Purification and cloning of an endogenous protein inhibitor of carp nephrosin, an astacin metalloproteinase

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摘要.

Abstract

Nephrosin is a newly discovered member of the astacin family. It is a secreted proteinase and is present in carp head kidney, kidney, and spleen, all of which are responsible for immune and hematopoietic functions in fish. A complex formed by nephrosin and its inhibitor was purified from carp kidney extract by heparin affinity column chromatography. The presence of the nephrosin-inhibitor complex in different tissues was examined by immunoblotting with polyclonal antisera against the purified nephrosin inhibitor and nephrosin. Both nephrosin and the nephrosin inhibitor were present mainly in gill, head kidney, kidney, and spleen. In addition, we have cloned the cDNA encoding the nephrosin inhibitor. There are two different cDNA clones possibly resulting from two different genes, and the long form contains unique tandem repeat sequences in the 3 ´ -end. The deduced primary structure of nephrosin inhibitor is similar to that of fetuin-A, a mammalian protein present in blood, liver, cerebrospinal fluid, and cerebral cortex during fetal development. Treatment with both N-glycosidase F and O-glycosidase removed the carbohydrate moiety of the nephrosin inhibitor and decreased the apparent molecular mass from 40 to 30 kDa. The nephrosin inhibitor seems to be synthesized in liver and then secreted to the blood as a precursor. When it was distributed into hematopoietic tissues, it was processed from 67 to 40 kDa and acquired inhibitory activity. This processing phenomenon of fetuin has not been reported elsewhere. Importantly, the presence of an endogenous inhibitor of nephrosin is the first report of this kind for astacin enzymes. It is very likely that endogenous tissue inhibitors may also be present for the regulation of other astacin enzymes.