Abstract

Sialidase is a glycohydrolytic enzyme that cleaves sialic acid residues from the non-reducing end of glycoproteins and glycolipids. In addition, desialylation by sialidases regulates cell survival, cell–cell communication, and pathogenesis of infectious diseases. While mammalian sialidase has been well studied, the physiological function of fish sialidase is not well understood. In this study, we focused on Nile tilapia, a member of the cichlid family with diverse morphology and ecology.

Among tilapia sialidase genes, two unidentified *neu1s* (*neu1a* and *neu1b*) and one *neu4* were cloned and analyzed for enzymatic properties. The results showed that tilapia Neu1a and Neu1b were localized to lysosomes similar to mammalian and medaka Neu1. Tilapia Neu1a showed the maximum activity against sialo-oligosaccharide, whereas Neu1b exhibited the activity only against the artificial substrate MU-Neu5Ac.

In contrast, tilapia Neu4 was active at acidic to neutral pH and used gangliosides and sialo-oligosaccharides as substrates. Tilapia Neu4 was localized at nucleus, which was very different from medaka (lysosome) and zebrafish Neu4 (ER).

As nuclear localized sialidase has not been reported to date, we examined the distribution of nuclear sialidase in fish. *In silico* analysis predicted a nuclear localization signal (NLS) in Acanthopterygii Neu4, but their actual localizations were mostly different from nuclear localization. Hence, we analyzed predicted 3D structure of tilapia Neu4. As the result, tilapia

Neu4 was found to possess low complexity (LC) region surrounding its NLS. The Neu4 mutant was generated and analyzed by fluorescence immunostaining and immunoprecipitation, which revealed that its LC region interacted importin, resulting in the nuclear localization. Only Perciformes Neu4 possesses NLS and LC region. Actually, amberjack Neu4 was confirmed to be localized at nucleus.

To investigate the physiological functions of tilapia sialidases during embryogenesis, the gene expression of each sialidase was analyzed by real-time PCR. As the results, each gene showed different expression pattern, suggesting that each sialidase has a different function during embryogenesis, and that tilapia Neu4 plays an important role in neurogenesis. Hence, the tilapia reared under aphotic condition, which exhibited the delay of retina development, showed the reduction of sialidase activity under neutral pH accompanied by the decrease of *neu4* gene expression. Furthermore, tilapia Neu4 accelerated neurite formation in neural cell lines, indicating that tilapia Neu4 is involved in neurogenesis during embryogenesis.