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As an extension of our interest in KDN-glycobiology, we have initiated studies to determine how these unique KDN-glycans are synthesized. We recently reported identification and characterization of a rainbow trout testis CMP-nonulosonate synthetase which catalyzes efficiently the reaction[1]:

$$\text{KDN} + \text{CTP} \rightarrow \text{CMP-KDN} + \text{PPi}.$$

We have used this enzyme to prepare CMP-[^{14}C]KDN as the donor substrate to search for KDN-transferases and found in the same tissue a highly active CMP-KDN: Galβ1→R α2→3-KDN-transferase. This Golgi-associated enzyme catalyzes the reaction:

$$\text{CMP-KDN} + \text{Gal}\beta1\rightarrow\text{R (acceptor)} \rightarrow \text{KDN}\alpha2\rightarrow3\text{Gal}\beta1\rightarrow\text{R} + \text{CMP}.$$

We have demonstrated the diversity of naturally occurring KDNosylated disaccharide sequences. Thus, KDN is frequently attached in the α-2→3 or α-2→6 linkage to Gal or GalNAc in the terminal sequences of glycoprotein and glycolipid oligosaccharides. KDN is also attached in the α-2→8-linkage to another KDN or sialic acid in the terminal or internal sequences of glycoprotein and possibly glycolipid glycans. Such diversity in KDN-glycan structure allows us to search for a variety of KDN-transferases which facilitate the synthesis of a wider variety of biologically relevant KDN-oligosaccharides and KDN-neoglycoconjugates.

We now report the enzymatic properties of rainbow trout testis CMP-KDN synthetase and α-2→3-KDN-transferase, as well as the results obtained by the use of these two enzymes to prepare α2→3-KDN substituted neoglycans and neoglycoconjugates. We obtained enzymatically several KDN-containing glycoconjugates by transferring the KDN residue(s) from CMP-[^{14}C]KDN to a variety of Neu5Ac-glycoconjugates and asialoglycoconjugates using the KDN-transferase activities.

1. T. Terada, S. Kitazume, K. Kitajima, S. Inoue, F. Ito, F. A. Troy and Y. Inoue (1993) *J. Biol. Chem.*, **268**, in press.

S18.17

Structural Elucidation of the α-2,8-Polysialylglycan Chains in Neural Cell Adhesion Molecules (N-CAM) in Embryonic Chick Brains and Characterization of the α-2,8-Polysialyltransferase Responsible for α-2,8-Polysialylation

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Although glycoproteins bearing α-2,8-linked polysialic acid, poly(Sia), residues have been identified on embryonic neural cell adhesion molecules (*N*-CAM), no detailed studies on the structures of the polysialylglycan chains have been reported. In this study, we first re-examined the structure of the glycans isolated from the embryonic chick brain *N*-CAM glycoprotein. Two glycopeptide fractions (GP-1 and GP-2), both containing poly(Sia) were obtained by pronase digestion, reductive carboxymethylation, re-digestion with pronase, gel filtration, ion-exchange, and gel chromatography. Based on structural analyses including chemical, enzymatic, and instrumental methods, two α-2,8-linked poly(Sia)-containing glycan structures different from that previously reported for the embryonic rat brain *N*-CAM were found. The smaller glycopeptide (GP-2) contained an α-2,8-poly(Neu5Ac) complex-type biantennary glycan. The GP-1 was a polysialylated triantennary glycan chain.

Secondly, we have characterized the enzymatic properties of α-2,8-polysialyltransferase (polyST) in embryonic chick brain, and examined the developmentally regulated expression of this activity. The preference of the polyST from embryonic chick brain and rainbow trout oocytes for exogenous acceptors and sugar nucleotides was also compared. The exogenous acceptor and sugar nucleotide specificity of the chick brain polyST differed from those previously reported for the trout enzyme. While colominic acid, an α-2,8-linked poly(Neu5Ac) was efficiently sialylated by the chick enzyme, the trout enzyme could not utilize this exogenous acceptor. Further, the trout oocyte polyST preferentially used CMP-Neu5Gc as a sugar nucleotide donor whereas this nucleotide was not used as a substrate by the chick brain enzyme.

The developmental expression of the α-2,8polyST during chick embryogenesis was studied and found to reach the highest level in the 15-day embryo. Expression of the polyST activity was correlated with the developmental expression of the poly(Neu5Ac) chains.

S18.18

Diversity in α-2→8-Linked Polysialyl Structures of Animal Polysialoglycoproteins (PSGP). Intra- and Inter-Genus Variation in Polysialic Acid Chains of Salmonid PSGPs

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Polysialoglycoproteins (PSGP) represent a series of α-2→8-linked oligo/polysialic acid-containing glycoproteins. Previous reports of the polysialylation of *N*-CAM and the related PSGP in an increasing number of animals have invariably described only poly(Neu5Ac) structures, and it now tends to be thought that different type of polysialic acid (PSA) structures are not expressed in animal PSGPs. It may be emphasized that the first demonstration of the PSGP molecule of animal origin was the rainbow trout *(Oncorhynchus mykiss)* egg PSGP which consisted solely of oligo/poly(Neu5Gc) residues. In fact, *Oncorhynchus* PSGPs contain only oligo/poly(Neu5Gc) residues. Thus, we studied the polysialylation patterns of PSGPs from 8 different species of 3 genera. Among *Salmonidae* fishes, lake trout *(Salvelinus namaycush)* PSGP represents the first documentation regarding the occurrence of Neu5Ac-containing oligo/polysialic acid chains. It was also shown that brook trout *(Salvelinus fontinalis)* PSGP possesses mixed segments such as