

**CHARACTERIZATION OF A 41-KDA INSULIN-LIKE GROWTH
FACTOR BINDING PROTEIN IN SALMON**

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EXTENDED ABSTRACT ONLY - DO NOT CITE

Insulin-like growth factor-I (IGF-I) is a potent mitogen that exerts its actions through endocrine, paracrine and autocrine fashions. In mammals, IGF-I is tightly associated with six IGF-binding proteins (IGFBPs). Since affinity of IGFBPs to IGF-I approaches or exceeds that of IGF receptor, IGFBPs are capable of controlling the availability of IGF-I to target tissues and thus act as important modulators of IGF-I actions. Recent findings also indicate IGF-independent actions of IGFBPs in multiple cell types. In mammals, about 80% of circulating IGF-I is bound to IGFBP-3. IGFBP-3 is a N-glycosylated protein with molecular mass of 41 kDa and its production is regulated by growth hormone (GH) and nutritional status. In salmon, a similar-sized (41 kDa) IGFBP has been found in serum. As in mammalian IGFBP-3, salmon 41-kDa IGFBP is up-regulated by GH treatment and down-regulated by fasting, suggesting that it is a homolog of IGFBP-3 (Shimizu et al., 1999). This IGFBP, however, has not been characterized due to lack of availability of intact protein. We have recently purified the 41-kDa IGFBP from serum of chinook salmon (*Oncorhynchus tshawytscha*). This study aimed to characterize the 41 kDa IGFBP.

The 41-kDa IGFBP was purified from serum by IGF-I affinity chromatography followed by reverse-phase chromatography. Purified IGFBP appeared as doublet bands at 43 and 41 kDa on electrophoresis and those bands were N-glycosylated (Figure 1). Since purified IGFBP gave a single N-terminal amino acid sequence, the doublet bands presumably

represent different glycosylated forms of IGFBP as is the case for mammalian IGFBP-3. The IGFBP family has cysteine rich domains in both N and C-terminal regions, which are essential for high-affinity IGF binding. Partial amino acid sequencing of the purified protein revealed that the N-terminal cysteine rich domain is conserved in the 41-kDa IGFBP. Although the 41-kDa IGFBP showed highest amino acid sequence homology with zebrafish IGFBP-2 (Duan et al., 1997), the highly conserved nature of the N-terminus makes it impossible to identify the type of IGFBP. However, based on physiological responses, molecular weight and type of glycosylation, we assume that this IGFBP is salmon IGFBP-3.

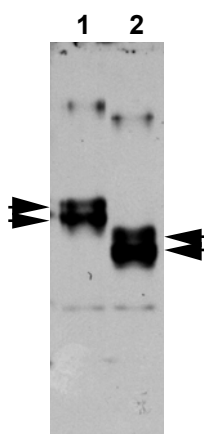


Figure 1. Western ligand blotting of purified 41-kDa IGFBP before (1) and after (2) enzymatic digestion. Forty nanograms of purified 41-kDa IGFBP was digested with glycopeptidase F under non-reducing conditions and subjected to Western ligand blotting using digoxigenin-labeled hIGF-I. Arrowheads indicate migration positions of 41-kDa IGFBP doublet bands.

On binding assay using ^{125}I -salmon IGF-I, purified 41-kDa IGFBP specifically bound salmon IGF-I, human IGF-I and human IGF-II, but neither Long R³IGF-I nor salmon insulin (Figure 2). No binding with Long R³IGF-I, which has little binding ability to mammalian IGFbps, indicates that binding characteristic of the salmon IGFBP is similar to those of mammalian IGFbps. Affinity constant (K_a) of 41-kDa IGFBP for salmon IGF-I was calculated as 4 nM^{-1} from a Scatchard plot derived from the competitive binding curve. The K_a value is comparable to those of fish type I IGF receptor preparations ($K_a = 2.3\text{-}7.7 \text{ nM}^{-1}$; Navarro et al., 1999). This

finding supports the concept that fish IGFBP is capable of modulating bioactivity of IGF-I in target tissues by competing with type I IGF receptor.

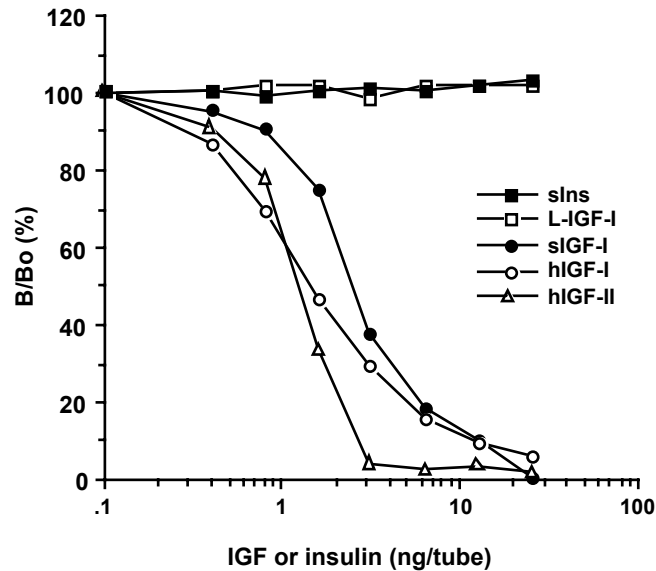


Figure 2. Binding specificity of 41-kDa IGFBP to IGFs and insulin. Ten nanograms of purified 41-kDa IGFBP was incubated with 15,000 cpm ¹²⁵I-salmon IGF-I and various amounts of unlabeled salmon IGF-I (sIGF I), human IGF-I (hIGF-I), human IGF-II (hIGF-II), Long R³IGF-I (L IGF-I), or salmon insulin (sIns). Binding (B/Bo) was expressed as a percentage of specific binding. All values are means of triplicate.

Acknowledgment

This work was supported by a grant from the U.S. Dept. Agriculture, NRICGP, Animal Growth and Nutrients Utilization Program.

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