Structure and Interactions of the C-terminal Domain of Insulin-like Growth Factor Binding Protein-2 (IGFBP-2)

By

Zhihe Kuang, B.Sc, M.Sc

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School of Molecular & Biomedical Science
The University of Adelaide
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Abstract

Insulin-like growth factor binding protein-2 (IGFBP-2) is the largest member of a family of six proteins (IGFBP-1 to 6) that bind insulin-like growth factors-I and -II (IGF-I/II) with high affinities. IGFBP molecules contain three domains of approximately equal length: the conserved cysteine-rich amino- and carboxyl-terminal domains, which are joined by a variable linker domain. The C-terminal domains of IGFBPs not only contribute to high-affinity IGF binding, but also confer binding specificity and have overlapping but variable interactions with many other molecules. At the time this project commenced, there was limited information on the structure-function relationships of the C-domain of IGFBPs.

In this thesis, the following N- and C-domain fragments were prepared in sufficient quantities for NMR studies: unlabelled, ^{15}N-labelled and ^{15}N/^{13}C-labelled $^{183-289}$IGFBP-2 (C-BP-2), unlabelled $^{141-289}$IGFBP-2 (Large-C-BP-2), and unlabelled and ^{15}N-labelled $^{1-138}$IGFBP-2 (N-BP-2). The IGF binding abilities of these fragments were assessed using BIAcore and cross-linking methods. Overall, the results indicated that C-BP-2 binds IGFs to only a limited extent, although the differences in IGF binding affinities among C-BP-2, N-BP-2 and full-length IGFBP-2 appeared to be larger in cross-linking studies than in BIAcore experiments.

The solution structure of C-BP-2 was determined using NMR spectroscopy. C-BP-2 has a thyroglobulin type 1 fold comprising an α-helix, a three-stranded anti-parallel β-sheet and three flexible loops. Compared to the structures of C-BP-6 (Headey et al., 2004a), C-BP-1 (Sala et al., 2005) and C-BP-4 (Sitar et al., 2006), which were reported during the course of the current study, the following structural differences that may affect IGF binding and have implications for other functional differences were found: C-BP-2 has (i) a longer disordered loop I, and (ii) an extended C-terminal tail, which is unstructured and very mobile. (iii) The length of the helix is identical to that of C-BP-6 but shorter than that of C-BP-1. (iv) An RGD motif is located in a solvent-exposed turn. The backbone dynamics of C-BP-2 were analysed using the reduced spectral density mapping approach based on the measured backbone amide ^{15}N relaxation parameters (R_1, R_2 and steady-state ^{15}N-{^{1}H} NOE), and the results were compared to the dynamic properties of C-BP-6 (Yao et al., 2004). C-BP-2 possesses significant fast time-scale

motions in the loops and termini, and may also have slow time-scale conformational or chemical exchange in the structured domain core and the loop II.

A more complete set of assignments for IGF-I ^{1}H-^{15}N HSQC spectra was obtained. The IGF and N-BP-2 binding sites on C-BP-2 were identified by NMR. Binding of C-BP-2 to the IGF·N-BP-2 binary complexes was significantly stronger than the binding of C-BP-2 to IGFs alone, switching from intermediate exchange to slow exchange, indicating there is cooperativity between N-BP-2 and C-BP-2 in IGF binding. Two possible structural mechanisms for this cooperativity were found: a possible conformational change of the Phe49-Leu54 region and the sidechain aromatic ring of Phe49 in IGF-I, and direct interaction between the N- and C-domains.

A pH-dependent heparin binding site on C-BP-2 was also identified by NMR. The heparin binding site is a patch containing the β-turn connecting the first and second strands, part of the third strand, and the beginning of the C-terminal tail. Lys227, His228, Asn232, Leu233, Lys234 and His271 are proposed to be the primary heparin binding residues. Protonation of His271 and His228 seems to be important for the binding, which occurs at slightly acidic pH (6.0) and is more significant at pH 5.5, but is largely suppressed at pH 7.4. Possible preferential binding of IGFBP-2 and its C-domain fragments to glycosaminoglycans in the acidic extracellular matrix of tumours may be related to their roles in cancer.