（学位第３号様式）

学 位 論 文 要 旨

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題　目	Biochemical Characterization of Tilapia (*Oreochromis niloticus*) Sialidases and their Significance in *Edwardsiella tarda* Infection (ティラピアシアリダーゼの生化学的解析とエドワードジエラタルダ感染におけるその意義)

Various enzymes have been extensively explored in mammals and are now very useful physiological indicators. Sialidases, which cleave sialic acids from glycoconjugates, are among the important proteins in nature that show significant degree of homology, exhibiting a primary structure with highly conserved regions. In humans four sialidases, Neu1, Neu2, Neu3 and Neu4 have been cloned and are used as markers of various physiological aspects. Studies of sialidase in fish are limited and their diversity, evolution and roles are still unclear. Therefore, sialidase exploration was extended to tilapia (*Oreochromis niloticus*), a widely cultured and economically important fish species.

Eight putative tilapia sialidases, two *neu1*-like, designated *neu1a* and *neu1b*, five *neu3*-like designated *neu3a*, *neu3b*, *neu3c*, *neu3d* and *neu3e* and one *neu4*-like were predicted in tilapia genome. Tilapia *neu1* genes amplified from brain cDNA yielded 1164 bp and 1218 bp for *neu1a* and *neu1b* while only three *neu*3 genes *neu3a*, neu3d, and *neu3e* yielded 1,227 bp, 1,194 bp and 1,155 bp, respectively. A single transcript of *neu4* genes was identified constituting 1,497 bp. All tilapia sialidase possessed conserved multiple Asp-boxes (SXDXGXTW), Y(V)RIP and VGPG motifs. Optimal sialidase activities were recorded at pH 4.5 and pH 4.2 for Neu1a and Neu1b, respectively. Neu1a showed preference towards 4-MU-NANA, 3-sialyllactose and colominic acid, while Neu1b showed narrow substrate specificity towards 4-methylumbelliferyl-N-acetyl-α-D-neuraminic acid (4-MU-NANA). Neu3a showed sialidase activity towards ganglioside mix optimally at pH 4.6. Neu3d and Neu3e showed no significant sialidase activities while transcripts for Neu3b and Neu3c were not observed. Neu4 enzymatic profiles were partially conserved with mammalian and medaka Neu4.

To determine the roles of tilapia sialidase in bacterial infection, Neu1a, Neu3a and Neu4 overexpressing cells were infected with *Edwardsiella tarda*. Increase in *E. tarda* infection was observed in Neu1a and Neu4 overexpressing Goldfish scale fibroblast (GAKS) cells, while Neu3a overexpression showed attenuated *E. tarda* infection. To clarify the mechanism of tilapia sialidases involvement in bacterial infection, *E. tarda* and its endogenous sialidase NanA roles in infection were examined. NanA activity assay *in vitro* showed that NanA significantly desialylated 3-sialyllactose and fetuin. GAKS cell pretreated with recombinant NanA showed up-regulation of *E. tarda* infection, suggesting that NanA is critical in *E. tarda* infection. Moreover, sialidase inhibitor-treated *E. tarda* showed a significantly reduced ability to infect GAKS cells. These results indicated that NanA-induced desialylation of cell surface glycoconjugates is essential in the initial step of *E. tarda* infection. GAKS cell treatment with recombinant NanA showed alteration of glycoconjugates only in α2-3 sialo-linked glycoprotein, but not in glycolipids and α2-6 sialo-linked glycoproteins. The up-regulation of *E. tarda* infection by Neu1a and Neu4 was possibly due to desialylation of α2-3 sialo-glycoprotein similar to NanA. The reason for *E. tarda* attenuation due to Neu3a could not be established, however, GM3, a major substrate of Neu3a, promoted *E. tarda* infection. This suggested that GM3 could be one of the key molecules in *E. tarda* infection and that its desialylation by Neu3a could be responsible for reduced *E. tarda* infection. This study has revealed that sialidases are an important group of enzymes, which if explored fully could help monitor and regulated important physiological functions in tilapia and other fish species.