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What's new in the IGF-binding proteins?

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Abstract

Since their initial discovery over 25 years ago as IGF carrier proteins, the insulin-like growth factor binding protein (IGFBP) family has grown to six members, ranging in size from 216 to 289 amino acids. The assumption over the years has been that this family of proteins, having higher affinities for IGF-I and IGF-II than does the IGF-IR, serves to block access of these ligands to the receptor. Although the need for such regulatory proteins is consistent with the constitutive secretion of IGFs from many cell types, it is not surprising that additional functions have begun to be uncovered for these proteins. This review will examine new and old actions of the IGFBPs from a biochemical and cell biological perspective.

Keywords

Insulin-like growth factor (IGF); IGF binding protein (IGFBP); IGF-I receptor (IGF-IR); Binding site; Structure–function

1. Historical introduction and overview

Over the last few years we have witnessed a renewed interest in the chemistry and biology of the IGFBPs. The new awareness of these proteins stems from numerous reports demonstrating that they exhibit a multitude of direct cellular actions that appear to be separate from their roles as IGF carrier proteins. A number of observations, which could not be explained on the basis of the paradigm of IGFBP action modulating IGF availability and function, led to the hypothesis that some of the IGFBPs have their own intrinsic biological activities, independent of their ability to interact with IGF-I and IGF-II. Considerable evidence has accumulated suggesting that IGFBPs directly regulate several cellular functions. What has been distilled from these studies is a broad description of both stimulatory and inhibitory actions for a given IGFBP. An in depth analysis of the literature encompassing the complete range of IGFBP actions is beyond the scope of this brief review, and the interested reader is referred to a number of recent summaries on the subject [1–4]. The focus of this mini-review will be limited to a discussion of recent advances in our understanding of IGFBP structure–function relationships, with emphasis on both new developments and new questions that have arisen in the field.

2. IGFBP structure: will the real IGF-binding domain please stand up?

The IGFBPs comprise a family of six soluble proteins ranging in length from 216 to 289 amino acids (Fig. 1). Based on their primary structures, each IGFBP may be divided into

three distinct domains of approximately equal size. The NH₂- and COOH-termini are highly conserved and contain 16–18 spatially conserved cysteine residues that form the various intra-domain di-sulfide bonds [1,5]. The middle or linker domain is the least conserved region of the protein family, exhibiting <30% primary sequence identity across the six IGFBPs [1]. Each IGFBP contains a GCGCCXXC motif and a CWCV sequence within their NH₂- and COOH-termini, respectively. Implicit in analysis of sequence homology has been the assumption that IGFBPs exhibit similar tertiary structures, including a “universal” IGF-binding domain.

Despite clues from structural homology among the six IGFBPs, the actual identification of the IGF binding domain has remained a subject of intense ongoing research and debate. Accumulating evidence indicates that sequences and residues important for IGF binding may vary across the IGFBP family. Based on investigation of mutations, truncations, and modifications of residues and regions of the NH₂- and COOH-termini, both domains have been implicated as participants in IGF binding (Fig. 1). Evidence supporting the NH₂-terminus as the site of IGF binding is based on studies using IGFBP fragments containing this region [6–8] and on the experimental determination of loss of IGF binding activity when the NH₂-terminal domain is deleted [9]. In addition, the crystal and NMR structures of an NH₂-terminal IGFBP-5 fragment as well as hydrophobic/hydrophilic interaction studies have strengthened the case for the NH₂-terminal region as the critical IGF binding domain for IGFBP-5 and IGFBP-3 [10–14]. In contrast, equivalently strong evidence exists supporting an essential role for the COOH-terminal domain for IGF-binding. COOH-terminal containing IGFBP peptides exhibit varying affinities for IGFs [15], while COOH-terminal deletion mutants typically show loss of binding activity [5,7]. When all of these data are considered, it appears that neither the NH₂- or COOH-terminus separately embodies the full, physiologically critical IGF binding domain. Rather, both regions contribute to high affinity IGF binding as independently organized segments that are spatially coordinated by the linker domain into the IGF binding site [9,16–18].

What falls out of this discussion is the question of whether one can generalize among the six IGFBPs in discussing structure–function attributes of the IGF binding pocket. For IGFBP-2, several studies indicate that its binding site is located primarily within the COOH-terminus [5,15,19,20], yet Carrick et al. [17] recently provided evidence identifying binding determinants in both the NH₂- and COOH-termini of bovine IGFBP-2. In contrast, for IGFBP-3 both the NH₂-[10–14] and COOH-termini [21,22] have been implicated in IGF binding. Importantly, the linker region has never been cited as part of the IGF-binding domain for any IGFBP. Rather, it is the site of proteolysis, of post-translational modifications, and perhaps also functions as a key region mediating IGF-independent actions.

As an initial step in defining a minimal high affinity IGF-binding fragment for IGFBP-2 we performed photoaffinity-labeling studies to identify contact sites for IGF-I. Using two different Gly-1 [1] derivatized IGF-I photoprobes we labeled two COOH-terminal segments, one located between residues 266–287, and the other at residues 212–227 (Fig. 1) [19]. These sites are on either side of the CWCV motif and flank the domain identified as essential for COOH-terminal binding by Forbes et al. [5]. Based on the collective findings from other laboratories and our results, we proposed a model to describe the molecular interactions between IGF-I and IGFBP-2 (Fig. 2) [19]. Based on the premise that both the NH₂- and COOH-termini of IGFBP-2 interact with ligand [17], as observed for IGFBP-3 [22–24], this model suggests that the COOH-terminus of IGFBP-2 contains a high affinity binding site for the NH₂-terminus of IGF-I (i.e., the IGFBP-binding domain [20]), and that the NH₂-terminus contains a lower affinity binding site for the IGF-IR-interacting region of IGF-I. This model departs from the proposed organized of IGF-I binding domains on

IGFBP-5, in which the receptor-binding region of the ligand faces away from the IGFBP [10,11], but the model provides a testable hypothesis of how IGFBP-2 inhibits IGF-I action that may be applicable to other IGFBPs (Fig. 2). It also raises the question, if both the NH₂- and COOH-termini of IGFBP-3 are involved in binding IGF-I [22–24], why does mutation of NH₂-terminal residues (Fig. 1, [12–14]) completely abrogate binding? Additional work, including solution of the three-dimensional structures of all six IGFBPs, should help resolve these questions.

To determine if residues downstream of the CWCV motif of IGFBP-2 contribute to IGF-binding, we have used the tryptophan-specific cleavage reagent, BNPS-skatole, coupled with the generation of equivalent recombinant truncation mutants. Preliminary results revealed that both the distal COOH portion of IGFBP-2 removed by BNPS-skatole and the remaining NH₂-terminal fragment (1–248) retain IGF binding activity [25,26].

3. Analysis of IGF-I:IGFBP interactions

Ligand blotting is a rapid, qualitative, and at least semi-quantitative technique for studying the IGFBPs [27]. This method additionally provides a means of examining “biologically relevant” IGFBPs, as misfolded, degraded or denatured proteins will not be detected. Few changes have been introduced into this method since its inception in 1986. A number of laboratories have applied the use of substituted biotinylated IGF-I or IGF-II in place of ¹²⁵I-labeled ligand, coupled with detection using an avidin-horseradish peroxidase conjugate [28–30]. This improvement has the potential to increase the sensitivity of this method; however, well-defined biotinylated IGF-I isoforms have not been described. Toward this end we have synthesized, purified, and characterized four distinct monobiotinylated IGF-I species, as well as tetrabiotinylated IGF-I in ligand blot, competition binding, and Biacore analyses [31,32]. The four species examined, N^αGly¹biotin-IGF-I, NH₂^eLys²⁷biotin-IGF-I, and a mixture of N^eLys⁶⁵biotin-IGF-I and N^eLys⁶⁸biotin-IGF-I, have a biotin present within the IGFBP-binding domain of IGF-I, within the IGF-IR-binding domain, and outside both of domains, respectively (Fig. 3). All monobiotinylated IGF-I forms have nearly the same affinities for IGFBP-2 and IGFBP-3 as native IGF-I based on competition binding analyses. In contrast, N^αGly¹biotin-IGF-I and N^eLys²⁷biotin-IGF-I yielded extremely weak signals in ligand blot assays, probably secondary to participation of these regions in IGF-I in IGFBP contact, rendering them inaccessible to avidin-peroxidase, while NH₂^eLys^{65/68}biotin-IGF-I displayed an equivalent signal to that exhibited by tetrabiotinylated-IGF-I, as this domain is not involved in growth factor-IGFBP contact [31,32].

4. IGFBP function: IGF-dependent vs. independent actions

Based on the exquisitely high affinities of IGF-I and IGF-II for IGFBPs, which exceeds the affinity toward the IGF-IR, the IGFBPs have been viewed as proteins involved in the sequestration of ligand away from the IGF-IR. Over the last few years, this concept has changed with the emerging idea that IGFBPs have additional activities that are independent of their IGF binding [33]. A variety of sometimes paradoxical and conflicting biological effects have been reported, including both stimulation and inhibition of cell migration, and enhancement of cell growth or cell death, making it difficult to reach a consensus.

IGFBP-1 has been shown to stimulate cell migration by engaging the α₅β₁ integrin (fibronectin receptor) via its COOH-terminal RGD motif [34,35]. More recently, Zhang and Yee [41] reported that addition of IGFBP-1 to the metastatic breast cancer cell line, MDA-231BO, inhibited cell motility, suggesting that some actions of IGFBP-1 are cell type or context specific. IGFBP-2 has been found to associate with the extra-cellular matrix [36], and to membranes of neuroblastoma cells [37], and olfactory bulb [38,39] via proteoglycan interactions. Comparison of IGFBP-2 binding to cell membranes in transgenic mice over-

expressing wild type protein versus mice expressing a mutant in which the RGD sequence was changed to RGE revealed no differences, suggesting an alternative mechanism of association with integrins in which the RGD motif was not involved [40]. IGFBP-2 also has been shown to inhibit DNA synthesis in Mv1 mink lung epithelial cells (CCL64) in an IGF-independent manner that may require cell sensitization by TGF β [42]. Additional studies on IGF-independent effects of these IGFBPs in transgenic mice have been reviewed recently [43].

Most studies on the IGF independent actions of IGFBPs have focused on IGFBP-3 and IGFBP-5. One of the initial key experiments demonstrated a growth inhibitory effect of over-expressed IGFBP-3 in fibro-blasts isolated from IGF-IR-deficient mice [44]. Subsequently, a link between anti-proliferative effects of IGFBP-3 and actions of TGF β 2 was established in breast cancer cells [45]. IGFBP-3 also was found to stimulate apoptosis in prostate cancer cells independent of both IGF-IR and p53 signaling, but required TGF β 1 [46]. Other studies have shown growth inhibitory and pro-apoptotic effects of IGFBP-3 in Hs578T breast cancer cells, which have “masked” IGF-IRs and are unresponsive to IGF-I [47,48]. Similar results have been observed in human retinal endothelial cells, where somatostatin agonist treatment led to a dose-dependent increase in IGFBP-3, implicating this IGFBP in growth inhibition in these cells [49].

IGFBP-5 shares considerable structural homology with IGFBP-3 [10]. IGFBP-5 has been reported to stimulate mesangial cell migration [50,51], and to enhance bone formation and osteoblast proliferation [52]. IGFBP-5-induced mesangial cell migration was attributed to a heparin-binding region comprising residues 201–218 [51,50]. A peptide containing this segment also was found to compete with ¹²⁵I-IGFBP-5 for binding to osteoblasts [53]. In contrast, amino acids 1–169 of IGFBP-5 were reported to stimulate osteoblast secretory activity [54]. A non-IGF binding mutant of IG-FBP-5 inhibited ceramide-induced Hs578T cell apoptosis, but was without effect in IGF-responsive MCF-7 cells [55]. On the other hand, a non-IGF binding mutant of IGFBP-3 enhanced ceramide-mediated apoptosis [56].

5. IGFBP cell biology: cell surface receptors, internalization and nuclear targets

In addition to cell surface interactions, considerable interest has been generated by observations of nuclear targeting of IGFBPs and in identification of potential nuclear proteins as binding partners in two-hybrid screening protocols. Fluorescent derivatives of IGFBP-3 and IGFBP-5 were observed to translocate to the nucleus [57] via an importin β -dependent pathway [58]. Once in the nucleus, these IGFBPs have been proposed to regulate transcriptional processes involved in the cell death response. This hypothesis remains to be proven as IGFBP-3 can cause growth inhibition and apoptosis in breast cancer cells without nuclear translocation [59]. Several putative nuclear targets for IGFBP-3 and IGFBP-5 have been described, including the transcription factors RXR and FHL2 [60], as have a number of other binding partners, including the Alzheimer’s survival peptide, humanin [61], and fibronectin [62].

The physiological significance of many of these interactions remains to be established, as does the existence of cell surface receptors for IGFBPs. A tacit assumption is that the IGFBPs are secreted and then taken up into cells. Outstanding questions to be addressed include: are IGFBPs internalized by receptor or non-receptor mediated mechanisms? How do vesicle-localized IGFBPs interact with nuclear pore proteins for transport to the nucleus? Along these lines, the search for IGFBP-specific cell surface receptors has yielded variable results. Specific receptors for IGFBP-3 have been described on Hs578T cells [63] and on endothelial cells [64]. In addition, a Type V TGF β receptor was reported to be an IGFBP-3

receptor [65,53]. This latter protein has now been shown to be identical to the low density lipoprotein receptor-related protein-1 (LRP-1)/ α 2 macroglobulin receptor, which functions in an endocytic role [66].

6. IGFBPs and cancer

Over the last few years the role of the IGF system in cancer has become an area of considerable interest. IGFBP-3 has been defined in epidemiological studies as a negative risk factor for breast, prostate and colorectal cancers, with low serum IGFBP-3 and high IGF-I levels posing the greatest statistical risk [67]. Consistent with this anti-cancer role are findings in cell culture models that IGFBP-3 inhibits neoplastic cell growth [14,68]. It has further been postulated that other IGFBPs also are negative risk factors for cancer on the basis of their IGF antagonist actions, yet recent microarray-based gene profiling studies have found increased IGFBP-2 expression in glioblastoma multiforme [69], glioblastoma [70], synovial sarcoma [71], brain tumors [72], and prostate cancer [73,74]. IGFBP-2 also has been directly associated in other studies with enhanced tumorigenicity [75]. Adding to this complexity is the observation that IGFBP-2 suppressed the growth of normal prostate epithelial cells while enhancing the growth of prostate cancer cells [74]. It thus remains difficult to generalize about a unified role for IGFBPs in cancer, underscoring the need for careful additional investigation.

7. The future of IGFBP-based therapeutics

As natural antagonists of the IGFs, IGFBPs logically could be significant therapeutic agents for inhibiting IGF actions in a variety of human diseases, including cancer [19], diabetic renal, vascular, and eye disease [76,77], and the “wet” form of age-related macular degeneration [78,79]. To this end, a number of investigators have designed potential small molecule IGFBP-mimetics capable of complexing with IGFs and blocking their access to the IGF-IR. Deshayes et al. recently reported the screening of a number of random libraries by phage display for peptides exhibiting high affinities for the IGFBP-binding domain of IGF-I [80]. One of the peptides identified, IGF-F1-1, blocked IGF-I stimulated insulin receptor autophosphorylation and inhibited ^{125}I -IGF-I binding to MCF-7 cells, thus serving as an excellent lead compound for further drug development. Comparison of the NMR structure of IGF-F1-1 with that of mini-IGFBP-5 [10] revealed considerable three-dimensional similarity between the proteins [81].

On the other end of the spectrum, because IGF-I has neuroprotective effects in ischemic injury, agents that block IGF:IGFBP interactions, and enhance access to the IGF-IR, have been proposed as therapeutic agents in the treatment of stroke [82]. A 14-residue peptidomimetic of IGF-I, bp-1-101 which was capable of displacing IGF-I from IGFBP-1 was developed by phage display technology [83]. The peptide was shown to increase insulin responsiveness in human HepG2 cells by raising free IGF-I levels, leading to activation of the IGF-IR and hybrid IGF-I:insulin receptors [84]. Using high throughput screening of chemical libraries, a number of isoquinoline analogs have been described that also bind to the IGFBPs at nM concentrations, and release bound IGF-I. These compounds have the additional advantage of being orally bioavailable [85,86]. Finally, through an in silico approach to the screening of small molecule inhibitors of the IGF-I:IGFBP-5 interaction, several lead compounds were identified based on the crystal coordinates of mini-IGFBP-5 [87].

8. Conclusions

Over the last several years the interest in IGFBPs has evolved from a focus on their roles as carrier proteins that modulate the levels of free IGF, to interest in a variety of other

biological effects. Clearly, many new and old questions remain to be answered in the field, including the three-dimensional structure of each IGFBP and the precise biochemistry of their interactions with IGFs, with extra-cellular matrix proteins, with putative receptors, and with intracellular targets. The potential roles played by the different IGFBPs in human disease remain to be elucidated, and new discoveries are likely to lead to a number of novel therapeutic opportunities. Once the three-dimensional structures of all six IGFBPs become available, the pace of discovery should be enhanced by the application of rational drug design to the field, and by the development of small molecule IGFBP-mimetics. IGFBP biology has much to look forward to in the future.

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Fig. 1.

Primary sequences of the NH₂- and COOH-terminal domains of IGFBPs 1–6. Key interaction domains are shown, as are the spatially conserved cysteine residues (shadow). Boxed sequences denote consensus CGCCXXC and CWCV motifs. Line at bottom denotes the thyroglobulin type-1 domain. Underlines in the IGFBP-1 COOH-terminus represent mAb epitopes causing binding inhibition. Arrowheads at bottom designate G/Q residues within the COOH-terminus mutated by Shand et al. [88], resulting in loss of IGFBP-5 binding activity. Open arrowhead indicates Y-60, important in IGF-binding [89]. RGD motifs in IGFBP-1 and 2 are in green. Grey underline in IGFBP-2 denotes residues 227–253 identified in truncation mutants to be important for IGF binding in bovine IGFBP-2 [5]. Dark underlines in IGFBP-2 are sites photoaffinity labeled by Horney et al. [19]. Underline in the NH₂-terminus of IGFBP-3 and IGFBP-5 denotes mutated residues within a common hydrophobic patch [10], which result in loss of IGF binding [12–14]. Grey residues in the IGFBP-3 COOH-terminus are the heparin binding domains. Domains swapped in IGFBP-3 and IGFBP-4 to identify endothelial cell binding region are underlined [64]. Underline in IGFBP-5 COOH-terminus (residues 201–218) represents extra-cellular matrix/heparin binding and mesangial cell migration regulating domain [51]. IGFBP-5 residues 1–169 have been defined as an osteoblast mitogen domain [54], which also inhibits IGF-I stimulated migration. The serine phosphorylation domain of IGFBP-3 (residues 105–119 [56]) is not shown. See text for additional details.

Fig. 2. IGF-1 binding model. As detailed in the text, this model incorporates work from many laboratories and is principally based on IGF-I:IGFBP-2 interactions [19].

Fig. 3.
Sites of IGF-I biotinylation. The sites of IGF-I biotinylation are underlined and the IGFBP-binding domain is shown in space filling mode, after Cooke et al. [90].