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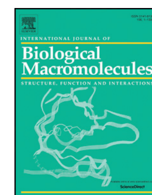
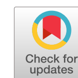


Exploring glycogen biosynthesis through Monte Carlo simulation



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ABSTRACT

Glycogen, a complex branched polymer of glucose (average chain length ~10 monomer units), is the blood-sugar reservoir in humans and other animals. Certain aspects of its molecular structure relevant to its biological functions are currently unamenable to experimental exploration. Knowledge of these is needed to develop future models for quantitative data-fitting to obtain mechanistic understanding of the biosynthetic processes that give rise to glycogen structure. Monte Carlo simulations of the biosynthesis of this structure with realistic macromolecular parameters reveal how chain growth and stoppage (the latter assumed to be through both the action of glycogen branching enzyme and other degradative enzymes, and by hindrance) control structural features. The simulated chain-length, pair-distance and radial density distributions agree semi-quantitatively with the limited available data. The simulations indicate that a steady state in molecular structure and size is rapidly obtained, that molecular density reaches a maximum near the center of the particle (not at the periphery, as is the case with dendrimers), and that particle size is controlled by both enzyme activity and hindrance. This knowledge will aid in the understanding of diabetes (loss of blood-sugar control), which has been found to involve subtle differences in glycogen molecular structure.

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1. Introduction

Glycogen is a complex hyperbranched glucose polymer, with essentially random branching, where individual chains (branches) are formed by α ᴅ glucosyl (anhydroglucose) units through (1 → 4) linkages; these chains are joined by (1 → 6) branch points (there is also a small but significant amount of bound protein [1]). The average chain length is ~10 monomer units. An important function of this molecule is to regulate blood-sugar levels in mammals and other organisms. Glycogen has multiple structural levels: that of the individual chains (branches), that of the whole branched molecules (which when isolated are termed β particles) and lastly tightly bound agglomerations of β particles, termed α particles [2]. Muscles (where there is a need for rapid energy release, *e.g.* during exertion) have more small particles, which degrade relatively quickly to glucose (to be more precise to glucose-6-phosphate, as muscle cells lack glucose-6-phosphatase) [3]. The largest single glycogen reservoir in the body is the liver, where (depending on the time after a meal [4]), glycogen can be found as the much larger α particles, which provide the slow and controlled glucose release needed, for example, during overnight fasting in diurnal animals.

It has been found that the molecular structure of the α particles of liver glycogen are more fragile in diabetic mice as compared to healthy mice [4–6]. Specifically, in the presence of dimethyl sulfoxide (DMSO, which disrupts H bonds), diabetic α particles fragment into smaller β particles *in vitro*, and the rate of enzymatic degradation to glucose of the latter is faster than of intact α particles [3]. This could well be related to the cause, the effect, or both, of the uncontrolled blood-glucose regulation which is a characteristic of diabetes.

Understanding the differences in molecular structure of this complex branched polymer between different samples is thus of significance for human health, given the rising incidence of diabetes worldwide. There are also other, much rarer, pathologies such as the different types of glycogen storage diseases [7] and Lafora disease [8] to which this study is relevant. Appropriate structural information includes the size distribution of the whole branched molecule, and of individual branches (chains – the chain-length distribution, CLD) following enzymatic debranching; accurate data for both of these are readily obtained experimentally. The number CLD is denoted N_{de} because it is found experimentally as the number of chains with a given DP following enzymatic debranching. Information pertaining to the molecular density of

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the whole (branched) molecule can also be obtained after size separation into relatively size-monodisperse samples, *e.g.* by SAXS, and by size-exclusion chromatography with dual detection. Quantitative interpretation of these data could lead to new understanding of the mechanisms of *in vivo* synthesis and degradation, and of the relations between structural features and properties such as degradation rate.

Such interpretation requires appropriate theory. A quantitative model for the CLD has been developed [9,10] for a molecule with many similarities to glycogen, namely amylopectin (one of the two glucose polymers in starch, which like glycogen is highly branched with (1 → 4) α linear links and (1 → 6) α branch points). Application of this model has indeed led to many insights. Given certain simplifications (such as the independence of enzyme activities on the degree of polymerization (DP) of the growing chain), relations for the CLD can be written down in terms of an infinite set of coupled first-order differential equations in terms of a single independent variable, the DP; this is completely analogous to the corresponding relations for the molecular weight distribution of unbranched polymers in free-radical polymerization. The resulting equations are complicated but can be readily solved in a form that is relatively easy to fit to data [9,10].

The main enzymes involved in the biosynthesis of both starch and glycogen (starch/glycogen synthases, branching enzymes and debranching enzymes) have many similarities. However, there are some important biosynthetic differences. The first is that the main starch production and storage organ in a plant, the endosperm, is a very different environment from that in the liver of an animal. The second is the existence of an additional mechanism in glycogen biosynthesis: chain stoppage by hindrance [11–13] as the molecule grows (as also occurs, for example, with synthetic dendrimers [14]); this is not significant in starch because the space between branch points is controlled by one of the starch debranching enzymes. A third difference is that although synthetic "glycogen" can be grown *in vitro* [15], these molecules do not arise from the complex regulatory environment in which animal glycogen is synthesized [16,17], and thus the insight into mechanisms for the *in vivo* biosynthesis of starch by using *in vitro* laboratory models may be less applicable to glycogen than they have been to amylopectin. A fourth problem is that sensitive *in vivo* experimental data on glycogen molecular structure are limited: for example, while good data on structural features such as pair distributions using SAXS have been obtained [18], and good CLD data are available (*e.g.*, work by the present group [11]), these data were not obtained for the same system, and moreover were for systems where there was inadequate size separation of the glycogen molecules in the samples.

These complexities result in the need for some guidance as to how these mechanisms can be put into the development of a model for glycogen CLD that will be analogous to (but significantly more complex than [17]) that previously developed [9,10] for amylopectin. The objective of this paper is to perform a Monte-Carlo simulation of the growth of a glycogen β particle; we do not consider binding of β particles into composite α particles, which involves steps additional to the growth of individual β particles [19]. This will build upon, but go beyond, earlier simulation work. Pioneering simulations were carried out by Melendez and colleagues [13,20–22], but these had a number of restrictions (necessitated in part by the limitations of computational power when this work was done). These included highly simplified inclusion of the enzyme kinetics, of which the understanding has significantly advanced in the intervening decades, and limited account of chain self-avoidance. A reverse Monte-Carlo simulation approach [23] had limitations in the problem of self-avoidance, and was such that the enzyme kinetics were assumed in the model by using actual CLDs (this has led to a useful model for randomly-branched polymers and for interpreting some types of experimental data [24–26]).These simulations did not take into account effects such as restricted access to an enzyme in a particular region due to the enzyme being unable to fit into the available space. The present paper goes beyond previous simulations by implementing a Monte-Carlo simulation of the growth of a glycogen molecule, using current knowledge of the dependences of the biosynthetic enzymes on the DP of the growing chain [10], and realistic inclusion of self-avoidance (which inherently simulates the effects of hindrance of both polymer and enzymes). We carry out semi-quantitative comparisons of the simulation results with extant, albeit limited, available data to see if the model is likely to be a reasonable representation of the actual molecule.

The types of questions which might be answered by these simulations are: whether the CLD attains a steady state in time during growth; how the conformation of chains within the branched molecule varies with total chain DP and the position of individual monomer units on that chain; the dependence of molecular density and of the CLDs on enzyme activities and distance from the center of the particle; and what mechanism stops β particles from growing beyond the observed size range. It is noted that the stoppage of growth of a dendritic polymer is by hindrance [14], and it has been suggested [21] (and often assumed) that the same is true of glycogen β particles. The knowledge from the simulation data could be used in subsequent work to develop heuristic models, as well as to understand experimental data.

2. Simulation method

The basic method is a Monte-Carlo approach to the growth of a whole molecule from a single monomer unit (the action of the starter protein, glycogenin [27], is implicitly included in this step), taking account of the kinetics of addition by propagation (the action of glycogen synthase, GS) and of formation of a branch (by glycogen branching enzyme, GBE). Mathematical evidence suggests [17] that the action of various degradative enzymes such as glycogen phosphorylase (GP), glycogen debranching enzyme (GDBE) and α-glucosidase (GAA) [16] must be considered in biosynthetic models to obtain structures consistent with experimental glycogen CLDs. GP can remove terminal glucose monomers from the non-reducing ends of chains until it reaches a point four residues away from a (1 → 6) branching linkage. GDBE removes chains with a DP of 4, by first removing the three terminal units together and placing these on the terminal end of a new branch, before removing the final unit from the original branch location. The function of GAA is similarly to remove the terminal unit by hydrolysis of (1 → 4) linkages but without the same stopping condition four residues away from a branch point that restricts the action of GP (while GAA can also hydrolyze (1 → 6) linkages at a low rate, this function is neglected in this method). Moreover, GAA is modeled as being unable to act on chains of DP 1 in this treatment, as action on a chain of DP 1 would represent hydrolysis of a (1 → 6) linkage.

The action of GBE is rather complicated. It seems to have the same mode of action as starch branching enzyme (SBE) [28] (which is reasonable given the similarities between starch and glycogen biosynthetic enzymes [29]). These branching enzymes act only on chains greater than some minimum DP (*e.g.* Guan et al. [30]), denoted $X_{\min}$; further, *in planta* studies [31] indicate that the chain remaining after this action of branching enzyme must have at least a minimum number of monomer units, denoted X_0. *In vitro* studies suggest the values for each of these parameters are likely 6 or 7 for GBE [28]. In the present simulations, $X_{\min}$ is set as 7 and X_0 as 6; only chains where DP ≥ $X_0 + X_{\min}$ can be acted on by GBE [28].

2.1. Self-avoiding walk (SAW) model

To take proper account of hindrance in the simulations, it is essential to make the chains self-avoiding. Rather than implementing a general treatment of self-avoidance, we use a lattice model (which enormously reduces the required computational resources, compared to using a spatial continuum), with the polymer growth simulated using a self-avoiding walk (SAW). Variants on this method have been used extensively. For example, Tries et al. [32] have mapped a polymer system in the melt onto a cubic lattice, and found a good quantitative match to

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