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General Aspects of Insulin-like Growth Factor Binding Proteins

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Insulin-like growth factor binding proteins (IGFBPs) belong to a family of at least six homologous proteins that bind insulin-like growth factors (IGFs) and modulate many of their biological actions. IGFBPs are produced by a wide variety of tissues and their circulating levels are regulated by both hormonal and metabolic factors. The binding activity of some IGFBPs is affected by phosphorylation, proteolysis, or association with extracellular matric (ECM) and plasma membrane. The IGFBPs may exert either inhibitory or potentiating effects on IGF actions. Recent evidence indicate that some IGFBPs are capable of direct actions on cells independent of the IGFs.

Key Words: IGFBPs; IGF; binding proteins.

Introduction

Insulin-like growth factor binding proteins (IGFBPs) are a family of structurally related proteins that bind insulin-like growth factor-I (IGF-I) and IGF-II with high affinity and specificity. They are critical regulators of the actions of IGFs. IGFBPs have been studied extensively in humans and rats, and demonstrated in other vertebrates such as amphibians *(1)*, fish *(2)*, and birds *(3)*. IGFBP activity has even been detected in lampreys, an agnathan species belonging to a primitive vertebrate class *(4)*. The finding suggests preservation and extension of the biological actions of IGFBPs during evolution. To date, six IGFBPs have been sequenced, characterized, and named IGFBP-1 to -6 *(5)*. A potential seventh IGFBP was recently identified and suggested to have a tumor suppressive role *(6)*. Additional IGFBPs have been postulated but not fully characterized.

Structure

The IGFBPs range in m.w. from 26 to 45 kDa and belong to a unique family of proteins as they show very little homology to other proteins. All binding proteins share highly conserved cystine-rich regions in the N- and C-terminal one-third with the middle portion exhibiting almost complete divergence. IGFBP-1 and IGFBP-2 each contain an Arg-Gly-Asp (RGD) sequence representing an integrin recognition sequence characteristic of several extracellular matrix (ECM) proteins, e.g., fibronectin. Only IGFBP-5 binds to hydroxyapatite. IGFBPs 1, 3 and 5 are phosphorylated which dramatically affects the activity of IGFBP-1. IGFBPs 3, 4, 5 and 6 are glycosylated, but the function of this modification is largely unknown. The six IGFBPs have varying binding affinity for IGFs with Ka range of $0.1–2.0 \times 10^{10}\ M^{-1}$, which equals or exceeds the affinity of type 1 IGF receptor binding ($1 \times 10^{9}\ M^{-1}$). IGFBP-2, -5, and -6 exhibit preferential binding to IGF-II *(7)*. The authors and others have previously shown that the binding determinants are present in both the N- and C-terminal portions of human IGFBP-3, with the latter showing less affinity for IGFs *(8)*. Because of the structural homology, these determinants are probably present on all IGFBPs.

Synthesis and Transport

IGFBPs are synthesized by a variety of cell types and found in plasma, lymph, milk, and intracellular fluid. In plasma, the IGFs are carried predominantly in the form of an ~150 kDa complex with IGFBP-3 and an ~85 kDa acid labile subunit (ALS) which does not bind IGFs directly. ALS binds IGFBP-3 after it has complexed with an IGF (any direct ALS to IGFBP-3 binding that occurs is insignificant). The complex sequesters approx 90% of the IGFs in circulation. Most of the rest of IGFs are bound to other IGFBPs as approximately 0.4% remains in the free form *(9)*. Some unsaturated IGFBP activity remains in plasma. Whereas complexes of IGF-I and IGFBPs in plasma can traverse the endothelial barrier into the extracellular millieu, the 150 kDa complex cannot. This causes most of the plasma IGFs to be confined in the circulation, which serves as a reservoir. The α-granules of platelets also transport IGFBP-3 and other IGFBPs with IGFs; lysis of α-the granules releases them into tissues *(10)*.

Regulation

Significant changes in the amounts of the different IGFBPs in plasma occur in response to stress, metabolic factors, trauma, and physiological changes associated

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with growth hormone (GH) status, pregnancy and aging. For instance, sustained reduction in plasma IGFBP-3 was reported in trauma patients during their stay in an intensive care unit *(11)*. By contrast, their IGFBP-1 levels increased threefold during the same period. Plasma IGFBP-1 levels are strongly regulated inversely by insulin, with levels varying by 10-fold or more in relation to meals and sleep *(12)*. Glucocorticoids, glucagon, and cAMP increase IGFBP-1 levels *(12)*. We have reported in abstract form that estrogen in postmenopausal subjects increases plasma IGFBP-1. Plasma IGFBP-2 levels are frequently inversely related to GH levels. However, GH increases the level of IGFBP-3. GH stimulates the synthesis of IGFBP-3 via IGF-I in rats, but in humans appears to act directly. IGFBP-3 production is stimulated by both retinoic acid and TGF-ß and probably accounts for their inhibitory actions on cell growth. The plasma level of ALS is GH-dependent and ALS is present in excess in plasma. Parathyroid hormone (PTH) increases the production of IGFBP-4, and IGFs have been reported to increase plasma IGFBP-5 levels (*see* ref. *12*, Chapter 11). The aging process is also accompanied by changes in the complexion of plasma IGFBPs. Gelato et al. have reported that IGFBP-1 increases in aging (*see* ref. *12*, Chapter 10). IGFBP-5 decreases and IGFBP-4 increases with aging *(13)*. The authors' unpublished data showed no significant change in IGFBP-3 with advancing years.

IGFBP proteases play an important role regulating plasma and tissue levels of IGFBPs 2–6. Degradation decreases or abolishes IGF binding resulting in increasing bioavailability of the IGFs. During late pregnancy in humans and rats, IGFBP-3 was found to undergo limited proteolysis, which impairs its ability to bind IGFs. Proteolysis of IGFBP-3 occurs postoperatively, during wound healing and in other disease states. Proteolysis of IGFBPs 4 and 5 are thought to be important in the regulation of bone formation *(13)*. Some of the multiple proteases have been characterized. The matrix metalloproteinases (MMPs), a family of zinc-dependent proteinases, are responsible for the pregnancy-associated proteolysis of plasma IGFBP-3 *(14)*. MMPs are also important in the proteolysis of IGFBP-5 resulting in fragments with reduced affinities to IGFs *(15)*. Proteases belonging to the kallikrein family that include plasmin and prostate-specific antigen (PSA) have also been shown to target IGFBP-3 *(16)*. Cathepsin D, a lysosomal acidic proteinase, represents another type found to cleave all IGFBPs except IGFBP-6 *(17)*. Synthesis of the IGFBP-4 protease is induced by IGFs (*see* Chapter 11).

Post-translational modifications modulate the bioavailability of IGFs by regulating the binding activity of IGFBPs. Phosphorylation of IGFBP-1 causes a four- to sixfold higher affinity for IGF-I and leaves only the inhibitory action of IGFBP-1, *see* below *(18)*. IGFBP-3 and IGFBP-5 also undergo phosphorylation, however, it is unclear if this affects their activity. Glycosylation of IGFBP-3 has consistently failed to affect binding, with only one questionable study to the contrary *(19)*. Glycosylation has been reported to inhibit the proteolysis of IGFBP-5 *(20)*.

Functions

In Plasma there are three general functions of IGFBPs

1. The IGFBPs transport IGFs to their receptor sites in the body.
2. The binding of IGFs prolongs their half-life as much as 30 min, or to 16 h when bound to IGFBP-3 in the 150 kDa form, compared to 10 min or less with IGFs alone (18).
3. Complexing with IGFBPs, especially IGFBP-3 in the 150 kDa complex, inhibits the insulin-like activity of IGFs and prevents the certain hypoglycemia that would otherwise lead to death.

Thus the plasma IGFBPs, especially the 150 kDa complex, provide an important, safe reservoir of IGFs for utilization by tissues.

In spite of the fact that all IGFBPs have prominent inhibitory effects on IGF activity, recent investigations have discovered that all IGFBPs, except IGFBP-4, can in certain circumstances potentiate the actions of IGFs. In vitro the potentiating action is usually IGFBP and tissue specific, also depending on the culture conditions, time course, and molar ratios. Potentiation has also been demonstrated in vivo. The authors' lab have reported that the addition of IGFBP-3 to IGF-I significantly augments the stimulatory action of IGF-I on wound healing in two different animal models. Others have shown that IGFBP-1 can substitute for IGFBP-3.

The mechanism for the potentiation is believed to lie in the property of the IGFBPs to associate with cells and ECM. IGFBP-1, -3, and -5 have been found to associate strongly with cells which create high concentrations of IGFs on the cell surface where they can interact with receptors in a facilitated manner *(18)*. In the case of IGFBP-1, it is the non-phosphorylated form that cells associates. The interaction of IGFBP-1 is mediated by integrin receptors, whereas IGFBP-3 and -5 binding involves cell surface glycosaminoglycans such as heparin *(21)*. A major feature of the binding of the IGFBPs to the cell surface is the lowering of their affinity for IGFs to a level where IGF binding to the IGF receptor becomes much more competitive. IGFBP-5 (and IGFBP-3 to a much lesser extent) associates with the ECM in skeletal tissues. ECM association, as in the case of membrane association, lowers the affinity of the binding protein for IGFs *(22)*. Both types of IGFBP association provide an IGF-rich reservoir juxtaposed to target cell IGF receptors that is capable of facilitating binding of IGF to its receptor. In addition this association protects both the IGFs and IGFBPs from degradation. The dual actions of IGFBPs are a reflection of the intricate mechanisms involved in the cellular actions of IGFs.

The IGFBP-3 gene has been identified as a target of the p53 suppressor gene product. This could mean a major role for IGFBP-3 in cancer *(23)*. IGFBP-5 appears to have a role in regulating muscle differentiation *(24)*.

IGFBPs are also capable of certain actions independent of IGFs *(18)*. IGFBP-3 was recently shown to exert significant inhibitory effect on Hs578T human breast cancer cells by directly binding to IGFBP-3 receptors on the cell membrane *(25)*. In another study, IGFBP-1 was shown to directly stimulate smooth muscle cells migration through binding to a vitronectin receptor *(26)*.

Summary

The IGFBPs are an unique family of binding proteins which possess the ability to dramatically alter the actions of IGFs. Therefore, in order to understand and modulate the actions of IGFs, the effects of the IGFBPs must be thoroughly appreciated.

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References

1. Cortizo, A. M., Braziunas, D., Jasper, H., and Gagliardino, J. J. (1993). *Gen. Comp. Endoc.* **92,** 242–249.
2. Kelley, K., Siharath, K., and Bern, H. (1992). *J. Exp. Zool.* **263,** 220–224.
3. Allander, S. V., Ehrenborg, E., Luthman, H., and Powell, D. R. (1995). *Prog. Growth Factor Res.* **6,** 159–165.
4. Upton, Z., Chan, S. J., Steiner, D. F., Wallace, J. C., and Ballard, F. J. (1993). *Growth Regu.* **3,** 29–32.
5. Shimasaki, S., Shimonaka, M., Zhang, H. P., and Ling, N. (1991). In: Spencer, E. M. (ed.). *Modern Concepts of Insulin-like Growth Factor,* Elsevier: NY, 343–358.
6. Swisshelm, K., Ryan, K., Tsuchiya, K., and Sager, R. (1995). *Proc. Nat. Acad. Sci. USA* **92,** 4472–4476.
7. Baxter, R. C. (1993). *Trends Endoc. Metab.* **4,** 91–96.
8. Spencer, E. M. and Chan, K. (1995). *Prog. Growth Factor Res.* 6, 209–214.
9. Frystyk J, Skjaerbaek C, Dinesen B, and Orskov H. (1994). *FEBS Lett.* **348,** 185–191.
10. Spencer, E. M., Tokunaga, A., and Hunt, T. K. (1993). *Endocrinology* **132,** 996–1001.
11. Wojnar, M. M., Fan, J., Frost, R. A., Gelato, M. C., and Lang, C. H. (1995). *Am. J. Physiol.* **2268,** R970–977.
12. Lee, P. D., Conover, C. A., and Powell, D. R. (1993). *Proc. Soc. Exp. Biol. Med.* **204,** 4–29.
13. Mohan, S., Farley, J. R., and Baylink, D. J. (1995). *Prog. Growth Factor Res.* **6,** 465–473.
14. Fowlkes, J. L., Suzuki, K., Nagase, H., Thrailkill, K. M. (1994). *Endocrinology* **135,** 2810–2813.
15. Fowlkes, J. L., Thrailkill, K. M., Serra, D. M., Suzuki, K, and Nagase, H. (1995). *Prog. Growth Factor Res.* **6,** 255–263.
16. Cohen, P., Peehl, D. M., Graves, H. C. B., and Rosenfeld, R. G. (1994). *J. Endocrinol.* **142,** 407–415.
17. Braulke, T., Claussen, M., Saftig, P., Wendland, M., Neifer, K., Schmidt, B., Zapf, J., Von Figura, K., and Peters, C. (1995). *Prog. Growth Factor Res.* **6,** 265–271.
18. Jones, J. I. and Clemmons, D. R. (1995). *Endocrine Rev.* 16, 3–29.
19. Cortizo, A. M. and Gagliardino, J. J. (1995). *J. Endocrinol.* 144, 119–126.
20. Arai, T., Arai, A., Busby, W. H. and Clemmons, D. R. (1994). *Endocrinology* **135,** 2358–2363.
21. Booth, B. A., Boes, M., Andress, D. L., Dake, B. L., Kiefer, M. C., Maack, C., Linhardt, R. J., Bar, K., Caldwell, E. E., and Weiler, J. (1995). *Growth Reg.* **5,** 1–17.
22. Jones, J. I., Gockerman, A., Busby, Jr. W. H., Camacho-Hubner, C., and Clemmons, D. R. (1993). *J. Cell. Biol.* **121,** 679–687.
23. Bertherat, J. (1996). *Eur. J. Endocrinol.* **134,** 426–427.
24. James, P. L., Stewart, C. E. and Rotwein, P. (1996). *J. Cell. Biol.* **133,** 683–693.
25. Oh, Y., Muller, H. L., Lamson, G., and Rosenfeld, R. G. (1993). *J. Biol Chem.* **268,** 14,964–14,971.
26. Jones, J. I., Doerr, M. E., and Clemmons, D. R. (1995). *Prog. Growth Factor Res.* **6,** 319–327.