

MS07-P20 | SELECTIVE INHIBITION OF ASTACIN METALLOPEPTIDASES BY MAMMALIAN FETUIN-B

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Mammalian fetuin-A and -B are abundant serum proteins with pleiotropic functions. Fetuin-B is a highly selective and potent inhibitor of metallopeptidases (MPs) of the astacin family, which includes ovastacin in mammals. By inhibiting ovastacin, fetuin-B is essential for female fertility. We determined the crystal structure of fetuin-B, unbound and in complex with archetypal astacin, and found that the inhibitor has tandem cystatin-type modules (CY1 and CY2). They are connected by an exposed linker with a rigid, disulfide-linked “CPDCP-trunk” and followed by an C-terminal region with little regular secondary structure (CTR). The CPDCP-trunk and a hairpin of CY2 form a bipartite wedge, which slots into the active-site cleft of the MP. These elements occupy the non-primed and primed sides of the cleft, respectively, but spare the specificity pocket so that the inhibitor is not cleaved. The trunk aspartate blocks the catalytic zinc of astacin while the CY2 hairpin binds through a QWVXGP motif. Module CY1 assists in structural integrity and the CTR is not involved in inhibition, as verified by *in vitro* studies with a cohort of mutants and variants. Overall, inhibition conforms to a novel “raised-elephant-trunk” mechanism for MPs, which is reminiscent of single-domain cystatins that target cysteine peptidases. Over 200 sequences from vertebrates are annotated as fetuin-B, which underpins its ubiquity and physiological relevance; accordingly, we found sequences with conserved CPDCP and QWVXGP-derived motifs from mammals to cartilaginous fishes. Thus, the raised-elephant-trunk mechanism is likely to be generally valid for the inhibition of astacins by fetuin-B orthologs.