主　論　文　の　要　約

論文題目　Discovery and biological significance of the sialate:*O*-sulfotransferases in vertebrates
（シアル酸:*O*-硫酸転移酵素の発見と脊椎動物における生物学的意義の解明）

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Sialic acids (Sias) are a family of negatively charged, nine-carbon sugars that modify the outermost part of glycan chains of *N*-glycans, *O*-glycans, and glycosphingolipids not only in vertebrates, but also in some types of invertebrates and bacteria. It is well known that Sia is an essential monosaccharide for survival of mammals, modifying glycoconjugates on cell surface and extracellular matrix to mediate and regulate cellular recognition and signaling events in vertebrates. In nature, more than 80 structurally different members of Sia derivatives are known, all of which arise from *N*-acetylneuraminic acid (Neu5Ac), *N*-glycolylneuraminic acid (Neu5Gc), deaminoneuraminic acid (Kdn), and their derivatives carrying one or more substitutions, such as *O*-acetyl, *O*-lactyl, *O*-methyl, *O*-sulfate, or *O*-phosphate groups, at the hydroxyl groups. However, metabolism and biological functions of those modified Sias have not been clarified, except for a limited knowledge of acetylated Sia. Sulfation of Sia residues is known to occur in various animals including mammals and sea urchin. It has been shown that sulfated Sia (SiaS) is involved in sperm-egg interaction and sperm motility in sea urchin. In contrast, nothing is known about the occurrence, biosynthesis, and function of SiaS in vertebrates. No enzyme responsible for sulfation of Sia has ever been described. Thus, the objective of my thesis is to elucidate biological significance of the SiaS. Following general introduction and the specific objective of the thesis in Chapter 1, three lines of experiments were performed: (1) Elucidation of expression profiles of SiaS in vertebrates (Chapter 2); (2) Identification and characterization of the sialate:*O*-sulfotransferases (SulT-Sias) (Chapter 3); (3) Biological significance of SiaS at animal level (Chapter 4). Finally, a summary of results and their significance in this thesis are discussed in general discussion (Chapter 5).

(1) Elucidation of expression profiles of SiaS in vertebrates (Chapter 2): Although only a limited distribution of SiaS has been documented in nature, nothing is known about the occurrence and distribution of SiaS in vertebrates, except in gangliosides from bovine gastric mucosa. First, several mammalian cell lines and tissues from mouse and medaka were analyzed

for the existence of SiaS not only by immunochemical methods (Western blotting and flow cytometry) with monoclonal antibody 3G9 recognizing 8-*O*-sulfated Neu5Ac residue, but also by the chemical method (DMB derivatization-fluorometric high performance liquid chromatography). SiaS were found to ubiquitously occur in vertebrate cells and tissues, although their expression was dynamically changed depending on the cell type and developmental stages. Second, the cell surface expression of SiaS were shown to be dependent on the cell density, as well as on the type of cell lines. Interestingly, SiaS was induced in Chinese hamster ovary (CHO) cells by the antibiotic G418 treatment. Taken together, this is the first observation that the expression level of SiaS is regulated by the intrinsic and extrinsic factors. It is thus suggested that there exists a sulfotransferase responsible for sulfation of Sia, whose gene expression and/or enzyme activation are regulated under various intrinsic and extrinsic factors.

(2) Identification and characterization of the sialate:*O*-sulfotransferases (SulT-Sias) (Chapter 3): No attention has been paid to the occurrence and biosynthesis of SiaS, irrespective of its prominent functional feature in sea urchin fertilization. No enzyme that catalyzes esterification of hydroxyl group with sulfate, or the sialate:*O*-sulfotransferase (SulT-Sia), has ever been found, either. Of those genes for possible sulfotransferases with unknown acceptor substrates, two sulfotransferase gene candidates, SulT-Sia1 and 2, were cloned from mouse. These two genes appear to stem from the same gene, sharing more than 50 % sequence identities. The transfection of CHO cells with these cDNAs showed an increase of the surface expression of SiaS by the immunochemical and chemical methods. On the other hand, when the cDNA for inactive form of SulT-Sia1 with mutations on the donor PAPS (3'-phosphoadenosine 5'-phosphosulfate)-binding domain was transfected, the surface SiaS expression remained unchanged or even decreased. In addition, cell surface SiaS-positive HEK cells that endogenously expressed SulT-Sia1 gene lost the SiaS expression, when transfected with the corresponding a short hairpin RNA (shRNA). Furthermore, *in vitro* activity was measured using recombinant enzymes obtained by over-expression of the SulT-Sia1 and 2. These two enzymes showed the SulT-Sia activity; however, their acceptor substrate specificity was different between them, suggesting that they have different roles in the sulfation of Sia.

(3) Biological significance of SiaS at animal level (Chapter 4): Among the Sia modifications, *O*-acetylation is the most documented on its significance in immune systems, cancer biology, and viral infections. In contrast, only a limited study on biological functions of *O*-sulfation have been done for invertebrates, such as sea urchin and starfish. Sulfated

sialoglycoproteins, flagellasialin, and sulfated gangliosides are implicated in sperm-egg interactions and subsequent signaling at fertilization. However, in vertebrates, almost no study has been performed. To understand biological significance of SiaS at animal level, medaka (*Oryzias latipes*) was chosen as a vertebrate model in which both reverse genetic and biochemical approaches are available. First, inherited frameshift mutations on the SulT-Sia1 or the SulT-Sia2 genes in medaka, SulT1-KO and SulT2-KO, respectively, were established using the gene editing method or the CRISPR/Cas9 technology. Both SulT1-KO and SulT2-KO were found to show low survival rates, and dead at young fry before maturation. They also showed different phenotypes than each other. SulT1-KO had defective in cardiac muscle function, while SulT2-KO were poor in innate immunity and showed delayed development especially in brain and eye. These results indicate that sulfation is one of the most critical modifications of Sia that affects the survival of animal.

Taken together, in this thesis I discovered and identified the sialate:O-sulfotransferases responsible for the biosynthesis of SiaS as the first example in nature, and demonstrated that critical importance of the enzymes in vertebrates.