(Neu5Ac, Neu5Gc)$_s$ separated by intervening segments of (Neu5Gc)$_n$ and (Neu5Ac)$_m$ of variable length depending on the particular brook trout PSGP subfraction, and the latter of which, i.e. (Neu5Ac)$_m$ would serve as epitopes for H.46 antibody. The Neu5Ac content in *Salvelinus* fish PSGPs ranges from 0 to 100%. Analysis of the structure of 8 PSGPs has thus shown that they can be classified into at least three groups on the basis of the PSA structures: **(a)** α-2→8-linked poly(Neu5Ac)-containing PSGPs; **(b)** α-2→8-linked poly-(Neu5Ac,Neu5Gc)-containing PSGPs; **(c)** α-2→8-linked poly-(Neu5Gc)-containing PSGPs. Therefore, they offer a model system in which to study the diversity of PSA structures of animal PSGPs. In addition to these PSA forms, we found recently another distinct type of PSA, i.e. α-2→8-linked oligo/poly(KDN) in the glycoprotein isolated from the vitelline envelope of rainbow trout.

In summary, the present study demonstrates that PSGPs from salmonid fish species are oligo/polysialylated in a species-specific manner and appear to contain α-2→8-linked oligo/poly(Sia) with diverse structures. Work is in progress to explore the possible use of these PSGP molecules in immunization against various types of poly(Sia) residues to produce monoclonal antibodies specific to each form of PSA in order to provide useful probes for identifying such PSA structures in animal species other than fish.

S18.19

A Ternary Complex Consisting of Cytochrome B5, CMP-*N*-Acetylneuraminic Acid Hydroxylase and its Substrate is Formed in the Hydroxylation Reaction

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The CMP-*N*-acetylneuraminic acid (CMP-NeuAc) hydroxylation reaction is mediated by an electron transport system including cytochrome b_5 (b_5), b_5 reducing factors and the CMP-NeuAc hydroxylase, which is the soluble terminal enzyme of the system (Kozutsumi, Y., Kawano, T., Yamakawa, T. and Suzuki, A. (1990) *J. Biochem.*, 108, 704–706). In order to study the reaction mechanism of CMP-NeuAc hydroxylase, the interaction between b_5 and the enzyme was elucidated with an affinity column to which b_5 was attached covalently. The enzyme activity from the b_5 column was retarded in the presence of the substrate, CMP-NeuAc, but not in the presence of the product, CMP-*N*-glycolylneuraminic acid (CMP-NeuGc). These findings suggest that a ternary complex consisting of b_5, the CMP-NeuAc hydroxylase and CMP-NeuAc is formed in the CMP-NeuAc hydroxylation reaction and after the conversion of CMP-NeuAc to CMP-NeuGc, the ternary complex is dissociated into each component. Due to the occurrence of b_5 in the soluble and microsomal forms, the molecular form of b_5 expressed in NeuGc-expressing tissues of mouse was determined by the polymerase chain reaction using the cDNAs and specific primers for the amplification of each form of b_5. In mouse liver, only the microsomal form of b_5 mRNA was detected, although both the microsomal and soluble forms of b_5 mRNAs were expressed in the erythrocyte fraction. These data imply that the ternary complex is membrane-anchored through the microsomal form of b_5 in mouse liver, and is both soluble and membrane-bound in the precursor cells of erythrocyte in which NeuGc-containing glycoconjugates are produced.

S18.20

Preparation of Two Kinds of Sialic Acid Specific Monomeric Monovalent Lectin Derivatives and Their Application to the Study of Cell Surface Glyco-Conjugates by Flow Cytometry

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Lectins are multivalent protein/glycoprotein having ability to bind carbohydrates. Therefore, they are able to agglutinate cells and precipitate polysaccharides and glycoproteins. However, the multivalent nature of lectins sometimes causes problems for their application, e.g., to flow cytometry/cell sorting.

We have established the stable subunits of *Sambucus sieboldiana* bark lectin (SSA; specific for Neu5Acα2-6Gal/GalNAc sequence[1]) and *Maackia amurensis* leukoagglutinin (MAL; specific for Neu5Acα2-3Galβ1-4GlcNAc/Glc sequence[2]) by the selective reduction of disulfide bridges between the subunits followed by alkylation with 4-vinyl-pyridine[3]. Both of these derivatives, MSSA and MMAL failed to agglutinate rabbit erythrocytes or CHO cells and also failed to precipitate fetuin. Analysis of their interaction with fetuin using ELISA which does not require the presence of multiple binding sites, however, revealed that MSSA and MMAL still retained the ability to bind carbohydrates including the original specificity toward the sialylated oligosaccharides.

Flow cytometric analysis showed that human histocytic lymphoma U937 cells were clearly stained with FITC-labeled MSSA (FITC-MSSA) without any detectable agglutination. Similarly, FITC-MMAL stained CHO cells too. This staining was inhibited with the oligosaccharides/glycoconjugates corresponding to the binding specificity of these lectins. Both of these monomeric derivatives could be used in a concentration range which gave enough bright staining for the detection whereas the native one suffered from the agglutination to get enough bright staining. Using these two fluorescent probes, differential expression of sialylated glycoconjugates on the cell surface of several cell lines could be shown. These observations demonstrate the usefulness of these monomeric derivatives of sialylated oligosaccharide-specific lectins as probes for analysis of cell surface glycoconjugates containing sialic acid by the technique of flow cytometry.

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