Insulin-like growth factor-binding proteins: functional diversity or redundancy?

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INTRODUCTION

The insulin-like growth factor-binding proteins (IGFBPs) represent a family of conserved proteins that share the ability to bind the insulin-like growth factors, IGF-I and IGF-II, with affinities comparable to their respective receptors. Unlike the transmembrane IGF receptors, the IGFBPs are secreted proteins. Although many of the IGFBPs are able to interact with cell surface membranes, there is no convincing evidence as yet that these binding sites function as signal transducers. The IGFBPs are present in the serum, virtually all biological fluids, tissue extracts and conditioned medium from a variety of cell lines. They appear to be present and conserved in lower species, suggesting a functionally important role throughout evolution.

Although the presence of IGFBPs was first suspected in the early days of somatomedin research in the 1960s, the elucidation of their nature and complexity had to await the development of molecular techniques. Six high-affinity members of this family of proteins have been identified and the cognate cDNAs for these binding proteins have been cloned from a variety of species. An additional four potential members of the IGFBP family have been identified through computer analysis of GenBank sequence data, on the basis of the presence of the IGFBP motif, GCGCCXXC (Rosenfeld 1998). These have considerably reduced binding affinity for the IGFs and, at the recent Fourth International IGF Symposium, the consensus opinion was that these proteins should be categorized as IGFBP-related proteins (IGFBP-RPs-1 to -4).

In the past decade, there has been a multitude of publications dealing with the IGFBPs. Whereas initial research focused on their role as modulators of IGF action, it is now appreciated that at least some of IGFBPs have additional IGF-independent functions. It is also important to remember that the vast majority of data concerning the biological functions of the IGFBPs have been obtained from in vitro cell culture conditions, in which concentrations of the IGFs and their binding proteins used may not match those encountered in vivo. Supraphysiological concentrations and non-physiological conditions may well account for the confusing and often contradictory results obtained in vitro: for example, both IGFBPs-1 and -3 have been shown to have stimulatory and inhibitory effects on IGF-I-induced actions in cell culture. In vivo studies of IGFBP action have been limited until recently by the lack of large quantities of purified IGFBP. With development of transgenic and knockout mouse models, some insights into the physiological role of the IGFBPs are now starting to emerge. However, the limited phenotype of the IGFBP-3-overexpressing mice (Murphy et al. 1995) and the IGFBP-2 and IGFBP-4 knockout mice (Wood et al. 1993, Pintar et al. 1997) have led to the need to reconsider the potential physiological roles of these binding proteins. In the absence of a clear understanding of the physiological role of the individual IGFBPs, some investigators have speculated that there may be considerable functional redundancy in the IGFBP family. Here, I will review some of the similarities and differences between the IGFBPs and some recent insights into the biological functions of these proteins.

STRUCTURAL CONSIDERATIONS

The amino-terminal and carboxy-terminal sequences of the IGFBPs are conserved between the various binding proteins, whereas the mid-region...
of the IGFBPs shows considerable variation between the different members of this gene family (Shimasaki & Ling 1991, Rechler 1993). Overall amino acid sequence similarity between the IGFBPs varies from 47 to 60%. IGFBPs-3 and -5 are the most similar. Secondary structure, contributed in part by the highly conserved cysteine residues located primarily at the amino- and carboxy-terminal ends, would appear to be very important in forming the molecular pocket for high-affinity binding of the IGFs. Truncated IGFBP molecules lacking the amino- or carboxy-terminal ends appear to have considerably reduced ability to bind IGFs-I and -II (Lalou et al. 1996, 1997).

The molecular structures of the genes encoding these proteins are also highly conserved, with IGFBPs-1-2, -4, -5 and -6 having four exons; IGFBP-3 has five exons (Rechler 1993). In each case, exons 1 and 4, containing the amino and carboxyl cysteines, are the most highly conserved. The additional exon in IGFBP-3 contains the unusually large 3' untranslated region that characterizes the IGFBP-3 mRNA (Wood et al. 1988). Each of the classical IGFBPs is approximately the same size consisting of 216-289 amino acids, but they are subject to varying types of post-translational modification, such as N-glycosylation for IGFBPs-3 and -4 (Wood et al. 1988, Ceda et al. 1991), O-glycosylation for IGFBP-6 (Bach et al. 1992) and phosphorylation for IGFBPs-1, -3 and -5 (Elgin et al. 1987, Frost & Tseng 1991, Hoeck & Mukku 1994, Coverley & Baxter 1997). The physiological significance of these post-translational modifications is not completely understood, but in the case of IGFBP-3 it may be important in prolonging the half-life of these molecules in the circulation. Whereas phosphorylation of IGFBP-3 does not appear to affect affinity for IGF-I (Coverley & Baxter 1997), phosphorylation of IGFBP-1 has been shown to enhance its affinity for IGF-I and may enhance the inhibitory effect of this binding protein in various in vitro situations. The latter has been reviewed extensively elsewhere (Jones & Clemmons 1995).

The affinities of the IGFBPs for IGFs-I and -II are comparable for most of the IGFBPs, with the exception that IGFBP-6 and, to a lesser extent, IGFBPs-2 and IGFBP-5, have a lower affinity for IGF-I and higher affinity for IGF-II than have the other IGFBPs (Roghani et al. 1991, Martin et al. 1990, Kiefer et al. 1992). This may be physiologically relevant, as IGFBPs-2 and -6 are abundant in cerebrospinal fluid (Roghani et al. 1991), and IGF-II is abundantly expressed in the central nervous system of many species.

The recently identified IGFBP-RPs, namely mac25 (previously known as IGFBP-7, but now more correctly referred to as IGFBP-RP-1), also called prostacyclin-stimulating factor and tumour-derived adhesion factor (Akaogi et al. 1994, Yamauchi et al. 1994, Swisshelm et al. 1995), connective tissue growth factor (IGFBP-RP-2), nov (IGFBP-RP-3) and cyr61 (IGFBP-RP-4) all share the IGFBP-binding motif and some structural homology with the amino-terminal end of the IGFBPs (Kim et al. 1997). Although there is conservation of many of the cysteine residues at the carboxy-terminal end of these proteins, structural difference in this region probably accounts for the highly reduced binding affinity these molecules have for the IGFs: it has been estimated that the affinities of these potentially new members of the IGFBPs for the IGFs are at least 100-fold less than those of the six classical IGFBPs. These proteins have been provisionally categorized as IGFBP-RP until further data are available to establish their physiological role as IGF binding proteins. The much lower IGF-binding affinity of the IGFBP-RPs suggests that their IGF binding is likely to be physiologically unimportant. However, IGFBP-RP-1 (IGFBP-7, mac25) has been found to bind insulin with high affinity – a property shared by the amino-terminal fragment of IGFBP-3 (Yamanaka et al. 1997). The discovery of these additional members of the IGFBP family may provide insights into other potential IGF-independent functions of the classical IGFBPs. It is now appreciated that many of the classical IGFBPs may be multifunctional molecules with IGF-independent functions. Indeed, the major role of some of the IGFBPs may be some as yet undiscovered IGF-independent function.

**BIOLOGICAL ACTIONS OF THE IGFBPS**

The biological actions of the IGFBPs can be considered under several categories: 1) modulation of the actions of IGF-I, IGF-II, or both, by competition with the receptor for ligand binding; 2) modulation of the actions of IGF-I, IGF-II, or both, by interacting directly with the IGF-I receptor; 3) storage and transport of IGFs; 4) interactions with other growth factor systems such as the transforming growth factor-β (TGF-β) system; and 5) IGF-independent actions on cellular proliferation and migration. These will be discussed in detail below.

**Modulation of the actions of IGF-I or IGF-II by competition with the receptor for ligand binding**

Potentially all the IGFBPs have the ability to inhibit the actions of the IGFs by reducing their
interaction with their receptors. Natural IGF variants and recombinant mutant molecules have indicated that there are subtle differences in the region of the IGF molecule that interacts with the binding proteins, compared with the receptor. For example the des (1-3) variant binds to the IGF-I receptor equally as well as the full-length molecule, whereas it has a markedly reduced affinity for the IGFBPs, indicating that the amino terminal of the IGF-I molecule is important for interaction with IGFBPs but not with the receptor (Bagley et al. 1989). However, there is no evidence that the IGF–IGFBP complex can interact with the IGF-I receptor; indeed, the converse appears to be true: that is, IGF bound to the IGFBP does not interact with the IGF receptor.

Data from a variety of in vitro cell culture experiments in which the effects of IGF-I have been examined indicate that, of the six classical binding proteins, IGFBPs-2, -4 and -6 are predominantly inhibitory, whereas IGFBP-5 potentiates IGF-I action (Jones et al. 1993a, Mohan et al. 1995). IGFBPs-1 and -3 can either inhibit or potentiate the actions of IGF-I, depending upon the cells and culture conditions used (Elgin et al. 1987, De Mellow & Baxter 1988). The growth-inhibitory effects of IGFBP-3 are easily demonstrated, but growth-stimulatory effects have also been reported; for example in PC-3 prostatic cancer cells, IGFBP-3 stimulates proliferation by a process that depends upon active proteolysis of the IGFBP-3 and the presence of IGF-I (Angelloz-Nicoud et al. 1996).

A commonly held view is that, whereas the insulin and the IGFs share a common ancestral origin derived from peptides that served both metabolic and growth promoting functions, in higher species the IGFs subserve predominantly growth promoting functions and insulin has a predominant role in regulating metabolism. As a logical extension of this hypothesis, the evolution of the binding proteins would have facilitated this differentiation of function predominantly by limiting the insulin-like metabolic activities of the IGFs. It is not clear why so many IGF binding proteins have evolved. Furthermore, this simple view does not account for the vast differences in the regulation of expression of the IGFBPs, or for the differences in the abundance and tissue distribution of the IGFBPs. Indeed, the complexity of regulation of the expression and action of the IGFBPs would indicate that these binding proteins serve diverse functions, rather than simple inhibition of IGF action.

In other highly conserved gene families such as the growth hormone–prolactin–placental lactogen family or the insulin–IGF-I–IGF-II family, individual members show tissue- or developmentally restricted expression. This often provides insight into the physiological functions of the individual members of the gene family. However, less insight into the functional role of the IGFBPs can be obtained from these types of data, as most tissues and even individual cell lines produce two or more IGFBPs. Surprisingly, cell lines derived from the same type of cancer can show differences, not only in the relative abundance of expression of individual binding proteins, but also in the regulation of expression of a particular IGFBP (Gong et al. 1992).

There does, however, appear to be some difference in the abundance of the IGFBPs in different tissues and body fluids. In serum, IGFBP-3 is by far the most abundant binding protein, with concentrations in the range of 100 nM, whereas concentrations of the other binding proteins vary from 2 to 15 nM (Mohan & Baylink 1996). IGFBP-3 is also the major binding protein in breast milk (Donovan & Odle 1994) and urine (Hasegawa et al. 1992), whereas IGFBP-1 predominates in amniotic fluid (Drop et al. 1979) and IGFBPs-2 and -6 are present in greater concentrations than IGFBP-3 in cerebrospinal fluid (Binoux et al. 1991, Baxter & Saunders 1992). In the liver, hepatocytes express predominantly IGFBP-1, but IGFBPs-2 and -4 are also expressed by these cells (Rechler 1993, Arany et al. 1994). In contrast non-parenchymal hepatic cells express IGFBP-3 (Arany et al. 1994).

In terms of serum concentrations, those of most of the IGFBPs are relatively stable, showing minimal change in response to physiological perturbations. The exception is IGFBP-1, the circulating concentrations of which show meal-related changes and are upregulated by fasting (Murphy et al. 1990, Ooi et al. 1990, Zapf et al. 1990). Circulating concentrations of IGFBP-1 are increased in a wide variety of clinical catabolic disorders, including malnutrition, poorly controlled diabetes, renal failure and AIDS wasting syndrome (Brismar et al. 1994, Frost et al. 1996, Powell 1997), and are also increased in a variety of animal models of fetal growth retardation (Unterman et al. 1990). Although multiple factors, including growth hormone, corticosteroids, glucagon, catecholamines and various cytokines (reviewed elsewhere, Lee et al. 1993, Murphy et al. 1993), insulin appears to be the major regulator. Insulin suppresses hepatic transcription via two insulin response elements in the 5' region of the gene (Suwanickul et al. 1993, Cichy et al. 1998). Protein kinase B/Akt appears to be involved in this insulin suppression of IGFBP-1.
transcription, as dominant negative PKB/Akt mutants disrupt this effect (Cichy et al. 1998). In addition, insulin enhances the clearance of IGFBP-1, but not that of IGFBPs-2 or -3, by promoting transcapillary movement (Bar et al. 1990). In man, IGFBP-1 concentrations are suppressed during oral glucose tolerance tests and show a marked stimulation after insulin-induced hypoglycaemia (Suikkari et al. 1988, 1989a, Baldwin et al. 1993). IGFBP-1 concentrations also increase during the earlier hours of the morning (Baxter & Cowell 1987) and are believed to be in some way associated with the insulin resistance that occurs at this time. The short half-life of IGFBP-1 in the circulation (Lewitt et al. 1992), is possibly due to the fact that, unlike some other IGFBPs, IGFBP-1 is not glycosylated, which therefore allows for rapid fluctuations in concentrations. Furthermore, IGFBP-1 has a Pro–Glu–Ser–Thr sequence, which is often present in proteins that have a rapid turnover (Julkunen et al. 1988).

Acute administration of IGFBP-1 to rodents can increase blood glucose (Lewitt et al. 1992), and transgenic mice that overexpress IGFBP-1 are hyperglycaemic and demonstrate glucose intolerance (Rajkumar et al. 1996). IGFBP-1 concentrations are increased in diabetic patients – even those considered to have acceptable glycaemic control (Brismar et al. 1994). In contrast, Bang et al. (1994) have reported normal IGFBP-1 in individuals with type 2 diabetes, but have suggested that the concentrations were inappropriate in view of the hyperinsulinaemia. The data derived from the transgenic mouse model generated in this laboratory (Rajkumar et al. 1996) suggest that increased IGFBP-1 concentrations may themselves contribute to disturbed glucose homeostasis in diabetic patients. The hypothesis that IGFBP-1 has a role in glucose homeostasis is supported by the observation that glucocorticoids and growth hormone – hormones known to have effects of glucose homeostasis – also regulate expression of IGFBP-1 (Luo et al. 1990, Murphy et al. 1991).

Unlike IGFBP-1 concentrations, plasma IGFBP-2 concentrations are not increased after an overnight fast and, in fasting subjects, are not suppressed by glucose infusion, which lowers IGFBP-1 (Clemmons et al. 1991). IGFBP-2 is present in greater concentrations than IGFBP-1 in plasma, but is still less than 10% of the concentration of IGFBP-3. After a prolonged fast in human subjects, a slight (1-7-fold) increase in IGFBP-2 concentrations has been observed (Busby et al. 1988, Clemmons et al. 1991). In rodents, both fasting and streptozotocin-induced diabetes markedly increase hepatic concentrations of IGFBP-2 mRNA, but appear to have minimal measurable effect on serum IGFBP-2 concentrations (Rechler 1993). The fact that IGFBP-2 concentrations are less responsive to acute metabolic changes may be due to the fact that it has a much longer half-life than IGFBP-1 in the circulation (approximately 30 h, compared with approximately 60 min; Rechler 1993). Although there is some evidence in both rodents and man that IGFBP-2 expression is regulated by insulin, it may have a less important role than IGFBP-1 in short-term glucose homeostasis; rather, IGFBP-2 may have a role in longer-term nutritional homeostasis. It is of interest that IGFBP-2 is developmentally downregulated in the rodent at about the time of weaning, when profound changes are occurring in the nutritional supply. The other IGFBPs do not appear to have a significant effect in glucose homeostasis.

Modulation of the actions of IGF-I or IGF-II by interacting directly with the IGF-I receptor

Recently, a novel mechanism whereby binding proteins may be able to modulate IGF action has been reported by Mohseni-Zadeh & Binoux (1997). They have shown, using the des (1–3) IGF-I variant, which has reduced affinity for binding to IGFBP-3, that IGFBP-3 is able to displace des (1–3) IGF-I from the IGF-I receptor in Chinese hamster lung cells. This displacement is presumably mediated by IGFBP-3 directly interacting with the IGF-I receptor. IGFBP-1 did not appear to share this action, and truncated fragments of IGFBP-3 were not effective. It remains to be determined whether IGFBP-3 has this effect at the mannose-6-phosphate–IGF-II receptor and whether other binding proteins such as IGFBP-5 share this action.

Storage and transport of IGFs

The serum concentrations of the other binding proteins are relatively stable, showing little change in response to short-term physiological perturbations. However, exercise has been reported to increase both IGFBP-3 concentrations and IGFBP-3 proteolytic activity (Suikkari et al. 1989b), possibly resulting in enhanced uptake of IGF-I into tissues. It has been suggested that the IGFBPs, particularly IGFBP-3, may function as a reservoir for the IGFs. Unlike classical hormones such as insulin, the IGFs do not appear to have significant tissue storage from which they can be released as required. The liver, which is believed to be the major site of synthesis of circulating IGFs-I and -II, does not appear to store IGFs in the same
fashion as a classical endocrine tissue. It is assumed that most of the transport of IGFs is from the circulation into peripheral tissues, and that there is little efflux of intact IGF from tissues; however, this has not been studied in detail.

When adult human serum is analysed by gel permeation chromatography under neutral conditions, the majority of IGF-I elutes as a 150–200-kDa ternary complex composed of IGF-I, IGFBP-3 and an acid-labile 100-kDa subunit. In the normal state, all the IGFBP-3 present in the circulation elutes as the 150–200-kDa complex (Martin & Baxter 1986). Under acidic conditions, the ternary complex is irreversibly dissociated and free IGF is liberated (Martin & Baxter 1986). Neutralization after acidification allows for the reassociation of IGF and IGFBP-3, but does not reconstitute the ternary complex. A smaller amount of IGF with an apparent molecular weight of 40 kDa elutes under neutral chromatography conditions, and this is believed to represent IGF-I bound to IGFBP-1, IGFBP-2, and possibly other low-molecular-weight binding proteins other than IGFBP-3. Approximately 0.5–2% of IGF-I elutes as free unbound IGF-I (Guler et al. 1989). Although IGFBP-3 is responsible for the majority of the IGF-binding capacity in the plasma, other binding proteins are present in plasma and there is evidence that the different binding proteins serve to compartmentalize the IGFs into pools with different functional half-lives and capacities. The major IGF-binding protein present in serum, IGFBP-3, is growth hormone-dependent. This growth hormone dependence appears to be mediated via IGF-I, as treatment of hypophysectomized rats with either growth hormone or IGF-I is able to restore the 150–200-kDa IGF-I complex (Zapf et al. 1989).

In serum from fetal and neonatal animals, the majority of IGFs-I and -II is associated with a 40–50-kDa complex, rather than the 150-kDa complex. In the fetal rodent, the predominant IGF-binding protein is IGFBP-2. In tissues other than the brain, the expression of this binding protein is reduced after the neonatal period, and little IGFBP-2 is detected in adult rat plasma (Donovan et al. 1989, Romanus et al. 1986).

Virtually all the IGFBP-3 in the ternary complex is saturated with IGF (Martin & Baxter 1986). IGFBP-3 is present in concentrations more than tenfold greater than those of any of the other binding proteins (Mohan & Baylink 1996). It has an affinity for the IGFs that is five- to tenfold greater than that of IGFBPs-1 and -2. However, IGFBPs-1 and -2, unlike IGFBP-3, are largely unsaturated (Lewitt & Baxter 1991). Thus IGFBP-3 may act as a relatively stable circulating reservoir of IGFs, whereas IGFBPs-1, -2, and possibly other low-molecular-weight IGFBPs present in the circulation, serve to mop up free IGF as it dissociates from this reservoir. However, there are as yet very few data that directly address the movement of IGFs between the various compartments, and whether IGF-I can enter the tissue space from each of the compartments has not been satisfactorily resolved. As there is unsaturated IGF-binding capacity in the plasma, it is not clear why any free IGF-I should be detectable. The measurement of apparently ‘free IGF’ in the circulation may be an artefact of the assays used to measure this component, although equilibrium dialysis and immunoradiometric assays yield very similar results (Nyomba et al. 1997, Frystyk et al. 1997). Alternatively, the immunoreactive IGF-I in the circulation may represent des (1–3) IGF-I, a variant that has markedly reduced affinity for most of the IGFBPs (Yamamoto & Murphy 1994).

Insight into the complexity of interdependence of the circulating binding proteins has been achieved through the detailed study of a relatively rare condition known as non-islet tumour-associated hypoglycaemia. The vast majority of non-islet tumour-associated hypoglycaemia is due to synthesis and secretion of a 15-kDa immunoreactive IGF-II-like molecule (Daughaday & Kapadia 1989). It has been suggested that there is a defect in the proteolytic processing of pro-IGF-II into mature IGF-II as a result of lack of the O-linked glycosylation of the E-domain of IGF-II (Daughaday et al. 1993). This condition also provides compelling evidence that the IGFs can function as hypoglycaemic agents, at least in pathological circumstances. The 15-kDa IGF-II most probably represents an incompletely processed pro-IGF-II, as antibodies raised against the E-domain of IGF-II recognize this molecule (Zapf 1994). In this non-islet tumour-associated hypoglycaemia, increased concentrations of ‘big IGF-II’ are associated with decreased concentrations of GH and, as a consequence, reduced concentrations of IGF-I, IGFBP-3 and the acid-labile subunit (Daughaday & Kapadia 1989). There is also evidence that tumour-derived big IGF-II, when complexed with IGFBP-3, may not be able to form the ternary complex with the acid-labile subunit (Baxter et al. 1995). This results in a decreased proportion of the IGFs present in the ternary complex and, presumably, increased concentrations of free IGFs, particularly free IGF-II, in the blood. It may be the abnormal distribution between the free and bound IGF-II, and possibly the direct suppressive effect of IGF on the counter-regulatory hormones such as glucagon and
growth hormone, rather than the absolute amount of pro-IGF-II, that is of critical importance in the development of hypoglycemia (Daughaday & Kapadia 1989, Zapf 1994).

The idea that circulating IGFs bound to IGFBPs acts as a storage reservoir of IGFs-I and -II is appealing, and may explain how IGF-I can be involved in the endocrine growth responses in addition to local growth responses in response to injury. However, there are few experimental data that directly address this storage role of the IGFBPs. IGFBP-2 null mutant mice are phenotypically normal (Wood et al. 1998). Although IGFBP-3 and IGFBP-4 null mutant mice have also been generated, the characterization of their phenotype has not been described in detail (Pintar et al. 1997). The generation of such mutant mouse models holds great potential for unravelling the complexity of function of the individual binding proteins.

In addition to their potential role as a storage reservoir for the IGFs, the IGFBPs may function to deliver IGFs-I and -II to their cell-surface receptors. Both IGFBPs-1 and -3 can potentiate the actions of IGF-I in vitro, and this is believed to be mediated through enhanced delivery of ligand to receptor. IGFBP-1 has an Arg–Gly–Asp sequence that can interact with the fibronectin receptor. IGFBP-3 has been shown to bind in a displaceable and saturatable fashion to cell-surface binding sites (Oh et al. 1993), one of which may be the type V TGF-β receptor (Leal et al. 1997). In PC-3 prostatic cancer cells, IGFBP-3 stimulates proliferation by a process that depends upon active proteolysis of the IGFBP-3 and the presence of IGF-I (Angeloz-Nicoud et al. 1996). IGFBP-5, which is abundant in bone and is able to interact with the hydroxyapatite and extracellular matrix, appears to have a predominantly potentiating effect on IGF-I action in in vitro experiments (Jones et al. 1993a, Mohan et al. 1995).

Antiproliferative effects and interaction with the TGF-β system

IGFBP-3 appears to be an important growth inhibitor in many diverse settings. Overexpression of IGFBP-3 is associated with the induction of senescence in human fibroblast cultures (Grigoriev et al. 1995) and with vitamin D-induced differentiation and growth arrest in a human osteosarcoma cell line (Velez-Yanguas et al. 1996). In these cases, simple inhibition of IGF action may be important, but some experiments have suggested that other mechanisms, such as interaction with other growth factor systems – for example, the TGF-β system or IGF-independent mechanisms – may be involved in IGFBP-3 growth inhibition.

Huynh et al. (1996) have shown that the anti-proliferative effect of antioestrogens in MCF-7 breast cancer cells is associated with increased transcription of IGFBP-3 and can be blocked with antisense oligonucleotides to IGFBP-3. Similarly, it has been demonstrated that the growth-inhibitory action of retinoic acid and tamoxifen on breast cancer cells (Huynh & Pollak 1995) and Ishikawa human endometrial cells (Karas et al. 1995) is mediated via enhanced expression of IGFBP-3, and that the growth-stimulatory effect of oestradiol on cellular proliferation is mediated, at least in part, by a reduction in expression of IGFBP-3. As these cells express low levels of IGFs-I or -II and can sequester IGFs from serum included in the culture medium, these antiproliferative effects may simply result from inhibition of IGF binding to the IGF-I receptor.

In a number of cell lines in which an increased IGFBP-3 expression is associated with reduced cellular proliferation, there is also increased TGF-β expression. For example, in human bronchial epithelial cells, retinoic acid induces growth arrest and enhances IGFBP-3 and TGF-β expression (Han et al. 1997). Although TGF-β was able to increase IGFBP-3 expression in these cells, the effect of retinoic acid appeared to be independent of TGF-β, as it could not be blocked by inactivation of TGF-β with latency-associated protein (Han et al. 1997). In a variety of cell lines, TGF-β has been shown to induce expression of IGFBP-3 and, in some circumstances, the growth-inhibitory effect of TGF-β appears to be inhibited by antisense oligonucleotides to IGFBP-3 (Martin & Baxter 1991, Oh et al. 1995, Gucev et al. 1996).

The association of IGFBP-3 overexpression with growth-arrested states in various cell lines would indicate that it is involved in some way in the inhibition of cellular proliferation. The importance of IGFBP-3 as a growth inhibitor has been confirmed by its recent identification as a p53-induced target gene (Buckbinder et al. 1995).

IGF independent actions

IGFBP-1 has been shown to facilitate migration of vascular smooth muscle cells in culture after disruption of the monolayer (Jones et al. 1993b). This action is independent of IGF-I, but requires the Arg–Gly–Asp sequence present in IGFBP-1, which interacts with the α5β1-integrin.

There are a number of clear examples in which the growth-inhibitory effects of IGFBP-3 can be explained by inhibition of IGF-I action. For
example, Schmid et al. (1991) have demonstrated that intact, but not truncated, IGFBP-3, which has reduced affinity for IGF-I, blocks IGF-I stimulation of DNA synthesis in osteoblasts. However, in addition to its ability to modulate the actions of IGF-I, IGF-independent growth inhibitory effects of IGFBP-3 have been suggested by some experiments. A growth-inhibitory effect of IGFBP-3 has been demonstrated in mouse fibroblasts derived from IGF-I receptor knockout mice (Valentinis et al. 1996). These cells are devoid of IGF-I receptors, so the growth inhibition cannot be explained by inhibition of an autocrine action of IGF-I, unless the latter mediates its effects via the insulin receptor. In this regard, the recent demonstration that a proteolytic fragment of IGFBP-3 can bind insulin (Yamanaka et al. 1997) is important.

Other experiments using serum-free media, breast and prostate cancer cell lines have demonstrated an inhibitory effect of IGFBP-3 under conditions devoid of IGFs-I and -II. In these experiments it was impossible to exclude inhibition of locally expressed IGFs (Gucev et al. 1996, Rajah et al. 1997). Further evidence for an IGF-independent growth inhibitory effect of IGFBP-3 has been provided recently by the observation that a proteolytic fragment of IGFBP-3, which does not bind the IGFs, inhibits fibroblast growth factor-induced mitogenesis in mouse fibroblasts devoid of IGF-I receptors (Zadeh & Binoux 1997). These data, obtained from the IGF-I receptor-deleted cell lines, clearly indicate that this growth-inhibitory effect is independent of IGFs-I and -II. However, the possibility remains that the growth-inhibitory effect of this IGFBP-3 fragment is due to interaction with insulin (Yamanaka et al. 1997).

In view of the fact that IGFBP-3 expression is enhanced in a variety of situations in which growth arrest occurs, and that this effect may be mediated in part by an IGF-independent function, there has been considerable interest in identifying specific receptors and signal transduction pathways that mediate this growth inhibition. Affinity cross-linking studies with radiolabelled IGFBP-3 using human breast cancer cell membranes have identified several proteins that appear to interact with IGFBP-3 (Oh et al. 1993). The interaction of IGFBP-3 with cell-surface binding sites was inhibited by exogenous IGF-I, suggesting that the IGFBP-3-IGF-I complex does not interact with IGFBP-3-specific binding sites, and that the IGFBP-3-IGF-I complex does not interact with the IGF-I receptor (Oh et al. 1995). As there is, at present, no convincing evidence that these IGFBP-3-binding sites transduce specific actions, it would be premature to classify them as ‘receptors’.

Interestingly, a recent publication reported convincing evidence that IGFBP-3 competes for binding to the type V TGF-β receptor (Leal et al. 1997). Although there is some evidence that this receptor is a transmembrane serine/threonine kinase like the other TGF-β receptors, the nature of this receptor remains unclear. Furthermore, it seems that, whereas the type I and type II receptors are necessary for TGF-β action, other TGF-β receptors may not be. Cell lines with mutated type I and type II receptors have been used to demonstrate the sequence of events involved in TGF-β action (Chen et al. 1997). TGF-β binding to the type II receptor results in phosphorylation of the occupied type I receptor, which then propagates the signal transduction cascade.

Recently, we have utilized a yeast two-hybrid system to investigate the interaction of IGFBP-3 with other proteins. Using this system we have identified a cDNA encoding latent TGF-β-binding protein 1 (LTBP-1) that interacts with IGFBP-3 (W Xu & L J Murphy, unpublished observations). LTBP-1 is a 160–240-kDa glycoprotein that is able to bind to the latency-associated protein that is non-covalently associated with mature TGF-β (Gleizes et al. 1996). It is not clear, as yet, whether binding of IGFBP-3 to the LTBP-1 could release TGF-β or vice versa. The type V TGF-β receptor has not been cloned, therefore the relationship between LTBP-1 and the type V TGF-β receptor remains unclear.

An additional mechanism by which IGFBP-3 may mediate a biological response involves the interaction of IGFBP-3 with nuclear proteins involved in gene activation. A putative nuclear localization signal, KKGFYKKQCRPSKGRK, is present in residues 215–232 of the human IGFBP-3 sequence (Radulescu 1994). IGFBP-3 has been localized to the nucleus of both kidney and lung cells and is possibly involved in the transport of IGF-I into the nucleus (Jacques et al. 1997, Li et al. 1997). The nature of the interaction of IGFBP-3 with nuclear components has not been elucidated. For example, does IGFBP-3 bind chromatin or non-chromatin proteins? Does it interact with specific DNA sequences, or simply interact with other proteins involved in gene regulation? Does it interact with specific nuclear transcription factors? A number of laboratories are preparing to investigate these questions.

**FUTURE DIRECTIONS IN BINDING PROTEIN RESEARCH**

Although we have learnt a considerable amount about the IGFBPs since their presence was first
suggested more than 30 years ago, the recent observations have raised further questions about their functional role as inhibitors of IGF action. Clearly, they have more important physiological actions than simple inhibition. The accumulating evidence would suggest that IGFBP-1 has a role in glucose homeostasis most probably mediated by its ability to modulate the concentrations of free IGF-I in the circulation. The recent demonstration that IGFBP-3 interacts with components of the TGF-β growth factor system indicates that this molecule may have evolved as a multifunctional inhibitor of cellular proliferation, mediating its actions through a variety of growth factor systems and mechanisms. The suggestion that IGFBP-3 has IGF-I-independent antiproliferative effects and that it interacts with specific binding sites has led to a considerable amount of controversy.

The development of null mutant mouse models, together with conditional knockout and targeted overexpression of the individual IGFBPs in specific tissues, is likely to provide insights into the functions of the IGFBPs. Like the investigations of the past decade, future experiments in the IGFBP field are likely to result in exciting and unexpected observations.

ACKNOWLEDGEMENTS

This work was supported by a grant from the Canadian Diabetes Association in honour of Edward C Cornes, and by the Medical Research Council of Canada. L J M is a recipient of an endowed Research Professorship in Metabolic Diseases.

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Insulin-like growth factor-binding proteins


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RECEIVED 6 April 1998