New Tools for Studying *O*-GlcNAc Glycosylation and Chondroitin Sulfate Proteoglycans and Studies on the Roles of *O*-GlcNAc Glycosylation on the Transcription Factor CREB

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Abstract

The addition and removal of the monosaccharide *N*-acetyl-D-glucosamine (GlcNAc) to serine and threonine residues of proteins has emerged as a critical regulator of cellular processes. However, studies of *O*-GlcNAc in such complex systems as the brain have been limited, in part due to the lack of tools. Here we report the development of new tools for studying *O*-GlcNAc, and the application of these and other tools for studying the roles of *O*-GlcNAc in the brain.

Working from a previously established chemoenzymatic method, we designed an isotopic labeling strategy for probing the dynamics of *O*-GlcNAc glycosylation using quantitative proteomics. With this tool, we show that *O*-GlcNAc is dynamically modulated on specific proteins by excitatory stimulation of the brain *in vivo*. Separately, we improved this chemoenzymatic strategy by integrating [3+2] azide-alkyne cycloaddition chemistry to attach biotin and fluorescent tags to *O*-GlcNAc residues. These tags allow for the direct fluorescence detection, proteomic analysis, and cellular imaging of *O*-GlcNAc modified proteins. With this strategy, we identified over 146 novel glycoproteins from the mammalian brain.

The transcription factor cAMP-response element binding protein (CREB) is critical for numerous functions in the brain, including neuronal survival, neuronal development, synaptic plasticity, and long-term memory. We show that CREB is highly glycosylated in the brain and discover new glycosylation sites on CREB in neurons. One

of these sites is dynamically modulated by neuronal activity and is important for regulating CREB. Removal of this glycosylation site accelerates axon and dendrite development *in vitro* and long-term memory consolidation *in vivo*. These studies are the first demonstration that *O*-glycosylation at a specific site on a specific protein is critical for neuronal function and behavior.

Chondroitin sulfates (CS) are sulfated linear polysaccharides important in neuronal development and viral invasion. Depending on their sulfation patterns, CS molecules differ dramatically in their functions. We developed a computational method to model the structure and function of CS. Using this approach, we show that different CS tetrasaccharides have distinct solution structures. We also modeled the CS binding site on a variety of proteins and discovered that CS may be important in modulating the interaction between specific growth factors and their receptors.

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