



Jennifer Kohler

Profile

Jennifer Kohler is a chemical biologist studying glycobiology. She obtained her A.B. in Chemistry from Bryn Mawr College in 1994. She attended graduate school at Yale University, receiving her Ph.D. in Chemistry in 2000. Her Ph.D. research was conducted in the laboratory of Prof. Alanna Schepartz and focused on protein-DNA interactions. From 2000-2004, Dr. Kohler was an American Cancer Society postdoctoral fellow with Prof. Carolyn Bertozzi at the University of California, Berkeley. In the Bertozzi lab, she studied the function and modularity of Golgi-resident glycosyltransferases. In 2005, Dr. Kohler established an independent research group in the Chemistry Department at Stanford University and, in 2007, her group moved to the University of Texas Southwestern Medical Center in Dallas, Texas. Currently, she is an Assistant Professor in the Department of Biochemistry at University of Texas Southwestern Medical Center. Dr. Kohler's awards include the Alfred P. Sloan Research Fellowship and the Camille and Henry Dreyfus New Faculty Award. Her research group studies the metabolism of monosaccharides and the function of glycoconjugates, with particular emphases on sialic acids and N-acetylglucosamine (GlcNAc). To conduct these studies, the research group employs a variety of experimental approaches, including chemical synthesis, biochemistry, and cell biology.

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Cellular metabolism of natural and unnatural sialic acids

PROGRAM 12

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Sialic acid is a critical component of many mammalian glycans and serves a variety of roles. Sialylated glycoconjugates mediate essential recognition events in the immune system and also act as receptors for many pathogens and their toxins. Sialylated molecules are abundant in the nervous system, where they coordinate cell-cell interactions. Finally, many recognized tumor markers are composed of sialylated molecules.

Given the critical roles of sialic acid, it is intriguing to note that humans differ from other primates in certain aspects of sialic acid biology. Notably, humans are incapable of synthesizing a hydroxylated version of sialic acid known as N-glycolylneuraminic acid (NeuGc) and make only the non-hydroxylated form, N-acetylneuraminic acid (NeuAc). The NeuGc deficiency in humans is attributed to a truncation in the gene encoding cytidine monophosphate sialic acid hydroxylase (CMAH). Despite their inability to make NeuGc, humans can incorporate this sialic acid into glycoconjugates through dietary acquisition. These NeuGc-containing glycoconjugates can be immunogenic in humans and are implicated in chronic inflammatory states, including those associated with cancer progression. Moreover, the presence of NeuGc-containing glycoconjugates predisposes humans to infectious diseases where the pathogens or toxin bind to NeuGc. Because the incorporation of NeuGc carries negative consequences for humans, we hypothesized that human cells may employ mechanisms to discriminate against NeuGc incorporation. To track the metabolism of NeuAc and NeuGc, we prepared cell-permeable forms of N-acetylmannosamine(ManNAc) and N-glycolylmannosamine(ManNGc). ManNAc and ManNGc serve as metabolic precursors to NeuAc and NeuGc. We discovered that both human and rodent cell lines preferentially metabolize ManNAc to NeuAc-containing glycoconjugates, while less efficiently metabolizing ManNGc to NeuGc-containing glycoconjugates. Discrimination appears to occur during several of the required enzymatic transformations. Notably, the rodent cell line was more efficient at metabolizing ManNGc than the human cell line was. Our results suggest that both humans and rodents may be under evolutionary pressure to exclude NeuGc from sialylated molecules, but that this pressure may be stronger in humans. Our current work in this area focuses on examining the NeuAc/NeuGc specificity of individual sialyltransferases, the enzymes that add sialic acids to glycoconjugates. Future work will delineate the functions of those sialvlated products and reveal why they might be subject to evolutionary forces.

In addition to examining the metabolism of naturally occurring sialylated molecules, we also exploit the sialic acid metabolic pathway to produce unnatural sialylated molecules. In particular, we developed a method to produce sialylated molecules that contain the diazirine photocrosslinking group. In this method, mammalian cell lines are cultured with a cell-permeable form of ManNAc, which has been chemically modified to include an N-acyl diazirine substituent. Cells metabolize the diazirine-modified Man-NAc compound to diazirine-modified sialic acid (SiaDAz) and then add this sialic acid analog to glycoproteins and glycolipids that are displayed on the cell surface. The diazirine can be activated with UV radiation, producing a highly reactive carbene. The carbene then inserts into neighboring molecules, forming a covalent bond between the sialylated molecule and its binding partner. These crosslinked complexes can be isolated and characterized using immunoblots or mass spectrometry analysis. In a proof-of-principle experiment, we demonstrated that a sialic acid binding Ig-like lectin, CD22, could be photocrosslinked to sialylated molecules, which include additional copies of CD22. Currently, we are using metabolically

Figure 1. Metabolic incorporation of natural and unnatural sialic acids. A NeuAc and NeuGc are two common forms of sialic acid. Cells cultured with cell-permeable metabolic precursors of NeuAc and NeuGc preferentially display NeuAc on the cell surface.

B Metabolic engineering enables the introduction of the diazirine photocrosslinker onto sialylated molecules displayed on the surface of mammalian cells. UV radiation activates the diazirine, resulting in covalent crosslinking between sialylated molecules and their binding partners.

incorporated SiaDAz to discover the interaction partners of sialylated proteins and the substrates of sialidases, enzymes that remove sialic acid.

We also demonstrated that SiaDAz is incorporated into gangliosides, sialic acid-containing glycoconjugates that are found in all animal tissues. We investigated whether metabolically incorporated SiaDAz could be used to covalently crosslink a ganglioside-protein complex. The ganglioside GM1a is recognized to be the receptor used by cholera toxin to bind to and invade host intestinal epithelial cells. We induced Jurkat cells to produce SiaDAz-containing gangliosides by culturing these cells with the cell-permeable form of diazirine-modified ManNAc. The cells were incubated with the B subunit of cholera toxin (CTxB) and irradiated to induce crosslinking. We observed effi-

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cient formation of a crosslinked complex containing GM1a and CTxB, which we characterized by immunoblot and mass spectrometry. Current efforts focus on discovering additional binding partners for cholera toxin and other toxins, using metabolic incorporation of SiaDAz in cell lines that mimic the physiological sites of intoxication.

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