



Technical Bulletin No. 8 *Last Updated: September 2004*
IGF Binding Proteins - Mini-Review
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Human Insulin-like Growth Factor Binding Proteins (IGFBPs)

IGF Binding Proteins (IGFBPs) are a family of six circulating proteins which bind Insulin-like Growth Factors-I and -II (IGF-I and IGF-II) with high affinity. By binding the IGFs the IGFBPs control their distribution, function and activity in various cells, tissues and body fluids, thereby modulating their metabolic and mitogenic effects *in vivo* (1-8).

All the IGFBPs bind IGF-I and IGF-II with an affinity equal to or greater than that of the IGF receptors (7). IGFBP-6 and to a lesser extent IGFBP-2 and IGFBP-5, have a higher affinity for IGF-II than IGF-I (5).

Besides the six “classical” IGFBPs, there are a further 8-10 IGFBP-related proteins (designated IGFBP-rP1 - IGFBP-rP10) which are structurally related to the IGFBPs especially in the N-terminal region. These related proteins bind IGF-I and IGF-II with relatively low affinity (11). Changes in the levels of these IGFBP-related proteins occur in many disease states (4).

Structural Motifs of the six IGFBPs

The six IGFBPs (designated IGFBP-1 - IGFBP-6) vary between 216 and 289 amino acids (24 - 31 kDa, non-glycosylated), and have no sequence homology with the Type 1 or Type 2 IGF Receptors. Structurally the IGFBPs have three distinct regions of approximately equal size. The highly conserved N-terminal region contains 12 (IGFBP-1 - IGFBP-5) or 10 (IGFBP-6) conserved cysteine residues and the highly conserved C-terminal region contains 6 conserved cysteine residues. Intra-domain disulphide bonds form between the cysteine residues within the C- or N-terminal regions (17, 25). In contrast the linking central domain of the IGFBPs (55-95 amino acids) is highly variable (8).

Binding of IGFs by the IGFBPs

BP fragments containing either the conserved N- or C-terminal regions can bind IGF but with reduced affinity (4). Current evidence suggests that for high affinity IGF binding both the N- and C-terminal regions are required (4).

Properties of IGF Binding Proteins

Despite their structural similarities, the six human IGFBPs have diverse functional properties:

	IGFBP-1	IGFBP-2	IGFBP-3	IGFBP-4	IGFBP-5	IGFBP-6
IGF Affinity	IGF-I ≥ IGF-II	IGF-II > IGF-I	IGF-I ≥ IGF-II	IGF-I > IGF-II	IGF-II > IGF-I	IGF-II >> IGF-I
Molecular Weight	25 kDa	31 kDa	43-45 kDa	24 kDa	29 kDa	28-30 kDa
Serum Concentration	2-15 nM (Variable)	2-15 nM	100 nM (with ALS)	2-15 nM	2-15 nM	2-15 nM
Bind ALS			+		+	
Nuclear Target Sequence			+		+	
Heparin Binding Residues			+		+	+
RGD Sequence	+	+				
ECM Binding		+			+	
N-glycosylation			+	+		
O-glycosylation					+	+
Serine Phosphorylation Sites	+		+		+	

RGD sequences are found in IGFBP-1 and IGFBP-2 and IGFBP-1 binds to α5β1 integrin through this region (7).

N-glycosylation sites are found in IGFBP-3 and IGFBP-4, and *O-glycosylation* sites in IGFBP-5 and IGFBP-6 (7). Glycosylation appears to protect the IGFBPs from proteolytic digestion by IGFBP specific proteases involved in the regulation of IGFBP function and turnover (24).

Nuclear targeting sequences are present in IGFBP-3 and IGFBP-5 and both these IGFBPs can bind an acid-labile subunit (ALS) involving their C-terminal region (28). For IGFBP-3 this binding depends on its glycosylation and sialylation status (19).

Heparin binding residues are found in IGFBP-3, IGFBP-5 and IGFBP-6. Binding of heparin or heparin sulphate (presumably to this site) inhibits the interaction between the ALS and both IGFBP-3 and IGFBP-5 (7). Binding of IGFBPs to extracellular matrix (ECM) or cell surface sites can also reduce their IGF binding affinity (7). For example the binding of IGFBP-5 to ECM reduces its affinity for IGF-I or IGF-II ~15 fold (2). By contrast the binding of IGF-I or IGF-II to IGFBP-2 enhances IGFBP-2 binding to ECM or heparin (9).

Serine phosphorylation sites are found in IGFBP-1, IGFBP-3 and IGFBP-5 (5). Phosphorylation of IGFBP-1 increases its affinity for IGF-I 5-fold (4). IGFBP-3 is secreted from the liver as the phosphorylated form with subsequent dephosphorylation leading to a 2-fold increase in its affinity for the ALS (16).

IGFBP Concentrations in Human Serum

IGFBP-3 (a 43-45 kDa glycoprotein) is the most abundant binding protein in serum, binding IGF-I or IGF-II in conjunction with an acid-labile glycoprotein subunit (ALS) to form a 150 kDa circulating complex at a serum concentration of about 100 nM. The IGF is released from the IGFBP-3 complex after limited proteolysis of the IGFBP-3. Concentrations of the other five IGFBPs in serum range from 2 - 15 nM and, with the exception of IGFBP-1, their concentrations are relatively constant in normal serum (8).

Where are the IGFBPs Produced?

The liver is the major source of circulating IGFs, IGFBPs and the ALS. Hepatocytes synthesize IGFBP-1, IGFBP-2, IGFBP-4 and the ALS (26). Hepatic Kupffer cells synthesize IGFBP-2 and IGFBP-3 (26). Growth hormone, IGFs and insulin all have a role to play in the production of IGFs, IGFBPs and the ALS by the liver. IGFBP-3, IGFBP-4 and IGFBP-6 are expressed in vascular smooth muscle cells (12), while IGFBP-4 and IGFBP-5 are produced by a wide range of osteoblast cells (23). Most cell types produce one or more members of the IGFBP family reflecting the ubiquitous nature of the IGF / IGFBP system.

Distribution of IGFBPs

A distinct pattern of IGFBPs is found in each tissue and biological fluid, due to cell-specific IGFBP expression (see below), as well as the unique localization determinants in the different IGFBPs. There are changes in IGFBP expression and concentration in different tissues in many pathological states (3).

Modulation of Activity by IGFBP Cleaving Proteases

There is increasing evidence that IGFBP specific proteases are involved in the regulation of IGFBP function and turnover. IGFBPs are cleaved at specific sites by a range of proteases including prostate-specific antigen (PSA) (15), matrix metalloproteases (18), cathepsin D (14), thrombin (30) and serine proteases (2). The majority of protease sensitive sites are localized in the middle non-conserved region. Following limited proteolysis, IGFBPs exhibit a dramatically reduced affinity for IGFs and some IGFBP fragments appear to have IGF independent activity (1).

IGFBP-1 (234 aa, 25 kDa protein) is produced mainly in the liver and is both hormonally and metabolically regulated. It is thought it may be involved in glucose homeostasis (5). It is in low concentration in serum where it has a short half-life (it contains the PEST sequence often present in proteins that have a rapid turnover). It is the predominant IGFBP in amniotic fluid where it is found in high concentration. Its IGF-I binding affinity can be up-regulated by serine phosphorylation (7).

IGFBP-2 (289 aa, 31 kDa protein) is widely expressed in the fetus where its expression closely follows that of IGF-II (29, 13). It preferentially binds IGF-II and is found in serum, milk, cerebrospinal fluid and seminal plasma. It is secreted by many cell types including hepatocytes.

IGFBP-3 (264 aa, 43-45 kDa glycoprotein) is the most abundant species in serum and milk. It is produced by non-parenchymal hepatic cells and circulates in serum, binding IGF-I or IGF-II in conjunction with an acid-labile subunit (ALS) to form a 150 kDa circulating complex at a serum concentration of about 100 nM. Free IGF-I has a half-life of ~8 min in serum. This can be increased to ~30 min if bound to IGFBP-3 and to ~15h in the ternary complex with IGFBP-3 and ALS (7). The IGF can be mobilized from this complex following limited proteolysis of IGFBP-3 to yield a C-terminal fragment that remains associated with the ALS (4) and a 30 kDa N-terminal fragment that has reduced affinity for IGF-I (50 fold) and IGF-II (20 fold). The degree of glycosylation and sialylation at different sites affects the efficacy of proteolytic attack (19). IGFBP-3 has a nuclear targeting sequence and recent evidence shows that, independent of the presence of IGF-I, proteolytic fragments of IGFBP-3 are translocated to the nucleus of actively dividing cells (20,22).

IGFBP-4 (237 aa, 24 kDa glycoprotein) is produced by mandibular, calvarial, vertebral, rib and stromal osteoblasts (23). It is found in serum and seminal plasma. IGFBP-4 does not associate either with the cell surface or the extracellular matrix and seems to act as a scavenger of IGFs and an inhibitor of IGF action, e.g. in smooth muscle cells (10).

IGFBP-5 (252 aa, 29 kDa glycoprotein) preferentially binds IGF-II. It potentiates the actions of IGF-I in smooth muscle cells, fibroblasts and osteoblasts. *In vitro* studies show IGFBP-5 down-regulates the stimulatory effects of IGFs by inhibiting their binding to the IGF Type 1 receptor (21). IGFBP-5 also binds with high affinity to extracellular matrix components which protect it from proteolysis but decrease its affinity for IGF-I by about 10-fold. IGFBP-5 is able to form a ternary complex with ALS but the significance of this interaction is not yet understood (28). IGFBP-5 has a nuclear targeting sequence and recent evidence shows that, independent of the presence of IGF-I, fluorescently labelled IGFBP-5 is translocated to the nucleus of actively dividing cells (27).

IGFBP-6 (216 aa, 28-30 kDa glycoprotein) has a 50-fold higher affinity for IGF-II than IGF-I (7). It is synthesized in liver and lung and is found in cerebrospinal fluid and detected by immunohistochemistry in skin, skeletal muscle, the meninges and pancreatic islets of Langerhans.

The Future

There is increasing interest in the IGF independent actions of the IGFBPs and in the specific IGFBP proteases. The key residues involved in the binding interactions between the IGFs and the IGFBPs are being determined by NMR and mutagenesis studies. In the clinical area changes in IGFBP profiles with disease states are under active investigation.

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