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Methods for Studying Glycogen Metabolism

Bartholomew A. Pederson[1]

[1] Indiana University School of Medicine-Muncie and Ball State University

Corresponding Author

Bartholomew A. Pederson
bapederson@bsu.edu

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Editorial

Glycogen, a branched glucose polymer, plays an important role in energy metabolism in prokaryotes and eukaryotes. Under conditions of nutrient limitation, bacteria and yeast utilize glycogen for cell survival[1]. In mammals, glycogen has tissue-specific roles, including local use in the muscles to fuel contraction and in the liver to regulate blood glucose levels[2]. The synthesis of glycogen is catalyzed by glycogen synthase, which joins glucose residues *via* alpha 1,4 linkages, and branching enzyme, which forms alpha 1,6 bonds, thus creating branches in the glycogen macromolecule. The utilization of this energy store is facilitated by glycogen phosphorylase in conjunction with the debranching enzyme, resulting in the remodeling of glycogen and the release of glucose-1-P. The structure of glycogen, including the particle size and branching, are of interest in part because the branching impacts the glycogen solubility, the size of the glycogen particles impacts their degradation rate, and glycogen structural instability has been implicated in diabetes. Furthermore, investigating the subcellular localization of glycogen is important in tissues like muscles to yield information about the role of this fuel during exercise.

The accumulation of glycogen is controlled by the relative activities of the glycogen-synthesizing enzymes and those that degrade the macromolecule. Wilson[3] describes techniques to measure the enzymatic activity of glycogen synthase, glycogen phosphorylase, branching enzyme, and debranching enzyme. These biochemical methods measure the production or consumption of NADH/NADPH or colorimetric change with a spectrophotometer. These are scalable approaches that circumvent the high cost, specialized equipment, and regulatory burden associated with the more sensitive method that utilizes radioisotopes. The described methods are most suitable for measuring enzyme activity with purified enzymes, though with sufficient homogenate dilution and proper controls, they have been used with tissue samples. Wilson[3] also describes a simple colorimetric assay for measuring glycogen branching. Though this method yields less information on the fine structure of glycogen than more laborious separation techniques, it does provide a simple and rapid measure of the relative degrees of branching.

Fermont et al.[4] describe the use of fluorophore-assisted carbohydrate electrophoresis (FACE), a method for determining the chain length distribution (CLD), which is a

reflection of the glycogen particle size and the percentage of branching. The structure of glycogen impacts the ability of bacteria to survive starvation, and abnormal glycogen accumulates in several glycogen storage diseases, thus highlighting the importance of methods for studying the size and branching of this fuel source. FACE involves the reductive amination of a hemiacetal group with 8-amino 1,3,6 pyrene trisulfonic acid (APTS). In addition to providing information about the CLD, FACE is useful for measuring the catalytic properties of branching enzymes from various sources. The primary competing technique, HPAEC-PAD, has the advantage of not requiring non-reducing ends, but this technique has a mass bias issue.

The degree of branching and the size of glycogen particles are both important characteristics of the glucose polymer. It has been observed using electron microscopy that glycogen exists in three structures: spherical beta particles (~20 nm), which include several gamma particles (3 nm), and larger rosette-shaped alpha particles (~300 nm) composed of beta particles[5]. Traditional methods for isolating glycogen are sufficient for measuring the glycogen concentration, but they compromise the fine structure of the glycogen, meaning the development of a milder technique is required. A cold-water extraction using sucrose density gradient centrifugation is described for use in bacteria by Wang, Wang et al.[6]. This technique is superior to other methods in preserving both larger glycogen molecules, as measured by size exclusion chromatography (SEC), and longer chains, as measured by fluorophore-assisted capillary electrophoresis (FACE)[6]. Wang, Liu et al.[7] also apply a similar method to the liver. The importance of understanding the glycogen structure is demonstrated by a recent report showing that, in diabetes, liver glycogen alpha particles are “fragile”, which results in faster degradation compared to stable alpha particles, thus possibly impacting blood glucose control. The limitations of these sucrose gradient-based isolation methods include the loss of glycogen in the supernatant, which may be mitigated by a lower sucrose concentration, and the enzymatic degradation of a portion of the glycogen due to the mild conditions used. The latter is mitigated by a brief boiling step, though this disrupts the associated proteins.

Finally, Jensen et al.[8] describe the use of transmission electron microscopy (TEM) to image the glycogen subcellular distribution and particle size in single skeletal muscle fibers. This method is the only one available for investigating subsarcolemmal, intermyofibrillar, and intramyofibrillar glycogen pools and has been used on muscle tissues from a variety of animals. As some subcellular processes preferentially utilize glycogen as a fuel, it is important to characterize the location of these fuel stores. In addition, this method provides the ability to examine glycogen metabolism in distinct muscle fiber types, which is important in exercise, where fatigue is glycogen-dependent in only select fibers. One limitation of the TEM method is the inability to detect small glycogen particles, and this is a concern particularly for conditions under which glycogen is being degraded, such as during muscle contraction.

In conclusion, this methods collection provides a sample of the techniques that are used to probe the structure of glycogen and the enzymes that regulate the anabolism and catabolism of this macromolecule. These methods will be instrumental in improving our understanding of glycogen metabolism in both physiologic and pathologic conditions.

Disclosures

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