Role of Insulin-Like Growth Factor Binding Proteins and Their Proteases

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Key words: GH, regulation, osteoblasts, IGF action

Introduction

Insulin-like growth factor-I (IGF-I) and IGF-II are the two most abundant polypeptide growth factors known to circulate in human plasma. IGFs were previously known by several names which include somatomedins, non-suppressible insulin-like activity, multiplication stimulating activity and sulfation factor (1). Although IGFs are structurally related to insulin, they differ from insulin in that IGFs circulate in plasma complexed to a family of structurally related binding proteins, the IGF binding proteins (IGFBPs). Although the existence of IGFBPs in circulation was suspected more than thirty years ago, it was only recently that the six known IGFBPs were cloned and sequenced (2-4). The principal focus of this review is to characterize IGFBPs in serum and other biological fluids and evaluate the serum regulation and functions of IGFBPs and their proteases.

General Characteristics of the IGFBPs

All six IGFBPs identified to date share structural homology with each other and specifically bind the IGFs, having negligible affinity for insulin. The amino terminal and carboxy terminal regions of IGFBPs are cysteine-rich and have substantial sequence homology (50-80%) within and between species. There are 18 cysteines in rat and human IGFBP-1, -2, -3 and -5 and their locations in the molecules are conserved (Fig. 1). Among the 18 cysteines, 12 are located at the N-terminal region of the molecule and the remaining 6 are present in the C-terminal region. IGFBP-4 contains two extra cysteine which are located in the variable middle region of the molecule while IGFBP-6 contains 2 and 4 less cysteine respectively in the rat and human species (2-4). Additional characteristics which distinguish the various IGFBPs include glycosylation, RGD sequence, phosphorylation, extracellular matrix and hydroxyapatite binding (2). The IGFBPs are capable of inhibiting or enhancing IGF effects and may even have ligand-independent effects (discussed later). Some of the IGFBPs bind both IGF-I and IGF-II with equal affinity while others bind IGF-II with preferential affinity. The functional significance of differences in IGF binding affinity of various IGFBPs is not known at this time.

IGFBPs in Serum and Other Body Fluids

In serum most of the IGFs (about 75%) cir-
IGFBP Structure

Fig. 1 IGFBP structure. IGFBP genes contain 4 exons and 3 introns. Exon 1 encodes for all of the conserved cysteine rich amino terminal region and part of the variable region, while exon 2 encodes the rest of the middle variable region of the IGFBP. Exon 3 and 4 encode for conserved cysteine rich carboxy terminal region of the IGFBP.

culate as a 150-200 kDa complex while the remaining IGFs circulate as 50 kDa complex (about 25%). Only a small amount of IGFs (less than 1%) circulate as free form (5-7). The 150 kDa complex consists of 7.5 kDa IGF-I or IGF-II plus 38-43 kDa IGFBP-3 and a 80-90 kDa non-IGF binding acid labile component termed acid labile subunit (ALS). With regard to the mechanism for formation of 150 kDa complex, Baxter and coworkers (5, 8, 9) have proposed that ALS does not bind to IGFBP-3 unless IGF-I or IGF-II is bound to either IGF-I or IGF-II. The estimated affinity constant for the formation of IGF-IGFBP-3 complexes (2-3 x 10^{-10} M) is considerably higher than the affinity of ALS for IGF-IGFBP-3 dimers (5 x 10^{-8} M). The question of whether ALS can form a binary complex with IGFBP-3 in the absence of IGF ligand, however, is controversial at this time based on recent reports. Bareca et al. (10) have demonstrated that incubation of recombinant human IGFBP-3 and ALS produces the appearance of a complex in the 150 kDa region in the absence as well as in the presence of IGF peptide. In addition, Rechler and coworkers have demonstrated two different IGFBP-3 complexes in the 150 kDa fraction of adult rat serum, one which binds IGF-I and -II with similar affinity and the other complex contains proteolytically nicked IGFBP-3 which has greater affinity for IGF-II (11). Thus the question of whether IGF is required for the formation of a complex between IGFBP-3 and ALS remains controversial.

With the development of improved RIAs and validation techniques for the various IGFBPs, it is now possible to measure the concentrations of IGF-I, IGF-II and their binding proteins in circulation. IGFBP-3 is the predominant form present in serum. Although other small molecular weight binding proteins add up to a total of 50% of that of IGFBP-3 concentration (12), they carry only 25% of IGFs compared to 75% by IGFBP-3. The concentrations of the small molecular weight
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binding proteins is found in increasing order (IGFBP-4>IGFBP-5>IGFBP-2>IGFBP-6>IGFBP-1) in human serum (12). There is a 50% molar excess of IGFBPs over IGFs in serum which implies that a very small percentage of the IGFs remain in the free form.

Of the two IGFs, the concentration of IGF-II is about 3 fold greater than that of IGF-I in adult human serum. There are a number of potential explanations why the concentration of IGF-II is more than that of IGF-I in human serum which include: 1) The production rate of IGF-II may be higher than that of IGF-I (2-4). 2) Three IGFBPs with higher affinity for IGF-II than IGF-I are present in human serum (2-4). 3) Binary complexes of proteolytically nicked IGFBP-3+ALS bind IGF-II with preferential affinity (11). These three mechanisms are not mutually exclusive and may together contribute to the observed differences in serum levels of IGF-II versus IGF-I in human serum. In any case, the finding that IGF-II circulates in great abundance in human serum and that IGF-II is produced by several human adult tissues is consistent with an important role for IGF-II in adult human physiology.

Besides serum, IGFBPs have been identified in a variety of body fluids including amniotic fluid, follicular fluid, cerebrospinal fluid, seminal fluid and milk (2). Characterization of the types and amounts of different IGFBPs present in various body fluids reveal evidence that extracellular fluids of certain tissues are enriched with specific IGFBPs. For example, IGFBP-1 is present in amniotic fluid in concentrations 100-500 fold higher than that found in the serum. IGFBP-2 and IGFBP-6 are present in high concentrations in cerebrospinal fluid while IGFBP-3 is the most abundant IGFBP present in follicular fluid (Table 1). Although the enrichment of various body fluids with one or more IGFBPs is consistent with the idea that IGFBPs may function locally to regulate IGF actions, further work is needed to verify the role of IGFBPs in various biological fluids.

Table 1 Distribution of IGFBPs in biological fluids

<table>
<thead>
<tr>
<th>Fluid/Tissue matrix</th>
<th>Major IGFBP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>IGFBP-3</td>
</tr>
<tr>
<td>Amniotic fluid</td>
<td>IGFBP-1</td>
</tr>
<tr>
<td>Follicular fluid</td>
<td>IGFBP-3</td>
</tr>
<tr>
<td>Cerebrospinal fluid</td>
<td>IGFBP-2, IGFBP-6</td>
</tr>
<tr>
<td>Bone matrix</td>
<td>IGFBP-5</td>
</tr>
</tbody>
</table>

IGFBP Proteases

IGFBP proteases are capable of cleaving the IGFBPs into forms that have either significantly reduced or no affinity for IGFs. Therefore, the biological response to IGFBPs may vary according to the presence or absence of IGFBP proteases in the local body fluid. One of the first identified proteases was a pregnancy associated IGFBP-3 specific protease that was active from the second trimester of pregnancy in humans (13, 14). The increased IGFBP-3 protease activity is also seen commonly during the catabolic state of an individual. For example, IGFBP-3 specific protease has been detected in patients with severe illness and in a catabolic state and negative nitrogen balance (15). Another example of a catabolic state is seen in acute and chronic renal failure patients. These patients showed an increase in urinary IGFBP-3 specific protease and this was found to be responsible for the complete absence of intact IGFBP-3 in the urine (16). Significant proteolytic activity of IGFBP-3 protease was observed in acute diseases of childhood such as Kawasaki disease, bacterial meningitis and mycoplasma pneumonia (17). In addition to IGFBP-3 protease in serum, amniotic fluid contains a IGFBP-1 specific protease and cerebrospinal fluid a IGFBP-2 protease. Thus, a number of IGFBP proteases have been identified from body fluids collected from a variety of pathophysiological situations, however, the physiological role of these
proteases remains to be determined.

Regarding the source of various IGFBP proteases in serum and other biological fluids, recent studies demonstrate a variety of cell types in culture secrete proteases capable of degrading IGFBPs. Some of these proteases are relatively specific to a given IGFBP while others are relatively less-specific. For example, bone cells in culture produce matrix metalloproteases and plasmin which degrade multiple IGFBPs as well as other proteins (18). In addition, human bone cells as well as fibroblasts in culture produce proteases that are relatively specific to IGFBP-5 (19, 20). The identification of IGFBP protease activities in the conditioned media of various cell types and in sera or body fluids of patients with a variety of pathophysiologic conditions and the recent findings that the rate of IGFBP proteolysis can be regulated by a variety of local and systemic factors suggest an important role for IGFBP proteases in regulating the level and thus the activity of IGFBPs in the local area.

One of the important modulators of IGFBP protease activity is the IGFs themselves. Porcine ovarian granulosa cells and fibroblasts in culture secrete serine-specific protease capable of degrading IGFBP-3 (21). The addition of IGF-I or IGF-II to these cultures greatly attenuated the degradation of IGFBP-3. Similarly, IGFBP-5 proteolysis is inhibited by the addition of IGFs in a variety of cell culture model systems (19, 22, 23) including fibroblasts, osteoblasts and granulosa cells (Table 2). In contrast to IGFBP-3 and IGFBP-5 proteolysis, IGFs stimulate IGFBP-4 proteolysis (19, 24). Studies on the mechanisms by which IGFs increase IGFBP-4 proteolysis revealed that addition of IGFs to cell-free conditioned medium increased IGFBP-4 proteolysis. Since IGFBP-4 proteolysis is not induced by the addition of insulin, des(1-3)IGF-I or des(1-6) IGF-II which bind IGFBP-4 at extremely low affinity, it has been proposed that the binding of IGF to IGFBP-4 may enhance the susceptibility of IGFBP-4 to proteolytic degradation (25). In contrast to these findings, we found that exogenous addition of IGF-II to cell free U2 human osteosarcoma cell conditioned media had no significant effect on IGFBP-5 proteolysis (26). Similarly, Nam et al. (20) have reported that addition of IGF-I or IGF-II caused minimal inhibition of proteolysis in cell free human fibroblast conditioned media. Based on these findings, two conclusions can be made: 1) IGFs may function to regulate IGFBPs as well as vice versa. 2) The mechanisms by which IGFs regulate IGFBP-4 and IGFBP-5 proteolysis are different.

Regarding the identity of IGFBP proteases present in various biological fluids, IGFBP protease activity has been attributed to a number of purified proteins including prostate-specific antigen, 7S nerve growth factor, plasmin, matrix metalloprotease and cathepsins (27-30). Some of these proteins degrade multiple IGFBPs although with different potencies. Studies on the specific activity, protein specificity and regulation of the various proteases are required in order for us to understand the functional role of these proteases in regulating IGFBP levels in a given tissue.

### Table 2 Effects of IGFs on IGFBP proteolysis

<table>
<thead>
<tr>
<th>IGFBP</th>
<th>IGF-I</th>
<th>IGF-II</th>
<th>Insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGFBP-1</td>
<td>Not known</td>
<td>Not known</td>
<td>Not known</td>
</tr>
<tr>
<td>IGFBP-2</td>
<td>Increase</td>
<td>Increase</td>
<td>No effect</td>
</tr>
<tr>
<td>IGFBP-3</td>
<td>Decrease</td>
<td>Decrease</td>
<td>No effect</td>
</tr>
<tr>
<td>IGFBP-4</td>
<td>Increase</td>
<td>Increase</td>
<td>No effect</td>
</tr>
<tr>
<td>IGFBP-5</td>
<td>Decrease</td>
<td>Decrease</td>
<td>No effect</td>
</tr>
<tr>
<td>IGFBP-6</td>
<td>Not known</td>
<td>Not known</td>
<td>Not known</td>
</tr>
</tbody>
</table>

**Regulation of IGFBP Levels**

If IGFBPs play an important role in regulating the actions of IGFs, then the levels of various IGFBPs should be regulated during various physiological and pathological situations. Recent studies demonstrate that various conditions including development, aging, pregnancy and
Table 3 Major regulators (hormonal and local) of IGFBP production

<table>
<thead>
<tr>
<th>Regulator</th>
<th>Major Regulators</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGFBP-1</td>
<td>Insulin, glucagon, glucocorticoid</td>
</tr>
<tr>
<td>IGFBP-2</td>
<td>Growth hormone, glucose, nutrition</td>
</tr>
<tr>
<td>IGFBP-3</td>
<td>Growth hormone, IGFs, glucocorticoid, TGFβ</td>
</tr>
<tr>
<td>IGFBP-4</td>
<td>PTH, Vitamin D₃, interleukin 1, prostaglandin</td>
</tr>
<tr>
<td>IGFBP-5</td>
<td>IGFs, glucocorticoid, BMP, progesterone</td>
</tr>
<tr>
<td>IGFBP-6</td>
<td>Retinoic acid, glucocorticoid</td>
</tr>
</tbody>
</table>

nutritional and hormonal status modulate the level of one or more IGFBPs in serum (31-33). For example, growth hormone deficiency is associated with increased serum levels of IGFBP-1 and decreased serum levels of IGFBP-3. Similarly, serum IGFBP-1 levels are increased while serum IGFBP-3 levels are decreased in patients with insulin-dependent diabetes. Serum levels of various IGFBPs have also been altered during renal failure. In addition to these pathological situations, serum IGFBP levels also change during normal physiological conditions such as exercise and nutrition. IGFBP levels have been reported to change during puberty as well as during aging (12).

The major regulators (effectors) of IGFBP synthesis appear to be different for the six IGFBPs (Table 3). Insulin appears to be the major regulator of IGFBP-1 production while IGFBP-2 production is regulated by growth hormone and nutrition (34-36). IGFBP-3 production is regulated by growth hormone and IGFs while IGFBP-4 production is regulated by PTH, 1,25 dihydroxyvitamin D₃ and interleukins (37, 38). IGFBP-5 production is regulated by IGFs, glucocorticoids, bone morphogenetic proteins and progesterone while IGFBP-6 production is regulated by retinoic acid (39-42). Based on the evidence that the various IGFBPs are regulated differently, it can be speculated that each of the IGFBPs may have specific roles in modulating the activity of IGFs in the target tissues.

The mechanisms which regulate the level of IGFBPs are complex involving both transcriptional and posttranscriptional (43). For example, IGFBP-1 production is regulated by insulin mainly at the transcriptional level in liver cells (2). There is evidence that IGFBP-3 levels in the conditioned medium of various cell types are regulated by mechanisms involving alterations in both synthesis and degradation (43). Similarly, the increase in IGFBP-5 level in human osteoblast cell conditioned medium by IGFs and bone morphogenetic protein-7 in human osteoblasts is regulated by both decreased proteolysis and increased gene transcription (42). Thus, some effectors appear to modulate the IGFBP level by regulating one control mechanism (e.g. proteolysis) while other effectors appear to modulate the IGFBP levels by regulating multiple control mechanisms (e.g. gene transcription, mRNA processing, mRNA stability, proteolysis). This complexity of IGFBP regulation may provide the required flexibility for modulating IGF actions by multitude of systemic and local effectors in various tissues.

**Functions of IGFBPs in Serum**

IGFs are present in the circulation and throughout the extracellular space in high concentration to provide a readily available reserve for their function as endocrine hormones. This function of IGFs as endocrine hormones is feasible because of the presence of IGFBPs. In addition to the endocrine effects, IGFs may func-
tion locally in a variety of cell types including brain, muscle, kidney, lung, pancreas and bone. Because IGFBPs can modulate the biological activity of IGFs both in a positive and negative manner and because IGFBPs are differentially regulated in various tissues, IGFBPs may also play a central role in the local regulation of IGF actions. More recently, it has been suggested that IGFBPs may also have a regulatory role independent of the IGFs (i.e. act through their own cell surface receptors). The potential role of IGFBPs in modulating the endocrine and local actions of IGFs are discussed below.

**Endocrine Actions**

1. To increase the half-life of IGFs: One of the important functions of plasma IGFBPs is to increase the half-life of IGFs in circulation and for this reason majority of IGFs circulate as 150 kDa complex. Both IGF-I and IGF-II when injected into hypophysectomized rats disappear in approximately 20-30 min, while in normal rats the half-life is extended to about 15-20 h (6). This difference is due to the fact that the majority of IGFs circulate in serum as 150 kDa ALS+IGFBP-3+IGF ternary complex as described previously. The stabilization of IGFs thus depend mainly on IGFBP-3, with other IGFBPs having minor role in this function.

2. To prevent insulin-like effects: The mean IGF concentration in human serum is about thousand fold higher than that of insulin. Despite the fact that insulin-like activity of IGFs is only 5% of insulin, the IGFs could in theory contribute 50 times more insulin-like activity than insulin alone, because of their abundance (5). However, this does not occur because the activity of IGFs is largely neutralized as a consequence of binding to the IGFBPs. Thus without the IGFBPs, the effect of IGFs would swamp any effect of insulin alone, and because IGF expression is not tightly controlled by blood glucose levels, blood glucose homeostasis would not be possible. Thus, by increasing the half-life of IGFs and protecting it from being rapidly degraded, IGFBP-3 alters the ratio of free IGF to bound IGF, which in turn regulates the metabolic actions of IGFs. The importance of 150 kDa complex in preventing the insulin-like effects of IGFs is evident in the case of hypoglycemia associated with nonislet cell tumors in which the formation of 150 kDa IGF complex is impaired (44-46). The hypoglycemic state in these patients is associated with an increase in serum level of pro IGF-II, a decrease in the circulating level of 150 kDa complex and a corresponding increase in the circulating level of 50 kDa complex. The altered distribution of IGFs between the 150 kDa and 50 kDa complexes is likely to be due to the failure of tumor-secreted pro-IGF-II to form a complex with ALS and IGFBP-3. The increase in 50 kDa IGF pool increases the bioavailability of IGFs (because 50 kDa and not the 150 kDa IGF complex can cross the vascular endothelium) to produce insulin-like effects in the target tissues. The association between decreased 150 kDa complex and glucose level in the serum of tumor induced hypoglycemia emphasizes the central role of 150 kDa IGF complex in glucoregulation (44).

3. To control the transport of IGFs from the vascular space into tissue space: The transportation of serum IGFs from the vasculature into the tissue space is necessary in order for IGFs to elicit hormonal growth stimulating responses. For this to be accomplished the IGFBPs bound to the IGFs in circulation have to be either degraded or should be able to cross the endothelial barrier. The majority of IGFs exist as ALS-IGFBP-3-IGF complex which does not cross the vascular barrier. However, IGFBPs 1-6 can cross the endothelial barriers in vivo (2, 3). The ability of these IGFBPs to cross the endothelial barriers intact, makes them important as transporters of IGFs to target tissues. Subsequent proteolysis of IGFBP in the target tissues may lead to an increase in the amount of IGFs to exhibit growth promoting effects. Based on the
above mentioned discussion, two mechanisms appear to be essential to increase the availability of free IGFs to local tissues: 1) Release of IGFs from 150 kDa complex by disruption of ALS+IGFBP-3+IGF complex (e.g. proteolysis of IGFBP-3), 2) Release of IGFs from 50 kDa inhibitory IGFBP+IGF complex by disruption of this complex (e.g. proteolysis of inhibitory IGFBP-4).

**Local Actions**

1. **To modulate local IGF actions:** IGFBPs have been shown to modulate IGF actions both in a negative and positive manner depending on culture conditions and cell type studied. Several IGFBPs including IGFBP-1, IGFBP-2, IGFBP-4 and IGFBP-6 have been shown to inhibit IGF action by binding to IGFs and preventing the binding of IGFs to IGF receptors (3). IGFBP-3 has been shown to both inhibit and potentiate IGF actions while IGFBP-5 has been shown to be stimulatory for a variety of cell types (2, 3, 37, 47). Thus it is clear that the different binding proteins modulate IGF action differently and the same binding protein can have both an IGF inhibiting and potentiating role under different conditions. The known factors that determine these differences include phosphorylation, proteolysis, cell surface association and ratio of IGF:IGFBPs.

2. **To facilitate storage of IGFs in extracellular matrices:** Another important role of IGFBPs is to help in the storage of IGFs. IGFs stored in extracellular matrices such as bone reveal that this is due to the role of IGFBP-5 in binding to the hydroxyapatite, which in turn fixes IGFs to the bone (48). This site-specific role of IGFBP-5 in fixing IGFs to bone cells, may help in promoting osteoblastic activity to replace bone lost following the phase of osteoclastic bone resorption. Similarly, IGFs stored in extracellular matrices of other tissues may have a role in wound healing.

3. **To exert IGF-independent effects of IGFBPs:** A layer of complexity is added by the ability of at least some of the IGFBPs to act in an IGF-independent manner. IGFBP-1, for instance, has been shown to stimulate smooth muscle cell migration possibly by binding to integrin receptors (49). IGFBP-3 and -5 have also been shown to inhibit or promote cell proliferation possibly through putative cell surface binding sites (47, 50, 51). These data support the unique possibility that locally produced IGFBPs may modulate their effects on target tissues depending on the local needs by not only regulating the IGF actions, but also by having IGF-independent effects on target tissues. Further experimental evidence is needed to verify this mode of IGFBP action.

**Acknowledgements**

This work was supported by funds from National Institutes of Health (AR31062), Veterans Administration, and the Departments Medicine and Nutrition, Loma Linda University.

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