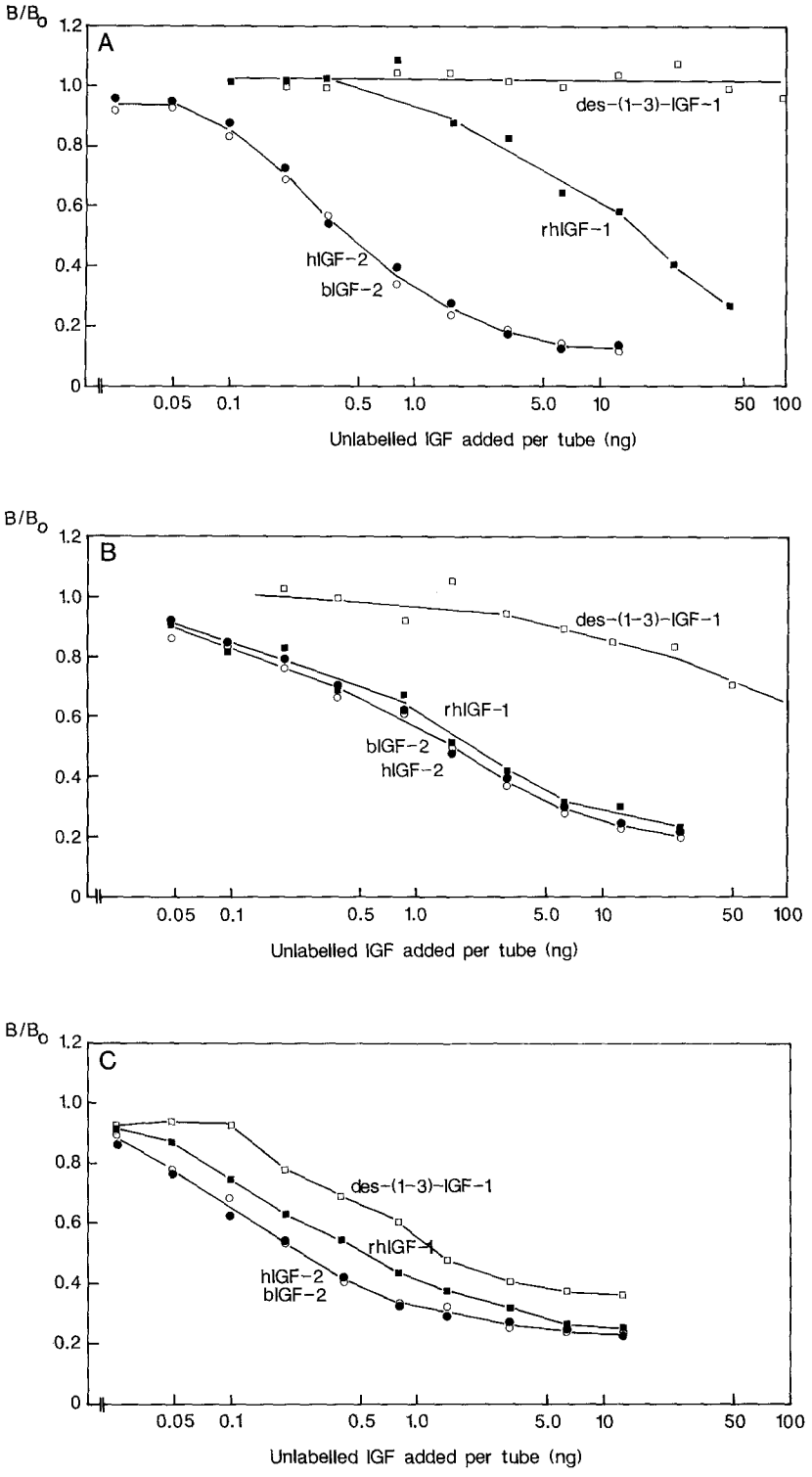

Figure 2. Competitive binding of bovine ^{125}I-IGF-2 tracer and rhIGF-1 (■), bIGF-2 (○), hIGF-2 (●) and des-(1-3)-IGF-1 (□) to three binding proteins. Competition with (A) 2.0ng MDBK BP per tube (Bo =30% total counts), (B) 2.5ng BP28 per tube (Bo =35% total counts), and (C) 1.0ng BP53 (Bo =50% total counts).

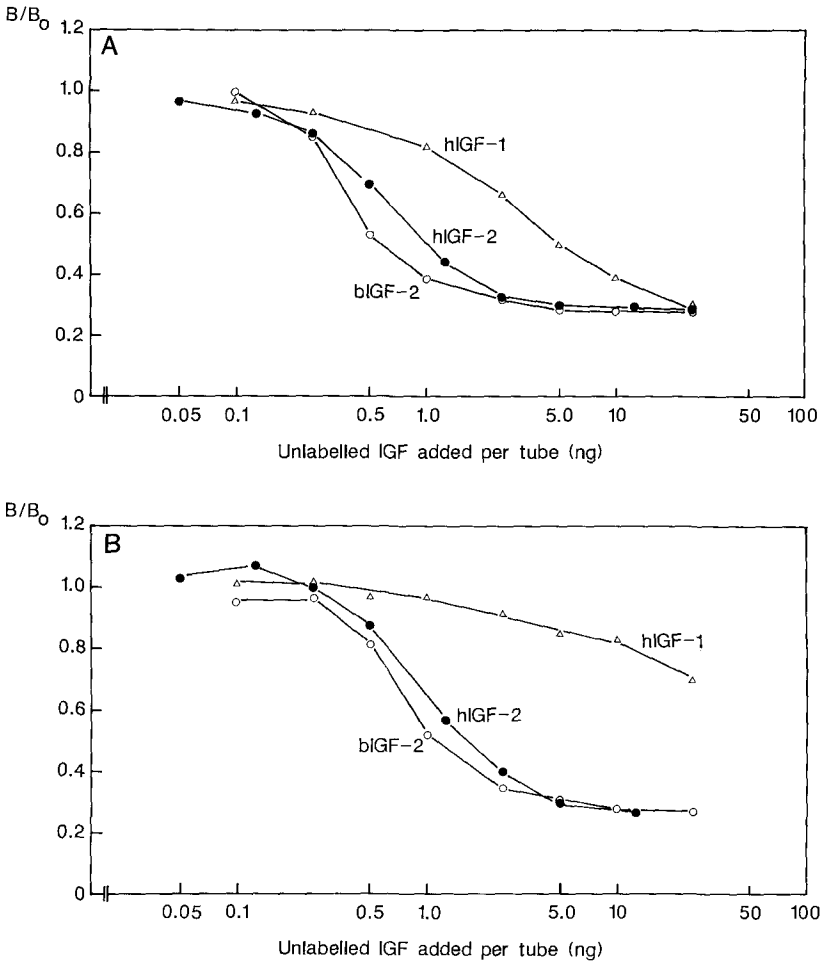

Figure 3. Competitive binding of human IGF tracers and hIGF-1 (△), bIGF-2 (○) and hIGF-2 (●) to the MDBK BP. Competition with **(A)** purified human ^{125}I IGF-1 tracer and 5.0ng MDBK BP per tube (Bo =28% total counts) and **(B)** with human ^{125}I IGF-2 tracer and 5.0ng MDBK BP per tube (Bo=46% total counts).

binding of either tracer to the MDBK BP. However, there was some competition for the binding of tracers to BP28, but up to 100 fold higher concentrations of des-(1-3)-IGF-1 than rhIGF-1 were required to achieve the same degree of competition. In contrast, the des-(1-3)-IGF-1 was able to compete for binding of both tracers to BP53 at only two to three fold higher concentrations than rhIGF-1.

DISCUSSION

We have compared the human serum BP53, human amniotic fluid BP28 and the bovine MDBK BP in a parallel study with the same batches of IGF peptides and have found distinct IGF binding patterns for each binding protein. A marked difference between the MDBK BP and the other binding proteins was evident when the binding curves were

measured with IGF-2 as radioligand. Thus rhIGF-1 competed at only high concentrations as compared to IGF-2 (Fig. 2A), while with the other binding proteins or even with the MDBK BP and IGF-1 tracer the competition curves were quite similar. This result is not compatible with the existence of a single binding site on the MDBK BP that recognises different IGF peptides with different affinities. The data can be explained either by the presence of two binding sites or by different affinities for iodinated and non-iodinated peptides. Notwithstanding which explanation proves to be valid, the effect distinguishes the MDBK BP from the other binding proteins because they do not show such a response. It is possible that this property of the MDBK BP indicates a similarity to the human cerebrospinal fluid binding protein because that form also shows a selective competition by IGF-2 only when IGF-2 is used as tracer (15,16).

The second difference in binding specificity between BP28, MDBK BP and BP53 is dramatically evident when des-(1-3)-IGF-1 is the competing ligand. This peptide competes well for the binding to BP53 of either labelled IGF-1 or IGF-2, poorly with BP28 and not at all with the MDBK BP. Taken together, these differences between the competitive binding results strongly suggest that each protein represents a distinct class of binding protein. Such an interpretation is consistent with the available sequence information which groups the BP53 from rat and human as one type (2), the BP28 as a second type (3-7) and the MDBK BP (8) with the rat BRL-3A (9) binding protein as a third type of binding protein.

The results reported here for des-(1-3)-IGF-1 indicate that the three amino-terminal residues of IGF-1 are crucial for IGF-1 binding to either class of small binding protein, but of only minor significance in the binding to BP53. Indeed, we have established that it is the removal of Glu at position 3 of IGF-1 that destroys binding to the small binding proteins (C.J. Bagley, B.L. May, L. Szabo, P.J. McNamara, M. Ross, G.L. Francis, F.J. Ballard and J.C. Wallace, unpublished observations). However, the amino-terminal region of IGF-1 must also be involved in the binding of the peptide to BP53. Thus although [Gln^3, Ala^4]IGF-1 is only slightly less effective as a competitor for labelled IGF-1 binding to total serum binding proteins (17), as would be expected from our data if BP53 accounts for most of the binding activity in serum, the additional substitution of Tyr for Gln^{15} and Leu for Phe^{16} virtually destroys binding. Moreover, the construction of insulin-IGF-1 hybrids in which the amino-terminal region of IGF-1 is replaced by the equivalent B chain of insulin, similarly prevents binding of the hybrid to the mixed binding proteins of serum (17,18).

ACKNOWLEDGMENTS: We thank Mr. G. L. Francis and Mr. C. J. Bagley for bIGF-2 and synthetic des-(1-3)-IGF-1 respectively. Support from the National Health and Medical Research Council of Australia is gratefully acknowledged.

REFERENCES

1. Martin, J. L. and Baxter, R. C. (1986) *J. Biol. Chem.* *261*, 8754-8760.
2. Baxter, R. C. and Martin, J. L. (1987) *Biochem. Biophys. Res. Commun.* *147*, 408-415.
3. Povoa, G., Enberg, G., Jornvall, H. and Hall, K. (1984) *Eur. J. Biochem.* *144*, 199-204.
4. Baxter, R. C., Martin, J. L., Wood, M. H. (1987) *J. Clin. Endocrinol. Metab.* *65*, 423-431.

5. Brewer, M. T., Stetler, G. L., Squires, C. H., Thompson, R. C., Busby, W. H. and Clemmons, D. R. (1988) *Biochem. Biophys. Res. Commun. 152,* 1289-1297.
6. Povoa, G., Isaksson, M., Jornvall, H. and Hall, K. (1985) *Biochem. Biophys. Res. Commun. 128,* 1071-1078.
7. Lee, Y.-L., Hintz, R. L., James, P. M., Lee, P. D. K., Shively, J. E. and Powell, D. R. (1988) *Mol. Endocrinol. 2,* 404-411.
8. Szabo, L., Mottershead, D. G., Ballard, F. J., Wallace, J. C. (1988) *Biochem. Biophys. Res. Commun. 151,* 207-214.
9. Mottola, C., MacDonald, R. G., Brackett, J. L., Mole, J. E., Anderson, J. K. and Czech, M. P. (1986) *J. Biol. Chem. 261,* 11180-11188.
10. Francis, G. L., Read, L. C., Ballard, F. J., Bagley, C. J., Upton, F. M., Gravestock, P. M. and Wallace, J. C. *(1986) Biochem. J. 233,* 207-213.
11. Francis G. L., Upton, F. M., Ballard, F. J., McNeil, K. A. and Wallace, J. C. (1988) *Biochem. J. 251,* 95-103.
12. Baxter, R. C. and De Mellow, J. S. M. (1986) *Clin. Endocrinol. 24,* 267-278.
13. Ballard, F. J., Francis G. L., Ross, M., Bagley, C. J., May, B. L. and Wallace J. C. (1987) *Biochem. Biophys. Res. Comm. 149,* 398-404.
14. Van Obberghen-Schilling, H. and Poussegur, J. (1983) *Exp. Cell. Res. 147,* 369-378.
15. Hardouin, S., Hossenlopp, P., Segovia, B., Seurin, D., Portolan, G., Lassarre, C. and Binoux, M. (1987) *Eur. J. Biochem. 170,* 121-132.
16. Hossenlopp, P., Seurin, D., Segovia-Quinson, B. and Binoux, M. (1986) *FEBS Lett. 208,* 439-444.
17. Bayne, M. L., Applebaum, J., Chicchi, G. G., Hayes, N. S., Green, B. G. and Cascier M. A. (1988) *J. Biol. Chem. 263,* 6233-6239.
18. De Vroede, M. A., Rechler, M. M., Nissley, S. P., Joshi, S., Burke, G. T. and Katsoyannis, P. G. (1985) *Proc. Natl. Acad. Sci. USA 82,* 3010-3014.