

Glycogen Synthase: An Old Enzyme with a New Trick

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Phosphorylation of glycogen has been known for decades; however, the basic metabolic pathways responsible for this modification are unknown. In this issue, [Tagliabracci et al. \(2011\)](#) report the enzyme responsible for incorporating phosphate and the chemical nature of the phosphate linkage, providing a framework for expanding our understanding of a devastating form of epilepsy.

Glycogen is a highly branched polymer of glucose and the major storage form of energy, from bacteria to mammals. It is composed of glucose residues connected by α -1,4-glycosidic linkages with α -1,6-glycosidic linkages forming branch points (Figure 1). Glycogen synthesis, in concert with its breakdown, is of great metabolic importance, and research in this field has led to the discovery of a variety of fundamental biochemical processes. As early as 1951, McArdle described the first glycogen storage disease, whose hallmark was a buildup of glycogen stores in muscle (McArdle, 1951) caused by a lack of glycogen phosphorylase, an enzyme that at the time was believed to both synthesize and degrade glycogen. This result triggered a search for an enzyme capable of synthesizing glycogen, and in the late 1950s, the Nobel laureate Luis Leloir described the synthesis of glycogen from UDP-glucose and glycogen synthase (Leloir et al., 1959). Since that time, many glycogen storage diseases have been described and the metabolic pathways responsible for glycogen synthesis and breakdown have been exhaustively characterized. Nevertheless, in this issue, [Tagliabracci et al. \(2011\)](#) report a new reaction carried out by glycogen synthase that may be a catalytic “error” underlying the fatal epilepsy, Lafora disease.

Lafora disease was first described by a student of Alois Alzheimer, Gonzalo Lafora (Lafora, 1911). It is an autosomal recessive neurodegenerative disorder resulting in severe epilepsy and death in the third decade. The hallmark of the disease is the accumulation of poorly branched insoluble carbohydrates called Lafora bodies (LBs) that are located in the cyto-

plasm of most cells (Collins et al., 1968). LB accumulation in neurons coincides with neuronal cell death and the number of seizures in patients. Consequently, LBs are hypothesized to be causative for the disease. Characterization of LBs demonstrates that they are composed of glucose polymers varying from normal glycogen in two important ways. First, LBs have the same general composition but contain fewer branch points, making them more similar to the plant starch amylopectin and rendering them insoluble. Second, LBs contain significantly more covalent phosphate than normal muscle glycogen (Tagliabracci et al., 2007). About 50% of Lafora patients have mutations in the *EPM2A* gene, which encodes the dual-specificity phosphatase laforin (Minassian et al., 1998), a glucan phosphatase that removes phosphate from naturally occurring carbohydrates and LBs (Tagliabracci et al., 2007; Worby et al., 2006). However, “how,” “why,” or “where” the phosphate is incorporated into glycogen was unknown. These questions are addressed by Tagliabracci et al. using various systems for analyzing glycogen synthase activity coupled with MALDI-TOF mass spectra and nuclear magnetic resonance (NMR) spectroscopy of purified glycogen.

The phosphate content in glycogen was attributed to C6 monoesters and C1–C6 phosphodiester presumably catalyzed by an uncharacterized UDP-glucose:glycogen glucose 1-phosphotransferase that transferred the β -phosphate of UDP-glucose to glycogen (Lomako et al., 1993). Tagliabracci et al. synthesized [β -³²P] UDP-glucose to assay and purify the proposed UDP-glucose:glycogen glucose 1-phosphotransferase. Unexpectedly, they

observed that a muscle extract from a genetically modified mouse, MGSKO, which lacks the muscle isoform of glycogen synthase, was unable to transfer the β -phosphate from UDP-glucose to glycogen, but a corresponding wild-type mouse muscle extract could. Similar results were seen with a yeast knockout strain lacking both yeast glycogen synthases (*gsy1gsy2*). Indeed, with purified glycogen synthases from multiple sources, the enzyme catalyzed the time-dependent addition of phosphate from UDP-glucose into glycogen at a constant relative rate with respect to glucose incorporation. These results pointed to glycogen synthase as the principal enzyme responsible for the phosphorylation of glycogen and the source of the β -phosphate of UDP-glucose. Glycogen synthase normally adds glucose residues from UDP-glucose to the nonreducing end of a glycogen molecule. However, these unanticipated results suggest that phosphate can also be introduced at a rate of one phosphate per 10,000 glucoses by glycogen synthase. Therefore, “how” the phosphate is incorporated into glycogen, i.e., the elusive glycogen “kinase” activity, has finally been attributed to glycogen synthase.

This raises the question of “where” the phosphate resides on glycogen which addresses the substrate specificity of laforin. In a series of technically demanding experiments, glycogen was purified from skeletal muscle by procedures designed to retain phosphate and was subjected to treatment with glycogen-hydrolyzing enzymes. The resulting phospho-oligosaccharides were subjected to MALDI-TOF mass spectrometry, high-performance anion exchange chromatography, and NMR spectroscopy. From these

experiments, the authors determined that the phosphate present in muscle glycogen was attached to the C2 and C3 carbons of the glucose residues as monoesters (Figure 1). There was no evidence for phosphodiester linkages or a C6 phosphomonoester as had been previously postulated. In addition, since laforin was able to remove the phosphate from these phospho-oligosaccharides, these results provide the first characterization of laforin's substrate selectivity in that laforin can hydrolyze C2 and C3 phosphomonoesters with a minimum requirement of a trisaccharide for activity.

Of the three questions posed above, only "why" the phosphate occurs in glycogen remains. The authors point out that the synthesis of other biopolymers such as DNA or RNA is carefully monitored for misincorporation of bases that results from polymerase error. Indeed, the cell has acquired specialized repair systems that improve the fidelity of DNA and RNA polymerases. Likewise, incorporation of phosphate into glycogen could result from an "error" of glycogen synthase with laforin serving as the repair enzyme. It is important to note that the rate of phosphate incorporation into glycogen ($\sim 10^{-4}$) is in the same range as the error rates for proofreading deficient DNA polymerases (10^{-3} – 10^{-6}). Alternatively, the C2 and C3 phosphates in glycogen could reflect an aspect of glycogen metabolism yet to be discovered. Whatever the case, it is clear that laforin is

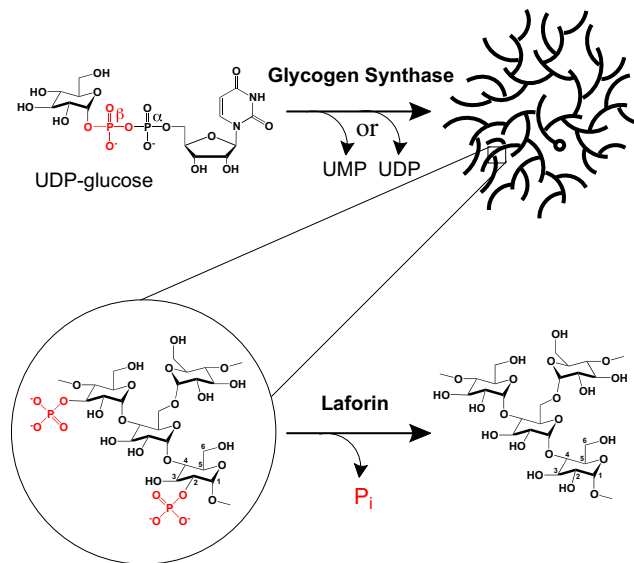


Figure 1. Incorporation of Phosphate into Glycogen

Glycogen synthase synthesizes glycogen by the sequential transfer of a glycosyl moiety from UDP-glucose to a nonreducing end of a glycogen molecule, forming α -1,4-glycosidic linkages concurrent with the release of UDP. Branches are formed by branching enzyme by cleaving an α -1,4-glycosidic linkage, excising a segment of existing oligosaccharide, and reforming an α -1,6 linkage. During synthesis, glycogen synthase adds the β -phosphate of UDP-glucose to the 2' and 3' hydroxyl groups of glucose at the rate of approximately one phosphate every 10,000 glucose residues. In this rare reaction, UMP is released. These phosphate residues are subsequently removed by the phosphatase laforin.

capable of removing these phosphate monoesters from glycogen and that when laforin's activity is lost, glycogen structure is compromised, resulting in insoluble LB.

Even though the molecular etiology of Lafora disease is becoming increasingly clear, what do these results suggest for the treatment of Lafora disease, a fatal disease for which there is no effective therapeutic treatment? The authors point out that MGSKO mice that lack brain glycogen appear to be normal, exercise as well as wild-type mice, and are capable of utilizing glucose while displaying no neurological

symptoms (Pederson et al., 2005). They suggest that inhibition of brain glycogen synthase, which would presumably preclude LB formation in neurons, "might provide a viable therapeutic intervention" for this rare but devastating disease.

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