The Insulin-like Growth Factor Binding Protein Superfamily: New Perspectives

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ABSTRACT. The insulin-like growth factor (IGF) binding proteins (IGFBPs) were initially identified as carrier proteins for IGF-I and IGF-II in a variety of biologic fluids. Their presumed function was to protect IGF peptides from degradation and clearance, increase the half-life of the IGFs, and deliver them to appropriate tissue receptors. The concept of IGFBPs as simple carrier proteins has been complicated, however, by a number of observations: 1) the six IGFBPs vary in their tissue expression and their regulation by other hormones and growth factors; 2) the IGFBPs are subjected to proteolytic degradation, thereby altering their affinities for the IGFs; 3) IGFBP-3 and IGFBP-5, in addition to binding IGFs, also can associate with an acid-labile subunit, thereby increasing further the half-life of the IGFs; 4) in addition to modifying the access of IGF peptides to IGF and insulin receptors, several of the IGFBPs may be capable of increasing IGF action; 5) some of the IGFBPs may be capable of IGF-independent regulation of cell growth; 6) some of the IGFBPs are associated with cell membranes or possibly with membrane receptors; and 7) some of the IGFBPs have nuclear recognition sites and may be found within the nucleus. Additionally, a number of cDNAs identified recently have been found to encode proteins that bind IGFs, but with substantially lower affinities than is the case with IGFBPs. The N-terminal regions of the predicted proteins are structurally homologous to the classic IGFBPs, with conservation of the cysteine-rich region. These observations suggest that these low-affinity binders are members of an IGFBP superfamily, capable of regulating cell growth by both IGF-dependent and IGF-independent mechanisms. Pediatrics 1999;104:1018–1020; insulin-like growth factor, insulin-like growth factor binding proteins.

ABBREVIATIONS. IGF, insulin-like growth factor; IGFBP, IGF binding protein; TAF, tumor adhesion factor; PSF, prostacyclin-stimulating factor; IGFBP-rP, IGFBP-related protein.

In serum and other biologic fluids, the insulin-like growth factors (IGFs) are bound to members of a family of high-affinity IGF binding proteins (IGFBPs).1–3 The affinity of these IGFBPs for IGF-I and IGF-II is sufficiently high ($K_d$, $10^{-11}$ to $10^{-10}$ mol/L) to ensure that virtually all circulating IGF is bound to IGFBPs. Six members of the IGFBP family have been identified, all encoded by distinct genes located on four separate chromosomes (Table 1). The IGFBPs were identified originally as carrier proteins for the IGFs in serum, but the existence of six distinct IGFBPs in all mammalian species studied to date, each encoded by separate, independent genes and regulated in a tissue-specific manner, has suggested that the biologic functions of the IGFBPs may be more complex than originally believed.

The physiologic roles of the IGFBPs may be divided into IGF-dependent and IGF-independent activities.4 The former refers to the ability of IGFBPs to 1) transport IGFs in plasma and other biologic fluids; 2) increase the half-lives of IGF peptides, typically from minutes to hours; and 3) modulate the access of IGF ligands to their receptors, thereby regulating IGF action. The latter, IGF-independent activities, refers to the ability of IGFBPs to regulate cell growth, migration, or metabolism in manners that are independent of IGF action, presumably through direct interactions of the IGFBPs with cellular receptors or “interacting proteins.”5 The observation that certain IGFBPs are proteolyzed under a variety of physiologic and pathologic conditions has complicated additional issues related to IGFBP action. Proteolysis of IGFBP’s first was shown in human pregnancy serum, in which circulating IGFBP-3 was found primarily in low-molecular-weight forms.6,7 These proteolytic fragments of IGFBP-3 were shown to bind IGF with lower affinity, potentially altering the release of IGF by IGFBPs to target receptors.

Subsequent studies have shown that proteolysis is not restricted to IGFBP-3 and that limited proteolysis of IGFBPs 2 through 5 may 1) alter the affinity of the IGFBP fragment for IGF, 2) modulate the release of IGF from IGFBPs to target receptors, and 3) produce IGFBP fragments that are capable of direct stimulatory or inhibitory action.

The multiplicity of potential biologic actions of IGFBPs and IGFBP fragments is, perhaps, best understood by recognizing that the IGFBP family is part of a protein superfamily, the members of which all preserve the cysteine-rich N-terminal domain that is characteristic of the six high-affinity IGFBPs (Fig 1).8–10 In addition to this cysteine-rich N-terminal domain, IGFBPs 1 through 6 also share homology in the C-terminal region, but vary in the midregion of the protein. In the N-terminal region, IGFBPs 1 through 5 conserve all 12 cysteines and IGFBP-6 has 10 of the 12 cysteines. Additionally, within the N-terminal region, IGFBPs 1 through 5 conserve a GCGCCxxC motif and IGFBP-6 substitutes a GCAE-
The AEGC motif (thereby accounting for the two “missing” cysteines). The significance of this highly conserved motif is not certain, and its role in the binding of IGF ligand is not clear. The IGFBPs, thus, may be considered modular proteins, with two conserved modules and one variable one. This concept is supported by the preservation of even numbers of cysteines in the N (10 or 12 cysteines) and the C (6 cysteines) regions, suggesting that covalent disulfide bonds form within each discrete module, leaving no free cysteines to interact between the two domains.

mac25 originally was identified as a cDNA that is expressed in normal leptomeninges, but not in certain meningiomas; similar subtractive hybridization studies in mammary tissue subsequently resulted in the identification of the same cDNA in senescent normal mammary tissue but not in some breast carcinomas. The deduced protein was predicted to conserve the N-terminal domain of the IGFBPs, including all 12 cysteines and the G/ACGC-CxxC motif. Oh et al synthesized the protein in a baculovirus expression system and found that mac25 bound IGF-I and IGF-II, but with considerably lower affinity than that observed with IGFBPs 1 through 6. This ability of mac25 protein to bind IGFs (albeit with less affinity) was consistent with the observation that N-terminal fragments of IGFBP-3 (both naturally occurring proteolytic fragments and synthetic peptides) retain the ability to bind IGF. Based on the structural homology with the IGFBPs and the conservation of the ability to bind IGFs, the mac25 protein was provisionally named IGFBP-7.

**TABLE 1. Characteristics of the Human IGFBP Superfamily**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Molecular Weight</th>
<th>Number of Amino Acids</th>
<th>Number of Cysteines</th>
<th>N-linked Glycosylation</th>
<th>Chromosome</th>
<th>mRNA (kb)</th>
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<tr>
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<tr>
<td>IGFBP-1</td>
<td>25,271</td>
<td>234</td>
<td>18</td>
<td>No</td>
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<td>1.6</td>
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<td>IGFBP-2</td>
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<td>289</td>
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<td>264</td>
<td>18</td>
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<td>237</td>
<td>20</td>
<td>Yes</td>
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<td>1.7</td>
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<tr>
<td>IGFBP-5</td>
<td>28,553</td>
<td>252</td>
<td>18</td>
<td>Yes</td>
<td>2q</td>
<td>1.7, 6.0</td>
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<td>IGFBP-6</td>
<td>22,847</td>
<td>216</td>
<td>16</td>
<td>No</td>
<td>12</td>
<td>1.1</td>
</tr>
</tbody>
</table>

| Low-affinity IGFBPs and IGFBP-rPs |                       |                     |                     |                        |            |           |
| IGFBP proteolytic fragments |                   |                       |                     |                        |            |           |
| IGFBP-rP1   | 26,400           | 251                   | 18                  | Yes                    | 4q         | 1.1       |
| IGFBP-rP2   | 35,500           | 349 (prepeptide)      | 39                  | Yes                    | 6q         | 2.4       |
| IGFBP-rP3   | 36,000           | 357 (prepeptide)      | 41                  | ?No                    | 8q         | 2.4       |
| IGFBP-rP4   | 39,500           | 381 (prepeptide)      | 35                  | ?No                    | 1p         | 2.5, 4.0  |

* Nomenclature for the IGFBP-rPs is given in Table 2.

**TABLE 2. Proposed Nomenclature for the IGFBP Superfamily**

<table>
<thead>
<tr>
<th>High-affinity IGFBPs</th>
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<td>IGFBP-6</td>
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</table>

| Low-affinity IGFBPs and IGFBP-rPs |                       |                     |                      |                      |            |            |
| IGFBP proteolytic fragments |                   |                       |                     |                      |            |            |
| IGFBP-rP1 | mac25 |                   |                       |                     |                      |            |            |
| IGFBP-rP2 | (CTGF) |                   |                       |                     |                      |            |            |
| IGFBP-rP3 | (novH) |                   |                       |                     |                      |            |            |
| IGFBP-rP4 | (cyr61) |                   |                       |                     |                      |            |            |

**Fig 1.** Schematic diagram of the IGFBP superfamily, consisting of IGFBPs 1 through 6 and IGFBP-rPs 1 through 4. The conserved N-terminal domains are shown as dark shaded bars. The conserved C-terminal domains in IGFBPs 1 through 6 are shown as light shaded bars. Nonconserved regions are indicated by open bars. Conserved cysteines are indicated by diagonal lines connecting the bars; other cysteines are indicated by vertical lines. The numbers to the right of the bars indicate the number of N-terminal conserved cysteines/total number of cysteines in the molecule.

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The growing understanding of the importance of the cysteine-rich N-terminal domain in IGF binding and its preservation in other modular proteins, such as mac25, led to a closer examination of the CCN family of proteins (C, connective tissue growth factor; C, CYR61, a growth factor-inducible immediately early gene; N, NOVH [nephroblastoma overexpression gene]). Like the high-affinity IGFBPs and mac25, the CCN family members are modular proteins, with the conserved cysteine-rich N-terminal domain, including the GCG/SCCxxC motif.15 Connective tissue growth factor and novH proteins now have been synthesized in baculovirus expression systems and, as with mac25, bind IGF-I and -II with low affinity.9

The high-affinity IGFBPs, mac25, and the CCN family thus are all modular proteins that conserve an N-terminal, cysteine-rich domain that appears to confer the ability of each protein to bind IGFs. The ability of IGFBPs 1 through 6 to bind IGF-I and -II with particularly high-affinity results, presumably, from the interaction of the N-terminal and the C-terminal domains; the conserved C-terminal domain is unique to the high-affinity IGFBPs. The crucial role of the cysteine-rich N-terminal domain is underscored by the fact that it is encoded by a single exon in genes for all these proteins; this strongly suggests that exon shuffling, with the dissemination of the DNA sequence that encodes the N-terminal domain, resulted in the distribution of this exon among various genes by a series of DNA recombinational events. The preservation of the cysteine-rich N-terminal module and the ability to bind IGFs have led to the proposal of an IGFBP superfamily, encompassing the high-affinity IGFBPs and the low-affinity IGFBP-related proteins (IGFBP-rPs) (Table 2; Fig 1).16

In addition to its role in binding IGFs, the cysteine-rich N-terminal domain rather unexpectedly has an affinity for insulin.17 Indeed, IGFBPs 1 through 6 are all capable of binding insulin, but with an affinity substantially lower than for IGF-I or IGF-II. On the other hand, IGFBP-rP1 appears to be capable of binding insulin with an affinity at least as great as its affinity for IGF peptides. Similarly, the cysteine-rich N-terminal domain of IGFBP-3 has less affinity for IGFs than does intact IGFBP-3, but greater affinity for insulin. Thus it is apparent that the secondary and tertiary structures of the IGFBPs, resulting from interactions between the N-terminal and the C-terminal domains, confer the capability of high-affinity binding of IGFs. Disrupting this tertiary structure, by a reduction of disulfide bonds or by proteolysis, results in a sharp reduction in affinity for IGFs, accompanied by an increased affinity for insulin.

The ability of the high-affinity IGFBPs to bind IGFs is clearly central to their IGF-dependent actions. Whether the binding of insulin by IGFBP, IGFBP proteolytic fragments, or IGFBP-rPs has physiologic significance remains uncertain at this time. It appears highly likely, however, that the preservation of the cysteine-rich N-terminal domain throughout the IGFBP superfamily is crucial to the IGF-dependent and IGF-independent actions of all these proteins. It is expected that additional investigation into structure-function relations among the members of the IGFBP superfamily will lead to further understanding of the importance of this highly conserved domain.

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